274P L-ARGININE ADMINISTRATION DOES NOT IMPROVE ENDOTHELIAL FUNCTION FOLLOWING BALLOON ANGIOPLASTY IN THE FROXFIELD HERITABLE HYPERLIPIDAEMIC RABBIT

C. Greenlees, C.L. Wainwright, R.M. Wadsworth, Deptartment of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW.

Angioplasty-induced vascular intimal injury is characterised by prolonged reduction of endothelial cell nitric oxide release which may be a factor in the development of restenosis (Moodie & Martin, 1991). The objective of this study was to test the hypothesis that supplementation of dietary L-arginine (to provide sustained elevation of NO production) would improve endothelial function following balloon angioplasty in the Froxfield Heritable Hyperlipidaemic (FHH) rabbit.

Nineteen FHH rabbits (16 weeks old) were anaesthetised with halothane/nitrous oxide and subjected to balloon injury of the left subclavian artery as described previously (Hadoke et al, 1995). Test animals were administered L-arginine 2 days prior to and for either 2 (n=6) or 4 (n=6) weeks post-angioplasty in their drinking water (5g/l). All animals were sacrificed 28 days after surgery. To assess the effect of balloon injury and L-arginine on endothelium-dependent relaxation, the left and right subclavian arteries were removed and cut into 3-4 mm rings for isolated blood vessel studies . Contractile responses to 5HT and KCl and relaxant responses to carbachol, calcimycin and Sin-1 were assessed. To assess the anti-aggregatory capacity of the vascular endothelium, blood was taken from the pulmonary artery for collagen-induced whole blood electronic impedance aggreometry studies in the absence and presence of rings from both injured and non-injured vessels as described previously (Greenlees et al, 1995).

In non-treated rabbits 28 days after balloon injury, maximum contractile responses to 5HT and KCl were similar in the injured (1.97 ±0.5 and 2.4 ± 0.56 g tension, respectively) and non-injured (2.7± 0.2 and 2.48 ± 0.3g tension) vessels. Relaxant responses to carbachol and calcimycin were also unaffected by the balloon injury although responses to Sin-1 were significantly greater in the injured vessel compared to the non-injured control (Table 1). Contractile responses to 5HT and KCl were not significantly different in the injured and non-injured vessels with either 2 or 4 weeks administation of L-arginine. L-arginine administration did however significantly impair the ability of the injured subclavian artery to relax to carbachol and calcimycin, although responses to Sin-1 were unaffected (Table 1)

Table 1 **Values as a % max relaxation**

	CONTROL		2 WEEK L-ARG	
	Non-injured	Injured	Non-injured	Injured
Carbachol	58.0 ± 3.2	66.6±14.7	77.8 ± 3.9	31.4±9.4*
Calcimycin	39.8 ± 9.4	46.9±12.9	65.0 ± 10.8	16.4±10.7*
Sin-1	60.5± 3.3	82.9±3.3*	77.7 ± 13.6	59.8±18.6

Values are given as a mean ±sem.; * P<0.05 compared to the non-injured vessel.

The presence of unstimulated rings from injured and non-injured vessels did not affect collagen-induced platelet aggregation. Stimulation of either vessel with carbachol or Sin-1 decreased the extent of collagen-induced aggregation to a similar degree. For example, with carbachol the extent of aggregation was 80.4±7.1% (injured) and 68.4±7,8% (non-injured) of that induced by collagen in the presence of the unstimulated vessel (P<0.05 for non-injured vessel). This demonstrates that the antiaggregatory capacity of the endothelium was not completely impaired 28 days after injury. L-arginine administration did not enhance the anti-aggregatory effect of the vessel stimulated with carbachol or sin-1 relative to the non-stimulated vessel. However, unstimulated vessels from 2-week L-arginine treated animals decreased collagen-induced aggregation. These results illustrate that L-arginine administration as a method for elevating endogenous nitric oxide production does not, in the FHH rabbit, improve endothelial function following balloon injury.

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Moodie, A. & Martin W. (1991) *Pharmacol. Revs.* 43,109-142.
Hadoke, P., Wainwright, C.L., Wadsworth, R.M., Butler, K. & Giddings, M.J. (1995) *Coronary Artery Disease. 6*, 403-415.
Greenlees, C., Wainwright, C.L, Wadsworth, R.M. (1994) Br. J. Pharmacol. 113, 75P.

275P INFLUENCE OF ATHEROSCLEROSIS AND ANATOMICAL POSITION ON LEUKOTRIENE C_4 REACTIVITY IN HUMAN EPICARDIAL CORONARY ARTERIES *IN VITRO*

D.Shah, J.R. Tippins, J.C. Kaski[1] & M. Alison[2], Dept of Biochemistry, Imperial College, London SW7 2AY, [1]St. George's Hospital Medical School, Cranmer Terrace, SW17 ORN, [2]Royal Postgraduate Medical School, Du Cane Rd, W12 ONN.

Vasospasm is commonly associated with an atherosclerotic lesion (Maseri, 1987), however the cause of vasospasm remains ill-defined. Allen *et al.* (1993) have shown that leukotriene C_4 (LTC_4) produces an enhanced contraction in atherosclerotic compared to non-atherosclerotic human epicardial coronary arteries. We have investigated LTC_4 reactivity in human epicardial coronary arteries in relation to the extent of atherosclerosis and anatomical position.

Left anterior descending (LAD) human coronary arteries were obtained from explanted hearts. The patients were diagnosed with either an obstructive lesion or a dilated cardiomyopathy (DCM). The LAD was divided into segments 6, 7 and 8 as set out by the AHA grading committee. 3mm rings were suspended in Krebs solution at 37°C for the measurement of isometric tension. Resting tension was adjusted according to the length/tension relationship of each tissue. Responses to 80mM KCl and either a single dose (10^{-7} M) or cumulative doses (10^{-9} M to 10^{-6} M) of LTC_4 were recorded. Tissues were then processed for light microscopy and intimal/medial ratios were determined.

Maximum responses (E_{max}) to 10^{-7} M LTC_4 in atherosclerotic segment 6, 7 and 8 were 15±4 (n=12), 21±5 (n=12) and 35±12 % KCl (n=10) respectively (p>0.05). EC_{50} values from additional cumulative dose-response curves were 43±12, 16±10 and 6±1nM (n=4; p>0.05). Non-atherosclerotic segment 6, 7 and 8 gave very weak E_{max} values of 0±0 (n=1), 1±1 (n=2) and 1±1 (n=2) respectively. All vessels, except those from DCM patients, showed eccentric atheroma formation. Individual atherosclerotic segments were graded into intimal/medial ratio groups of between 1-5, 6-10 and >11, which had mean E_{max} values (10^{-7} M LTC_4) of 39±13, 18±6 and 0 % KCl respectively, p<0.05.

The results indicate that LTC_4 reactivity in human atherosclerotic LAD is not significantly influenced by anatomical position along the length of the vessel but by the extent of atherosclerosis present in the particular segment.

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Maseri, A (1987) J. Am. Coll. Cardiol. 9, 249-262
Allen, S.P., Dashwood, M.R., Chester, A.H. *et al.* (1993).Cardiosci. 4, 47-54.

276P ROLE OF CALCIUM IN THE ALTERATIONS OF DAMPING CAPACITY IN A RAT MODEL OF ELASTOCALCINOSIS

S. Boutinet, P. Giummelly, C. Capdeville-Atkinson & J. Atkinson, Groupe d'Etude de la Physiopathologie du Vieillissement Cardiovasculaire, Faculté de Pharmacie-UHP Nancy 1, BP 403, 54001 Nancy Cedex, France.

Vascular calcium overload occurring for example in ageing and hypertension increases arterial stiffness and reduces damping capacity. This effect could be due to a change in the wall extra- or intracellular calcium. A technique to study simultaneously the capacity of the rat carotid artery to dampen a phasic pressure signal and intracellular calcium levels has been developed (Boutinet *et al.*, 1994) and applied to a rat model of elastocalcinosis (VDN model; Atkinson, 1992). Two-month old male Wistar rats received vitamin D_3 (300,000 iu/kg im) and nicotine (2x25 mg/kg po) (VDN, n=12), controls (12-month old rats, n=11) received 0.15 M NaCl im and distilled water (5 ml/kg po). Total wall calcium (acid digestion and atomic absorption) was 507±52 and 75±7 µmol/g in VDN and controls, respectively. Carotid artery segments (1.4-cm long) mounted in a spectrofluorometer (Fluorolog F1T11, Spex, USA) and connected to the pulsatile pressurization system, were subjected to increases in mean intraluminal pressure of 25 mmHg at 5-min intervals from 25 to 150 mmHg. Damping capacity was calculated as (pulse pressure at the exit of the arterial segment/pulse pressure at the entry)x100. Carotid segments were next loaded with Fura-2/AM (5 µM) for 105 min at 25 mmHg, and washed with Krebs solution for 30 min (Capdeville-Atkinson *et al.*, 1993). The fluorescent signals were recorded at 340 or 380 nm (excitation) and 510 nm (emission) and their ratio (R') calculated. Noradrenaline (10 µM) was superfused for 10 min followed by Krebs containing 2 µM ionomycin and 4 mM calcium for 7 min, at 25 mmHg mean intraluminal pressure. Results are given as means±s.e.mean in Tables 1 and 2 (* P<0.05 *versus* controls; Student's t test).

Table 1. Carotid damping capacity at increasing mean intraluminal pressures (MIP, mmHg).

MIP	25	50	75	100	125	150
Damping capacity controls	33±3	35±4	65±5	99±5	104±3	106±2
Damping capacity VDN	92±8	97±6	104±2	105±3	103±2	103±2

Table 2. Effects (% baseline) of noradrenaline and ionomycin on damping capacity and intracellular calcium level (R').

Noradrenaline		Ionomycin	
Controls	VDN	Controls	VDN
Damping capacity			
42.8±4.7	1.5±1.6*	37.6±4.1	3.1±1.7*
R'			
32.2±3.8	-0.8±1.0*	404±48	146±47*

With stepwise increases in mean pressure ≤ 100 mmHg the pulsatile pressure signal was dampened in controls but not in VDN (Table 1). Noradrenaline and ionomycin produced an increase in the intracellular calcium signal and a decrease in damping capacity in controls but not in VDN (Table 2). Vascular calcium overload induced by hypervitaminosis D and nicotine leads to a loss in carotid artery damping capacity which involves both extra- and intracellular calcium.

Atkinson, J. (1992) *Drugs* 44, 710-714.
Boutinet, S., *et al.* (1994) *Br. J. Pharmacol.* 112, 587P.
Capdeville-Atkinson, C., *et al.* (1993) *Am. J. Physiol.* 265, C1689-C1702.

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277P NARROWING OF THE CEREBROVASCULAR SECURITY MARGIN IN A RAT MODEL OF ELASTOCALCINOSIS

I. Lartaud-Idjouadiene, C. Capdeville-Atkinson & J. Atkinson, Groupe d'Etude de la Physiopathologie du Vieillissement Cardiovasculaire, Faculté de Pharmacie-UHP Nancy 1, BP 403, 54001 Nancy Cedex, France.

We have suggested previously that a decrease in the distensibility of the common carotid arteries could participate in the age-related impairment of cerebral blood flow (CBF) autoregulatory capacity (Lartaud *et al.*, 1994). In order to test this hypothesis, CBF autoregulation was evaluated in a normotensive rat model of carotid arterial stiffness, induced by arterial elastocalcinosis following treatment of 2-month old, male Wistar rats with vitamin D_3 (300,000 iu/kg, im) plus nicotine (2x25 mg/kg, po; VDN). Such treatment produces a decrease in *in situ* carotid compliance and in carotid dilatory capacity reserve (Atkinson *et al.*, 1994). Basal CBF (ml/min/100g) was measured in the frontal cortex of awake Wistar rats (VDN, n=11; controls, n=15) by the hydrogen clearance technique, and mean arterial blood pressure (MAP, mmHg) *via* a chronically implanted femoral artery cannula. The lower limit of CBF autoregulation (LL, mmHg) was determined following hypotensive hemorrhage (21 ml/kg; Lartaud *et al.*, 1994). The security margin (SM, %) was calculated as (MAP-LL)x100/MAP. The carotid artery calcium content (Calcium, µmoles/g dry weight) was measured by atomic absorption spectrometry. Results (Table 1, means±s.e.mean) show that the security margin was narrower in VDN.

In another series of 11 awake unrestrained VDN rats, arterial blood pressure lability, evaluated as the coefficient of variation (CV, %) of systolic and diastolic arterial blood pressures recorded on-line for 2 h, was found to be substantially increased (CVsystolic: 5.7±0.5, P<0.05 *versus* controls 4.0±0.1, n = 10; CVdiastolic: 8.7±0.7, P<0.05 *versus* controls 5.8±0.3).

In conclusion we would propose the following working hypothesis. Following a decrease in the distensibility of the carotid influx arteries, a diminution in their dilatory capacity leads to a fall in the dilatory reserve capacity of the downstream cerebral arterioles and so attenuates CBF autoregulatory capacity. The increase in lability associated with the narrowing of the cerebrovascular security margin will increase the risk of periodic, hypotension-induced cerebral ischemia. The VDN rat, therefore, may provide an interesting model for the study of aged-linked changes in CBF autoregulatory mechanisms.

Table 1.

Calcium	Basal CBF	Basal MAP	LL	SM
Controls				
33±4	80±2	107±2	70	35
VDN				
507±109*	85±3	105±2	90*	14*

* = P<0.05 *versus* controls (Student's t test).

Atkinson, J., *et al.* (1994) *Am. J. Physiol.* 266, H540-H547.
Lartaud, I., *et al.* (1994) *Am. J. Physiol.* 267, R687-R694.

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278P TERMINATION OF MITOGEN-ACTIVATED PROTEIN KINASE ACTIVITY ON STIMULATION WITH URIDINE TRIPHOSPHATE IN EAhy926 ENDOTHELIAL CELLS

A. Graham, A. McLees, K. Malarkey & R. Plevin. Department of Physiology & Pharmacology, Strathclyde University, 204 George St, Glasgow G1 1XW.

Stimulation of endothelial cells with UTP leads to activation of mitogen-activated protein kinase (MAP kinase) and this is thought to occur through interaction with an extracellular P_{2U} purinoceptor (Graham *et al.*, 1995: O'Connor *et al.*, 1991). Sustained activation is thought to be important in stimulating mitogenesis. Thus stimulation of MAP kinase may have a role in the mitogenic action of nucleotides on endothelial cells. Recently several MAP kinase phosphatases which dephosphorylate and inactivate MAP kinase have been identified (Sun *et al.*, 1993) and we therefore investigated the relative roles of receptor desensitization and MAP kinase phosphatases on termination of the MAP kinase signal in EAhy 926 endothelial cells.

Experiments were performed on EAhy 926 cells cultured on six well plates and rendered quiescent by serum deprivation for 40 h. Desensitization of the MAP kinase response was determined by incubating the cells for 1 h with agonist then washing some cells while leaving others for 1 or 2 h, followed by reapplication of agonist for 2 min. The effect of *de novo* protein synthesis on MAP kinase activation was assessed by pretreating the cells with cycloheximide prior to agonist activation. MAP kinase activation was determined by Western blotting using specific antibodies and *in vitro* kinase assay (BIOTRAK assay kit, Amersham). MAP kinase phosphatase-1 (MKP-1) was identified following immunoprecipitation of [^{35}S]-methionine labelled cells using a specific MKP-1 antibody.

In EAhy 926 cells, UTP stimulated MAP kinase activity 3-4 foldas assessed by in vitro kinase assay in pmol Pi min^{-1} mg protein^{-1} (mean±S.D.) (control - 3.08±0.13; UTP - 2 min 10.64±2.86) with activity returning to basal levels within 1 h, (UTP 60 min - 3.8±0.05) and could not be restimulated when the agonist was left in contact with the cells even up to 3 h after initial stimulation (vehicle - 2.72±0.02, UTP - 3.56±0.14). In contrast if agonist was removed from the cells after 1 h, full MAP kinase activation was seen on restimulation after a further 2 h (vehicle - 2.13±0.1^1; UTP washed - 9.86±1.17). However, phorbol ester stimulation of MAP kinase was unaffected indicating the receptor is desensitized. A similar pattern was seen by Western blotting. Although receptor desensitization is occurring, pretreatment of the cells with cycloheximide (100µg ml^{-1}, 1 h) prolonged the MAP kinase signal in response to UTP, even up to 3 h after addition of agonist indicating that *de novo* protein synthesis is also required for termination of the MAP kinase signal. Antibodies against MKP-1 identified a protein in UTP -stimulated cells which was not present in cells pretreated with cycloheximide. However, induction of MKP-1 alone was not sufficient to maintain a MAP kinase signal unless agonist was present since removal of UTP under these conditions led to loss of activation.

These results support a role for both receptor desensitization and induction of MKP-1 in the transient activation of MAP kinase in response to UTP in EAhy 926 endothelial cells.

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Graham, A., McLees, A., Kennedy, C. & Plevin, R. (1995) Brit. J. Pharmacol. Proceedings supplement (Canterbury).
O'Connor, S.E., Dainty, I.A. & Leff, P. (1991) Trends Pharmacol. Sci. **12**, 137-141.
Sun, H., Charles, C.H., Lau, L.F. & Tonks, N. (1993) Cell **75**, 487-493.

279P PHOSPHORYLATION OF CALDESMON BY MITOGEN-ACTIVATED PROTEIN KINASE WITH NO EFFECT ON CALCIUM SENSITIVITY IN SMOOTH MUSCLE

G.F. Nixon, T.A.J. Haystead#, A.P. Somlyo* & A.V. Somlyo* (introduced by S.D. Logan), Dept. of Biomedical Sciences, Marischal College, University of Aberdeen, Aberdeen AB9 1AS and Depts. of *Molecular Physiology and Biological Physics and #Pharmacology, University of Virginia, Charlottesville, VA 22908, USA.

Caldesmon (CaD), an actin associated protein, has been reported to have an inhibitory effect on smooth muscle contraction (Sobue & Sellers,1991). CaD can be phosphorylated *in vitro* by several enzymes such as mitogen-activated protein kinase (MAPK) (Adams & Hathaway,1993) and phosphorylation has been proposed to reverse the inhibition (Walsh,1991). The purpose of this study was to determine if p42^{mapk} can phosphorylate CaD in permeabilized smooth muscle and alter the calcium (Ca^{2+}) sensitivity of contraction.

The endogenous concentration of p42^{mapk} in intact smooth muscle was estimated to be 2.8µM by comparing immunoblots of portal vein against purified p42^{mapk} standards. Strips (200µm wide) of phasic (rabbit portal vein) or tonic (rabbit femoral artery) smooth muscle were permeabilized in 0.1% Triton-X 100. Strips were incubated in relaxing solution containing 3.3µM recombinant, activated p42^{mapk} and [^{32}P]-ATP for 1h at 24°C, homogenized and proteins separated by 2-dimensional gel electrophoresis. Gels tranferred onto nitrocellulose membranes were exposed to x-ray film and immunoblotted with CaD antibody. Permeabilized strips were incubated as above with p42^{mapk} and activated with Ca^{2+} solutions; pCa 6.5,6.3 and 6.0, while tension was monitored. Controls were treated identically, but had no p42^{mapk}.

In both smooth muscles, p42^{mapk} phosphorylated several proteins compared to controls as revealed by autoradiography of 2-dimensional gels. A phosphorylated protein of molecular weight 140kD was easily visible in both smooth muscles. A protein of the same mobility was also stained in immunoblots with CaD antibody, identifing this phosphorylated protein as CaD. Using these same conditions no change was observed in baseline tension or Ca^{2+} sensitivity of p42^{mapk} treated muscles compared to controls [i.e. portal vein, 0.34±.0.05 (treated) v's 0.36±0.03 (control); femoral artery, 0.75±0.10 (treated) v's 0.77±0.13 (control) - pCa 6.3 responses normalized to maximum contraction, n=3]. The rates of rise of tension were also unchanged [half times for pCa6.5 response, femoral artery: 227±31s (treated) v's 198±18s; portal vein: 156±28s (treated) v's 144±13s, n=3].

We have demonstrated that p42^{mapk} can phosphorylate CaD in permeabilized smooth muscle. However, this had no effect on the Ca^{2+} sensitivity of contraction and, therefore, phosphorylation of caldesmon by p42^{mapk} is unlikely to be a signalling pathway involved in regulating smooth muscle contractile responses.

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Sobue,K. & Sellers,J.R. (1991)J.Biol.Chem.266,12115-12118.
Walsh,M.P. (1991) Biochem. Cell Biol. 69, 771-800.
Adam,L.P. & Hathaway,D.R. (1993)FEBS Lett.322,56-60.

280P USE OF REVERSE TRANSCRIPTION-POLYMERASE REACTION TO IDENTIFY PROSTANOID RECEPTOR mRNA IN HUMAN MYOMETRIUM

Michelle Senchyna & D.J. Crankshaw, (introduced by R.A. Coleman) Dept of Obstetrics and Gynecology, McMaster University, Hamilton, Ontario, Canada.

Pharmacological studies suggest that non-pregnant human myometrium possess all of the currently defined prostanoid receptors, except EP_4 (Senior *et al.*, 1991, 1992; Brown & Crankshaw, 1995). This characterization remains tentative due to the lack of selective antagonists and the fact that most prostanoid agonists have activity at more than one receptor. Additional support for the characterization could come from a demonstration that mRNA for each receptor is expressed. We have investigated the expression of mRNA for human DP, EP_1, EP_2, EP_3, EP_4, FP, IP and TP receptors in non-pregnant human myometrium via reverse transcription (RT) followed by polymerase chain reaction (PCR).

Total RNA from non-pregnant human myometrium was extracted by the method of Chomczynski & Sacchi (1987) and converted to cDNA by RT. The cDNA was amplified by Taq polymerase using oligonucleotide primers designed against portions of each of the reported human prostanoid receptor cDNA sequences. PCR products were analysed by gel electrophoresis.

Initial experiments using sense primers overlapping putative transmembrane regions (PTMR) and antisense primers falling before the sixth PTMR produced a large number of non-specific amplification products, but did suggest the presence of mRNA for all the prostanoid receptors. However, PCR products were also obtained when the RT reaction was performed in the absence of reverse transcriptase. This result suggested that genomic DNA was being amplified. Based on the observation that the TP receptor gene possesses an intron at the end of the sixth PTMR (Nüsing *et al.*, 1993), a second set of primers was designed for each receptor, so that each sense primer fell between PTMRs, and each antisense primer was targeted at the carboxy terminal. In the case of the EP_3 receptor, the antisense primer overlapped each of the currently identified splice variants (Regan *et al.*, 1994).

With this second set of primers we obtained appropriate RT-PCR products for all eight receptors in four separate RNA preparations. The identity of each product was verified by endonuclease digestion. With the exception of the EP_3 receptor message, no PCR products were obtained when the RT reaction was performed in the absence of reverse transcriptase and there were few non-specific products.

We conclude that, despite the lack of functional evidence for EP_4 receptors in non-pregnant human myometrium (Brown & Crankshaw, 1995) it does express EP_4 mRNA, as well as mRNA for DP, EP_1, EP_2, EP_4, FP, IP and TP receptors. The genomic structure of each of these receptors likely possesses an intron at the end of the sixth PTMR. Close attention to primer design is crucial in the use of RT-PCR for the identification of human prostanoid receptor mRNA.

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Brown, C.J. & Crankshaw, D.J. (1995) This meeting.
Chomczynski, P. & Sacchi, N. (1987) *Analyt. Biochem.* **162**, 156.
Nüsing, R.M. *et al.* (1993) *J. Biol. Chem.* **268**, 25253.
Regan, J.W. *et al.* (1994) *Br. J. Pharmacol.* **112**, 377.
Senior, J. *et al.* (1991) *Br. J. Pharmacol.* **102**, 747.
Senior, J. *et al.* (1992) *Br. J. Pharmacol.* **107**, 215.

281P IL-1β INDUCES ARACHIDONIC ACID RELEASE AND CYCLOOXYGENASE 2 EXPRESSION IN A CONCERTED FASHION, BY A COMMON SIGNALLING PATHWAY INVOLVING PLC AND PKC

S.P. Newman, J.D. Croxtall, Q. Choudhury and R.J. Flower.
Department of Biochemical Pharmacology, The William Harvey Research Institute, The Medical College of, St. Bartholomew's Hospital Charterhouse Square, London, EC1M 6BQ, UK.

IL-1ß stimulates production of pro-inflammatory mediators such as arachidonic acid(AA) and PGE_2 (Dinarello, 1988). In A549 cells we have previously shown that treatment with IL-1ß up regulates PGE_2 release and induces cyclooxygenase 2 (Cox 2) (Newman *et al.*, 1994). The signal transduction pathway of IL-1ß receptor stimulation is unclear but activation of PKC, PLC and the activation of nuclear factors such as AP-1 and NF-κB are involved (Dinarello, 1991). Here we demonstrate that IL-1ß signalling in A549 cells involves the activation of PKC, PLC and NFκB.

Human lung adenocarcinoma A549 cells kept in log phase growth.were cultured in DMEM containing 10% fetal calf serum and 1% pen/strep. Prior to experimentation cells were incubated in serum free media for a further 48h. Cytokine-treated cells were lysed with PBS containing 10mM EDTA and 0.1% Triton 100 to extract intracellular proteins. Proteins from treated cells were then analysed by Western blotting using specific primary antibodies (Cox 2 1:2000, $cPLA_2$ 1:2000). Protein bands were visualised with either diaminobenzidine (DAB) or enzyme chemiluminescence (ECL, first Ab 10x DAB dilution) and quantified by densitometric analysis. To measure arachidonic acid release cells were pre labelled with ^3H AA overnight, treated with IL-1ß (1ng/ml) and the amount of labelled AA released into the media measured.

IL-1ß (1ng/ml) induces Cox 2 in A549 cells (Newman *et al.*, 1994), we now demonstrate that this induction can be reversed by various inhibitors of cell signalling, Table 1.

Inhibitor		% change in Cox 2
H7	100μM	-45%
GF 109203X	0.1μM	-40%
D609	10μM	-54%
A.P.	100μM	-46%

Table.1 The effect of inhibitors of cell signalling on the IL-1ß (1ng/ml) induction of Cox 2; percentages are the reduction in densitometric readings relative to IL-1ß controls (n=2-3).

In addition to inducing Cox 2 IL-1ß activates $cPLA_2$ by phosphorylation, as seen on a western blot, leading to an increase in AA release of over 100±7% (4h) reaching a maximum of 400±2% (8h) compared to untreated cells. Both the PKC inhibitors H7 and GF 109203X completely reversed this stimulation of AA release. The PKC activator PMA (10^{-8}M) mimicked the actions of IL-1ß as it induces Cox 2, increases AA release (+500% ±10 after 4h) and phosphorylates $cPLA_2$.

This study demonstrates that IL-1ß acts through protein kinase C to induce Cox 2 and activate $cPLA_2$ with subsequent AA release. The PLC inhibitor D609 inhibits Cox 2 induction which indicates that IL-1ß acts through its receptor to activate PLC which in turn activates PKC. Certain isoforms of PKC activate NF-κB (Siebenlist, 1994). Ammonium pyrroline-dithiocarbamate (AmP; an inhibitor of NF-κP) blocks the induction of Cox 2, suggesting that IL-1ß acts through PKC to activates NF-κB and subsequent Cox 2 gene transcription.

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Dinarello, C. (1988) FASEB J. **2** 108-115.
Dinarello, C. (1991) The cytokine handbook (Thomson, A.W., ed), 47-83.
Newman, S., *et al.*, (1994) BBRC **202**, 931-939.
Siebenlist, U., *et al.*, (1994) Annu. Rev. Cell Biol. **10**, 405-455.

282P CHARACTERIZATION OF THE GALANIN-MEDIATED INCREASE IN INTRACELLULAR CALCIUM ($[Ca^{2+}]_i$) IN THE RAT PANCREATIC ACINAR TUMOUR CELL LINE AR42J

R.D.Pinnock, P.Daum, G.N.Woodruff. & C. E. Otley†. Parke-Davis Neuroscience Research Centre, Addenbrookes Hospital Site, Hills Rd, Cambridge CB2 2QB. †Dept of Pharmacology, Tennis Ct Rd, Cambridge CB2 1QJ

Galanin is a 29 amino acid peptide that was isolated from porcine intestine. It has been shown to be distributed in the intrinsic nervous system of the pancreas and *in vivo* to inhibit amylase secretion. The effects of the peptides galanin (1-29), galanin (1-16) and galanin (21-29) on $[Ca^{2+}]_i$ levels in a transplantable rat acinar tumour cell line (AR42J) were studied using the fluorescent Ca^{2+} indicator Fura-2. The cells were plated at a density of $3x10^5$ cm^{-2} on glass coverslips, loaded with Fura-2 and perfused with a Krebs buffer at 23°C. Drugs were applied via the perfusing solutions. 60 sec application of galanin (1-29) above a threshold of 1nM produced a concentration dependent, transient increase in $[Ca^{2+}]_i$,. The mean pEC_{50} was 8.09 (Range of EC_{50} values 1.39 to 26.5nM n=14). Galanin (1-16) produced a similar increase in $[Ca^{2+}]_i$ but only above a threshold of 3μM. In 4 paired experiments the mean pEC_{50} for galanin (1-29) was 7.99 (range of EC_{50} values 2.8nM to 25.6nM) and the mean pEC_{50} for galanin (1-16) was 5.06 M (range of EC_{50} values 1.27 to to 22.8μM). 60 second applications of galanin (21-29) at concentrations up to 1.0μM did not produce a increase in $[Ca^{2+}]_i$ .The mean pEC_{50} value for galanin (1-29) obtained in these experiments (n=3) was 8.27 (range of EC_{50} values 1.40 to 26.5nM). Galanin 21-29 (1.0μM) applied for a period of 7 minutes had no effect on the size of a control response to galanin1-29. The galanin receptor antagonist galantide (Lindskog *et al* ., 1992) was found to be equipotent to galanin at mobilizing $[Ca^{2+}]_i$. The response to galantide but not pentagastrin or galanin (1-29) could be blocked by the rat NK-1 tachykinin receptor specific antagonist RP 67580 (0.1μM). The galanin receptor antagonist C7 (1.0μM) (Crawley *et al.*, 1992) did not significantly alter the response to galanin (1-29). Supramaximal concentrations of pentagastrin (10nM) or galanin (1-29) (1.0μM) blocked responses to subsequent applications of both galanin and pentagastrin. The response to both peptides could be recovered on prolonged washing and the response to pentagstrin but not galanin was blocked by the CCK_B receptor antagonist CI-988 (1.0μM) (n=3). Perfusion of a Ca^{2+} free buffer caused a slight fall in the resting $[Ca^{2+}]_i$ level and an immediate decrease in the size of the response to galanin (1-29). Ryanodine (10μM) had no effect on the galanin (1-29) response. In contrast the phospholipase C inhibitor U-73122 (1-10μM) produced block of the galanin response, that could not be overcome by supramaximal concentrations of galanin. The effects of the U-73122 did not reverse on washing for 30 min.

Thus the increase in $[Ca^{2+}]_i$ in response to galanin probably resulted from activation of a conventional G-protein linked receptor and second messenger cascade releasing Ca^{2+} from inositol sensitive rather than cADPribose/Ca^{2+}-activated Ca^{2+} stores.

Crawley, J. N., Robinson, J. K., Langel, U. *et al.* (1993), *Brain Res.* **600**, 268.-274
Lindskog, S., Ahren, B., Land, T., *et al.* (1992), *Eur.J.Pharmacol.* **210**, 183-189.

283P STUDIES ON THE UPTAKE OF N^1-ACETYLSPERMIDINE BY HUMAN CANCER CELLS

Heather M Wallace , Ruth E Lock and Kirstie M Nicoll Departments of Medicine & Therapeutics and Biomedical Sciences, University of Aberdeen, Polwarth Building, Foresterhill, Aberdeen AB9 2ZD

The polyamines, putrescine, spermidine and spermine, are important growth regulatory factors found in all living cells (Pegg, 1986). Their intracellular content is regulated at several levels including biosynthesis, catabolism and transport both into and out of the cell. Uptake of the free polyamines is energy and temperature dependent and is saturable suggesting a carrier mediated transport system. In some cells more than one system for polyamine uptake exists: one for spermidine and spermine and one for putrescine (Khan *et al.*, 1991). N-acetylpolyamines are also found in mammalian cells, in particular tumour cells, although little is known of how they enter cells. The aim of this project was to determine whether N-acetylpolyamines are taken up by cells and if they use the same systems as the free amines.

Human colonic cancer cells (HT115) were grown in culture under standard conditions in DMEM supplemented with 10% (v/v) horse serum. Cells were incubated with N^1-acetylspermidine and intracellular content measured by HPLC (Wallace *et al.*, 1988).

N^1-Acetylspermidine was taken up by HT115 cells. Uptake increased from 0 to about 45 min and was temperature dependent being decreased at both 20°C and 4°C (Table 1). When cells were incubated with putrescine and N^1-acetylspermidine, both amines were taken up, suggesting that two separate transporters are used. Analysis of individual polyamine content showed increases in spermidine, spermine and total polyamines in cells treated with N^1-acetylspermidine alone or in combination with putrescine.

Table 1: Temperature dependence of N^1-acetylspermidine uptake in human colonic carcinoma cells

Time (min)	Uptake of N^1-acetylspermidine (nmol)		
	37°C	20°C	4°C
10	0.37 +/- 0.01	0.17 +/- 0.01*	0.13 +/- 0.01*
45	0.43 +/- 0.02	0.32 +/- 0.01*	0.30 +/- 0.03*

Values are mean +/- SD (n=3) *p < 0.01 compared with 37°C

Results show that N^1-acetylspermidine is taken up by human tumour cells, possible in an energy dependent manner and the transporter is probably separate from that of other polyamines.

Khan, N.A., Quemener, V. & Moulinoux, J.P. (1991) *Cell Biol. Int. Rep.* **15**, 9-24
Pegg, A.E. (1986) *Biochem. J.* **234**, 249-262
Wallace, H.M., Nuttall, M.E. & Robinson, F.C. (1988) *Biochem. J.* **253**, 223-227

284P EFFECTIVE REFRACTORY PERIOD AND CONTRACTILITY OF ISOLATED CARDIAC MUSCLE IN THE PRESENCE OF 5-HT_2 AND THROMBOXANE ANTAGONISTS, ALONE AND IN COMBINATION

Susan J. Coker & Andrew J. Batey, Department of Pharmacology & Therapeutics, University of Liverpool, Liverpool L69 3BX.

Previously, it has been reported that combined administration of a 5-HT_2 antagonist and a thromboxane antagonist significantly reduced reperfusion-induced arrhythmias in anaesthetized rats, whereas the effects of either drug administered alone were not significant (Shaw & Coker, 1992). Further experiments have now been performed to determine whether the drugs have direct effects on cardiac muscle which could explain their antiarrhythmic activity when administered in combination. Male Wistar rats (270g to 395g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.). Hearts were removed rapidly and left ventricular papillary muscles and right ventricular strips were dissected free, and set up in a Krebs solution (mM: NaCl 119, KCl 3.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 10, $CaCl_2$ 1.9) in organ baths maintained at 37°C and gassed with 95% O_2 / 5% CO_2. Preparations were suspended under a resting tension of 1g, paced at 1Hz with square wave pulses of 5ms duration at twice threshold voltage and developed tension measured isometrically. After equilibration for 1 hr, the effective refractory period (ERP) was measured by interpolation of extrastimuli. Three control measurements of ERP were obtained at 5 min intervals, and then at 5, 10 and 15 min after the addition of each concentration of drug. In each preparation a concentration response curve was constructed to either the 5-HT_2 antagonist ICI 170,809 (Blackburn *et al.*, 1988), or the thromboxane antagonist ICI 192,605 (Jessup *et al.*, 1988) given alone or in the presence of a fixed concentration of the other drug.

In left papillary muscles, concentrations up to 10^{-4}M ICI 192,605 did not alter the developed tension (230±40 to 220±40 mg) or ERP (see Figure 1) whereas ICI 170,809 alone and in the presence of 10^{-4}M ICI 192,605 increased ERP. Measurements of ERP could not be obtained at 10^{-4}M ICI 170,809 because of reductions in developed tension. Similar results were obtained in right ventricular strips.

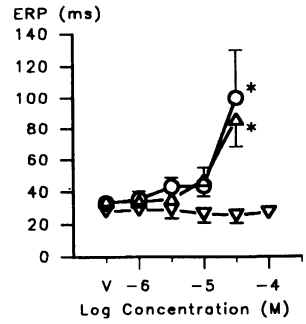

Figure 1. ERP measured 10 min after addition of vehicle (V) then increasing concentrations of ICI 170,809 (△), ICI 192,605 (▽) and ICI 170,809 in the presence of ICI 192,605 10^{-4}M (○) in isolated left papillary muscles (n=6). Values are means with s.e. mean. *P<0.05 compared with corresponding vehicle control, Wilcoxon test.

Although the 5-HT_2 antagonist ICI 170,809 increased ERP this effect was not significantly enhanced in the presence of the thromboxane antagonist ICI 192,605. These results suggest that direct effects of the drugs on cardiac muscle are unlikely to account for the significant antiarrhythmic activity observed previously with combined drug administration.

Blackburn, T.P., Thornber, C.W., Pearce, R.J. & Cox, B. (1988) *FASEB J.*, **2**, A1404.
Jessup, C.L., Jessup, R. & Wayne, M. (1988). *Br. J. Pharmacol.*, **95**, 676P.
Shaw, L.A. & Coker, S.J. (1992) *Br. J. Pharmacol.*, **107**, 126P.

285P THE EFFECT OF 8-EPI PROSTAGLANDIN $F_{2\alpha}$, AN F_2 ISOPROSTANE, ON PORCINE AND OVINE CORONARY ARTERIES *IN VITRO*

B.M.Kromer & J.R.Tippins, Department of Biochemistry, Imperial College of Science, Technology & Medicine, London SW7 2AY.

8-epi $PGF_{2\alpha}$, an isoprostane produced *in vivo* by non enzymatic oxidation of arachadonic acid, has been shown to be present in human plasma and urine (Awad *et al.*, 1993) and to be a potent renal and pulmonary vasoconstrictor. 8-epi $PGF_{2\alpha}$ also constricts bovine coronary arteries, an action which is reported to be mediated via thromboxane A_2 (TxA_2) receptors (Ogletree, 1992). In an attempt to elucidate further the mechanism of action of this novel compound within the coronary vasculature, the effects of a thromboxane mimetic (U46619), 8-epi $PGF_{2\alpha}$ and $PGF_{2\alpha}$ were compared on porcine and ovine coronary arteries.

3 mm rings of segments 6, 7 and 8 (AHA Grading Committee, 1975), mean circumferences 11.56±0.63, 9.35±0.92 and 6.76±0.59mm (n=8) and 13.02±0.39, 9.81±0.68 and 7.05±0.72mm (n=14) respectively, of the left anterior descending coronary artery from ovine and porcine hearts were suspended in Krebs at 37°C. Tension was adjusted by the length/tension relationship for each tissue and responses to 40mM KCl recorded, followed by cumulative concentration-response curves for U46619, 8-epi $PGF_{2\alpha}$ and $PGF_{2\alpha}$. U46619 produced concentration-dependent contraction in both ovine (EC_{50} values: 538±100, 363±115 and 383±9nM, (n=4) for segments 6, 7 and 8 respectively) and porcine rings (EC_{50} values: 18±1, 18±3 and 29±8nM, (n=7) for segments 6, 7 and 8 respectively). 8-epi $PGF_{2\alpha}$ and $PGF_{2\alpha}$ caused concentration-dependent contraction of porcine coronary artery rings, with EC_{50} values of 560±98, 331±115 and 613±168nM (n=7) and 900±84, 910±422 and 1790±639nM (n=7) for segments 6, 7 and 8 respectively. However, at concentrations up to 10μM, neither 8-epi $PGF_{2\alpha}$ nor $PGF_{2\alpha}$ produced a response in ovine coronary arteries. Prior incubation with 2.8μM indomethacin had no effect on responses to any of the agonists in either species.

Since neither 8-epi $PGF_{2\alpha}$ nor $PGF_{2\alpha}$ contracted the ovine coronary artery, these results suggest that coronary artery contraction induced by 8-epi $PGF_{2\alpha}$ is either independent of the thromboxane receptor, or a consequence of its partial agonist activity. Although many actions of isoprostanes have been reported to be inhibited by TxA_2 receptor antagonists, the responses may be mediated through receptors other than the TP receptor.

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AHA Grading Committee (1975) Circulation 51, 7-40
Award J.A., Morrow J.D., Takahashi K. *et al.* (1993) J. Biol. Chem. 268, 4161-4169
Ogletree M. (1992) Circulation 86, I-298

286P BENEFICIAL EFFECTS OF Z1046, A SELECTIVE DOPAMINE RECEPTOR AGONIST, DURING MYOCARDIAL ISCHAEMIA

J.R. Parratt*, A. Vegh, C. Semeraro+ & J. Gy Papp, Department of Pharmacology, Albert Szent-Gyorgyi Medical University, Szeged, Hungary, and *Department of Physiology and Pharmacology, University of Strathclyde, Glasgow +Cardiovascular Research, Zambon Group, Milan, Italy.

Z1046, (S)-6-[(6-[(2-(2-methoxyphenoxy)ethyl]amino]hexyl] propylamino]-5,6,7,8-tetrahydro-1,2-naphtalene-diol diHCl) is a new dopamine agonist with both D_1 and D_2-like activities (Pocchiari et al., 1994) which reduces arterial blood pressure and increases renal and femoral blood flows in anaesthetised dogs (Marchini et al., 1994). Because stimulation of D_2-like receptors inhibits neuronal noradrenaline (NA) release, and because of the involvement of NA release in the mechanisms of early ischaemia-induced, life-threatening ventricular arrhythmias, we have investigated the effects of this compound in a large animal model of myocardial ischaemia and reperfusion.

25 dogs in excess of 17 kg (mean weight 24.2 ± 1.4 kg; 17 male, 8 female) were anaesthetised with a mixture of chloralose and urethane (60 and 200 mg kg^{-1} respectively given i.v.) ventilated with air, subjected to a left thoracotomy and the anterior descending branch of the left coronary artery (LAD) prepared for occlusion. Epicardial ST-segment changes and the degree of inhomogeneity of activation were measured from the left ventricular wall distal to the proposed occlusion as previously described (Vegh et al., 1992). Z1046 was given in a dose of 10 μg kg^{-1} over a 2 min period and the coronary artery occluded 30 min later. The occlusion time was 25 min and the ischaemic area was then reperfused. Z1046 lowered mean arterial blood pressure (from 94±6 mmHg to 72±7 mmHg just prior to occlusion), heart rate from 132±7 to 115±7 beats min^{-1}) positive LVdP/dt (from 2780±207 to 1956±202 mmHg s^{-1}) and left circumflex coronary flow (117±7 ml min^{-1} to 84±4 ml min^{-1}). The above haemodynamic changes were significant at a level of $P < 0.01$.

When the LAD coronary artery was occluded in dogs administered Z1046 the severity of ventricular arrhythmias was markedly reduced compared to controls (e.g. ventricular premature beats 40±23 over the 25 min occlusion period versus 435±72 in the controls ($P < 0.05$), episodes of ventricular tachycardia (VT) 0.4 ± 0.3 versus 7.8 ± 2.4 episodes per dog ($P < 0.01$), a lower incidence of both VT (56% versus 88%) and ventricular fibrillation (18% versus 43%) and a higher survival (36% versus 7%). Other indices of ischaemia severity were also reduced (e.g. the change in the degree of inhomogeneity at 5 min was 132±16 ms compared to 185±15 ms in controls ($P < 0.05$) and the increases in ST-segment elevation at the same time were 13±2 mV versus 17.8±1.0 mV in the controls ($P < 0.05$).

Z1046 thus has marked cardioprotective effects, presumably due to inhibition of noradrenaline release through activation of presynaptic dopamine receptors.

Pocchiari, F., Allievi, L., et al. (1994) Canad. J. Phys. Pharm. 72, Suppl 1, 132.

Marchini, F., Pradella, L., Miragoli, G., Semeraro, C. (1994) Canad. J. Phys. Pharm. 72, Suppl 1, 131.

Vegh, A., Komori, S., Szekeres, L., Parratt, J.R. (1992) *Cardiovascular. Res.* 26, 487-495.

287P THE EFFECT OF LODOXAMIDE, A CARDIAC MAST CELL STABILISER, ON MYOCARDIAL ISCHAEMIC PRECONDITIONING IN THE RAT

R.A. Humphreys, K.A. Kane & J.R. Parratt, Department of Physiology and Pharmacology, University of Strathclyde, 204 George Street, Glasgow Gl lXW.

It has been suggested that release of mediators from cardiac mast cells may play a role in the phenomenon of myocardial ischaemic preconditioning (Linden, 1994). The aim of this study was to investigate whether stabilization of cardiac mast cells modified the antiarrhythmic effect of preconditioning in anaesthetised rats. Male Sprague Dawley rats (300 - 350 g) were anaesthetised with pentobarbitone sodium (60 mg kg^{-1} i.p.) and, following thoracotomy and commencement of artificial ventilation the left coronary artery was occluded for either 30 min (control) or 3 min followed by 10 min reperfusion before the permanent 30 min occlusion (preconditioned; Vegh *et al.*, 1992). In some animals lodoxamide, tromethamine (20 mg kg^{-1} h^{-1} i.v.)was infused for 30 min before and throughout the occlusion period to stabilise cardiac mast cells (Jolly *et al.*, 1982). The severity of arrhythmias occuring during the 30 min occlusion period, heart rate and mean arterial blood pressure were measured. Data is expressed as mean ± s.e.m. or as a % incidence. The incidences of arrhythmias were compared using a Fishers exact test and mean data compared using a Student's 't' test. A P value of less than 0.05 was considered to be significant.

In untreated rats, preconditioning had a marked antiarrhythmic effect reducing significantly the number of ectopic beats from 1232 ± 105 (n = 10) to 490 ± 139 (n = 8) and the incidence of ventricular fibrillation from 40 to 0%. In non-preconditioned animals administered lodoxamide neither the number of ectopic beats (1208 ± 90 n = 8) nor the incidence of ventricular fibrillation (75%) was significantly different from control rats. In lodoxamide treated rats, preconditioning still had a pronounced antiarrhythmic effect, reducing the number of ectopic beats to 603 ± 105 (n = 8) and the % incidence of ventricular fibrillation to 20%. Heart rate and mean arterial blood pressure prior to coronary artery occlusion were similar in all groups of animals. These values in control animals were 340 ± 16 beats min^{-1} and 112 ± 8 mmHg respectively. The haemodynamic consequences of coronary artery occlusion and preconditioning were also similar in all groups and consisted of a transient fall in mean arterial blood pressure but no change in heart rate. It is concluded that stabilization of cardiac mast cells with lodoxamide tromethamine does not modify the antiarrhythmic effect of preconditioning in anaesthetised rats.

We thank Upjohn Pharmaceutical Company, Kalamazoo, USA for the gift of lodoxamide tromethamine.

Linden, J. (1994). *TIPS* 15, 298-306.

Jolly, S.R., Abrahams, G.D. & Ramson, J.L. (1982). *J. Cardiovasc. Pharm.* 4, 441-448.

Vegh, A., Komori, S. *et al.* (1992). *Cardiovasc. Res.* 26, 487-495.

288P DELAYED MYOCARDIAL PROTECTION BY PACING-INDUCED PRECONDITIONING: A POSSIBLE ROLE FOR BRADYKININ

A. Vegh, K. Kaszala, J. Gy Papp, J.R. Parratt*, Dept of Pharmacology, Albert Szent-Györgyi Medical University Szeged, Hungary and *Dept of Physiology & Pharmacology, University of Strathclyde, Glasgow. G1 1XW

Preconditioning can be defined as the cardioprotective effect of short periods of ischaemia on the consequences of a subsequent more prolonged period of ischaemia. This protection is only short-lasting (usually < 1h) but returns several hours later ('second window' of protection; Yellon & Baxter 1995). This delayed protection can also be induced by rapid ventricular pacing (Vegh *et al.* 1994a).

There is some evidence (Vegh *et al.* 1994b) that bradykinin is involved in classical preconditioning in dogs. We aimed to determine whether it plays any role in the delayed protection afforded by cardiac pacing. Dogs (mean weight 28 ± 4 kg; 16 male, 8 female) were lightly anaesthetised and paced for four 5 min periods at a rate of 220 beats min^{-1} by way of a pacing electrode in the right ventricle. In some dogs the bradykinin B_2 receptor blocking drug icatibant (300 $\mu g\ kg^{-1}$ i.v.) was given 10 min prior to pacing. The dogs were allowed to recover from the anaesthetic and the following day were re-anaesthetised (chloralose and urethane, 60 and 200 mg kg^{-1} i.v.) thoracotomised and the left anterior descending coronary artery (LAD) occluded for 25 min (Vegh *et al.* 1994b).

In control dogs (n=8) there was marked ventricular ectopic activity following occlusion (ventricular premature beats (VPBs) 474±139; episodes of ventricular tachycardia (VT) 9.4±5.4; incidence of fibrillation (VF) on occlusion 63% and survival from the combined ischaemia-reperfusion insult 13%). In contrast, those dogs paced in the absence of icatibant had significantly ($P<0.05$) less severe arrhythmias when the LAD was occluded 24h after pacing (VPBs 77 ± 32; VT episodes 3.5±3.1; VF on occlusion 10% and survival 60%). Those dogs paced in the presence of icatibant and occluded 20-24 h later had rather more VPBs (185±73), a higher incidence of VF on occlusion (43%) and a lower survival (29%). This difference was mirrored by changes in epicardial ST-segment elevation and in the inhomogeneity of activation within the ischaemic area (Vegh *et al.* 1994b) Thus, the ST-segment changes at 25 min were 17.9±2.9 mV in the controls, only 9.1±1.0 mV ($P<0.01$) in the paced dogs but 15.3±1.8 mV (ns) in those dogs paced in the presence of icatibant. The changes in inhomogeneity at this time were respectively 97±22 ms, 46 ± 3 ms ($P<0.01$) and 105±5 ms (ns).

These results suggest that early bradykinin release is a trigger for the delayed protective effects of cardiac pacing perhaps by releasing nitric oxide and prostacyclin and/or the translocation of protein kinase C to the membrane (Parratt 1994).

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Parratt, J.R. (1994) *Trends in Pharmacol. Sci.* 15, 19-25.
Vegh, A. *et al.*(1994a) *Br. J. Pharmacol.* 113, 1081-1082.
Vegh, A. *et al.* (1994b) *Br. J. Pharmacol.* 113, 1167-1172.
Yellon, D., Baxter, G. (1995) *J. Molec. Cell. Cardiol.* 27, 1023-1034.

289P EXAMINATION OF THE CARDIOVASCULAR EFFECTS OF WAY-100802, A SELECTIVE 5-HT_{1A} ANTAGONIST, IN ANAESTHETIZED CATS

A.G. Ramage & V. Mirtsou-Fidani[1], Departments of Pharmacology, Royal Free Hospital School of Medicine, London NW3 2PF, U.K. and [1]Aristotelian University, 54635 Thessaloniki, Greece

WAY-100802 [(R)-2,3,4,5,6,7-hexahydro-1(4-(1-(4-(2-methoxyphenyl)piperazinyl))-2-phenyl)butanoyl-1H-azepine] HCl has a high affinity for 5-HT_{1A} binding sites (IC_{50} 1.4 nM cf. α_1-adrenoceptor 682 nM) and antagonises the action of 5-carboxamidotryptamine in the guinea-pig ileum with a pA_2 of 9.1 (cpd 49; Cliffe et al., 1993) indicating that it is a highly selective 5-HT_{1A} antagonist. This study was carried out to investigate its cardiovascular actions and to determine if it could reverse the central sympathoinhibitory actions of the selective 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin, 8-OH-DPAT.

Male cats (2.5-3.4 kg) were anaesthetized (i.v.) with a mixture of α-chloralose (70 mg kg^{-1}) and pentobarbitone sodium (6 mg kg^{-1}) and artificially ventilated after neuromuscular blockade with vecuronium bromide (200 $\mu g\ kg^{-1}$). Simultaneous recordings of whole nerve activity were made from the inferior cardiac (CNA), splanchnic (SNA) and renal nerves (RNA) along with brachial arterial pressure (BP), heart rate (HR) and femoral arterial flow from which conductance (FAC) was derived (Ramage & Wilkinson, 1989). The above variables were recorded for 20 min before a cumulative dose (30 μg - 3 mg kg^{-1};i.v.; n = 5) response curve was constructed for WAY-100802. In another set of experiments (n=5) 64 $\mu g\ kg^{-1}$ i.v. of 8-OH-DPAT was administered and 5 min later cumulative doses of WAY-100802 (10-100 $\mu g\ kg^{-1}$) were given i.v. In two experiments the time course of the effects 8-OH-DPAT were followed and were found to remain constant for at least 60 min. Changes caused by drugs were compared to normalised baseline values using a one way ANOVA and the least significant difference test; $P<0.05$ was considered significant.

WAY-100802, after 300 $\mu g\ kg^{-1}$, caused a significant fall in BP decreasing to 28 ± 7 mmHg (mean ± s.e.mean) by 3 mg kg^{-1}. This was associated with an increase in FAC of 67 ml min^{-1} $mmHg^{-1}$ x 10^{-3} and a decrease in activity in all the nerves, CNA -52 ± 11 %; SNA, -48 ± 11% and RNA, -11 ± 49%. However, only changes in CNA and SNA were significant and after the 300 $\mu g\ kg^{-1}$ dose. At all doses WAY-100802 had little effect on HR. 8-OH-DPAT caused falls in BP of 56 ± 7 mmHg, HR of 58 ± 10 beats min^{-1}, CNA, 77 ± 6%, SNA 89 ± 7%, RNA 95 ± 3% and no change in FAC. After a dose of 30 $\mu g\ kg^{-1}$ WAY 100802 the changes in these variables caused by 8-OH-DPAT had returned to near baseline values i.e. 15 min later. WAY 100802 alone also caused a dose related transient decrease in BP and increases in HR, sympathetic nerve activity and FAC.

These experiments indicate that WAY-100802 is a potent 5-HT_{1A} antagonist which has minimal cardiovascular effects up to a dose of 300 $\mu g\ kg^{-1}$ and above that causes sympathoinhibition and a fall in BP which could be attributed to its affinity for α_1-adrenoceptors. WAY-100802 also has an unexpected transient vasodilator action. The present data indicates that blockade of central sympathoinhibitory 5-HT_{1A} receptors does not produce central sympathoexcitation, as observed for central sympathoinhibitory α_2-adrenoceptors (Tomlinson & Ramage, 1985). One interpretation of this data is that central 5-HT_{1A} receptors involved in the control of sympathetic tone are not under tonic activation in anaesthetized cats.

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Cliffe,I.A., Fletcher,A & Dourish,C.T.(1993)Curr. Drugs 1, 99-124
Tomlinson,A & Ramage,A.G..(1985)Eur.J.Pharmacol. 109, 153-160
Ramage,A.G. & Wilkinson,S.J.(1989)Br.J. Pharmacol. 98, 1157-1164

290P INVOLVEMENT OF A 5-HT$_1$ RECEPTOR IN THE RESPONSE TO 5-HT IN THE FIRST BRANCH PULMONARY ARTERY OF THE RAT

C Brown & A.M. Shaw, Department of Biological Sciences, Glasgow Caledonian University, Cowcaddens Road, Glasgow G4 OBA.

Contractile responses of the first branch pulmonary artery of the rat to 5-hydroxytryptamine (5-HT) are relatively insensitive to ritanserin and methysergide (Brown & Shaw, 1995). This study investigated the role of 5-HT$_1$ receptors in the contractile response to 5-HT in this vessel. This study examined the contractile responses to 5-carboxytryptamine (5-CT), sumatriptan and α-methyl-5-HT (5-HT$_1$ agonists). The effect of methiothepin, a 5-HT$_1$ receptor antagonist, on the concentration response curve to 5-HT was also examined. Since 5-HT$_1$-mediated contractile responses are tone-dependent the responses to 5-HT and the 5-HT$_1$ agonists were examined in the absence and presence of tone. The study also examined the effect of 5-HT on the cyclic AMP content of this artery.

Male Wistar rats (250 - 300 g) were killed by intraperitoneal injection of sodium pentobarbitone (100 mg kg $^{-1}$). Artery rings, with an intact endothelium, (1.2 - 1.5 mg) from the first branch pulmonary artery were suspended on stainless steel hooks in Krebs buffer (37°C) under a resting tension of 1 g and gassed with a mixture of O_2:CO_2 (95%/5% v/v). The tissues were allowed to equilibrate for 1 hour before the addition of agonists. To examine the effect of methiothepin two concentration response curves (CRCs) to 5-HT were performed, the second in the presence of the antagonist. Untreated tissues acted as time controls. In some tissues U46619 (0.2 nM) was added to produce an increase in the basal tone. cAMP was measured after incubation with 5-HT for 2 min and in the presence of IBMX (10μM). In some experiments forskolin (100nM) was added 1 min. after the addition of 5-HT. The rings were then rapidly immersed in liquid nitrogen. Cyclic nucleotides were extracted in ethanol (90%) and measured by RIA. Results are expressed as the mean values ± s.e. mean. The significance of differences was determined using the Student's *t*-test. 5-HT (0.1μM-1mM) produced a concentration-dependent contractile response which was inhibited by methiothepin (1nM-10nM) in a non competitive manner. In the presence of tone, 5-HT (10nM-1mM) produced a biphasic CRC. The initial contractile response displayed a plateau between 100 nM and 1 μM. High concentrations of 5-CT (10μM-1mM) produced a small contractile response (20% of the maximum response to 5-HT). In the presence of tone 5-CT, in some preparations, produced a transient contractile response. Sumatriptan was inactive in the absence of tone. In the presence of tone sumatriptan (0.1nM-10nM) produced a contractile response in some preparations (8 out of 14) and at higher concentrations (100nM-100μM) induced a concentration-dependent relaxation. In the absence of tone α-me-5-HT (0.1μM-1mM) produced a contractile response that was similar to 5-HT. In the presence of tone the response to α-me-5-HT was biphasic, low concentrations (1nM-1μM) producing a contractile response whereas higher concentrations induced relaxation.

In the presence of IBMX the basal cAMP content was 4.68 ± 0.13 pmol/mg protein (n=6). 5-HT (100μM) produced a transient fall in the basal cAMP content. The reduction in basal cAMP content was greatest at 10 sec (2.46 ± 0.14 pmol/mg protein, n = 4, $p < 0.001$) and returned to 78% of the basal level by 120 sec. In the presence of forskolin the cAMP content was 46.4 ± 1.1pmol/mg protein (n=4). 5-HT produced a concentration-dependent fall in the cAMP content. The maximum inhibition (17.2±1.6 pmol/mg n=6, $p < 0.001$) was observed at 100 μM 5-HT.

These results indicate that a 5-HT$_1$ receptor is present in this vessel and that increased basal tone exposes the contractile response mediated by this receptor. The biphasic nature of the responses to sumatriptan and α-me-5-HT may also suggest the presence of an inhibitory receptor for 5-HT in this tissue.

Brown, C & Shaw A.M. (1995) *Br. J. Pharmacol.*, in press

291P RAT LUNG MICROVASCULAR ENDOTHELIAL CELLS IN CULTURE EXPRESS A 5-HT TRANSPORTER BUT NOT A NORADRENALINE TRANSPORTER

L.J. Bryan-Lluka[1], K.T.Oldham[2], K.S. Guice[2] & H. Bönisch[1] (Introduced by V.G. Wilson, Dept of Physiology & Pharmacology, Queen's Medical Centre, Nottingham NG7 2UH), [1]Dept of Pharmacology, University of Bonn, D-53113 Bonn, Germany and [2]Dept of Surgery, Duke University Medical Center, Durham 27710, U.S.A.

Previous histological evidence has shown that, in the lungs, the removal of 5-hydroxytryptamine (5-HT) and catecholamines, such as noradrenaline (NA), from the circulation occurs mainly in pulmonary microvascular endothelial cells (PMVEC). A simplified technique has recently been described for the isolation and pure culture of PMVEC from rat lungs (Magee *et al.*, 1994). Hence, the aim of this study was to examine the properties of the transporters for 5-HT and NA in these cells.

PMVEC from rat lungs were isolated, cultured and characterised as described previously (Magee *et al.*, 1994) and used between passages 6 and 10. Four days after subculturing, the culture medium was replaced by Krebs-Hepes buffer, containing selective inhibitors of 5-HT uptake or uptake$_1$ (the transporter for NA in perfused lungs of rats; Bryan-Lluka *et al.*, 1992) as appropriate, and incubated initially for 15 min at 37°C. [^{3}H]-5-HT or [^{3}H]-NA (10 nM) was then added and the cells were incubated for 1, 2, 5 or 10 min. The cells were washed with ice-cold buffer and, after lysis, their [^{3}H] and protein contents were determined.

There was a marked time-dependent 5-HT uptake which reached a steady-state level of 2570 (s.e.mean 360) fmol/mg protein (n=3) after an incubation time of 5 min. An incubation time of 2 min was used for further experiments. The uptake of 5-HT (1950, s.e.mean 190, n=3) was markedly inhibited ($P<0.001$, analysis of variance; n=3 for each treatment) when NaCl in the buffer was replaced by LiCl (by 86%) or when the selective 5-HT uptake inhibitors, citalopram (10 or 0.1 μM) or paroxetine (0.1 μM), were present (by 97%, 96% and 97%, respectively), but was affected much less by the selective uptake$_1$ inhibitors, desipramine and nisoxetine (0.1 μM) (by 39% ($P<0.001$) and 15% ($P>0.05$), respectively). In contrast, there was very little uptake of NA (45.5 fmol/mg protein, s.e.mean 6.2, n=6) and 10 μM desipramine had no significant effect (34.6 fmol/mg protein, s.e.mean 8.5, n=6; $P>0.05$, *t*-test).

Hence, the results show that 5-HT uptake occurs in cultured PMVEC from rat by a Na^+-dependent transporter with a potency order for inhibitors that is typical for a 5-HT transporter, so these cells are suitable for further studies on this transporter in the lungs. However, under the conditions used in these experiments, rat PMVEC did not express a NA transporter, despite avid NA uptake in intact lungs where there is histological evidence that the site of uptake is the endothelial cells. The reason for this discrepancy is not clear, but it is essential to determine whether a NA transporter is expressed in rat PMVEC in primary culture.

Bryan-Lluka, L.J., Westwood, N.N. & O'Donnell, S.R. (1992) *Naun.-Schmied. Arch. Pharmacol.* 345, 319-326.
Magee, J.C., Stone, A.E., Oldham, K.T. *et al.* (1994) *Amer. J. Physiol.* 267, L433-L441.

292P I_{KRP}: CHARACTERIZATION AND PHARMACOLOGICAL SEPARATION OF A NOVEL POTASSIUM CURRENT IN RABBIT PULMONARY ARTERY MYOCYTES

A.M. Evans, O.N. Osipenko, & A.M. Gurney. Department of Pharmacology, U.M.D.S., St. Thomas's Hospital, Lambeth Palace Road, London SE1 7EH

Until now four K channel subtypes were thought to coexist in smooth muscle cells of the pulmonary artery, namely those carrying the ATP-inhibited ($I_{K(ATP)}$: Clapp & Gurney, 1991), Ca^{2+}-activated ($I_{K(Ca)}$: Clapp & Gurney, 1990), A-like ($I_{(A)}$: Clapp & Gurney, 1990) and delayed rectifier ($I_{K(V)}$: Gelband *et al*, 1993; Evans *et al*, 1994) K currents. Using standard patch clamp techniques (methods: Clapp & Gurney, 1990), we have now characterized a fifth and previously unidentified K current in rabbit pulmonary artery myocytes, which we have denoted $I_{K(RP)}$, because it sets the resting membrane potential (RMP). This K current was found in smooth muscle cells from the main and resistance vessels of the pulmonary arterial tree, but was absent from smooth muscle cells isolated from the aorta and mesenteric artery from the same species. Furthermore, we have identified this current in rat (n=4), pig (n=6) and human (n=7) pulmonary artery myocytes. $I_{K(RP)}$ is a voltage-gated current, activation threshold close to -65mV. It activates slowly, without delay and does not inactivate during prolonged depolarization (≤30min). Current activation was described by a single exponential, with a time constant of 1.6 ± 0.4 s at -60mV (mean ± s.e.m.; n=4), which displayed only limited voltage-sensitivity. Current deactivation was described by a single exponential, with a time constant of 37 ± 7 ms at -110mV (n=3), which displayed little or no voltage-sensitivity over the range -110 to -60mV.

$I_{K(RP)}$ can be pharmacologically separated from $I_{K(ATP)}$ and $I_{K(Ca)}$, as it is not inhibited by glibenclamide (10μM), a selective blocker of $I_{K(ATP)}$, or tetraethylammonium (≤125mM), a blocker of $I_{K(Ca)}$. In common with $I_{K(V)}$ and $I_{(A)}$, $I_{K(RP)}$ is blocked by 4-aminopyridine (0.1-10mM). In contrast, however, $I_{K(RP)}$ is relatively insensitive to concentrations of quinine (≤ 100μM) which virtually abolish $I_{(A)}$ and $I_{K(V)}$. At 10μM quinine blocked $I_{K(V)}$ by 50%, but was without effect on either $I_{K(RP)}$ or the RMP. As $I_{K(RP)}$ appears to set the RMP of pulmonary artery myocytes (Evans *et al*, 1995) and its expression may be restricted to the pulmonary vascular tree, modulation of its expression and/or activity may underlie some forms of pulmonary hypertension. $I_{K(RP)}$ could, therefore, provide a useful therapeutic target, as activators of this current could produce pulmonary specific vasodilation.

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Clapp, L.H. & Gurney, A.M. (1991) *J. Exp. Physiol.* **76**, 677-693
Clapp, L.H. & Gurney, A.M. (1992) *Am. J. Physiol.* **262**, H916-920.
Evans, A.M., Clapp, L.H. & Gurney, A.M. (1994) *Br. J. Pharmacol.* **111**, 972-974.
Evans, A.M., Osipenko, O.N. & Gurney, A.M. (1995) *Proc. Physiological Soc. Oxford meeting.*
Gelband, C. H., Ishikawa, T., Post, J.M., Keef, K.D. & Hume, J.R. (1993) *Circ. Res.* **73**, 24-34.

293P CDP840 INHIBITS ANTIGEN-INDUCED AIRWAY RESPONSES IN THE NEONATALLY IMMUNISED RABBIT

N.Gozzard, C.M.Herd[1], S.M.Blake, M.Holbrook, B.Hughes & C.P.Page[1]. Celltech Therapeutics Ltd., Slough, SL1 4EN and [1]Dept Pharmacology, Kings College, London, SW3 6LX.

The phosphodiesterase type IV (PDE IV) inhibitor rolipram inhibits antigen-induced airway hyperresponsiveness and pulmonary eosinophilia in the immunised rabbit (Gozzard *et al*, 1995). In this study we report the effects of the novel potent and selective PDE IV inhibitor CDP840 (R-(+)-4-[2-(3-cyclopentoxy-4-methoxyphenyl)-2-phenylethyl] pyridine) in this model.

Two groups of 9 litter-matched NZW rabbits (2.4-3.4Kg), immunised within 24h of birth and until 12 weeks of age with *Alternaria tenuis* antigen (Ag) in Al(OH)3 gel (*i.p.*), were pre-treated twice daily on days 1-3 with CDP840 (1mg kg^{-1} *i.p*) or vehicle (acidified saline: pH4.5 with HCl, *i.p.*), (2ml kg^{-1}). On day 3, rabbits were sedated with diazepam (2.5 mg kg^{-1}) and Hypnorm (0.4 ml kg^{-1}), the concentration of inhaled aerosolised histamine required to provoke a 50% increase (PC_{50}) in total lung resistance (R_L) and 35% decrease (PC_{35}) in dynamic compliance (C_{dyn}) was then determined and bronchoalveolar lavage (BAL) performed. On day 4, rabbits were given a final dose of CDP840 or vehicle 1h prior to Ag aerosol (20,000 PNU ml^{-1} in saline for 20 min). On Day 5, PC_{50} and PC_{35} values were determined and BAL performed as on day 3 (Herd *et al*,1994).

Treatment with CDP840 did not alter basal lung function. (R_L 37.5±2.2 *vs*.vehicle 35.4±2.0 $cmH_2O^{-1}L^{-1}sec^{-1}$ and C_{dyn} 4.4± 0.1 *vs* vehicle 4.1±0.2 ml cmH_2O^{-1}) prior to Ag-challenge.

Ag-induced acute bronchoconstriction was reduced by CDP840 pre-treatment (% increase in R_L 19.7±3.5 *vs* vehicle 46.4±4.2; % decrease in C_{dyn} 23.6±3.1 *vs* vehicle 41.0±3.0; P<0.05).

In vehicle-treated rabbits, Ag-induced airway hyperresponsiveness (table 1) was evidenced by reduced PC_{50} and PC_{35} values to inhaled histamine 24h post Ag. This hyperresponsiveness was abolished by CDP840 pre-treatment.

Table 1. Histamine PC_{50} and PC_{35} values pre and post Ag.

Treatment	PC_{50}(mg ml^{-1})		PC_{35}(mg ml^{-1})	
	Pre	Post	Pre	Post
vehicle n=9	21.5±1.3	9.1±1.2†	14.6±1.2	7.0±1.1†
CDP840 n=9	23.3±1.2	21.7±1.2#	20.3±1.3#	20.4±1.2#

†P<0.05 *vs* Pre (paired t-test); #P<0.05 *vs* post vehicle (unpaired t-test)

Total leucocytes recovered in BAL 24h post Ag challenge were increased in vehicle-treated rabbits only (Table 2). Ag-induced increases in eosinophils were reduced (85%) with CDP840 pre-treatment (P=0.002). Mononuclear cells, but not neutrophils, were reduced in CDP840-treated rabbits 24h after Ag challenge.

Table 2. Total and differential cell counts in BAL (10^4 cells ml^{-1}) pre and post Ag.

Treatment	Total		Mono		Neut		Eosin	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Vehicle	34.1 ±3.6	41.4† ±3.2	33.4 ±3.5	33.6 ±3.4	0.6 ±0.2	5.7† ±1.5	0.04 ±.02	2.0† ±0.3
CDP840	25.2 ±3.0	25.2# ±3.0	23.9 ±2.8	18.8# ±2.7	1.0 ±0.7	6.1† ±2.2	0.01 ±.01	0.3†# ±0.1

†P<0.05 *vs* Pre; #P<0.05 *vs* post vehicle (Wilcoxon test), *n*=9 per group.

These data demonstrate that the novel PDE IV inhibitor CDP840 attenuates Ag-induced acute bronchoconstriction, airway hyperresponsiveness and pulmonary eosinophilia in the neonatally immunised rabbit.

Gozzard, N. *et al.* (1995) *Br. J. Pharmacol.* 114, A54.
Herd, C.M. *et al.* (1994) *Br. J. Pharmacol.* 112, 292-298.

294P INHIBITION BY LACIDIPINE OF THE RESPONSIVENESS OF BASILAR ARTERY ENHANCED BY HIGH SALT DIET IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

C. Dessy, S. Salomone, O. Feron, N. Morel & T. Godfraind, Laboratoire de Pharmacologie, Université Catholique de Louvain, Avenue Hippocrate 54, UCL 5410, B-1200 Bruxelles, Belgium

When stroke-prone spontaneously hypertensive rats (SHR-SP) are submitted to a high salt diet, they show motor abnormalities due to stroke lesions and die after 8 weeks treatment. It has been reported that lacidipine, a 1,4-dihydropyridine calcium antagonist, exerts a protective effect by preventing motor alterations and death (Cristofori *et al.*, 1994). The present experiments were designed in order to examine if changes in basilar artery reactivity preceeded the stroke lesions and if the protective effect of lacidipine was related to an action on cerebrovascular bed or to its systemic antihypertensive effect. Therefore, we examined the response to the Ca^{2+} channel activator Bay K 8644 of basilar arteries and of mesenteric resistance arteries obtained from 14-week-old SHR-SP untreated or lacidipine-treated (0.3 and 1 mg/kg.day), receiving during 6 weeks either normal water (SHR-SP NS) or water containing 1% NaCl (SHR-SP SL).

The systolic blood pressure (SBP, tail-cuff method) after 6 weeks treatment was affected neither by high salt diet (SHR-SP NS, 243.0 ± 4.5 mm Hg, n=23; SHR-SP SL, 244.3 ± 6.2 mm Hg, n=24) nor by 0.3 mg/kg.day lacidipine (SHR-SP NS, 241.4 ± 5.3 mm Hg, n=8; SHR-SP SL, 265.3 ± 8.9 mm Hg, n=8), whereas 1 mg/kg.day lacidipine had a significant antihypertensive effect (SHR-SP NS, 209.1 ± 7.0 mm Hg, n=14; SHR-SP SL, 208.5 ± 7.8 mm Hg, n=14; P<0.01 vs non-lacidipine-treated).

Rats were killed by decapitation. Basilar and mesenteric arteries were mounted in physiological solution in a wire myograph (Mulvany & Halpern, 1977). After normalization the vessels were washed for 20 min with 100 µM N-ω-nitro-L-arginine and then were exposed to 30 nM Bay K 8644. When their contractile response had reached a steady-state, the KCl concentration in the bath was cumulatively increased up to 20 mM. The contractile response was expressed in percentage of the maximal response to KCl.

We observed that the sensitivity to Bay K 8644 was enhanced after high salt diet. For instance, in basilar arteries bathed in 9 mM KCl, the response was equal to 44.2 ± 12.3 % (n=10) in SHR-SP SL and to 9.7 ± 6.7 % (n=10) in SHR-SP NS (P<0.05). By contrast, the contractile responses of mesenteric resistance arteries (300 µm normalized diameter) from SHR-SP SL (n=6) and SHR-SP NS (n=11) were not significantly different. When rats had been treated with 0.3 mg/kg.day lacidipine, responses of basilar arteries were similar in SHR-SP SL and SHR-SP NS (respectively, 17.4 ± 7.9 %, n=7 and 15.3 ± 11.1 %, n=5), and they were not significantly different from untreated SHR-SP NS. In mesenteric resistance arteries, the responsiveness to Bay K 8644 was not changed by lacidipine treatment.

These results show that, in SHR-SP, salt load did not change the SBP but increased the responsiveness of basilar arteries to Bay K 8644. This augmented responsiveness of basilar arteries was blunted by 0.3 mg/kg.day lacidipine treatment, this inhibition was blood pressure independent, since this dose of lacidipine was not sufficient to lower SBP. We have observed that the expression of preproendothelin-1 mRNA, increased in hearts from salt-loaded SHR-SP, is blunted by lacidipine (Feron *et al.*, 1995). Furthermore, exogenous threshold concentrations of endothelin-1 (ET-1) potentiate the action of Bay K 8644 (Godfraind *et al.*, 1989), therefore, experiments are in progress in order to see if endogenous ET-1 could be responsible for the action of the high salt diet.

Cristofori, P., Sbarbati, A., Accordini, C. *et al.* (1994). *J. Submicrosc. Cytol. Pathol.* 26, 331-341.
Feron, O., Salomone, S. & Godfraind, T. (1995). *Biochem. Biophys. Res. Commun.* 210, 219-224.
Godfraind, T., Mennig, D., Morel, N. *et al.* (1989). *J. Cardiovasc. Pharmacol.* 13 (suppl 5), S112-S117.
Mulvany, M.J. & Halpern, W. (1977). *Circ. Res.* 41, 19-26.

295P ENDOTHELIAL MODULATION OF ANGIOTENSIN RESPONSE IN CANINE BLOOD VESSELS

C. FORSTER Department of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8, Canada.

The endothelium plays a dynamic role in regulating vascular tone. This is brought about by a) a barrier function, b) acting as an adhering surface and c) production and release of substances which cause either dilation of contraction. Angiotensin converting enzyme is located in endothelial cells allowing the formation of angiotensin II. Whether the endothelium modulates the vascular effects of AII is unclear. Until recently, only angiotensin II and angiotensin III were considered biologically active and angiotensin I was considered to be inactive. However, with the development of non-peptide antagonists, two distinct receptors have been characterised. This allows better definition of the effects of various angiotensin analogues. This study was conducted to observe if the endothelium modulated the effects of angiotensin analogues in canine dorsal pedal artery and saphenous vein.

Dorsal pedal artery and saphenous vein segments were obtained from up to ten male mongrel dogs and cut into 6 five mm rings. Alternate rings had their endothelium denuded, rings were mounted in organ baths and equilibrated. Cumulative concentration effect curves were constructed to angiotensin I, II and III on pairs of rings (one intact and one denuded) in the absence and presence of losartan (10^{-8} - 10^{-6} M). Curves were analysed by the logistic equation: $Y=\{(a+d)/[1+(X/c)^b]\}+d$ (Parker & Waud, 1971). The table shows maximum response (E in grams.mm^{-2}; mean ± s.e.mean), EC_{50} (nM; geometric mean with 95% confidence limits) and * P < 0.05 denuded versus intact.

Table 1 Efficacy and sensitivity for angiotensins

	DORSAL PEDAL ARTERY		SAPHENOUS VEIN	
	Intact	*Denuded*	*Intact*	*Denuded*
AI E	8.0±0.9	7.4±1.5	19.3±2.7	15.0±1.4
EC_{50}	220(110-380)	170(100-470)	23(18-28)	7(3-13)*
AII E	6.3±0.7	9.4±1.0*	7.8±1.2	16.7±3.5*
EC_{50}	10(5-19)	3(2-4)*	4(3-7)	0.4(0.3-0.7)*
AIII E	6.0±0.7	10.5±1.9	16.0±2.3	16.0±3.1
EC_{50}	700(430-970)	26(21-50)*	11(6-23)	33(9-74)

The results show that both efficacy and sensitivity to angiotensin II were enhanced in denuded vessels. The EC_{50} for angiotensin I in the denuded saphenous vein was decreased and the EC_{50} to angiotensin III was decreased in the denuded dorsal pedal artery. The saphenous vein was always more sensitive, with angiotensin II being the most potent. In the saphenous vein, losartan antagonised *all* analogues but was *only* competitive against angiotensin I (pA_2 = 8.4 and 8.9 [intact and denuded]) and angiotensin II (pA_2 = 8.6 and 9.1 [intact and denuded]). In *only* denuded artery preparations, losartan antagonised competitively angiotensin II (pA_2 = 9.0).

These results indicate that the endothelium regulates the response to angiotensin. That losartan's ability to be a competitive antagonist is also endothelium-regulated, blood vessel dependent and angiotensin analogue dependent, suggests that the response involves more than one receptor.

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Parker, R.B. & Waud, D.R. (1971) *J. Pharmacol. Exp. Ther.* 177, 1-12.

296P INFLUENCES OF DIFFERENCES IN ENDOTHELIN (ET)$_A$ AND ET$_B$ RECEPTOR SUBTYPE PROPORTIONS ON ET-1-INDUCED CONTRACTION IN PORCINE ISOLATED BRONCHUS AND TRACHEA

R.G. Goldie, A.C. D'Aprile, R. Cvetkovski, P.J. Rigby & P.J. Henry, Department of Pharmacology, University of Western Australia, Perth, Nedlands, Western Australia, 6907

Both ET_A and ET_B receptors can mediate contraction of airway smooth muscle (Henry, 1993). Furthermore, the relative contributions of ET_A and ET_B receptors to contraction may be different at different levels within the respiratory tract (Hay *et al.*, 1993). We evaluated ET_A and ET_B receptor-mediated contraction in porcine trachea and 4th generation bronchus in the light of estimates of the relative densities of ET_A and ET_B binding sites in these tissues.

Quantitative autoradiography revealed specific binding of [^{125}I]-ET-1 (0.3 nM; 180 min; 37°C) in tracheal and bronchial airway smooth muscle. Nonspecific binding was assessed in the combined presence of the ET_A receptor-selective ligand BQ-123 (1 μM) and the ET_B receptor-selective ligand sarafotoxin S6c (Stx S6c; 100 nM). Specific binding in tracheal smooth muscle was inhibited in the presence of BQ-123 (1 μM) by 25 ± 6% and Stx S6c (100 nM) by 67 ± 3% (n=3 animals), indicating a greater number of ET_B than ET_A receptors. In sharp contrast, in porcine 4th generation bronchial smooth muscle, ET_A sites predominated, since BQ-123 reduced specific [^{125}I]-ET-1 binding by 73 ± 2%, whereas 100 nM Stx S6c reduced binding by only 32 ± 8% (n=3 animals). Thus, the relative proportions of ET_A:ET_B receptors in tracheal and bronchial smooth muscle were about 70:30 and 30:70 respectively.

Carbachol, ET-1 and Stx S6c caused concentration-dependent contraction of porcine tracheal and bronchial airway smooth muscle. ET-1 has similar potency (concentration producing 30% of the maximum contraction to carbachol; Cmax) in trachea (34 nM; 95% confidence limits (c.l.) = 10-110 nM; n=6) and bronchus (22 nM; c.l.=9-55 nM; n=6). ET-1 produced comparable maximal contractions in trachea (53 ± 6% Cmax; n=6) and bronchus (65 ± 4% Cmax; n=6). However, maximum contraction induced by Stx S6c was greater in trachea (53 ± 7% Cmax; n=6) than in bronchus (21 ± 5% Cmax; n=6). Furthermore, BQ-123 (3 μM) shifted the ET-1 concentration-effect curve to the right in bronchus (4.3 fold; $P<0.05$), but not in trachea. The ET_B receptor-selective antagonist BQ-788 (1 μM) reduced Stx S6c potency (e.g. by 50 fold in trachea; c.l. = 14-180; n = 6; $P<0.05$), but did not affect the potency of ET-1 in either tissue. Thus, both ET_A and ET_B receptors can mediate ET-1-induced contraction in porcine tracheal and bronchial airway smooth muscle, although this response was mediated predominantly via the ET_A receptor subtype in bronchus. In summary, changes in ET_A and ET_B receptor subtype proportions at different airway levels in the pig were associated with significant changes in contractile function.

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Hay, D.W.P., Luttmann, M.A., Hubbard, W.C. & Undem, B.J. (1993) *Br. J. Pharmacol.*, 110, 1175-1183.
Henry, P.J. (1993) *Br. J. Pharmacol.*, 110, 435-441.

297P RESPONSES OF PERMEABILIZED SMOOTH MUSCLE CELLS IN THE RABBIT MESENTERIC ARTERY TO ENDOTHELIN

N.J. Yard, A.G. Roach* and C.J. Garland Department of Pharmacology, University of Bristol, Bristol BS8 1TD and *Rhone-Poulenc Rorer Ltd, Dagenham, Essex RM10 7XS

In the porcine coronary artery, smooth muscle contraction to ET-1 followed the combined action of calcium influx from the extracellular space, calcium release from intracellular storage sites and an increase in the sensitivity of the contractile proteins to calcium (Ushio-Fukai *et al.*, 1995) However, in contrast ET-3 induced vasoconstriction seemed to be almost entirely mediated by calcium influx from the extracellular space, in spite of the fact that both ET-1 and ET-3 appeared to act through ET_A receptors. In the rabbit mesenteric artery, ET-1 induces sensitization of the contractile proteins to calcium (Nishimura *et al.*, 1994) but there have been no reports of the mechanism by which ET-3 causes contraction in this vessel. The present study was designed to determine if ET-3 could also stimulate sensitization of the contractile proteins to calcium in the rabbit mesenteric artery, and investigate the receptors involved.

Helical strips, 100-150μm wide and around 2mm long, were cut from a third order branch of rabbit mesenteric artery from which the endothelium had been removed. The strip was attached to a force transducer and stretched to a resting tension of 0.05mN. The strip was then placed in mock intracellular solution (0 Ca^{2+}, 10mM EGTA) and permeabilized by incubation with *Staphylococcus aureus* α-toxin for 15 minutes at room temperature. The extent of permeabilization was then assessed by the ability of calcium to stimulate contraction in concentrations (0.03-100μM) which had no effect before exposure to α-toxin (Seager *et al.*, 1994).

Maximal myofilament sensitization to calcium was obtained with 10nM ET-1, and represented an increase in contraction of 34.7 ± 1.6 % (mean ± s.e. mean; n=3) on top of the contraction to 10μM GTP and 0.3μM calcium (0.34 ± 0.04mN). The sensitization was concentration-dependent over the range 0.003 to 10nM. ET-3 (0.3-300nM) induced a similar, concentration-dependent myofilament sensitization in strips pre-contracted with 0.3μM calcium and 10μM GTP (26.9 ± 4.0%; n=4), although it was around 100 times less potent than ET-1, a rank order of potency which is consistent with the involvement of the ET_A receptor subtype. BQ-123 (10μM), a selective ET_A receptor antagonist, completely inhibited the sensitization induced by a supra-maximal concentration of ET-1 (100nM; n=3). The selective ET_B receptor agonist, sarafotoxin S6c (10nM-1μM), failed to induce any myofilament sensitization (n=3).

These observations show that both ET-1 and ET-3 can cause myofilament calcium sensitization in the rabbit mesenteric artery, which contrasts with observations in the porcine coronary artery (Ushio-Fukai *et al.*, 1995). As ET-3 was less potent than ET-1 in the induction of sensitization, this indicates the involvement of the ET_A receptor subtype. This suggestion is supported by the inhibition of ET-1 induced sensitization by the selective ET_A receptor antagonist BQ-123, and the failure of sarafotoxin S6c to induce myofilament sensitization to calcium.

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Nishimura, J., Moreland, S., Yul Ahn, H. *et al* . (1992) *Circ. Res.* 71, 951-959.
Seager, J.M., Murphy, T.V. & Garland, C.J. (1994) *Br. J. Pharmacol.* 111, 525-532.
Ushio-Fukai, M., Nishimura, J., Kobayashi, S. & Kanaide, H. (1995) *Br. J. Pharmacol.* 114, 171-179.

298P COLD-INDUCED POTENTIATION OF THE CONTRACTILE RESPONSE TO AN α_1-ADRENOCEPTOR AGONIST IN RAT RESISTANCE ARTERIES: THE ROLE OF ENDOTHELIN

Paula J.W. Smith[1,2], Daniel S. McQueen[1] & David J. Webb[2] The University of Edinburgh, [1]Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ & [2]Department of Medicine, Western General Hospital, Edinburgh EH4 2XU.

People who suffer from Raynaud's disease, characterised by digital vasospasm induced by cold or emotional stress, have an enhanced responsiveness to α–adrenoceptor agonists *in vivo* (Freedman *et al.*, 1989). The present study examined the effect of cooling on the response to phenylephrine (α_1–adrenergic agonist, PE) in isolated rat mesenteric resistance arteries, and the potential involvement of the potent endothelium-derived vasoconstrictor, endothelin (ET), in the response.

Mesenteric vascular beds were removed from male Wistar rats (11-16 weeks) and placed in a dissecting dish containing cold physiological salt solution. Second and third order resistance arteries with a mean diameter of 310±6μm (n=36) were excised and cannulated in a small vessel arteriograph (Living Systems Instrumentation, USA) at 37°C. A pressure servo unit maintained intraluminal pressure, without flow, at 60mmHg, and the lumen diameter was measured using a video dimension analyser. Following 60-90 min equilibration, three doses of PE (10^{-5}M) were given to produce a contraction to < 35% of resting lumen diameter. Acetylcholine (ACh, 10^{-6}M) was given during the third contraction to assess endothelial integrity. Removal of endothelium was achieved by passing air through the lumen of the vessel and confirmed by the loss of ACh (up to 10^{-4}M)-induced relaxation during constriction to a fourth dose of PE. Arteries were cooled by passing the superfusate through a rapid heat-exchange element (Moor Instruments, U.K.) before it entered the arteriograph. Six sets of experiments (n=5-7 per set) were carried out: at 37°C or 24°C, with endothelium either intact or removed, and at 37°C or 24°C in the presence of bosentan (mixed $ET_{A/B}$ antagonist, 10^{-6}M). PE (10^{-8} to 10^{-5}M) concentration-response curves were obtained for each set. Responses are expressed as a percentage of the maximum contraction to the PE 'wake-up' dose. EC_{50} and E_{max} values are shown as mean ± s.e.mean.

In arteries with an intact endothelium, cooling to 24°C caused a 4-fold increase in sensitivity to PE (EC_{50} = 5.9±1.0 x 10^{-7}M at 37°C vs 1.4±0.2 x 10^{-7}M at 24°C, $P<0.01$; unpaired t-test). After denudation, this leftward shift of the concentration-response curve to PE was still apparent (EC_{50} = 5.7±1.0 x 10^{-7}M at 37°C vs 2.9±5.0 x 10^{-7}M at 24°C, $P=0.05$), but was significantly reduced compared to the intact arteries at 24°C ($P<0.05$). Similarly, the presence of bosentan reduced the cold-induced increase in sensitivity such that the leftward-shift of the concentration-response curve to PE no longer reached significance (EC_{50} = 7.6±1.6 x 10^{-7}M at 37°C vs 4.9±1.2 x 10^{-7}M at 24°C, $P=0.34$). At 37°C, endothelial removal or bosentan had no significant effect on the sensitivity of arteries to PE, compared to intact control vessels. However, at 24°C, removal of the endothelium or addition of bosentan resulted in a 2-($P<0.05$) and 3-($P<0.05$) fold decrease in sensitivity to PE, respectively, compared to the intact control vessel. There was no significant difference in the E_{max} for PE between any of the sets studied.

The present study shows cooling increases the sensitivity to PE in resistance arteries with an intact endothelium. This effect appears to be partly mediated by an endothelium-derived vasoconstrictor, probably ET, since denudation or addition of an $ET_{A/B}$-receptor antagonist, reduces the cold-induced increase in sensitivity to PE. These findings are in contrast to studies carried out in large arteries and veins, where cooling augments contractile responses to α_2– but not α_1–adrenergic agonists (Flavahan *et al.*, 1985). This may reflect differences between large vessels and resistance arteries. The present study suggests that ET might contribute to the cold-induced potentiation of α–adrenoceptor-mediated contraction, and may be a useful target for investigation as a cause of the prolonged vasospasm of Raynaud's Disease.

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Freedman, R.R., Sabharwal S.C., Desair, N., Wenig P. & Mayes, M. (1989) *Arthritis Rheum.* 32, 61-65

Flavahan, N.A., Lindblad, L.E., Verbeuren, T.J., Shepherd, J.T. & Vanhoutte, P.M. (1985) *Am. J. Physiol.* 249, H950-H955

299P REGULATION BY ANGIOTENSIN II OF $[Ca^{2+}]_i$ IN HUMAN NEUROBLASTOMA (SH-SY5Y) CELLS TRANSFECTED WITH THE RAT AT_{1A} RECEPTOR

R.L. McDonald, P.F.T. Vaughan, A.J. Balmforth & C. Peers. Institute for Cardiovascular Research, Leeds University, Leeds LS2 9JT.

Angiotensin II (AII) is known to have various effects in sympathetic neurones, including the ability to modulate noradrenaline release (Reid, 1992). In this study we have used the human neuroblastoma SH-SY5Y as a model for a sympathetic neurone (Vaughan *et al.*, 1995), stably expressing the recombinant rat AT_{1A} receptor (Balmforth *et al.*, 1994) to investigate the regulation of $[Ca^{2+}]_i$ by AII. Cells were loaded with Fura-2 by incubation with 4μM Fura-2-AM for at least 1h at room temperature. They were then transferred to a perfusion chamber on the stage of an inverted microscope and $[Ca^{2+}]_i$ was measured in groups of approximately 5 cells using dual excitation methods as previously described (Vaughan *et al.*, 1993). The perfusate was of composition (in mM): NaCl 135, KCl 5, $CaCl_2$ 2.5, glucose 6, $MgCl_2$ 0.6, HEPES 10mM (pH 7.4, 21-24°C). All values reported are means ± SEM.

Bath applied AII (10nM) evoked rapid rises of $[Ca^{2+}]_i$ in SH-SY5Y cells, from basal levels of 56±8nM to a peak of 664±110nM, and on prolonged exposure levels declined to a plateau value of 250±22nM (n=5 groups of cells) by 3 min. Second applications of AII evoked similar patterns of response, but increases were always reduced (e.g. peak levels 288±29nM, n=5). In Ca^{2+}-free solutions (1mM EGTA added), AII evoked similar rises in $[Ca^{2+}]_i$ (peak levels 516±45nM, n=5), but these rises were transient and $[Ca^{2+}]_i$ always fell to basal levels within 3 min. Subsequent applications of AII always failed to evoke a rise of $[Ca^{2+}]_i$ (n=5). In Ca^{2+}-free solutions, application of 100μM carbachol (CCh, to activate M_3 receptors; Murphy *et al.*, 1991) evoked transient rises of $[Ca^{2+}]_i$ (to peak levels of 688±42nM, n=5) and subsequent exposure to AII failed to raise $[Ca^{2+}]_i$. Similarly, exposure to AII occluded responses to CCh (n=5). However, if 1mM Ca^{2+} was applied to and then removed from the cells between agonist applications, this in itself caused a rise of $[Ca^{2+}]_i$ (to a maximum of 246±30nM following CCh (n=3) and 190±5nM following AII, n=3), and subsequent responses to either AII or CCh could be observed.

Our findings suggest that activation of recombinant AT_{1A} receptors stimulates Ca^{2+} release from the same intracellular store(s) as are coupled to M_3 receptors as well as influx across the plasma membrane. Furthermore, in the absence of agonist, store depletion itself appears to activate Ca^{2+} uptake in SH-SY5Y cells.

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

Balmforth, A.J., Bajaj, B.P.S. & Ball, S.G. (1994) *J. Hypertension* 12, S179.

Murphy, N.P., Vaughan, P.F.T., Ball, S.G. & McCormack, J.G. (1991) *J. Neurochem.* 57, 2116-2123.

Reid, I.A. (1992) *Am. J. Physiol.* 262, E763-E778.

Vaughan, P.F.T., Kaye, D.F., Reeve, H.L., Ball, S.G. & Peers, C. (1993) *J. Neurochem.* 60, 2159-2166.

Vaughan, P.F.T., Peers, C. & Walker, J.H. (1995) *Gen. Pharmacol.* (in press).

300P PHARMACOLOGICAL STUDIES WITH A-61603 AND RS 17053 EXPOSE A PUTATIVE α_{1A} ADRENOCEPTOR IN CAUDAL ARTERY OF RAT

W.G. Lachnit, D.E. Clarke & A.P.D.W. Ford, Center for Neurobiology, Roche Bioscience, Palo Alto, CA, U.S.A.

Three distinct subtypes of α_1-adrenoceptor (AR) (α_{1A}, α_{1B} & α_{1D}) have been defined fully, and existence of a fourth, the putative α_{1L}-AR has been proposed based on functional studies (see Ford *et al.*, 1994). A functional α_{1A}-AR has been defined in perfused vascular beds in kidney and mesentery of rat (see Blue *et al.*, 1995). However, evidence for other functional α_{1A}-AR preparations remains equivocal (Ford *et al.*, 1994). In this study we have examined α_1-ARs mediating contraction of the caudal artery of rat.

Caudal arteries from male Sprague-Dawley rats were denuded of endothelium and mounted as helical strips (0.5 - 1.0 cm) at 4 mN in waterjacketed tissue baths (37°C) for measurement of isometric tension in oxygenated (95% O_2, 5% CO_2) Krebs' solution supplemented with the following: ascorbate (100 µM), cocaine (30 µM), corticosterone (30 µM), indomethacin (10 µM), propranolol (1 µM), and idazoxan (300 nM). All values shown are mean ± s.e.mean.

Cumulative concentration-effect (E/[A]) curves were constructed to noradrenaline (NA) and yielded pEC_{50} of 5.60 ± 0.11 (n=6). Prazosin caused concentration-dependent, parallel, dextral shifts of NA E/[A] curves yielding a pA_2 value of 8.8 ± 0.2 (n=6). RS 17053 (10, 30 and 100 nM), a selective α_{1A}-AR antagonist (Ford *et al.*, 1995), produced biphasic dextral shifts of E/[A] curves to NA suggesting more than one subtype of α_1-AR. Single point analysis (assuming Schild slope=1) of the high affinity site yielded a pA_2 value of 9.2 ± 0.3 (n=15).

A-61603, a novel imidazoline agonist, is reportedly selective for the α_{1A}-AR subtype (Hancock *et al.*, 1994). In caudal artery strips, A-61603 behaved as a full agonist relative to NA. Cumulative additions of A-61603 resulted in monophasic E/[A] curves with a pEC_{50} of 7.45 ± 0.05 (n=19). Prazosin (3-300 nM) and RS 17053 (3-300 nM) caused concentration-dependent, parallel, dextral shifts of A-61603 E/[A] curves yielding pA_2 estimates of 9.2 ± 0.2 (n=15) and 9.2 ± 0.2 (n=28), respectively.

Preliminary experiments with the α_{1A}-AR antagonists, SNAP 5089 (Wetzel *et al.*, 1995) (30 nM), tamsulosin (3 nM), and 5-methylurapidil (30 nM) revealed parallel dextral shifts of the A-61603 E/[A] curves. Single point analyses (assuming Schild slope=1) yielded pA_2 esimates of 9.5 ± .05, 10.5 ± .03, 8.6 ± .03, for SNAP 5089, tamsulosin, and 5-methylurapidil, respectively (n=6). The affinity profile obtained with these antagonists, plus prazosin and RS 17053, *best* reflects that described for the α_{1A}-AR (Ford *et al.*, 1994, Blue *et al.*, 1995).

In conclusion, caudal artery of rat contracts in response to NA via activation of at least 2 α_1-AR subtypes. One of these subtypes appears to display the pharmacology of the α_{1A}-AR. Use of the novel selective agonist, A-61603, allows for pharmacological isolation of this receptor for analytical studies.

Blue, D.R., Bonhaus, D.W., Ford, A.P.D.W. *et al.* (1995) *Br. J. Pharmacol.* **115**, 283-294.
Ford, A.P.D.W., Arredondo, N.F., Blue, D.R., *et al.* (1995) *Br. J. Pharmacol.*, **114**, 24P.
Ford A.P.D.W., Williams, T.J., Blue, D.R. & Clarke, D.E. (1994) *Trends Pharmacol. Sci.*, **15**, 167-170.
Hancock, A.A., *et al.* (1994) *IUPHAR Satellite: Pharmacology of Adrenoceptors,* King of Prussia, PA, U.S.A.
Wetzel, J.M., Miao, S.W., Forray, C. *et al.* (1995) *J. Med. Chem.* **38**, 1879-1881.

301P ROLE OF POTASSIUM CHANNELS IN THE RESPONSE TO HYPOXIA IN RAT INTRAPULMONARY ARTERY RINGS

V.S.N.M.R. Karamsetty, R. M. Wadsworth & K. A. Kane, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW

Acute hypoxia has been reported to inhibit Ca^{2+}-dependent (Post et al., 1992) and Ca^{2+}-independent (Yuan et al., 1993) K^+-channels in single dispersed pulmonary artery smooth muscle cells. The aim of the present study was to investigate the role of K^+-channels in hypoxia-induced contraction and relaxation of intact rat pulmonary artery (PA) rings. The response to hypoxia was recorded isometrically from the phenylephrine (PE) EC_{50} contracted rat PA rings (i.d.=2.4±0.05 mm) that were incubated for 30 min with the K^+-channel blockers: glibenclamide (a K_{ATP} channel blocker), charybdotoxin (CHTX, a Ca^{2+}-activated K^+-channel blocker), 4-aminopyridine (4-AP, a voltage dependent K^+-channel blocker) and the non-specific K^+-channel blocker tetraethylammonium (TEA). The response to hypoxia in the drug treated PA rings was compared with a parallel control or vehicle treated PA rings. Tissues were made hypoxic (PO_2=7±1 mmHg) for 30 min by altering the gas mixture aerating the Krebs-Henseleit solution from 20%O_2-5%CO_2-75%N_2 to 0%O_2-5%CO_2-95%N_2. Tissues were reoxygenated at the end of hypoxia.

Acute hypoxia generated a triphasic response which comprised (1) a rapidly developing phasic contraction followed by (2) a transient relaxation and (3) a slowly developing tonic contraction. Table 1 summarises the three phases of the response to hypoxia in control and pretreated IPA rings measured relative to the PE-induced tone. Glibenclamide (10µM) markedly diminished the PE-induced contraction during normoxia (from 35±9 to 10±2 $g.cm^{-2}$, $P<0.05$), without modifying the response to hypoxia. 4-AP at 5 mM concentration produced a small transient contraction followed by a complete relaxation to the base line tone. However, 4-AP (5 mM) had no effect on the PE-induced tone or on the response to hypoxia. CHTX (50 nM) had no effect on either baseline or the PE-induced tone during normoxia nor did it modify the response to hypoxia. Similarly, TEA 20 mM did not modify the baseline or the PE-induced tone during normoxia or the response to hypoxia. It can be concluded from this data that the hypoxia-induced contraction and relaxation of intact rat pulmonary artery rings is not dependent on 1) K_{ATP} channels, 2) voltage activated K^+-channels or 3) Ca^{2+} activated K^+-channels.

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Table 1. Three phases of the response to hypoxia in rat PA rings. The hypoxic response was measured relative to the PE induced tone and expressed as $g.cm^{-2}$. Mean values ± s.e.mean are shown.

Treatment	*n*	*Hypoxic Contraction*		*Hypoxic Relaxation* (2)
		Phasic (1)	Tonic (3)	
Control	6	28±9	36±10	-3±6
Glibenclamide 10µM	4	30±4	48±7	11±3
Control	5	24±5	15±7	-8±4
4-aminopyridine 5mM	5	19±4	8±4	-6±4
Charybdotoxin 50nM	5	17±3	19±3	3±4
Control	6	32±7	39±7	8±3
TEA 20mM	6	47±14	58±17	8±3

Post, JM;Hume, JR;Archer, SL;Weir, EK (1992) *Am. J. Physiol.* 262, C882-C890.
Yuan, XJ;Goldman, WF;Tod, ML;Rubin, LJ;Blaustein, MP (1993) *Am. J. Physiol.* 264, L116-L123.

302P EFFECTS OF CYTOSKELETAL MODIFYING AGENTS ON SYSTEMIC HYPOXIC VASOCONSTRICTION AND OEDEMA FORMATION IN THE RAT ISOLATED PERFUSED LUNG

R.M. Smith, A.G.Roach*, K.I. Williams & B. Woodward, School of Pharmacy and Pharmacology, University of Bath, BA2 7AY, and *Rhone-Poulenc Rorer, Dagenham, Essex, RM10 7XS.

We have provided evidence that endothelin(s) are involved in mediating the response of rat isolated perfused lungs to systemic hypoxia (Smith *et al.*, 1995). We have now examined the role of the endothelial cytoskeleton in the pulmonary responses to systemic hypoxia by utilising the F-actin stabiliser phalloidin (Cooper, 1987), and the microtubule disrupting agent colchicine (Borisy and Taylor, 1967).

The perfused rat lung model used allows simultaneous measurement of pulmonary perfusion pressure (PPP), pulmonary inflation pressure (PIP) and lung weight (LW), as a measure of fluid accumulation (Lal *et al.*, 1994). Lungs were isolated, ventilated with room air and perfused with Krebs solution gassed with 95%O_2/5%CO_2 and allowed to stabilise for 30 min prior to the onset of hypoxia (HYP, Krebs solution gassed with 95%N_2/5%CO_2). In the drug studies lungs were perfused with the relevant agent for 15 min prior to and for the duration of the hypoxic period (90 min).

Systemic hypoxia led to a slow increase in PPP, which peaked at 70 min, LW also increased. Table 1 shows that the hypoxia-induced increases in PPP and LW were attenuated by the F-actin stabiliser phalloidin. However, with the lower concentration (10nM) there was a selective inhibition of the increase in PPP. After 90 min hypoxic perfusion in the presence of 50nM phalloidin it was still possible to increase PPP with bradykinin (50nmol) or endothelin-1 (400pmol). Colchicine (100nM) also abolished the hypoxia-induced increases in LW and PPP.

Table 1. Effects of 70 min hypoxic perfusion ± phalloidin, or colchicine, on PPP and Lung Weight in the rat isolated lung.

Treatment	PPP ± SEM (mmHg)	ΔLW ± SEM (g)
Normoxic Control	9.7 ± 0.7	0.53 ± 0.15
Hypoxic Control	15.8 ± 3.2 *	3.9 ± 1.0 *
HYP + 10nM Phalloidin	8.8 ± 1.1 #	4.0 ± 0.74
HYP + 50nM Phalloidin	9.9 ± 0.780 #	0.66 ± 0.17 #
HYP + 100nM Colchicine	8.4 ± 0.62 #	0.24 ± 0.06 #

* p<0.05 vs. Normoxic Control. #p<0.05 vs. Hypoxic Control. n=4

These data indicate that cytoskeletal components are involved in the hypoxia-induced increases in PPP and LW seen in this rat lung model. Our previous data implicated endothelins in the hypoxic response (Smith *et al*, 1995). Therefore the present data suggests that cytoskeletal components may also be involved in mediating the release of endothelin(s) following the onset of hypoxia. The low concentration phalloidin study also shows that increases in LW can be separated from changes in PPP.

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Borisy,G.G. & Taylor,E.W. (1967), J.Cell Biol., **34**, 525-533.
Cooper,J.A. (1987), J.Cell Biol., **105**, 1473-1478.
Lal, H. *et al.*, (1994), Pulmonary Pharmacol., **7**, 271-278.
Smith,R.M. *et al.*, (1995) Oral communication, BPS Oxford.

303P CATECHOL-O-METHYLTRANSFERASE ACTIVITY AND ITS SENSITIVITY TO INHIBITION BY TOLCAPONE IN TWO RENAL CELL LINES AND IN RAT AND PIG KIDNEY TISSUES

P. Soares-da-Silva, M.A. Vieira-Coelho, M.P. Serrão & C. Marques. Institute of Pharmacology & Therapeutics, Faculty of Medicine, 4200 Porto, Portugal.

Catechol-O-methyltransferase (COMT) is of major importance in the metabolic inactivation of catecholamine neurotransmitters, catechol steroids and xenobiotic catechols. Until recently, there have been no selective, non toxic inhibitors of COMT and the present availability of such compounds opens new therapeutic possibilities. Tolcapone, a new COMT inhibitor, is an electron deficient catechol derivative which is believed to bind well to the enzyme, but appears to be sufficiently deactivated so that methyl transfer from S-adenosyl-methionine (SAM) does not occur (Zürcher et al., 1990). The liver has been considered to be endowed with the highest levels of enzyme activity, closely followed by that in renal tissues. However, previous work form our laboratory has shown that despite similar V_{max} and K_m values for liver and kidney COMT activities, the form of the enzyme in renal tissues reveals a 4-fold increased sensivity to inhibition by tolcapone (Soares-da-Silva & Vieira-Coelho, 1993). The present work has studied COMT acitivity in the rat and pig renal cortex and in two kidney cell lines, OK cells (derived from the opossum kidney) and LLC-PK_1 cells (derived from the pig kidney); the sensitivity to tolcapone was also studied. COMT activity, evaluated by the ability to methylate adrenaline into metanephrine, was determined in homogenates prepared in 0.5 mM phosphate buffer (pH=7.8). The homogenates were incubated for 30 min at 37° C with increasing concentrations of adrenaline (0.5-500 μM) in the absence of oxygen; the incubation medium contained pargyline (0.1 mM), $MgCl_2$, EGTA (1 mM) and SAM (0.1 mM). Some experiments were performed in the presence of increasing concentrations (0.5 to 5000 nM) of tolcapone. The kinetics of COMT are indicated in the table.

Kinetic parameters (V_{max} in pmol mg protein^{-1} h^{-1}; K_m in μM) of COMT activity in homogenates of OK and LLC-PK_1 cells and homogenates of renal cortex obtained from pig and rat kidneys in control conditions and in the presence of tolcapone (n=4).

	V_{max}	K_m
OK cells		
Control	4329±184	3.6 (3.5, 3.7)
Tolcapone (2.5 nM)	1705± 91	5.1 (4.1, 6.4)
LLC-PK_1 cells		
Control	354± 45	18.0 (9.3, 34.7)
Tolcapone (2.5 nM)	146± 23	39.2 (17.6, 87.3)
Pig kidney		
Control	984± 53	67.4 (44.9, 101.1)
Tolcapone (100 nM)	536± 29	182.5 (117.3, 284.1)
Rat kidney		
Control	6676±531	19.9 (11.5, 34.5)
Tolcapone (100 nM)	3564±295	27.1 (16.5, 44.4)

The IC_{50} (in nM) values (n=4) for inhibition by tolcapone were as follows: OK cells, 3.0 (2.6, 3.5); LLC-PK_1 cells, 1.5 (0.5, 4.9); pig kidney, 161.2 (130.8, 198.6); rat kidney, 176.7 (130.5, 239.3). In conclusion, tolcapone was found to be an effective and potent inhibitor of renal COMT, particularly that present in OK and LLC-PK_1 cells. In all preparations used, the type of inhibition caused by tolcapone was found to be a non-competitve one, though differences in the affinity of COMT for the substrate do exist between them.

Soares-da-Silva, P. & Vieira-Coelho (1993) J. Am. Soc. Nephrol. 4:896.
Zürcher et al. (1990) J. Neural Transm., [Suppl] 32, 375-380.
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304P THE DOPAMINE REUPTAKE BLOCKER NS 2214 INCREASES LOCOMOTOR ACTIVITY BUT DOES NOT PRODUCE DYSKINESIAS IN MPTP-TREATED COMMON MARMOSETS

R.K.B. Pearce, T. Banerji, J. Scheel-Krüger[1] & P. Jenner, Neurodegenerative Diseases Research Centre, Pharmacology Group, King's College, London SW3 6LX, U.K. and [1]NeuroSearch, DK-2600 Glostrup, Denmark

The common marmoset (*Callithrix jacchus*) shows parkinsonian motor disability following acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) exposure and develops dyskinesias during chronic L-DOPA dosing and on rechallenge with L-DOPA and other dopamine agonist compounds (Pearce *et al.*, 1994). We now report the effects of the potent dopamine reuptake blocker NS 2214 [(*E*)-(1*R*,2*R*,3*S*)-3-(3,4-Dichlorophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carbaldehyde *O*-methyloxime, sulphate (1:1 salt)] upon parkinsonian disability, locomotor activity and dyskinesias in this model.

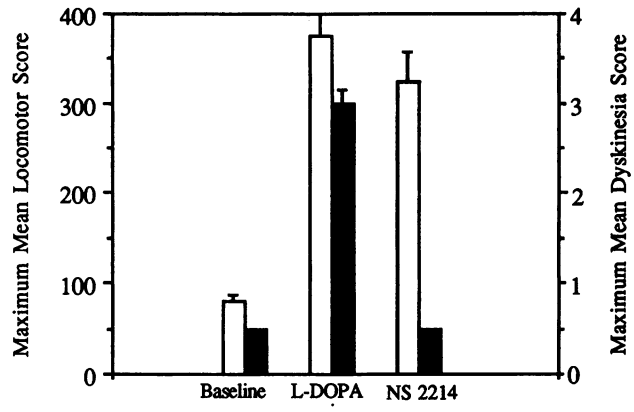
Common marmosets received MPTP (2.0 mg/kg sc for 5 days) and were subsequently treated with L-DOPA (12.5 mg/kg bid po) plus carbidopa (12.5 mg/kg bid po) until they showed dyskinesias. Several months later they received L-DOPA plus carbidopa for two days followed by NS 2214 (0.5 mg/kg po) for 11 days. The animals were monitored in computer-linked infrared beam-equipped cages for a quantitative assessment of locomotor activity. Motor behaviour was graded on a visual scoring system and dyskinesias were rated on a semi-quantitative scoring system (0= absent to 4= severe).

L-DOPA reversed MPTP-induced akinesia but also produced dyskinesias, with prominent limb chorea and dystonia, accompanied by climbing, stereotypy and hyperactivity. NS 2214 administration also reversed parkinsonian akinesia with a prolonged duration of effect. However, NS 2214 did not cause the appearance of dyskinesia, stereotypy or hyperactivity (Figure 1). Tremor was less effectively relieved than akinesia and a mild weight loss (approximately 10%) was detected over the period of NS 2214 administration.

The ability of NS 2214 to produce a prolonged and naturalistic antiparkinsonian response without eliciting previously primed dyskinesia suggests a potential role for this compound in the early treatment of Parkinson's disease.

Pearce R.K.B., Jackson M., Smith L. *et al.* (1994) *Br. J. Pharmacol.* 112, 150P.

Figure 1. Maximum locomotor activity counts (open columns) collected over 10 min segments and maximum mean dyskinesia scores (filled columns) in MPTP-treated common marmosets at baseline, after L-DOPA (12.5 mg/kg po) plus carbidopa (12.5 mg/kg po) and after NS 2214 (0.5 mg/kg po). Bars = S.E.M.



305P CHRONIC SUPRANIGRAL INFUSION OF ISOQUINOLINE DERIVATIVES IN RATS DOES NOT CAUSE NIGROSTRIATAL TOXICITY

K. St.P. McNaught, P. Jenner, B. Testa, A. Carotti & C.D. Marsden, Neurodegenerative Diseases Research Centre, Pharmacology Group, Biomedical Sciences Division, King's College, London, UK.

Isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 1-methyl-4-phenylpyridinium (MPP^+) potently inhibit mitochondrial function, but are moderate to poor substrates for the dopamine re-uptake system and only weakly toxic to PC12 cells (Suzuki *et al.*, 1990; McNaught *et al.*, 1995a,b). However, the *in vivo* neurotoxicity of these compounds is unclear. Consequently, we have studied the effects of chronic supranigral infusion of some isoquinoline derivatives and MPP^+ in rats.

Chronic indwelling cannulae were stereotaxically implanted above the right substantia nigra (P - 5.2 mm, L - 2.4 mm relative to bregma; V - 7.2 mm from skull surface; Paxinos & Watson, 1986) in male Wistar rats (270-310g) under chloral hydrate (400 mg/kg i.p.) anaesthesia. Vehicle (0.9% saline), MPP^+ (33 nmol/24 hr) or isoquinoline derivatives (150 nmol/24 hr) (see legend to Fig. 1) were continuously infused (0.5 µl/24 hr) using Alzet osmotic minipumps. Seven days post-surgery, rats were assessed for spontaneous and drug-induced motor abnormalities in 40 cm hemispherical bowls, then anaesthetised with sodium pentobarbitone (60 mg/kg i.p.) and transcardially perfused with ice-cold 0.1M phosphate buffer (pH 7.4). Brains were immediately removed and transected coronally at the infundibular stem. Striata were rapidly dissected and stored at -70 °C, and monoamine levels determined by HPLC. Nigral blocks were immediately fixed, cryoprotected and stored at -70°C. Coronal sections (20 µm) were cut on a cryostat and stained with 0.1% cresyl violet or used for tyrosine hydroxylase immunohistochemistry.

MPP^+-treated rats were bradykinetic and showed ipsilateral asymmetry. Administration of (+)-amphetamine (5 mg/kg i.p.) or apomorphine (0.25 mg/kg s.c.) to MPP^+-treated rats produced robust ipsilateral and contralateral rotation, respectively. In contrast, rats treated with isoquinoline derivatives or saline did not show any spontaneous or drug-induced motor changes. Cresyl violet staining and TH immunohistochemistry revealed, that while MPP^+ produced marked dopaminergic cell death in the ipsilateral substantia nigra pars compacta (SNc), the isoquinoline derivatives only produced minor SNc cell loss and saline had no effect. MPP^+ depleted the ipsilateral striatal dopamine content by >95% but the isoquinoline derivatives had a less marked and more variable effect (0-52%) and saline was ineffective (Fig. 1). In conclusion, at the concentrations employed in this study, isoquinoline derivatives exert limited toxicity to the nigrostriatal pathway.

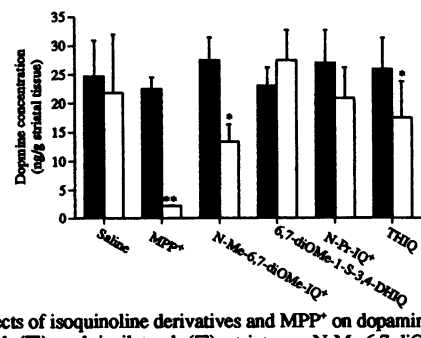


Fig. 1. Effects of isoquinoline derivatives and MPP^+ on dopamine content in contralateral (■) and ipsilateral (□) striatum. N-Me-6,7-diOMe-IQ$^+$, N-methyl-6,7-dimethoxyisoquinolinium; N-Pr-IQ$^+$, N-*n*-propylisoquinolinium; THIQ, 1,2,3,4-tetrahydroisoquinoline; 6,7-diOMe-1-S-3,4-DHIQ, 6,7-dimethoxy-1-styryl-3,4-dihydroxyisoquinoline. Values are mean ± SEM (n = 6). * $p < 0.05$ and ** $p < 0.01$ compared to contralateral striatum of same animal; Student's *t*-test.

Supported by the Wellcome Trust.

McNaught K.St.P. *et al.* (1995a). *Brit. J. Pharmacol. (in press).*
McNaught K.St.P. *et al.* (1995b). *Brit. J. Pharmacol. (in press).*
Paxinos G. and Watson C. (1986). *The rat brain.* Academic Press Inc. USA.
Suzuki K. *et al.* (1990). *Neurochem. Res.* 7, 705-710.

306P EFFECTS OF ADENOSINE RECEPTOR ANTAGONISTS ON CCPA-STIMULATED GUANOSINE-5'-0-(3-[^{35}S]-THIO)TRIPHOSPHATE BINDING TO RAT CEREBRAL CORTICAL MEMBRANES

H. Ito, T. Maemoto, K. Finlayson[1], H.J. Olverman[1] and S.P. Butcher, Fujisawa Institute of Neuroscience and [1]University Department of Pharmacology, Edinburgh, EH8 9JZ, U.K.

We have previously reported the effects of adenosine receptor agonists on the binding of guanosine-5'-O-(3-[^{35}S]-thio)-triphosphate ([^{35}S]GTPγS) to rat cortical membranes (Ito *et al.*, 1994). In the present study, we have examined the effects of adenosine receptor antagonists in this assay. P_2 membranes (7.5μg/sample) from rat cerebral cortex (Sprague Dawley, male) were incubated with 0.2nM [^{35}S]GTPγS (1245 Ci/mmol; NEN) at 25°C for 45 min in 250μl of 50mM Tris-HCl buffer (pH 7.4) containing 1mM EDTA, 5mM $MgCl_2$, 1mM dithiothreitol, 100mM NaCl, 0.2iu/ml adenosine deaminase, 0.5% BSA, 10μM GDP and test drugs. Binding was initiated by addition of membranes and terminated by rapid filtration using a Brandell Cell Harvester. Receptor binding experiments were performed using [^{3}H]DPCPX as a ligand as previously described (Maemoto *et al.*, 1994).

[^{35}S]GTPγS binding was stimulated by 2-chloro-N^6-cyclopentyladenosine (CCPA), an adenosine A_1 receptor agonist, with the maximal 2-fold increase over basal levels at 1μM. Adenosine receptor antagonists inhibited CCPA (0.1μM) - stimulated [^{35}S]GTPγS binding, with the rank order of IC_{50} values corresponding to K_i values determined in receptor binding studies (Table 1; mean ± s.e.mean; n ≥ 3). In order to estimate antagonist affinity, concentration shift studies were performed. Concentration response curves for CCPA were shifted to the right with increasing concentrations of antagonists without a significant change in the maximal response. Schild analyses provided K_B values (Table 1), and each slope was close to unity suggesting competitive antagonism (Table 1). Comparison of functional and receptor binding data indicate a similar rank order of potency in terms of absolute affinity. The [^{35}S]GTPγS binding assay may therefore be a useful determinant of the functional activity of drugs acting at adenosine A_1 receptors.

Ito, H., Olverman, H.J. and Butcher, S.P. (1994) Br. J. Pharmacol. 113, 109P

Maemoto, T., Finlayson, K., Atkins, J.M. *et al.* (1994) Br. J. Pharmacol. 113, 53P

Table 1: IC_{50}/K_B and Ki values determined in [^{35}S]GTPγS and [^{3}H]DPCPX binding assays.

Receptor Antagonists	[^{35}S]GTPγS IC_{50} (nM)	[^{35}S]GTPγS K_B (nM)	[^{35}S]GTPγS Slope	[^{3}H]DPCPX Ki (nM)
FK453 ((R)-1-((E)-3-(2-phenylpyrazolo(1,5-a)pyridin-3-yl)acryloyl)-2-piperidine ethanol)	4.62 ± 1.27	0.84	1.08	0.54 ± 0.09
DPCPX (8-cyclopentyl-1,3-dipropylxanthine)	2.93 ± 0.09	0.99	1.18	0.29 ± 0.01
CGS15943 (9-chloro-2-(2-furyl)[1,2,4]triazol[1,5-c]quinazolin-5-amine)	27.5 ± 5.7	3.58	0.94	1.5 ± 0.3
CPT (8-cyclopentyl-1,3-dimethylxanthine)	81.8 ± 24.4	16.5	1.04	5.9 ± 0.7
DPX (1,3-diethyl-8-phenylxanthine)	573 ± 76	N.D.	N.D.	32 ± 2
8-PT (8-phenyltheophylline)	730 ± 265	N.D.	N.D.	44 ± 2

307P MEASUREMENT OF ADENOSINE RECEPTOR ANTAGONISTS IN RAT BRAIN FOLLOWING INTRAPERITONEAL ADMINISTRATION USING A MODIFIED RADIORECEPTOR ASSAY

K. Finlayson, J. Sharkey[1], H.J. Olverman and S.P. Butcher[1], University Department of Pharmacology and [1]Fujisawa Institute of Neuroscience, Edinburgh, EH8 9JZ, U.K.

Ex vivo binding has been used to measure the ability of a variety of drugs, including adenosine antagonists, to penetrate into brain (Baumgold *et al.*, 1992). Since this assay uses each animal as its own control, we have developed a modified radioreceptor assay for measuring the blood brain barrier permeability of adenosine receptor antagonists.

Male Sprague-Dawley rats (280-300g; Charles River) were injected intra-peritoneally (i.p.) with vehicle or drug. After 20 min, animals were anaesthetised with 5% halothane, blood samples taken from the vena cava and the vasculature flushed with saline for 30 sec. Independent experiments using [^{14}C]inulin found the residual blood content of brain to be 4.5μl/g tissue. The cortex was homogenised in 9 vol of 50mM Tris-HCl buffer (pH 7.4). Protein denaturation (80°C, 15 min) removed the binding capacity of vehicle and drug treated cortical homogenates without affecting the stability of any drug tested. The denatured homogenate was incubated (1h, 37°C) with adenosine deaminase (6iu/ml), and aliquots (50μl) of this homogenate were used as the 'competing drug' in a standard 1ml [^{3}H]DPCPX binding assay using rat brain P_2 membranes (Maemoto *et al.*, 1994). The brain concentration of adenosine receptor antagonists was calculated from a standard curve generated for each drug in the presence of denatured homogenate from vehicle treated animals. A similar procedure was adopted with serum samples diluted 9-fold with buffer.

Evaluation of the assay using DPCPX revealed a detection limit of 6nM in brain and serum, corresponding to 10% inhibition in the standard curve. Brain entry of DPCPX was dose (0.01-1mg/kg) and time (20-180min) dependant with a brain:serum ratio of 0.63 at 20min using 0.03mg/kg. All other antagonists were injected at a dose known to reverse hypolocomotion induced by the adenosine receptor agonist CPA (N^6-cyclopentyladenosine) (Marston *et al.*, 1994). The peripheral adenosine receptor antagonist, 1,3-dipropyl-8-*p*-sulfophenylxanthine (5.6mg/kg; DPSPX) was undetectable in brain but was present in serum at 63μM. An association between receptor affinity and the estimated brain concentration at an equipotent behavioural dose was apparent ($R^2 = 0.86$).

Baumgold, J. *et al.* (1992) Biochem. Pharmacol. 43, 889-894.
Maemoto, T. *et al.* (1994) Br. J. Pharmacol. 113, 53P.
Marston, H.M. *et al.* (1994) Br. J. Pharmacol. 113, 110P.

Table 1: (mean ± s.e.mean; n ≥ 3; N.D. = not detectable)

	Concentration (nM) Brain	Serum
8-PT (1mg/kg; 8-phenyltheophylline)	3831 ± 1457	2790 ± 344
CPT (3mg/kg; 8-cyclopentyl-1,3-dimethylxanthine)	2981 ± 449	10126 ± 925
DPX (0.1mg/kg; 1,3-diethyl-8-phenylxanthine)	1221 ± 449	N.D
MDL102234 (3mg/kg; (R)-3,7-dihydro-8-(1-phenylpropyl)-1,3-dipropyl-1*H*-purine-2,6-dione)	570 ± 55	758 ± 195
FK453 (0.1mg/kg; (R)-1-((E) -3-(2-phenylpyrazolo(1,5-a)pyridin-3-yl)acryloyl)-2-piperidine ethanol)	103 ± 13.5	30.9 ± 1.6
KW3902 (0.03mg/kg; 8-(3-noradamanytyl)-1,3-dipropylxanthine)	23.3 ± 2.54	N.D.
DPCPX (0.03mg/kg; 8-cyclopentyl-1,3-dipropylxanthine)	16.8 ± 3.3	26.5 ± 5.4

308P PHARMACOLOGICAL CHARACTERISATION OF ADENOSINE A_1 RECEPTORS ON HUMAN BRAIN MEMBRANES USING A [^{3}H]DPCPX BINDING ASSAY

T. Maemoto, K. Finlayson[1], H.J. Olverman[1] and S.P. Butcher, Fujisawa Institute of Neuroscience and [1]University Department of Pharmacology, Edinburgh EH8 9JZ, U.K.

Species differences in the pharmacological profile of brain adenosine A_1 receptors have been reported (Klotz *et al.*, 1991). In the present study, we have examined the pharmacology of adenosine A_1 receptors in human brain membranes using [^{3}H]DPCPX, an adenosine A_1 receptor antagonist ligand.

P2 membranes (7µg/sample) prepared from frozen human parietal cortex (Brodman area 7) of 5 male patients who died of acute cardiovascular problems (age range 47-62; 16-60h postmortem delay) were incubated with 0.7nM [^{3}H]DPCPX (NEN; 109Ci/mmol), 0.1iu adenosine deaminase and test drug for 120 min at 25°C in 50mM Tris-HCl (pH 7.4). Assays were terminated by rapid filtration using a Brandell Cell Harvester. Non specific binding was defined using 100µM CPA.

The receptor affinity (K_D) of the human brain adenosine A_1 receptor was 2.49 ± 0.85 nM and the B_{max} was 5.68 ± 1.25 pmol/mg protein. Ki values for agonists and xanthine-based antagonists were similar to values reported previously in guinea-pig, but were approximately 10-fold higher than in rat and mouse (Table 1; Maemoto *et al.*, 1994). Agonist Hill coefficients were invariably less than unity, and addition of 100µM Gpp(NH)p reduced the affinity of CCPA to 380 ± 40 nM (nH = 0.78 ± 0.02). Interestingly, the non-xanthine adenosine receptor antagonist, FK453 exhibited a similar Ki in rat and human membranes (Table 1), suggesting differences in the structural determinants for binding of xanthine and non-xanthine antagonists to adenosine A_1 receptors.

Klotz, K-N., Vogt, H., Tawfik-Schlieper, H. (1991) Naunyn-Schmiedeberg's Arch.Pharmacol. 343, 193-201.
Maemoto, T., Finlayson, K., Atkins, J.M. *et al.* (1994) Br. J. Pharmacol 113, 53P.

Table 1: Receptor affinity (Ki) and Hill coefficient (nH) in human and rat brain membranes (mean ± s.e.mean; n ≥ 3)

	Human		Rat	
Receptor Agonists	Ki (nM)	nH	Ki (nM)	nH
CCPA (2-chloro-N^6-cyclopentyladenosine)	48 ± 11	0.50 ± 0.06	3.6 ± 0.4	0.65 ± 0.02
CPA (N^6-cyclopentyladenosine)	64 ± 13	0.46 ± 0.05	4.3 ± 0.6	0.65 ± 0.03
R-PIA (R(-) N^6-(2-phenylisopropyl)adenosine)	88 ± 21	0.53 ± 0.04	5.2 ± 0.3	0.61 ± 0.02
CHA (N^6-cyclohexyladenosine)	184 ± 27	0.58 ± 0.04	6.9 ± 0.8	0.59 ± 0.03
NECA (5'-N-ethylcarboxyamidoadenosine)	310 ± 68	0.61 ± 0.04	33 ± 5	0.59 ± 0.04
Receptor Antagonists				
DPCPX (8-cyclopentyl-1,3-dipropylxanthine)	2.2 ± 0.3	0.91 ± 0.10	0.29 ± 0.01	1.02 ± 0.01
CPT (8-cyclopentyl-1,3-dimethylxanthine)	49 ± 3	1.31 ± 0.13	5.9 ± 0.7	0.99 ± 0.05
DPX (1,3-diethyl-8-phenylxanthine)	470 ± 120	0.91 ± 0.07	32 ± 2	1.03 ± 0.03
8-PT (8-phenyltheophylline)	730 ± 140	0.86 ± 0.05	44 ± 2	1.03 ± 0.04
FK453 ((R)-1-((E) -3-(2-phenylpyrazolo(1,5-a)pyridin-3-yl)acryloyl)-2-piperidine ethanol)	0.50 ± 0.07	0.90 ± 0.16	0.54 ± 0.09	1.06 ± 0.04

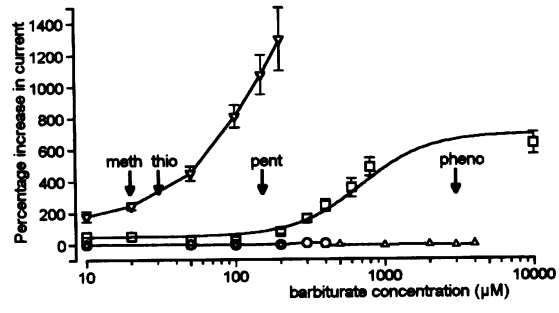
309P THE EFFECT OF BARBITURATES ON RAT GLYCINE RECEPTORS AND RECEPTOR SUBUNITS EXPRESSED IN *XENOPUS* OOCYTES

C.J. Shelton, R.J. Roberts, S.M. Bishop and S. Daniels
(Introduced by K.J. Broadley)
Pharmacology Department, Welsh School of Pharmacy, University of Wales College, Cardiff, Cardiff. CF1 3XF.

In an oocyte expression study we have shown that pentobarbitone acts by inhibiting the current through kainate type glutamate receptors and by potentiating the current through $GABA_A$ type receptors at clinical concentrations. Strychnine sensitive glycine receptors were not affected by pentobarbitone at saturating concentrations of agonist (Daniels *et al.* 1995). By comparison, in dissociated neurons from the CNS it was shown that the volatile anaesthetics halothane and enflurane potentiate glycine receptor current by shifting the glycine concentration response curve to the left in a similar manner to their effect upon $GABA_A$ receptor channels (Wakamori *et al.* 1991). We have expressed strychnine sensitive glycine receptors and also homomeric glycine α_1 receptor subunits in *Xenopus* oocytes in order to study the effects of pentobarbitone, phenobarbitone, methohexitone and thiopentone on the isolated glycine receptor-channel. In our initial studies with glycine receptors expressed from adult rat spinal cord mRNA we found that barbiturates depressed the maximally activated (1mM glycine) receptor current by less than 10% at anaesthetic concentrations. With cDNA derived homomeric glycine receptor α_1 subunits similar results were obtained. Glycine concentration response curves prepared from heteromeric and homomeric receptor-channels again indicated a small depression by barbiturates at saturating glycine concentrations.

With homomeric α_1 receptor subunits pentobarbitone (100µM) and thiopentone (20µM) potentiated the current at low (50µM) glycine by 36 ± 10 % and 245 ± 23 % respectively (n = 6). Phenobarbitone and methohexitone had no measurable effect at the anaesthetic concentrations shown in Figure 1.

Figure 1



Effect of increasing barbiturate anaesthetic concentration on the membrane current elicited by a low glycine concentration (50µm) in *Xenopus* oocytes expressing the α_1 glycine receptor subunit (n=6). □ pentobarbitone, ○ methohexitone, △ phenobarbitone, ▽ thiopentone. Marked arrows denote anaesthetic ED_{50} (tadpole) for each barbiturate.

Daniels, S., Roberts, R.J., Shelton, C.J. (1995). B.J.P. in press (Oxford meeting).
Wakamori, M., Ikemoto, Y. and Akaike, N. (1991). J. Neurophysiol. 66, 2014 - 2021.

310P COMPARISON OF THE INTERACTION OF AGMATINE AND CRUDE METHANOLIC EXTRACTS FROM BOVINE LUNG AT α_2-ADRENOCEPTORS ON GUINEA-PIG CORTEX SLICES

D. Pinthong, V.G. Wilson and D.A.Kendall. Dept of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH.

Partially purified 'clonidine displacing substance' (CDS) from bovine lung has been reported to displace [^{3}H]-clonidine from α_2-adrenoceptor binding sites on rat and bovine cerebral cortex membranes (Atlas & Burstein,1984), and to activate α_2-adrenoceptors in human platelets (Diamant *et al.*, 1987) and prejunctional α_2-adrenoceptors on the rat vas deferens (Diamant and Atlas,1986). Agmatine has recently been suggested to be an endogenous CDS since it occupied α_2-adrenoceptor binding sites on rat cerebral cortex membranes (Li *et al.*, 1994). We have recently shown that both agmatine and crude methanolic CDS extracts from bovine brain and lung produced a concentration-dependent inhibition of [^{3}H]-clonidine binding to bovine cerebral cortex membranes (Pinthong *et al.*, 1995a). Agmatine was found to be devoid of activity at either peripheral or central α_2-adrenoceptors (Pinthong *et al.*, 1995b) whereas no data are available to indicate whether these partially purified CDS extracts can activate central α_2-adrenoceptors.

In this present study, we have compared the effects of bovine lung methanolic CDS extracts and agmatine with a known α_2-adrenoceptor agonist, UK14304, on forskolin-stimulated cAMP accumulation in guinea-pig cerebral cortex slices in order to examine whether these putative CDS have any effect on the activity of α_2-adrenoceptors.

Cerebral cortex slices were prepared from male guinea pigs (*Dunkin Hartley*, 300-700 g) and incubated for 60 min in modified Krebs-Henseleit buffer and then labelled with [^{3}H]-adenine (74 kBq.ml^{-1}) for 45 min. After washing away the unincorporated [^{3}H]-adenine, the slices were incubated for 10 min with or without the α_2-adrenoceptor antagonist, idazoxan (10^{-6}M), and then incubated for 10 min with agmatine (10^{-7}-10^{-4} M) or with lung methanolic CDS extracts (0.14,1.4 and 8.2 units; 1 unit of CDS activity is equal to the amount of the extract that produced 50% inhibition of [^{3}H]-clonidine (1nM) binding to bovine cerebral cortex membranes in a total volume of 1 ml) or with UK14304 (10^{-9}-10^{-6}M) before adding 30μM forskolin. After 10 min the incubations were terminated by the addition of 1M HCl and [^{3}H] - cAMP was isolated by sequential Dowex50/alumina chromatography and quantified by using liquid scintillation counting.

In the presence of 30μM forskolin, [^{3}H]-cAMP accumulation was increased by 4.9±1.7 fold (n=5) of the basal cAMP generation. The α_2-adrenoceptor agonist, UK14304, inhibited forskolin-stimulated cAMP accumulation in a concentration-dependent manner with an IC_{50} of 3.1±1.0x10^{-8}M (n=3). The maximum inhibition was 72.1±3.2 % of the forskolin response (n=3). The effect was prevented by the α_2-adrenoceptor antagonist, 10^{-6}M idazoxan, and idazoxan alone had no effect on the forskolin-induced response. Agmatine (10^{-7}-10^{-4}M) did not produce any significant effect ($p>0.05$, Student's t-test) on forskolin-stimulated cAMP accumulation whereas the bovine lung methanolic extract increased the effect of forskolin stimulation at lower concentrations (0.14 and 1.4 units) to 2.9±0.4 and 4.0±0.6 fold (n=3) of the forskolin-response. However, at a higher concentration of the extract (8.2 units) there was no significant effect on the forskolin response. The effects of this partially purified extract were not modified by idazoxan. However, the stimulatory effect of the lower concentration of the extracts was inhibited by a combination of 10^{-6}M mepyramine and 10^{-4}M cimetidine.

This study provides evidence that neither agmatine nor the bovine lung extract represent a biologically-active endogenous CDS; both failed to mimic the inhibitory effect of the α_2-adrenoceptor agonist, UK-14304, on cAMP accumulation. In addition, the stimulatory effects of the lung extract on cAMP accumulation may be due to contamination by histamine.

Atlas, D. and Burstein, Y. (1984) Eur. J. Pharmacol., **144**, 287-293.
Diamant, S. and Atlas, D. (1986) Biochem. Biophys. Res. Comm., **134**, 184-190.
Diamant, S., Eldor, A. and Atlas, D. (1987) Eur. J. Pharmacol.,**144**, 247-255.
Li, G., Regunathan, S., Barrow, C.J., Esharghi, J., Cooper and Reis, D.J. (1994) Science **263**, 966-969.
Pinthong, D., Hussain, J.F., Kendall, D.A. and Wilson, V.G. (1995a) Br. J. Pharmacol., **115**, 689-695.
Pinthong, D., Wright, I.K., Hanmer, C., Millins, P., Mason, R., Kendall., D.A. and Wilson, V.G. (1995b) Naunyn-Schmiedeberg's Arch. Pharmacol., **351**, 10-16.

311P MODULATION OF THE POLYSYNAPTIC REFLEX OF THE RAT HEMISECTED SPINAL CORD BY XYLAZINE AND DETOMIDINE *IN VITRO*

E.S.L. Faber, R.H. Evans & J.P. Chambers, Department of Pharmacology, School of Medical Sciences, University of Bristol. BS8 1TD.

The dorsal root evoked ventral root potential (DR-VRP) in the rat hemisected spinal cord produces a polysynaptic reflex (PSR) which has a duration of between twenty and forty seconds (Nussbaumer et al 1989). The polysynaptic reflex is thought to be dominated by C fibre afferent input and to reflect synaptic activity in a nociceptive pathway. This reflex has been shown to be depressed by a number of agents which are analgesic in man including opioids (Nussbaumer et al 1989) and α2 adrenoceptor agonists (Kendig et al 1991). The monosynaptic compound action potential of motoneurons (MSR) in the in vitro spinal cord preparation is unaffected by analgesic drugs (Nussbaumer et al 1989). In the present experiment the actions of two other α2 adrenoceptor agonists xylazine and detomidine, which also have analgesic activity clinically, have been compared with clonidine.

Hemisected spinal cord preparations from Wistar rats aged between three and six days (unsexed, approximately 10g in weight) were bathed in a Ringer's solution containing 1.25mM Mg^{2+} and 1.5mM Ca^{2+}. The L4 or L5 ventral root was recorded from following stimulation of the corresponding dorsal root at 16 times threshold to evoke the PSR and at 3 times threshold to evoke the MSR.

Clonidine, xylazine and detomidine were each applied cumulatively to different preparations. Each of these drugs produced a selective dose-dependent depression of the PSR with IC_{50} values (expressed as mean ± sem) of 42 ± 1.41 nM (n=4), 1 ± 1.07 μM (n=4) and 51.3 ± 1.13 nM (n=4) respectively. These values are comparable with the relative analgesic potencies reported from writhing tests (Skingle et al 1982, Virtanen 1986). The depressant effects in the current study were fully reversed by the α2 adrenoceptor antagonist atipamezole (1μM). The MSR was not significantly affected by any of these drugs. Thus the present results with xylazine and detomidine confirm the close correlation which appears to exist between analgesic activity and depression of the PSR in the in vitro spinal cord preparation.

Supported by the Wellcome Trust and the Medical Research Council.

Nussbaumer, J-C., Yangisawa, M. & Otsuka, M. (1989) Br. J. Pharmacol., 98, 373-382
Kendig, J.J., Savola, M.K.T., Woodley, S.J. & Maze, M. (1991) Eur. J. Pharmacol., 192, 293-300
Skingle, M., Hayes, A.G. & Tyres, M.B. (1982) Life Sciences, 31, 1123-1132
Virtanen, R. J. vet. Pharmacol. Therap. (1986) 9, 286-292

312P DEMONSTRATION OF LOW THRESHOLD ACTIVATION OF ANALGESIC-SENSITIVE SYNAPTIC REFLEXES IN THE RAT SPINAL CORD *IN VITRO*

R.H.Evans Department of Pharmacology, School of Medical Sciences, University of Bristol BS8 1TD & Tocris Cookson Ltd, Churchill Bldg, Langford House, Langford, Bristol BS18 7DY.

The synaptic excitation recorded in ventral roots (DR-VRP) which persists for tens of seconds following supramaximal electrical stimulation of dorsal roots in the in vitro spinal cord of the rat is considered to be dominated by C fibre primary afferent input and in consequence to be selectively depressed by opiate (Nussbaumer et al 1989) and alpha$_2$-adrenoceptor agonists (Kendig et al 1991). However, in vivo these drugs potently depress spinal reflexes which do not involve the activation of primary afferent C fibres (Clarke et al 1988). The present experiments show that these drugs can depress reflexes of the in vitro spinal preparation evoked at stimulus intensities too low for activation of C fibres.

Hemisected spinal cord preparations from 3 to 9 day old Wistar rats of either sex were bathed in medium containing 1.5 mM $Ca2^{+}$ and 1.25 mM Mg^{2+}. DR-VRP was recorded from L4 or L5 ventral root following a 0.5 msec duration cathodal pulse applied to the central end of the dorsal root.

DR-VRPs evoked at stimulus intensities of up to three times threshold consisted of an initial population compound action potential (MSR) of motoneurones superimposed on a population epsp which had a total duration usually of less than 5 seconds. On three preparations application of morphine (1 µM) for 15 to 25 min produced from 15 to 46% depression of the area under the curve of the population epsc. The depression could be abolished by naltrexone (1 µM). On two preparations tizanidine (0.2 µM) produced idazoxan (1 µM) reversible depression of 42 and 45%. The MSR was not significantly altered by these drugs. These observations show that it is not necessary to excite C fibre afferents in order to evoke analgesic-sensitive spinal reflexes in vitro. This is consistent with the behaviour of in vivo spinal preparations.

The experiments were carried out during an award under the Senior Academics in Industry Scheme between Tocris Cookson Ltd and the Department of Trade and Industry.

Clarke, R.W., Ford, T.W. & Taylor, J.S.(1988) J.Physiol.(Lond.) 404, 407-417.
Kendig, J.J., Savola, M.K.T., Woodley, S.J. & Maze, M.(1991) Eur.J.Pharmacol.192, 293-300
Nussbaumer, J.-C., Yanagisawa, M. & Otsuka, M.(1989) Br.J.Pharmacol., 98, 373-382.

313P INVOLVEMENT OF NMDA RECEPTORS IN THE MONOSYNAPTIC SEGMENTAL REFLEX OF THE RAT SPINAL CORD *IN VITRO*

R.H.Evans, Department of Pharmacology, School of Medical Sciences, University Walk, Bristol BS8 1TD & Tocris Cookson Ltd, Churchill Bldg, Langford House, Langford, Bristol BS18 7DY.

The monosynaptic component of segmental reflexes in motoneurones (MSR) produced by single supramaximal shocks to dorsal roots at intervals of several seconds has been reported to be characteristically sensitive to AMPA-, but resistant to NMDA-receptor antagonists (Long et al, 1990). The present experiments show that this characteristic does not appear to hold for all conditions of stimulation.

Hemisected spinal cord preparations from 3 to 9 day old Wistar rats of either sex were bathed in medium containing 1.5 mM Ca^{2+} and 1.25 mM Mg^{2+} at 25 ^{o}C. MSRs measured as the area under the oscillographic trace of the initial monosynaptic population spikes of motoneurones were recorded from the L5 ventral root proximal to the spinal cord in response to electrical stimulation of the corresponding dorsal root. Square pulses of 0.5 msec duration were applied to a cathode in contact with the bathing medium close to the central end of the dorsal root and an anode in contact with the distal end of the dorsal root.
The MSR is maximal at approximately four times threshold (T) stimulus intensity. In three preparations MSRs evoked by stimulus intensities of less than 2T were depressed by at least 25% in the presence of 50 µM AP5 (D-2-amino-5-phosphonopentanoate). In agreement with previous results AP5 had no significant depressant action when a supramaximal stimulus intensity of 16T was used. Furthermore when paired pulses at an intensity of 3T were applied at 200 msec interval then the second MSR, which was superimposed on the population epsp evoked by the first pulse, was depressed by 64% in the presence of AP5. When the first pulse of the pair was switched off the MSR was unaffected by AP5.

These observations show how the relationship between NMDA and non-NMDA receptors in the synaptic excitation of motoneurones is not fixed but is context dependent. Such context dependence may explain differences in susceptibility to NMDA antagonists between excitatory responses produced at different spinal sites or by different types of stimuli (Headley, Parsons & West 1987).

The experiments were carried out during an award under the Senior Academics in Industry Scheme between Tocris Cookson Ltd and the Department of Trade and Industry.

Headley, P.M., Parsons, C.G. & West, D.C.(1987) J.Physiol.(Lond.) 385, 169-188.
Long, S.K., Smith, D.A.S., Siarey, R.J. & Evans, R.H.(1990) Br.J.Pharmacol., 100, 850-854.

314P DESENSITIZATION OF δ-OPIOID MOBILIZATION OF INTRACELLULAR CALCIUM IN SH-SY5Y CELLS

M. A. Connor, M.J. Keir & G.Henderson, Dept. Pharmacology, University of Bristol, University Walk, Bristol, BS8 1TD.

In the human neuroblastoma cell line SH-SY5Y, δ and μ opioid receptor agonists mobilize intracellular Ca^{2+} when applied in the presence of carbachol (Connor & Henderson 1994). There is good evidence *in vivo* for subtypes of δ receptor, although the δ receptor cloned from SH-SY5Y cells exhibits a mixed δ_1/δ_2 pharmacological profile (Simonin *et al*. 1994). Furthermore, we have observed that both the δ_1 "selective" agonist DPDPE and the δ_2 "selective" agonist deltorphin II mobilize Ca^{2+} in these cells, and that the mobilization by either agonist is potently inhibited by both δ_1 and δ_2 selective antagonists (Connor *et al*. 1995). We have now examined whether there is cross desensitization between δ agonists in mobilizing Ca^{2+} in SH-SY5Y cells.

Intracellular Ca^{2+} concentrations ($[Ca^{2+}]i$) were measured in confluent monolayers of undifferentiated SH-SY5Y cells loaded with the Ca^{2+} sensitive dye Fura 2. Standard ratiometric measurements of $[Ca^{2+}]i$ were made. Data are presented as mean ± s.e.mean. All the applications of test drugs were made in the continuing presence of carbachol (1 μM).

A brief (30s) application of a maximally effective concentration of DPDPE (1 μM) elevated $[Ca^{2+}]i$ by 30 ± 4 nM and a second 30s application 30min later elevated $[Ca^{2+}]i$ by 28 ± 4 nM. In parallel experiments addition of a maximally effective concentration of deltorphin II (1 μM) together with the second application of DPDPE resulted in an increase of $[Ca^{2+}]i$ that was not greater than the initial elevation of $[Ca^{2+}]i$ by DPDPE alone (101 ± 7 %, n=4). Prolonged (5 min) application of DPDPE (1 μM) increased $[Ca^{2+}]i$ from 62 ± 2 nM to 104 ± 6 nM (n=17) but this elevation was not sustained for the duration of the drug exposure. After 5 min in DPDPE the $[Ca^{2+}]i$ had returned to predrug levels (58 ± 2 nM). Similarly, deltorphin II (1 μM) elevated $[Ca^{2+}]i$ from 49 ± 7 to 99 ± 6 nM (n=3), after 5 min in deltorphin II $[Ca^{2+}]i$ had again declined to predrug levels (50 ± 2 nM). Deltorphin II (300 nM) applied at the end of a 5 min DPDPE (1 μM) exposure caused essentially no elevation of $[Ca^{2+}]i$ (62 ± 3 nM to 63 ± 3 nM, n=7). DPDPE (300 nM) applied at the end of a 5 min deltorphin II (1 μM) application also failed to raise $[Ca^{2+}]i$ (n=3).

This failure of either DPDPE or deltorphin II to further elevate $[Ca^{2+}]i$ in the continued presence of the other opioid agonist could reflect desensitization at the level of the receptor, signal transduction pathway or an exhaustion of intracellular Ca^{2+} stores. To test the last possibility we applied agonists at other receptors which also mobilize intracellular Ca^{2+} only in the presence of carbachol. NPY (30 nM) applied at the end of 5 min exposure to DPDPE (1 μM) elevated $[Ca^{2+}]i$ by 26 ± 10 nM, which was not different from the elevation of $[Ca^{2+}]i$ caused by NPY alone (28 ± 4 nM, n=4). Similarly, the μ opioid agonist DAMGO (1 μM) elevated $[Ca^{2+}]i$ by 11 ± 2 nM when applied at the end of a 5 min DPDPE exposure, this was not significantly different from the elevation caused by DAMGO alone (15 ± 2 nM, n=6).

The elevations of $[Ca^{2+}]i$ caused by the δ-opioid agonists DPDPE and deltorphin II are not additive and appear to cross desensitize. This desensitization does not result from exhaustion of intracellular Ca^{2+} stores or from a generalized inactivation of signal transduction through Gi/Go coupled receptors. Together with previous studies using selective δ_1 and δ_2 receptor antagonists this study suggests that deltorphin II and DPDPE may act through a single population of δ receptors to mobilize intracellular Ca^{2+} in SH-SY5Y cells.

Connor, M.A. & Henderson, G (1994) *Brit. J. Pharmacol.* 114:136P
Simonin, F. *et al*. (1994) *Mol. Pharm*. 46:1015-1021
Connor, M.A., Keir, M.J. & Henderson, G. (1995) *Analgesia, In Press*

315P HYPERALGESIA IN MONOARTHRITIC RATS: ENHANCEMENT BY $GABA_B$ RECEPTOR ANTAGONISM

S. A. Towers, M. Malcangio, H.M. da Silva & N. G. Bowery, Dept of Pharmacology, School of Pharmacy, Brunswick Square, London WC1N 1AX.

It has been reported that the inflamed paw of mono-arthritic rats develops a low threshold to thermal (Malcangio and Bowery, 1994), and mechanical (Iadarola *et al.* 1988) stimuli which is maximal two days after inoculation with Freund's adjuvant and declines during the subsequent 28 days. The pain threshold is further decreased by administration of $GABA_B$ antagonist CGP 36742 (100 mg kg^{-1}, i.p.) in both the inflamed and contralateral paws at 21 days following induction of mono-arthritis (Malcangio & Bowery, 1994). This dose of the antagonist has been shown to block, *in vivo*, the effects of the $GABA_B$ agonist, baclofen (Bittiger *et al.* 1992).

The aim of this study was to further investigate the effect of CGP 36742 (3-aminopropyl-*n*-butyl-phosphinic acid) on monoarthritis-induced hyperalgesia at various day intervals (7, 14, and 21 days) after inoculation with Freund's adjuvant. For comparison a $GABA_B$ antagonist CGP 56999 ([3-{[1-(R)-(3-carboxyphenyl)ethyl]amino}-2-(S)-hydroxy-propyl]cyclohexyl-methyl-phosphonic acid) with at least 1000 fold higher affinity for the receptor was also examined.

Monoarthritis was induced in male Lewis rats (190-240 g) via the intradermal injection of 500μg *Mycobacterium tuberculosis* /100μl mineral oil (complete Freund's adjuvant, CFA) into the left hind paw. Controls were injected only with mineral oil (incomplete Freund's adjuvant, IFA) as described by Malcangio & Bowery (1994).

Paw withdrawal latencies (PWL) in response to thermal stimuli were measured in s and pre-injection latencies were subtracted from 15, 30, and 45 min post-drug administration latency values. CGP 36742 (30 min after injection of 100mg kg^{-1} i.p.) significantly reduced CFA rat ipsilateral PWL by 4.0 ± 0.4 s (*n*=14), 4.0 ± 0.7 s (*n*=9), and 5.6 ± 0.7 s (*n*=7) at 7, 14 and 21 days after inoculation respectively. The reduction in PWL at all times was statistically significant ($P<0.05$) using ANOVA. CGP 36742 did not modify PWL of IFA treated rats, but did significantly reduce PWL in contralateral paws of CFA rats at 14 and 21 days.

CGP 56999 (1 mg kg^{-1}, i.p.)·30 min after administration also significantly reduced ipsilateral PWL in CFA treated rats by 4.5 ± 0.5 s (*n*=6, $P<0.01$), 6.8 ± 0.5 s (*n*=6, $P<0.01$), and 4.3 ± 0.6 s (*n*=5, $P<0.05$) at 7, 14 and 21 days after inoculation respectively. Neither of these $GABA_B$ antagonists were hyperalgesic in naive rats.

The potentiation of Freund's adjuvant induced hyperalgesia in the inflamed paw by these $GABA_B$ antagonists suggests the presence of GABAergic tone in the primary afferent input to the dorsal spinal cord, whereby removal of the inhibitory action of GABA leads to increased excitability and hence an increase in nociception. GABAergic tone appears to be present at least 7 days following injection of Freund's adjuvant and is important up to at least 21 days post-injection.

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Bittiger, H. *et al.* (1992) Pharmacol. Commun. 2, 70-74.
Iadarola, M., *et al.* (1988) Brain Res. 455, 205-212.
Malcangio, M. & Bowery, N.G. (1994) Br. J. Pharmacol. 113, 1561-1566.

316P IMMUNOLOGICAL ASSESSMENT OF GLYCOSYLATION SITES IN HUMAN CORTICOSTEROID BINDING GLOBULIN (CBG)

H.J. Harris & R.J. Flower. *Department of Biochemical Pharmacology, The William Harvey Research Institute, The Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, UK. EC1M 6BQ.*

To test the hypothesis that leukocyte elastase (LE) cleaves the steroid transporter CBG at sites of inflammation (Hammond *et al.*, 1987; Pemberton *et al.*, 1988), we generated a novel anti-human CBG antibody (anti $H_{337-344}$ antibody) which preferentially recognised cleaved CBG (Harris & Flower, 1994). Here we investigate the mechanism of this differential recognition using western blot analysis and novel epitope-selective antibodies. In particular, we test whether carbohydrate attachment to the N-glycosylation sites on human CBG (hCBG) influences the exposure of these critical epitopes.

We deglycosylated hCBG (a gift from Allelix Biopharmaceuticals Inc., Canada) using endoglycosidase F/N-glycosidase F (Endo-F; Boehringer Mannheim UK) at a protein:enzyme ratio of 1µg:0.05U overnight at 37°C in deglycosylation buffer (0.1M Na_2HPO_4, pH8.0, 0.2% triton-X100, 0.1% SDS, 0.01M EDTA, 0.01M dithiothreitol). 10%SDS-PAGE followed by western blotting (using anti $H_{337-344}$ antibody and novel anti rat CBG antibodies, anti $R_{144-151}$ and anti $R_{339-348}$ at 0.5µgIgG/ml) revealed changes in hCBG recognition which we quantified by densiometric analysis (values given as median absorbance with range). We compared these changes to the increased signal observed when anti $H_{337-344}$ antibody detects cleaved hCBG *in vitro* (Harris & Flower, 1994).

Anti $R_{144-151}$ and anti $R_{339-348}$ antibodies against rat CBG residues 144-151 and 339-348 failed to recognise homologous epitopes in intact hCBG despite an estimated inter-species sequence identity of 88% and 40%, respectively. However, after deglycosylation of hCBG, recognition by these antibodies increased significantly (**P<0.01 compared to intact hCBG; Mann Whitney U- unpaired test). Endo-F treatment of hCBG did not alter the recognition of hCBG by anti $H_{337-344}$ antibody (Table 1).

	Anti $R_{144-151}$ antibody n=8	Anti $R_{339-348}$ antibody n=7	Anti $H_{337-344}$ antibody n=4
Intact hCBG	0 (0-0.047)	0 (0-0.031)	0.029 (0.015-0.065)
Endo-F-treated hCBG	0.027** (0.021-0.089)	0.036** (0-0.073)	0.044 (0.028-0.094)

Table 1: Densiometric analysis of western blots; values represent median absorbance (range).

Treatment of hCBG with LE significantly increased the recognition of hCBG by anti $H_{337-344}$ antibody from 0.029 median absorbance (range 0-0.069) to 0.195 (0.031-0.449) n=12, P<0.05, for the major bands.

Using anti rat CBG antibodies we show that homologous regions in hCBG (residues 152-159 and 348-357 of hCBG, respectively) are shielded by carbohydrate, probably attached at Asn_{154} and Asn_{347}, respectively. Clipping of hCBG by LE removes a C-terminal fragment (Thr_{345}-Val_{383}; Pemberton *et al.*, 1988) which includes Asn_{347}. However, we propose that the enhanced exposure of epitope 337-344 after the action of LE does not result from removal of this carbohydrate moiety *per se* because Endo-F treatment does not increase the signal to hCBG blotted with anti $H_{337-344}$ antibody. Thus the enhanced signal to cleaved hCBG results from removal of the polypeptide chain itself and cannot be attributed to removal of the carbohydrate on the leaving fragment. These results suggest that epitope 337-344 may serve as a marker for cleaved hCBG and that anti $H_{337-344}$ antibody could be used to test biological fluids for intact and cleaved hCBG.

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Hammond, G.L., *et al.*, 1987. Proc. Natl. Acad. Sci. USA., **84**, 5153-5157.
Harris, H.J. & Flower, R.J., 1994. Br. J. Pharmacol., **111**, 281P.
Pemberton, P.A., *et al.*, 1988. Nature, **336**, 257-258.

317P EFFECTS OF OXIDISED LDL ON BASAL AND STIMULATED NITRIC OXIDE RELEASE IN RAT AORTA

K.L. McPherson, C.A. Hamilton, A.F. Dominiczak, M. McIntyre, J.L. Reid. Department of Medicine and Therapeutics, Western Infirmary, Glasgow, G11 6NT.

Altered reactivity of vascular smooth muscle and impaired endothelium-dependent relaxation are well documented in the development of atherosclerotic disease. Risk factors such as elevated cholesterol levels have been linked to these alterations (Kugiyama *et al.*, 1990), however exact mechanisms are not fully understood (Rosenfeld, 1991). The aim of this study was to examine the effects of oxidised low density lipoproteins (ox-LDL) on basal and stimulated nitric oxide release.

LDL was prepared via sequential density gradient ultracentrifugation from the freshly drawn blood of healthy, normolipidemic volunteers. Samples were oxidised by addition of copper chloride (4uM) and incubation at room temperature for 8 hours. A minimum of 5 individual preparations were used per experiment type. Aortic rings 3-4mm wide were prepared from male Sprague Dawley rats (> 6 weeks). Rings were incubated for 5 hours, at 37°C, 5% CO_2 95% air, in control, 100ug/ml, or 500ug/ml human ox-LDL solutions. Following incubation rings were mounted under 1g tension for recording of isometric tension. Concentration-response curves were constructed to phenylephrine (10^{-8} - $3x10^{-5}$M), carbachol (10^{-8} - $3x10^{-5}$M), and potassium chloride (10^{-2} - 10^{-1}M). Tissues were then contracted to EC_{20} phenylephrine and the nitric oxide synthase inhibitor L-NAME (200uM) added at the plateau of phenylephrine contraction. The additional contraction obtained after L-NAME treatment was taken as a measurement of basal nitric oxide release. Statistical analysis was carried out using repeated measures ANOVA and Bonferroni paired multiple comparison tests.

No shift in concentration-response to phenylephrine (n=11) was observed on ox-LDL treatment, however, treated tissues showed increased maximum contraction (control 29.7g tension/0.1g tissue +/-4.5, 100ug/ml 42.4 +/-5.1*, 500ug/ml 38 +/-4.3). A significant decrease in the carbachol relaxation response (n=10) was observed (maximum response as % of contraction; control 92.83 +/-2.6, 100ug/ml 91.88 +/-2.7, 500ug/ml 73.84 +/-7.9), however there was no significant alteration in the potassium chloride response (n=11). Contraction in response to L-NAME (n=5) was significantly reduced in ox-LDL treated tissues, this response being concentration dependent (as a % 10^{-1}M KCl; control 207.82 +/-20, 100ug/ml 118.98 +/-14.3*, 500ug/ml 50.56 +/-11.9*).

The ox-LDL-induced attenuation of basal nitric oxide release may account for the increased response to phenylephrine observed, and potentiation of agonist responses reported during the early development of atherosclerosis (Kolodgie *et al.*, 1990).
Basal release was shown to be more sensitive to ox-LDL, than carbachol stimulated release, consistent with suggestions that alterations in contractile responses preceed decreased endothelium-dependent relaxation.

*Highlights significant difference from control (p<0.05)

Kolodgie F.D., Virmani R., Rice H.E., Mergner W.J. (1990) Circ. Res. 66, 1112-1126
Kugiyama *et al.* (1990) Nature 344, 160-162
Rosenfeld M.E. (1991) Circulation 83(6), 2137-2140

318P EFFECTS OF CYTOCHROME P_{450} INHIBITORS ON NITRIC OXIDE SYNTHASE-DEPENDENT RESPONSES IN RAT AORTA AND ANOCOCCYGEUS MUSCLE

M.J. Rand & C.G. Li, Pharmacology Research Laboratory, Department of Medical Laboratory Science, Royal Melbourne Institute of Technology, Victoria 3001, Australia

Nitric oxide synthases (NOS) have a close homology to cytochrome P_{450} reductase (CPR) (Bredt *et al.*, 1991). The formation of NO by NOS involves Nω-hydroxy-L-arginine (OH-Arg) as an intermediate reactant (Stuehr *et al.*, 1991) and OH-Arg can be converted to NO and citrulline not only by NOS but also by CPR (Boucher *et al.*, 1992). If an appreciable portion of OH-Arg was converted to NO by CPR, relaxations induced by EDNO and the nitrergic transmitter might be reduced by CPR inhibitors. This was tested using relaxations induced by acetylcholine (ACh) in rat aortic rings and by nitrergic nerve stimulation (NNS) in rat anococcygeus muscles with the CPR inhibitors troleandomycin (Trol), which inhibited citrulline formation (Boucher *et al.*, 1992), and 7-ethoxyresorufin (7ER) (Tassaneeyakul *et al.*, 1993), which inhibits conversion of [^{3}H]-L-arginine to [^{3}H]-citrulline (Li & Rand, 1993).

In aortic rings, Trol (10 - 100 μM) had no effect on ACh-induced relaxations, but 7ER (0.3 - 2 μM) inhibited them in a concentration-dependent manner; however, it also inhibited responses to NO. SOD (100 U/ml) restored responses to NO, suggesting that superoxide had been formed during metabolism of 7ER, but the relaxant action of ACh was not restored by SOD, suggesting 7ER inhibited NO production.

In anococcygeus muscles, Trol (10 - 100 μM) did not affect NO- or NNS-induced relaxations. 7ER (2 μM) inhibited NO- but not NNS-induced relaxations. A higher concentration of 7ER (10 μM) did reduce relaxations to NNS, but SOD restored both them and NO-induced relaxations.

The findings indicate: 1) a lack of correspondence between inhibition of nNOS in a biochemical system and a functional test (failure to reduce NNS-induced relaxations in the presence SOD); 2) that the functional importance of the production of superoxide by 7ER differs between responses to EDNO and the nitrergic transmitter; 3) a trol- or 7ER-sensitive CPR pathway is unlikely to be involved in NOS-dependent responses in the tissue studied.

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Boucher, J.L., Genet, A., Vadon, S. *et al.* (1992) *Biochem. Biophys. Res. Commun.* 187, 880-886.
Bredt, D.S. Hwang, P.M., Glatt, C.E. *et al.* (1991) *Nature* 351, 714-718.
Li, C.G. & Rand, M.J. (1993) *Clin. Exp. Pharmacol. Physiol.*, Suppl. 1, 43.
Stuehr, D.J., Fasehun, O.A., Kwon, N.S. *et al.* (1991) *FASEB J.* 5, 98-103.
Tassaneeyakul, W., Birkett, D.J., Veronese, M.E. *et al.* (1993) *J. Pharmacol. Exp. Ther.* 265, 401-407.

319P RESPONSES TO AGONISTS IN BOVINE PULMONARY CONVENTIONAL AND SUPERNUMERARY ARTERIES: EFFECT OF ENDOGENOUS NITRIC OXIDE

D. Bunton*, A. Fisher, A. MacDonald, A.M. Shaw. Department of Biological Sciences, Glasgow Caledonian University, Cowcaddens Road, Glasgow. G40BA.

The pulmonary circulation consists of two populations of arteries; conventional arteries which divide with and accompany the airway and supernumerary arteries which leave the conventional artery at 90° and are unaccompanied by an airway. The supernumerary arteries are smaller, more muscular arteries than the conventional arteries and have a muscular sphincter at their origin (Hislop & Reid, 1973). The role of supernumerary arteries in the pulmonary circulation is unclear but these vessels may be recruited to accommodate an increased cardiac output (CO) and as a consequence may contribute to the fall in pulmonary vascular resistance as CO increases. The present study investigated the effects of 5-hydroxytryptamine (5-HT) and the thromboxane-mimetic U46619 on the responses of the conventional and supernumerary arteries and the influence of endogenous nitric oxide on these responses.

Bovine lungs were obtained fresh from the abattoir. Segments of conventional artery (diameter 4-5 mm) and their associated supernumerary arteries (diameter 0.5 - 1 mm) were dissected from the lung and freed of surrounding connective tissue. The vessels were then weighed and suspended between stainless steel hooks in Krebs buffer (37°C) under a tension of 2 g for conventional arteries and 1 g for supernumerary arteries and gassed with a mixture of O_2:CO_2 (95%/5% v/v). The tissues were allowed to equilibrate for 1 hour before carrying out two concentration response curves (CRC) to 5-HT and U46619. The second in the presence of 100 μM L-Nitro arginine methyl ester (L-NAME). Paired tissues acted as time controls. Results are means ± s.e. mean. The significance of differences was determined using Student's t-test.

In conventional arteries 5-HT (1nM-1mM) and U46619 (0.1nM-1μM) produced concentration-dependent contractile responses.. L-NAME (100μM) did not significantly alter the maximum tensions (252±73mg/mg, 5-HT; 155±48 mg/mg, U46619) or the sensitivity of the arteries to 5-HT and U46619.

In supernumerary arteries L-NAME (100μM) increased the maximum responses to 5-HT from 675 ± 185 mg/mg to 942 ± 130 mg/mg n = 14, $p < 0.005$). L-NAME did not alter the sensitivity of the vessels to 5-HT (pD_2 values before and after L-NAME: 8.99± 0.66 and 8.76± 0.65, p = 0.97). In contrast, U46619 (0.1nM-1μM) produced a concentration-dependent contractile response in the supernumerary arteries which was unaffected by L-NAME (max. tension (mg/mg) before and after L-NAME: 838 ± 140 and 853 ± 156, n = 9, p = 0.86).

Sodium nitroprusside (SNP) produced a concentration-dependent relaxation in both conventional (1nM-10μM) and supernumerary (10nM-0.1mM) arteries preconstricted with U46619 (EC_{60-80}). Supernumerary arteries were 36 fold less sensitive to SNP than conventional vessels (pD_2: 7.37 ± 0.18 (conventional); 5.81 ± 0.26 (supernumerary) n= 10, $p< 0.001$).

The results show that endogenous nitric oxide selectively inhibits 5-HT-induced responses in supernumerary arteries. This may suggest that 5-HT stimulated the synthesis of NO in supernumerary arteries. However the fact that SNP was much less sensitive in supernumerary arteries suggests that these vessels have a higher basal release of NO than conventional arteries.

Hislop, A. & Reid, L. (1973) Thorax. 28: 129 - 135.

320P INVESTIGATION OF THE PGE (EP-) RECEPTOR SUBTYPE LINKED TO INHIBITION OF SUPEROXIDE ANION GENERATION IN HUMAN EOSINOPHILS

Elodie Talpain & Roma A Armstrong, Department of Pharmacology, University of Edinburgh, EH8 9JZ.

The aim of the present study was to determine if similar to human neutrophils, eosinophils express receptors for PGE_2 which are positively coupled to adenylate cyclase and mediate inhibition of superoxide anion (O_2^-) generation.

Polymorphonuclear leukocytes were isolated as described previously (Talpain *et al.*, 1995) and eosinophils were magnetically separated from contaminating neutrophils using CD 16 microbeads (Hansel *et al.*, 1991). Eosinophils (1.5×10^6 cells/ml) were preincubated in PBS (37°C) for 10 min, with PGE_2, EP-agonists or vehicle in presence of cytochalasin B (5mg/ml) and cytochrome C (1mg/ml) prior to stimulation with C5a (30 nM) for 35 min. O_2^- was assayed by spectrophotometric evaluation of the reduction of cytochrome C (A550nm). Cyclic AMP levels were determined in eosinophils (1×10^6 cells) suspended in Hanks buffer (1 ml) containing 0.25 mM isobutyl methyl xanthine after a 5 min incubation with EP-agonist at 37°C.

PGE_2 caused both a concentration-related inhibition of C5a-induced O_2^- (EC_{50} of 0.12 ± 0.04 µM; max inhibition of 59.2 ± 4.3%, n=18) and increase in eosinophil cyclic AMP (EC_{50} of 0.23 ± 0.11 µM; max of 8565 ± 1752 fmol cyclic AMP /10^6 cells, n=16). Table 1 shows the ability of EP-agonists to inhibit O_2^- generation.

Table 1 The ability of EP agonists (0.001-10 µM) to inhibit O_2^- generation induced by C5a (30 nM), n≥4

analogue	EC_{50} (µM)	max inhibition (%)
PGE_2	0.12 ± 0.04	59.2 ± 4.3
butaprost	0.38 ± 0.19	43.0 ± 0.2
AH 13205	0.10 ± 0.01	62.0 ± 7.5
misoprostol	0.88 ± 0.88	54.0 ± 9.8
sulprostone	3.48 ± 3.26	41.7 ± 8.1
GR 63799X	4.28 ± 2.38	36.8 ± 6.5
MB 28,767	3.62 ± 3.20	25.9 ± 2.3

The EP_2 selective agonists butaprost and AH 13205 are slightly less active than PGE_2 as inhibitors of O_2^-, and increase cyclic AMP levels, EC_{50}s of 2.0 ± 0.1 and 5.0 ± 2.8 µM, max increases of 6820 ± 1602 and 7956 ± 5103 fmol cyclic AMP/10^6 cells respectively, n=4-6.

However, the EP_3 agonists sulprostone, GR 63799X and MB 28,767 are more potent inhibitors of O_2^- than would be predicted, being 20-30 times less active than PGE_2, which is surprising because EP_3 receptor activation is normally linked to inhibition rather than stimulation of adenylate cyclase. However recent reports that splice variants of the EP_3 receptor can couple positively to adenylate cyclase may be relevant here (Regan *et al.*, 1994). The ability of EP_3 agonists to increase eosinophil cyclic AMP levels, as well as a causal link between cyclic AMP and inhibition of O_2^- are under investigation.

Hansel H.*et al.*, (1991). J. Immunol. Meths., 145, 105-110.
Regan J. *et al.*, (1994). Br. J. Pharmacol., 112, 377-385.
Talpain E. *et al.*, (1995). Br. J. Pharmacol., 114, 1459-1465.

321P INVESTIGATION OF THE ABILITY OF SELECTIVE EP- AGONISTS TO INHIBIT SUPEROXIDE ANION GENERATION IN HUMAN MONOCYTES

Stuart Milne, Roma Armstrong & [1]David Woodward Department of Pharmacology, University of Edinburgh, Edinburgh EH8 9JZ, and [1] Allergan Inc.

We have previously suggested that the EP- receptor expressed by human monocytes is of the EP_4 subtype (Milne *et al.*, 1994). The aim of the present study was to examine the ability of selective EP-agonists to inhibit monocyte superoxide anion (O_2^-) generation induced by both phorbol 12, 13 dibutyrate (PdBu) and formyl-methionyl-leucine-phenylalanine (FMLP).

Monocytes were isolated from human blood, and separated from contaminating mononuclear cells by adhesion as described previously (Milne *et al.*, 1994). O_2^- was determined by spectrophotometric evaluation of the reduction of ferricytochrome C (A550nm) after incubating cells ($1\text{-}2 \times 10^5$/well) in RPMI buffer (37°C) for 10 min, with EP-agonists or vehicle in the presence of cytochrome C (1mg/ml) prior to stimulation with PdBu or FMLP for 1h.

Stimulating human monocytes with PdBu gave a concentration-dependent increase in O_2^- generation (EC_{50} = 33.9 ± 6.1 nM; max increase of 121.7 ± 9.2 nmol O_2^- per 10^6 cells, n=7) which was greater than that observed with FMLP (EC_{50} = 126.3 ± 110.4 nM; max increase of 90.6 ± 10.6 nmol O_2^- per 10^6 cells, n=4). PdBu (30 nM) directly activates protein kinase C and is not inhibited by PGE_2 (0.001-10 µM). In contrast, PGE_2 and EP_4 agonists inhibit O_2^- generation induced by

Table 1 The ability of EP agonists (0.001-10 µM) to inhibit O_2^- generation induced by FMLP (1 µM) (n≥4)

analogue	EC_{50} (µM)	max inhibition %
PGE_2	0.56 ± 0.20	78.1 ± 7.5
16,16 dime PGE_2	0.25 ± 0.19	97.1 ± 2.2
11-deoxy PGE_1	0.56 ± 0.35	99.5 ± 0.4
misoprostol	0.14 ± 0.06	98.3 ± 1.1
butaprost	3.6 ± 1.9	98.8 ± 0.6
AH 13205	2.8 ± 1.7	92.6 ± 24.3
nocloprost	1.70 ± 1.10	50.8 ± 11.0

FMLP, as is shown in Table 1. The finding that EP_4 receptor agonists such as 11-deoxy PGE_1 and 16,16-dimethyl PGE_2 (Milne *et al.*, 1995) are 10-20 fold more potent at inhibiting O_2^- generation than the EP_2 selective agonists, butaprost and AH 13205 supports the involvement of EP_4 receptors. Unfortunately, this conclusion cannot be substantiated using the EP_4 receptor antagonist AH 23,848 (10-100 µM), which was found to potentiate rather than block, inhibition of O_2^- observed with PGE_2 (EC_{50} with 10 µM AH 23,848 is < 10 nM, n=4). AH 23,848 also potentiates inhibition of O_2^- with both PGE_2 and the non-prostanoid, 5'-N-ethylcarboxamido-adenosine in human neutrophils (Talpain *et al.*, 1995). The nature of this effect of AH 23,848 is not clear, as no partial agonist activity can be detected.

Milne, S. *et al.*, (1994). Br. J. Pharmacol. Proc. Suppl., 113, 7P.
Milne, S. *et al.*, (1995) Prostaglandins, in press.
Talpain, E. *et al.*, (1995), Br. J. Pharmacol, 114, 1459-1465.

322P INVESTIGATION OF THE ROLE OF NITRIC OXIDE AND CYCLIC GMP IN SUPEROXIDE ANION GENERATION BY HUMAN NEUTROPHILS

Roma A Armstrong & Amir Rafi, Department of Pharmacology, University of Edinburgh, EH8 9JZ.

Recent evidence suggests that increased neutrophil cyclic GMP levels are associated with chemotaxis (Belenky *et al.*, 1993) and that nitric oxide (NO) production is responsible for this observed increase in cyclic GMP (Kaplan *et al.*, 1989). The aim of this project was to investigate the role of NO and cyclic GMP in superoxide anion (O_2^-) generation by human neutrophils. Neutrophils were isolated using a discontinuous percoll gradient, and O_2^- generation was measured by spectrophotometric evaluation of the reduction of ferricytochrome C, as described previously for both (Talpain *et al.*, 1995).

Pre-incubation of neutrophils with the NO synthase inhibitor L-n-monomethyl arginine (L-nMMA 100μM, 30 or 45 min) did not inhibit the production of O_2^- induced by formyl methionyl leucyl phenylalanine (FMLP), EC_{50}s of 24.6 ± 4.7; 22.9 ± 1.7 and 19.1 ± 0.12; 17.6 ± 0.6 nM respectively, n=3.

In contrast, the cyclic GMP kinase inhibitor KT 5823 (1-10 μM) markedly inhibited O_2^- generation induced by FMLP, as shown in Table 1. O_2^- induced by phorbol 12, 13 dibutyrate (PdBu) was inhibited by KT 5823 to a lesser extent. The protein kinase C (PKC) inhibitor calphostin C (1 μM, n=4) did not inhibit O_2^- induced by FMLP (EC_{50}s of 32.1 ± 6.7 and 30.6 ± 3.9 nM) or PDBu (EC_{50}s of 64 ± 13.1 and 31.8 ± 7.9 nM).

In conclusion, the results with L-nMMA suggest that NO is not involved in O_2^- generation by FMLP, although the effect of KT 5823 would support a role for cyclic GMP. Results with calphostin C suggest that KT 5823 is unlikely to be acting nonselectively to inhibit PKC. However, a possible explanation is that KT 5823 is inhibiting phospholipase D, which can be directly stimulated by PdBu, and has a major role in ensuring neutrophil diacylglycerol levels are sustained.

Belenky, S. *et al.*, (1993). J. Lab. Clin. Med., 122, 388-394.
Kaplan, S. *et al.*, (1989). Blood, 74, 1885-1887.
Talpain, E. *et al.*, (1995). Br. J. Pharmacol., 114, 1459-1465.

Table 1. Inhibition of O_2^- induced by FMLP and PdBu, by the cyclic GMP kinase inhibitor KT 5823 (n=4-5).

FMLP	control	KT 5823 (1 μM)	control	KT 5823 (10 μM)
EC_{50} (nM)	30.9 ± 7.1	>150	16.4 ± 5.2	>300
max O_2^- (nmol/10^6 cells)	22.2 ± 0.7	12.2 ± 1.4	25.1 ± 3.4	6.6 ± 2.1
PdBu				
EC_{50} (nM)	90.1 ± 37.0	342.2 ± 220	87.5 ± 38.1	451.9± 214.9
max O_2^- (nmol/10^6 cells)	37.4 ± 8.3	26.0 ± 1.9	35.8 ± 9.1	27.2 ± 8.2

323P BLOCKADE OF NITRERGIC RELAXATION IN BOVINE RETRACTOR PENIS MUSCLE BY HYDROQUINONE BUT NOT HYDROXOCOBALAMIN IS ENHANCED BY DIETHYLDITHIOCARBAMATE

W. Martin & K. Paisley. Clinical Research Initiative, West Medical Building, University of Glasgow, Glasgow, G12 8QQ.

Although superoxide anion generating agents inhibit the relaxant actions of NO but not nitrergic nerves in the bovine retractor penis (BRP), we have recently reported (Martin et al., 1994) that neurotransmission can be blocked following inhibition of endogenous superoxide dismutase (SOD) with diethyldithiocarbamate (DETCA). Thus, high levels of SOD appear to protect the neurotransmitter from destruction by superoxide anion. In this study we have examined in the BRP whether inhibition of SOD with DETCA affects the actions of hydroquinone and hydroxocobalamin, two additional agents reported to inhibit the relaxant actions of NO but not nitrergic nerves in the anococcygeus muscle (Gibson et al., 1992; Rand & Li, 1994).

BRP muscle strips were mounted for isometric tension recording within Ag/AgCl ring electrodes. Adrenergic motor responses were blocked and the tone raised using guanethidine (30 μM). Electrical field stimulation (4 Hz, 10 s) was delivered with a pulse width of 0.5 ms at supramaximal voltage. Endogenous SOD activity was inhibited following treatment with DETCA (3 mM) for 2 hours.

In control strips of BRP hydroquinone (0.01-1 mM) induced a concentration-dependent inhibition of nitrergic relaxation (4 Hz, 10 s): maximum blockade 57.0 ± 4.7 %. The sensitivity of nitrergic relaxation to blockade by hydroquinone was enhanced 29-fold following treatment with DETCA (3 mM): maximum blockade 85.6 ± 9.8 %. The ability of DETCA to potentiate the actions of hydroquinone was largely blocked following treatment with exogenous SOD (250 u ml^{-1}). Hydroxocobalamin (0.001-1 mM) induced a concentration-dependent inhibition of nitrergic relaxation: maximum blockade 99.3 ± 0.7 %. The sensitivity of nitrergic relaxation to blockade by hydroxocobalamin was unaffected by treatment with DETCA (3 mM): maximum blockade 96.4 ± 3.6.

In contrast to findings in the anococcygeus muscle (Gibson et al., 1992; Rand & Li, 1994), both hydroquinone and hydroxocobalamin inhibit nitrergic relaxation in the BRP. Potentiation of the inhibitory action of hydroquinone following inhibition of SOD with DETCA, and its reversal with exogenous SOD, is consistent with destruction of NO by superoxide anion. The effect of DETCA is highly selective since it has no effect on blockade by the NO-binding agent, hydroxocobalamin.

Gibson, A., Babbedge, S.R., Brave, S.L., Hobbs, A.J., Tucker, J.F. & Wallace, P. (1992). *Br. J. Pharmacol.*, 107, 715-721.
Rand, M.J. & Li, C.G. (1993). *Eur. J. Pharmacol.* 241, 249-254.
Martin, W., McAllister, K.H.M. & Paisley, K. (1994). *Neuropharmacol.*, 33, 1293-1301.

324P ROLE OF CATALASE AND HYDROGEN PEROXIDE IN THE FORMATION OF NITRIC OXIDE FROM SODIUM AZIDE AND HYDROXYLAMINE

K. B. Mian & W. Martin, Clinical Research Initiative, University of Glasgow, Glasgow, G12 8QQ.

Catalase, the enzyme that promotes the breakdown of hydrogen peroxide to water and oxygen, also has peroxidase activity, i.e. it catalyses the oxidation of various substrates in the presence of hydrogen peroxide (Theorell & Ehrenberg, 1952). The metabolism of two such substrates, the nitrovasodilators, azide and hydroxylamine has been proposed to result in the formation of nitric oxide (Murad, 1994). We have already reported (Mian & Martin, 1995) that 3-amino-1,2,4-triazole (AT), an inhibitor of catalase (Margoliash & Novogrodsky, 1958), inhibits the relaxant activity of azide and hydroxylamine in isolated rings of rat aorta. Here, we have investigated the ability of catalase to generate nitric oxide, from azide and hydroxylamine in the presence of hydrogen peroxide, and have examined the ability of AT to inhibit this action.

The generation of nitric oxide from azide and hydroxylamine by the peroxidase activity of catalase was assessed indirectly by measuring nitrite, its major stable breakdown product. Briefly, reaction mixtures containing phosphate buffer (pH 5.6, 0.08 M), bovine liver catalase (300 or 1000 u ml^{-1}), hydrogen peroxide (1 mM) together with azide (10 µM-3 mM) or hydroxylamine (10 µM-30 mM) were incubated at 37 °C for 1 or 3 h in a final volume of 80 µl. The nitrite content of the samples was measured by the formation of a diazo product using a variant of the method of Green *et al.* (1982), i.e. the samples were mixed with 80 µl of 1 % sulphanilic acid in 2M HCl. After 5 min, 80 µl of 1 % aqueous N-(1-napthyl) ethylenediamine dihydrochloride was added, and the absorbance of the pink complex determined at 550 nm using a microplate reader.

Following incubation for 1 h at 37 °C, catalase (1000 u ml^{-1}) failed to generate nitrite from azide (3 mM) or hydroxylamine (30 mM). However, when incubated in the presence of hydrogen peroxide (1 mM), catalase (1000 u ml^{-1}) led to a concentration-dependent generation of nitrite from both azide (10 µM-3 mM) and hydroxylamine (10 µ M-30 mM): maximum formation of nitrite was 23.3 ± 0.7 µM and 8.7 ± 0.2 µM, respectively. Furthermore, if AT (1-100 mM) was present during the 1 h incubation, the catalase (1000 u ml^{-1})-dependent formation of nitrite from azide (0.3 mM) in the presence of hydrogen peroxide (1 mM) was inhibited in concentration-dependent manner: maximum inhibition was 36.4 ± 1.2 % with AT at 100 mM. Extending the incubation to 3 h together with a reduction of the catalase concentration to 300 u ml^{-1} enhanced the ability of AT to block formation of nitrite from azide (maximum inhibition 77.7 ± 6.3 % with AT at 100 mM).

The data confirm that peroxidase activity of catalase is indeed required for the metabolism of azide and hydroxylamine to nitric oxide. The ability of AT to inhibit the generation of nitric oxide probably accounts for its ability to block the vasodilator actions of azide and hydroxylamine (Mian & Martin, 1995).

Green, L.C., Wagner, D.A., Glogowski, J., *et al.*, (1982). *Analytical Biochemistry*, 126, 131-138.
Margoliash, E. & Novogrodsky, A. (1958). *Biochem. J.* 68, 468-475.
Theorell, H. & Ehrenberg, A. (1952). *Arch. Biochem. Biophys.* 41, 462-474.
Mian, K.B. & Martin, W. (1995). Abstract, Oxford BPS meeting.
Murad, F. (1994). *Advances in Pharmacology.* 26, 19-33.

325P THE EFFECTS OF N^G-NITRO-L-ARGININE METHYL ESTER AND 3-MORPHOLINOSYDNONIMINE UPON LOCAL CEREBRAL BLOOD FLOW IN AN IMPLANTATION GLIOMA MODEL IN THE RAT

G.P. Malcolm, P.A.T. Kelly, I.M. Ritchie & I.R. Whittle, Clinical Neurosciences, University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh EH4 2XU.

Nitric oxide (NO) plays an important role in the physiological control of cerebral blood flow *(Kelly et al. 1994)*, but its significance with respect to tumour blood flow remains to be established. The purpose of the present study was to investigate whether the known abnormalities of tumour blood vessels might be reflected in altered vascular responsiveness to either an NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) or an NO donor, 3-morpholinosydnonimine (SIN-1).

Male Wistar rats (n = 12) were anaesthetized (2% halothane in 70% N_2O: 30% O_2) for the unilateral, stereotactic injection of a suspension of C6 cells (10^6 in 10µl mock CSF) into the striatum. Twelve days after implantation, rats were treated with either a single *i.v.* injection of L-NAME ($30mg.kg^{-1}$ in saline; n = 4) ,.or with a constant *i.v.* infusion of SIN-1 ($1.8mg.kg^{-1}.h^{-1}$; $40µl.min^{-1}$; n = 4). A final group (n = 4) were injected with saline. The injections were given, or the infusion commenced, 20min prior to the measurement of local cerebral blood flow (LCBF) using the fully quantitative [^{14}C]-iodoantipyrine autoradiographic technique *(Sakurada et al., 1978)*. Mean arterial blood pressure (MABP) and rectal temperature were measured throughout the experiments, and blood gas status measured prior to any treatment, and again immediately before the measurement of LCBF. Measurements of LCBF were taken from the tumour itself, and from "normal" brain tissue contralateral to the tumour placement. Data (presented as mean ± s.e.m.) were analysed using Student's t-test, with acceptable levels of significance set at $P < 0.05$.

L-NAME produced a significant increase in MABP, from 121 ± 4 mmHg before treatment to 148 ± 4mmHg at the time of LCBF measurements, with a concurrent decrease in heart rate (428 ± 23 to 333 ± 15 $beats.min^{-1}$). In contrast, SIN-1 produced a significant decrease in MABP, from 126 ± 4mmHg to 103 ± 6mmHg, with an increase in heart rate (417 ± 20 to 512 ± 12 $beats.min^{-1}$). There were no significant changes in any of the other physiological variables measured.

In the host brain tissue contralateral to the tumour site, L-NAME produced a 19% decrease in LCBF compared to control (from 89 ± 5 to $72ml.100g^{-1}.min^{-1}$), whilst SIN-1 produced a 42% increase (to 126 ± $8ml.100g^{-1}.min^{-1}$). However within the tumour itself, LCBF appeared to be more sensitive to the actions of the two drugs, with L-NAME producing a 42% decrease in tumour perfusion (to $35ml.100g^{-1}.min^{-1}$), and SIN-1 increasing flow in the tumour by 162% (to $157ml.100g^{-1}.min^{-1}$). The pronounced effects of SIN-1 were apparent from visual inspection of the autoradiograms, where the tumour appeared as a clearly defined area of increased grain density.

The blood vessels supplying implanted C6 gliomas show appropriate constrictor and dilator responses to NO inhibitors and donors respectively. However, there is evidence that the responses to these agents are enhanced in tumour vessels. These experimental findings may have implications for the chemotherapy of brain tumours.

Kelly, P.A.T., Thomas, C.L., Ritchie, I.M., et al. (1994) *Neuroscience*, **59**, 13-20.
Sakurada, O., Kennedy, C., Jehle, J., et al. (1978) *Am. J. Physiol.*, **34**, 487-499.

326P INDUCTION OF NITRIC OXIDE SYNTHASE IS ASSOCIATED WITH CIRCULATORY AND RESPIRATORY FAILURE ELICITED BY STAPHYLOCOCCAL PEPTIDOGLYCAN AND LIPOTEICHOIC ACID IN THE ANAESTHETISED RAT

M. Kengatharan, S.J. De Kimpe, C. Thiemermann and J.R. Vane. The William Harvey Research Institute, St Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ.

Peptidoglycan (PepG) and lipoteichoic acid (LTA) are major cell wall components of gram-positive organisms such as *Staphylococcus aureus* (which do not contain endotoxin). An enhanced release of nitric oxide (NO) following the induction of nitric oxide synthase (iNOS) contributes to the circulatory shock (severe hypotension and vascular hyporeactivity to vasoconstrictor agents) elicited by LTA (De Kimpe *et al.*, 1995). Here, we have investigated the effect of a combined administration of PepG and LTA on mean arterial blood pressure (MAP), heart rate (HR), pressor responses to noradrenaline (NA) and the arterial oxygen pressure (P_aO_2) in the anaesthetised rats.

Male Wistar rats (250-325g) were anaesthetised with thiopentone sodium (120 mg/kg, i.p). The trachea was cannulated to facilitate spontaneous respiration. The carotid artery was cannulated to monitor MAP, HR and to obtain arterial blood samples for the measurement of blood gases. The jugular vein was cannulated for administration of compounds. The pressor response to noradrenaline (1μg/kg, i.v.) was assessed prior to and every 60 min after the injection of the bacterial components (time 0). At 360 min, rats were killed and lungs removed to determine iNOS activity by measuring the conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline in lung homogenates (De Kimpe *et al*, 1995).

Intravenous injection of LTA (3mg/kg) together with PepG (10mg/kg), from *Staphylococcus aureus,* resulted in a delayed circulatory failure (fall in MAP and a reduced pressor response to NA) and respiratory failure (decrease in P_aO_2) which were greater than in animals treated with PepG or LTA alone (Table 1). At 360 min, the increase in iNOS activity in the lungs from animals treated with PepG and LTA was significantly greater than in animals given LTA or PepG alone. Moreover, the effects of PepG and LTA on heart rate, vascular hyporeactivity, respiratory function and iNOS induction is significantly greater ($p<0.05$) than the additive effects of the PepG or LTA administered alone, suggesting synergy between these two cell wall components.

We suggest that cell wall components of gram-positive organisms act in concert to induce iNOS activity, which contributes to the circulatory and respiratory failure in gram-positive septic shock.

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De Kimpe, S.J., Hunter, M.L., Bryant, C.E., Thiemermann, C. & Vane, J.R. (1995). *Br. J. Pharmacol.* **114**, 1317-1323.

Table 1. The effect of PepG and LTA on the lung iNOS activity, MAP, responses to NA and the P_aO_2 in the anaesthetised rats.

Treatment	MAP (360min) (mmHg)	HR (360min) (bpm)	Pressor response to NA (360min) (mmHg·min)	P_aO_2 (360min) (mmHg)	iNOS activity (360 min) (pmol/min/mg protein)
sham control	113±4	405±10	48±4	78±2	0.3±0.1
LTA	97±6	430±14	29±2$^{\#}$	74±5	3.2±0.9
PepG	85±2	410±12	35±6	72±3	2.5±0.4
LTA + PepG	73±11$^{\#}$	492±10$^{\#*\$}$	5±2$^{\#*\$}$	55±4$^{\#*\$}$	22 ±4.0$^{\#*\$}$

Values are given as mean ± s.e.mean (n=5-6); # $P<0.05$ vs sham control, * $P<0.05$ vs LTA and $ $P<0.05$ vs PepG, by ANOVA (Bonferroni's test).

327P LIPOCORTIN 1 SUPPRESSION OF THE LIPOPOLYSACCHARIDE INDUCTION OF INDUCIBLE NITRIC OXIDE SYNTHASE: COMPARISON WITH THE RESPONSE TO INTERFERON γ

C.E. Bryant, A. Paul*, J.D. Croxtall & R.J. Flower.
*Department of Biochemical Pharmacology, The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London, EC1M 6BQ and *Dept. Physiology and Pharmacolcogy, University of Strathclyde, 204, George St., Glasgow, G1 1XW.*

Administration of lipopolysaccharide (LPS) to macrophages induces nitric oxide synthase which can be suppressed by pre-treatment with dexamethasone (DEX; Radomski et al., 1991) acting through the production of lipocortin 1 (Wu et al., 1995). The site of action of LC1 in this process is unknown. The aim of this study was to determine if LC1 could modulate the expression of iNOS in response to mediators other than LPS in macrophages.

J774.2 macrophages were cultured to 95% confluence in DMEM containing 10% fetal calf serum, 1%penicillin-streptomycin and 2mM L-glutamine. NOS activity was measured by the detection of nitrite (μM) in the medium using the Griess reaction. Cells were lysed in a 1% Triton X-100-EDTA (10mM) solution containing a mixture of protease inhibitors and protein levels were measured by Western blot analysis using a primary iNOS antibody dilution of 1:2000 (Bryant et al., 1995). The blots were quantified using densiometric analysis (expressed as optical density (OD) units). Cells were exposed to increasing ammounts of interleukin 1β (0.1ng-1μg/ml), tumour necrosis factor α (0.1nM-1μM), interferon γ (1-1000IU/ml; IFN), dibutyrladenosine cyclic monophosphate (0.001-10mM; cAMP) or LPS (1μg/ml) for 24h. Inhibitory curves for pre-treatment (-1h) with DEX were constructed at a dose of mediator which gave maximal iNOS induction. The role of LC1 was investigated using specific anti-LC1 antisera (1:60) to reverse the dexamethasone effects as described by Wu et al., 1995.

Nitrite release was detected for all mediators, but consistent dose response curves were only obtained with IFN and cAMP (n=4). The ED_{50} values for the induction of nitrite are shown in the table 1. LPS, IFN and cAMP all cause an induction of iNOS protein synthesis by Western blot analysis (n=3). Pre-treatment with DEX (1nM-1μM) inhibited the release of nitrite in response to all mediators at the same potency (see table 1; n=4). DEX also caused a suppression in iNOS protein induction for each mediator (n=3). Anti-LC1 antibodies reversed the effects of DEX on iNOS protein expression in LPS, but not IFN treated cells (see table 2; n=3). An inhibitor of cytosolic phospholipase A_2 (AACOCF$_3$; 10nM-1μM; Bartoli et al., 1994) failed to inhibit iNOS activity in response to LPS (n=3).

Mediator	EC_{50}	DEX IC_{50}
LPS	nd	0.031μM
IFN	3.4IU/ml	0.035μM
cAMP	0.23mM	0.029μM

Table 1: EC_{50} and IC_{50} values

Mediator	% inhibition DEX	% inhibition DEX+anti LC1
LPS (1μg/ml)	55±3.5%	19.5±2.5%
IFN (300 IU/ml)	39±11.4%	33±5.6%

Table 2: Effect of anti-LC 1 antibodies on iNOS expression

We conclude that LPS, IFN and cAMP, but not IL1 or TNF consistently induce both iNOS activity and protein in J774.2 macrophages. DEX can suppress induction of iNOS to LPS, IFN and cAMP with a similar potency. The effects of dexamethasone on iNOS expression are partially mediated by LC1 in response to LPS, but not IFN. This suggests that LC1 might inhibit iNOS induction blocking a signal transduction pathway not stimulated by IFN.

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Bartoli, F., et al., 1994, J. Biol. Chem. **265**, 15625-15630
Bryant et al., 1995, J. Endocrinol. ***(In press)***
Radomski, M.W. et al., 1991, Proc. Natl. Acad. Sci. **87**, 10043-10047
Wu, C.C., et al., 1995, Proc. Natl. Acad. Sci. **92**, 3473-3477

328P NITRO-TYROSINE-LIKE IMMUNOREACTIVITY IN HUMAN PLASMA OF CIGARETTE SMOKERS AND NON-SMOKERS

J. Khan, D.M. Brennand[1], K. R. Bruckdorfer[2] & M. Jacobs. Departments of Pharmacology and Biochemistry[2], Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF and [1]Thrombosis Research Institute, Emanuelle Kaye Building, Manresa Road, London SW3 6LX

Nitric oxide (NO) combines with superoxide anions in vitro to form peroxynitrite, a potent oxidant which modifies tyrosine residues in proteins to form nitro-tyrosine and -phenylalanine (Van der Vliet et al.1994). Recently, the formation of peroxynitrite has also been demonstrated in blood cells and endothelial cells (Beckman & Crow, 1993; Kooy et al, 1994), it is therefore possible that plasma proteins are exposed to peroxynitrite causing the modification of the tyrosine (phenylalanine) residues of plasma proteins. Furthermore, increased exposure of plasma to peroxynitrite may occur through the presence of NO, O_2^- and peroxynitrite in cigarette smoke. These possibilities have been examined by the development of competitive ELISA method for the detection of protein-nitro-tyrosine (phenylalanine) residues and its use in the determination of their concentration in the plasma of smokers and non-smokers.

Fasted - blood samples were obtained from 10 healthy human volunteers (non-smokers) and 12 cigarette smokers (after 12 h of abstaining and after smoking two cigarettes) and plasma isolated by centrifugation. Immuno-reactive nitrotyrosine (phenylalanine) residues in plasma were quantified by competitive ELISA assay performed using 96 - well plates coated with 4 μg ml^{-1} nitrated bovine serum albumin (NO_2-BSA). A standard curve was constructed by co-incubating serial dilutions of NO_2-BSA in the coated wells with anti-rabbit polyclonal anti-nitrotyrosine IgG (TCS Biologics Ltd) for 2h at 37°. Competition assays were performed by adding appropriate dilutions of the plasma samples, serum albumin or nitrated amino acids instead of NO_2 - BSA and inhibition of antibody binding determined from the standard curve as ng ml^{-1} NO_2 - BSA equivalents (mean ± s.e.mean). NO_2 -BSA was prepared by reaction of BSA with peroxynitrite (Van der Vliet et al.1994).

Antibody binding was blocked by 3-nitrotyrosine (10 mM) and 4-nitrophenylalanine (10 mM) but not by BSA (100 μg ml^{-1}). The plasma of non-smokers contained 2204 ± 105 (1774-2931) ng ml^{-1} whereas that of the smokers was significantly lower at 1743 ± 100 (1199-2393) ng ml^{-1} ($P<0.005$, Student's t-test). After smoking, the value was 1906 ± 142, (1227-2799) ng ml^{-1}, not significantly different from the control or pre- smoking value. It is concluded that immuno-reactive nitro-tyrosine (phenylalanine) residues are detected in human plasma of healthy volunteers and the concentration is decreased in smokers, an observation consistent with the attenuation of endothelial NO by cigarette smoking (Celermajer et al. 1993).

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Van der Vliet, A., O'Neill, C.A., Halliwell et al. (1994). FEBS Lett. 339,89-92.
Beckman, J.S. & Crow, J.P. (1993). *Biochem. Soc. Trans.* 21, 330-334.
Kooy, N.W. & Royall, J.A. (1994). *Arch. Biochem. Biophys.* 310, 353-359.
Celermajer, D.S., Sorensen, K.E., Georgakopoulos, D. et al. (1993). *Circulation* 88, 2149-2155.

329P SPHINGOLIPID-ACTIVATED SIGNAL TRANSDUCTION PATHWAYS IN AIRWAY SMOOTH MUSCLE

S. Pyne & N.J.Pyne. Dept of Physiology & Pharmacology, Strathclyde University, Royal College, Glasgow G1 1XW.

We have previously demonstrated that, in quiescent cultured guinea-pig tracheal smooth muscle cells (TSM), bradykinin, phorbol 12-myristate 13-acetate (PMA) and, to a lesser extent, platelet-derived growth factor (PDGF), stimulate cyclic AMP formation and that the latter limits the magnitude of activation of extracellular-signal-regulated kinase 2 (ERK-2) (Stevens *et al.*, 1994; Pyne *et al.*, 1994). Agonist-stimulation of sphingomyelinase elicits the hydrolysis of plasma membrane sphingomyelin to produce ceramide which can be further metabolised to sphingosine and sphingosine phosphate. Each of these has been shown to elicit biological effects in a variety of cell types and the mechanism of action of ceramide is the most clearly defined (Hannun, 1994). In this study, we have examined the ability of sphingomyelin-derived 'second messengers' to modulate TSM function.

TSM (grown to passage 3, 18-25 days from initial isolation in DME/10% foetal calf serum/10% donor horse serum) were quiesced in DME/1% FCS/1% DHS for 18 hr prior to experiments. Cells were stimulated with cell-permeable ceramides, sphingosine and sphingosine phosphate and assayed for activation of ERK-2 (by SDS-PAGE shift), [^{3}H]thymidine incorporation and cyclic AMP (radioligand binding assay). With regard to the latter, the quiescing medium contained cholera toxin (0.5 ng/ml) which activates a small proportion of the cellular pool of $G_{s\alpha}$ and elevates basal cyclic AMP levels from 0.6 ± 0.1 to 4 ± 2.5 pmol/0.25 x 10^6 cells.

A short acyl chain length ceramide, N-hexanoylsphingosine (C_6-Cer), elicited the sustained formation of cyclic AMP in a concentration-dependent manner in cholera toxin pre-treated TSM whereas sphingosine (S) induced a transient response (C_6-Cer, 29* ± 5; S, 11* ± 1 pmol/0.25 x 10^6 cells at 10 min using 125 μM). Detection of these responses required the coincident activation of a limited pool of $G_{s\alpha}$. In contrast, sphingosine phosphate (SP) reduced cyclic AMP (basal 2.25 ± 0.25; SP (125 μM, 10 min) 1.35* ± 0.25 pmol/0.25 x 10^6 cells) whereas the biologically inactive form of ceramide, N-acetyldihydrosphingosine, was without effect. Application of exogenous sphingomyelinase also elevated cyclic AMP in a concentration-dependent manner. ERK-2 was fully activated by PDGF, partially activated by sphingosine phosphate and more weakly by C_2-Cer whereas sphingosine was without effect. ERK-2 activation by PDGF was weakly attenuated by cholera toxin pre-treatment whereas the sphingosine phosphate and C_2-Cer responses were abolished. [^{3}H]thymidine incorporation into DNA was increased by sphingosine and sphingosine phosphate (basal, 11540 ± 1850, S, 16400* ± 1800; SP, 18160* ± 1250 dpm) whereas C_6-Cer was non-mitogenic (12500 ± 600 dpm). However, C_6-Cer, sphingosine and sphingosine phosphate were all able to potentiate the maximal PDGF-stimulated response (PDGF, 100100 ± 12800; PDGF/S, 178600* ± 5700; PDGF/SP, 202500* ± 24700; PDGF/C_6-Cer, 147600* ± 9000 dpm). This suggests that DNA synthesis can be enhanced by these agents, over and above the maximal PDGF response, in a manner that is apparently independent of direct ERK-2 activation.

Data expressed as means ± S.D., n=3 for representative experiments. * $p < 0.05$ (Student's t-test).

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Stevens, P. *et al.* (1994) *Biochem. J.* 297, 233-239.
Pyne, N.J. *et al.* (1994) *Biochem. J.* 304, 611-616.
Hannun, Y.A. (1994) *J. Biol. Chem.* 269, 3125-3128.

330P EFFECTS OF THE 5-HT_{2B} RECEPTOR AGONIST, BW 723C86, IN RAT SOCIAL INTERACTION, ELEVATED X-MAZE AND GELLER-SEIFTER MODELS OF ANXIETY

G.A. Kennett, F. Bright, B. Trail, T.P. Blackburn, G.S. Baxter, SmithKline Beecham Pharmaceuticals, Psychiatry Research Department, New Frontiers Science Park, Harlow, Essex CM19 5AW, U.K.

The 5-$HT_{2C/2B}$ receptor agonist m-chlorophenylpiperazine (mCPP) has anxiogenic-like properties while the 5-$HT_{2C/2B}$ receptor antagonist SB 200646A has anxiolytic-like effects in the rat social interaction (SI) and other tests (Kennett et al. 1994, 1995). As it is unclear whether 5-HT_{2C} or 5-HT_{2B} receptors mediate these actions, the effects of BW 723C86 (BW), a putative selective 5-HT_{2B} receptor agonist (Baxter et al. 1995), on behaviour in the rat SI, elevated x-maze and Geller-Seifter (GS) models of anxiety was studied.

Male Sprague Dawley rats (200-250 g) were held singly (SI) or in groups of 6 (x-maze), under a 12 h light cycle, (lights on (0700 h), with free access to food and water. In the SI test, rats were placed with a weight matched pair mate in a white perspex arena and total interaction and locomotion scored for 15 min under bright white light (Kennett et al., 1994). In the x-maze test, rats were placed in the centre of the apparatus lit by white light for 5 min (Blackburn et al., 1993). In the GS procedure, rats (400-500 g), fed a restricted diet, were trained to press a lever for a food reward, and to associate a light cue with both a high level of reward and a contingent footshock, in 5 x 3 min periods. These were alternated with 5 x 3 min unpunished periods with rewards at variable intervals (see Kennett et al., 1995). BW and chlordiazepoxide (CDP) were given s.c. 30 min pretest in saline. All data is cited as means ± s.e.m., n=12-18 (SI, x-maze) or 6 (GS) per group. Significance was tested by 1 way ANOVA and Dunnetts test (SI), Kruskal-Wallis ANOVA and Mann-Whitney U test (x-maze) or 2-way ANOVA (treatments x subjects) in the GS test where comparisons were made with levels of responding on two preceding vehicle treated tests and expressed as % change.

BW and CDP increased social interaction scores (s) (saline 54.1 ± 5.5, BW 3 mg/kg 104.4 ± 7.8 $p<0.01$, 10 mg/kg 104.1 ± 7.3 $p<0.01$, CDP 5 mg/kg 125.2 ± 10.2 $p<0.01$) but had no effect on locomotion. In the x-maze test, BW tended to increase % time spent on the open arms (vehicle 23.7 ± 3.4, BW 2 mg/kg 31.4 ± 3.7 ns, 10 mg/kg 29.9 ± 2.8 ns, CDP 4 mg/kg 45.5 ± 2.5, $p<0.01$) without affecting other parameters. In the GS test, BW and CDP increased punished (BW 1 mg/kg +33.8 ± 18.0 % $p<0.05$, 5 mg/kg +49.9 ± 29.1 % $p<0.05$, 50 mg/kg +66.1 ± 19.7 % $p<0.01$, CDP 5 mg/kg +335.9 ± 101.3% $p<0.01$) without altering unpunished responding.

BW has efficaceous anxiolytic-like actions only in the SI test. If this effect is 5-HT_{2B} receptor mediated, 5-HT_{2C} receptors are likely to account for both the anxiogenic profile of mCPP and the anxiolytic-like properties of SB 200646A.

Baxter, G.S., Kennett, G.A., Blaney, F., Blackburn, T.P. (1995) T.I.P.S. 16, 105-110.
Blackburn, T.P., Baxter, G.S., Kennett, G.A. et al. (1993) Psychopharmacology 110, 257-264.
Kennett, G.A., Wood, M.D., Glen, A., et al. (1994) Brit. J. Pharmacol., 111, 797-802.
Kennett, G.A., Bailey, F., Piper, D.C., and Blackburn, T.P. (1995) Psychopharmacology 118, 178-182.

331P INTRA-AMYGDALA INJECTION OF THE 5-HT_{2B} RECEPTOR AGONIST BW 723C86 PRODUCES ANXIOLYSIS ON THE ELEVATED PLUS-MAZE IN THE RAT

M.S. Duxon, S.R. Beckett, G.S. Baxter[1], T.P. Blackburn[1] & K.C.F. Fone, Department of Physiology & Pharmacology, Queen's Medical Centre, Nottingham University, NG7 2UH. [1]SmithKline Beechams Pharmaceuticals, New Frontiers Science Park, The Pinnacles, Harlow, Essex, CM19 5AW.

We have recently shown immunohistochemical evidence for the presence of 5-HT_{2B} receptor protein-like immunoreactivity (5-HT_{2B}-LI) on neurones in discrete rat brain regions including the medial amygdala (Duxon *et al.*, 1995). Few 5-HT agonists show selectivity for any of the three 5-HT_2 receptor subtypes identified, however, both 1-[5-(2-thienylmethoxy)-1H-3-indolyl]propan-2-amine HCl (BW723C86) and α-methyl-5-HT (α-Me-5HT) possess approximately 10-fold higher affinity for 5-HT_{2B} than either 5-HT_{2C} or 5-HT_{2A} receptors in ligand binding studies (Baxter *et al.*, 1995). Therefore, the current study examined the behavioural consequence of injection of these three 5-HT agonists directly into the medial amygdala to investigate the CNS function of 5-HT_{2B} receptors.

Indwelling guide cannulae were bilaterally implanted 2 mm above the medial amygdala (A -2.8; L ±3.8; V -6.6 mm from bregma) in male Lister Hooded rats (280 - 400 g, n = 6 - 12 per group) under halothane (1.5 % (v/v) with O_2/N_2O, 1:1) anaesthesia. After seven days recovery, rats received bilateral injections (500 nl over 30 sec) of either 0.154 M saline or 1-(3-chlorophenyl)piperazine (mCPP, 3.1 nmol, whose rank order of affinity is 5-$HT_{2C} \geq$ 5-HT_{2B} > 5-HT_{2A}), or α-Me-5-HT (3.1 nmol) or BW723C86 (0.09 - 3.1 nmol), one minute prior to being placed on an elevated plus maze (250 lux) where behaviour was monitored for 5 min using a computer tracking system. In all rats, the site of injection was confirmed by histology following injection of pontamine sky blue (500 nl, 0.5 % w/v) immediately after the behavioural studies. Results were analysed using one way ANOVA followed by Duncan's New Multiple Range and are expressed as mean ± s.e.mean.

BW723C86 produced a bell-shaped increase in the percentage time on the open arms which was significantly greater ($P < 0.05$ following ANOVA $F_{4,32} = 2.682$, $P = 0.0491$) than saline (20.8 ± 2.7 %) with 0.31 nmol (44.6 ± 9.5 %) and was accompanied by a bell-shaped decrease in stretch attend postures (from 3.3 ± 0.7 to 1.7 ± 1.1 with 0.31 nmol, ANOVA $F_{4,32} = 2.730$, $P = 0.0462$) suggesting that this compound produced anxiolysis. BW723C86 did not produce any significant increase in the number of head dips (from 8.8 ± 2.0 to 19.0 ± 3.9 at 0.31 nmol) nor did it alter locomotor activity compared with that in saline controls. The increase in percentage time spent on the open arms produced by α-Me-5-HT (35.8 ± 6.9 compared with 21.3 ± 4.6 % after saline) and mCPP (37.7 ± 11.0 compared with 20.3 ± 8.0 %) were not significant. In contrast, when injected 0.5 mm above the medial amygdala BW723C86 (0.31 nmol) failed to alter behaviour on the elevated plus-maze.

These data suggest that activation of 5-HT_2 receptors in the medial amygdala causes anxiolytic behaviour on the elevated plus maze. The use of selective 5-HT_2 receptor antagonists in future studies will confirm whether 5-HT_{2B} receptors (which are present on medial amygdala neurones) are the 5-HT_2 subtype responsible for mediating this behavioural response.

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Baxter, G.S., Kennett, G., Blaney, F. *et al.*, (1995) Trends Pharmacol. Sci. 16, 105-110.
Duxon, M.S.*et al.*, (1995) Br. J. Pharmacol. 115, 105P.

332P $GABA_B$ RECEPTOR DOWN-REGULATION FACILITATES MUSCARINIC OR METABOTROPIC AGONIST-DEPENDENT BURST FIRING IN RAT OLFACTORY CORTICAL NEURONES, *IN VITRO*

V. Libri, A. Constanti & N.G. Bowery. *Department of Pharmacology, The School of Pharmacy, London WC1N 1AX.*

Interest in the role played by $GABA_B$ receptors in epileptogenesis has grown since the demonstration that selective $GABA_B$ receptor agonists can decrease the occurrence of convulsive seizures induced experimentally (Karlsson *et al.*, 1992) and exacerbate the appereance of non-convulsive seizures in models of absence epilepsy (Marescaux *et al.*, 1992). We have recently observed that $GABA_B$ receptor mechanisms in rat brain can be functionally up- or down- regulated by chronic administration of $GABA_B$ receptor antagonists or agonists, respectively (Malcangio *et al.*, 1995).
We have now investigated the effects of the $GABA_B$ receptor down-regulation on muscarinic and metabotropic agonist induced responses in rat olfactory cortical neurones, *in vitro*.

Male Wistar rats were injected daily for 21 days with the $GABA_B$ agonist (-) baclofen (10 mg/kg) or saline (10 ml/kg). Brains from treated or control rats were quickly removed 24 hours after the last injection and placed into Krebs'solution (4°C). Transverse olfactory cortex slices were prepared as previously described (Constanti *et* al., 1993). Stable intracellular recording were made from neurones in the pyramidal cell layer using microelectrodes filled with 4 M potassium acetate.

As previously reported (Constanti & Libri, 1992; Constanti *et* al., 1993) a 2 min bath-application of the muscarinic receptor agonist oxotremorine-M (OXO-M; 10 μM; 2 min; n=5) or the metabotropic-glutamate receptor agonist (1S-3R)-ACPD (10 μM; n=5) induced in control neurones, a strong membrane depolarization (9.2 ± 0.6 mV), an increase in input resistance (14 ± 4%, measured at -70 mV), a sustained neuronal discharge and a slow post-stimulus after depolarization (sADP) in response to a 1.5 s depolarizing command. Spontaneous bursts of action potentials were never evident in response to muscarinic or metabotropic receptor activation. In slices from (-)baclofen pretreated rats (n=4), bath-application of OXO-M (10 μM) or (1S,3R)-ACPD (10 μM) evoked similar excitatory effects, consisting of prolonged membrane depolarization (8.9 ± 0.8 mV), increase in input resistance (13 ± 6%), repetitive neuronal discharge and sADP. However, when the depolarization was offset by applying maintained hyperpolarizing current, the cells began to show spontaneous epileptiform bursts of action potentials. These consisted of rhythmic, large amplitude (30-50 mV) depolarizations lasting for 80-150 ms, separated by membrane afterhyperpolarizations and occurring at a frequency of 0.2-1.7 Hz. This bursting activity disappeared when the membrane potential was held more negative than -80 mV, or artificially depolarized to -60 mV, and persisted only as long as the muscarinic or metabotropic effects lasted.

These data suggest that when $GABA_B$ receptors are down regulated, activation of intrinsic muscarinic and/or metabotropic receptors may co-operate in generating epileptic burst discharges in a selective population of cortical neurones.

Constanti, et al. (1993). *Neuroscience* **56**, 887-904.
Constanti, A. & Libri, V. (1992). *Eur. J. Pharmacol.* **214**, 105-106.
Karlsson, G. et al. (1992). *Neuroscience* **47**, 63-68.
Malcangio, M. et al. (1995). *NeuroReport*, **6**, 399-403.
Marescaux, C. et al. (1992). *J. Neural Transm., Suppl.* **35**, 37-69.

333P 3,4-DIAMINOPYRIDINE AND APAMIN FAIL TO IMPROVE SCOPOLAMINE-INDUCED DEFICITS IN WORKING MEMORY IN RATS

Poorheidari G, Stanhope KJ* & Pratt JA Dept. of Physiology & Pharmacology, University of Strathclyde, Glasgow, G1 1XW. *Wyeth Research (UK) Ltd, Taplow, Berkshire, SL6 OPH.

3, 4-Diaminopyridine (DAP, a voltage-dependent K^+ channel blocker) stimulates acetylcholine release, reverses age-related motor deficits and improves age-related memory deficits in rodents (Davis et al., 1983). Apamin, a blocker of Ca^{2+}-activated K^+ channels, increases the firing of central cholinergic neurons and facilitates learning and memory processes (Messier et al., 1981). In the present study, the effect of 4-aminopyridine (4-AP, a DAP homologue which crosses the blood brain barrier) and apamin were investigated on working memory in rats. The effect of these compounds was assessed on either the baseline performance or the reversal of an impairment induced by scopolamine (Scop, 0.1 mg kg^{-1}, s.c. 30 min) in the delayed matching to position (DMTP) task (Dunnett, 1985). The doses of drugs employed were based upon those used in previous studies (Davis et al., 1983; Messier et al., 1981) and upon preliminary locomotor activity data. The new cholinesterase inhibitor, E2020, was employed as a positive control (Dawson & Iversen, 1993).

Male Sprague-Dawley rats (280-320g) were trained to remember the position of a lever to obtain a food reward. At the start of a trial, one of the two levers (sample) was inserted into the box. Following a lever press response, the lever was retracted. After a delay of 0, 4, 8 or 16s both levers were inserted into the box. Pressing the correct lever (same as sample) resulted in a food reward whereas pressing the other lever did not.

The parameters measured were percentage of correct choices and latency to initiate the next trial (ITI), see Table 1. Data analysis was conducted using one- or two-way analysis of variance and, when appropriate, post-hoc Student Newman Keuls tests.

When administered alone, neither E2020 nor the K^+ channel blockers had an effect on choice accuracy but some motor/motivational side-effects were observed with apamin. The drugs did not reverse the Scop-induced impairment in choice accuracy, apart from E2020 which, at the largest dose, significantly attenuated the Scop-induced deficit. When apamin was co-administered with Scop the side-effects observed were greater than when either of these compounds was administered alone. These results demonstrate that K^+ channel blocker drugs, unlike a cholinesterase inhibitor, do not attenuate Scop-induced deficits in the DMTP task. Further experiments are required to investigate the actions of these compounds in paradigms not dependent on cholinoceptor blockade.

Davis, H.P., Idowu, A. & Gibson, G.E. (1983) *Exp. Aging Res.*, 9, 211-214.
Dawson, G.R. & Iversen, S.D. (1993) *Behav. Brain Res.*, 57, 143-153.
Dunnet, S.B. (1985) *Psychopharmacol.*, 87, 357-363.
Messier, C., Mourre, C., Bontempi, B., et al. (1981) *Brain Res.*, 551, 322-326.

Table 1. The effect of E2020, 4-AP and apamin on performance of rats in the DMTP task.

Treatment (mg kg^{-1} s.c. t-20)	%Correct	ITI (s) log_e	Treatment (mg kg^{-1} s.c. t-10)	%Correct	ITI (s) log_e	Treatment (mg kg^{-1} s.c. t-60)	%Correct	ITI (s) log_e
Saline/Saline	89±2.6	3.49±0.02	Saline/Saline	93±1.3	3.49±0.02	Saline/Saline	91±1.9	3.49±0.01
Saline/Scop	70±2.6*	3.74±0.14	Saline/Scop	73±4.2*	3.52±0.04	Saline/Scop	66±2.3*	3.94±0.26
E2020 0.1/Scop	71±2.7*	3.65±0.09	4-AP 0.001/Scop	76±2.8*	3.66±0.11	Apamin 0.1/Scop	67±4.4*	3.91±0.15
E2020 0.3/Scop	72±2.8*	3.69±0.18	4-AP 0.01/Scop	75±2.8*	3.72±0.16	Apamin 0.2/Scop	67±2.4*	4.54±0.40
E2020 1.0/Scop	80±2.4*+	3.56±0.04	4-AP 0.1/Scop	74±2.7*	3.83±0.18	Apamin 0.4/Scop	--	5.29±0.40*+

Results are means±s.e.mean, *$p<0.05$ compared with saline- and +$p<0.05$ compared with Scop-treated group. n = 7-12. t=pretreatment time (min).

334P BEHAVIOURAL COMPARISON OF NOVEL, FULL EFFICACY DOPAMINE 'D_1-LIKE' AGONISTS: DIHYDREXIDINE AND A 68930

Aaron M. Deveney* & John L. Waddington, Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, St. Stephen's Green, Dublin 2, Ireland.

The search for full efficacy agonists at the 'D_1-like' dopamine receptor has generated the isochroman A 68930, which has high affinity and ~100 fold selectivity (DeNinno *et al*, 1991) and the phenanthridine dihydrexidine, which has lower affinity and selectivity for 'D_1-like' over 'D_2-like' receptors (Mottola *et al*, 1992). As the behavioural profiles of A 68930 (Daly & Waddington, 1993) and of dihydrexidine (Darney *et al*, 1992) appear to show differences as well as similarities, we have conducted a systematic comparison of these two agents.

Using previously described procedures (Daly & Waddington, 1993; Deveney & Waddington, 1995a,b), rats [n= 8 per group] were challenged s.c. with vehicle, A 68930 [0.0625-4.0 mg/kg] or dihydrexidine [0.25-16.0 mg/kg] and resultant behavioural responses assessed; 0.5 mg/kg A 68930 and 4.0 mg/kg dihydrexidine were used to examine the effects of pretreatments with the selective 'D_1-like' antagonist SCH 23390 or the selective 'D_2-like' antagonist YM 09151-2. A 68930 readily induced grooming and intense grooming [maximal mean ± s.e mean behavioural counts: vehicle, 0.9±0.4; 1.0 mg/kg A 68930, 5.3±0.6, $P<0.01$] with some vacuous [$P<0.01$] and directed [$P<0.01$] chewing; sniffing and rearing were stimulated at higher doses but no locomotion was induced. All of the responses to A 68930 except vacuous chewing were blocked [$P<0.01$] by 0.01-1.0 mg/kg SCH 23390, while the effects of 0.005-0.5 mg/kg YM 09151-2 were considerably more variable. Conversely, dihydrexidine readily induced [$P<0.01$] grooming but less so intense grooming [maximal counts: vehicle, 0.1±0.1; 16.0 mg/kg dihydrexidine, 2.6±0.8, $P<0.01$ vs vehicle, $P<0.05$ vs A 68930]; sniffing and directed but not vacuous chewing were induced [$P<0.01$] while there was no consistent stimulation of rearing or locomotion. All of these responses to dihydrexidine were blocked [$P<0.05$] by SCH 23390 and YM 09151-2.

Dihydrexidine evidenced a behavioural profile distinct from that of A 68930 including less prominent induction of intense grooming, the most widely accepted model of 'D_1-like' stimulation, and of vacuous chewing, a more controversial model thereof (Daly & Waddington, 1993); furthermore, the antagonist profile for dihydrexidine was less specific than for A 68930. The extent to which these differences derive from the reduced 'D_1-like'/'D_2-like' selectivity of dihydrexidine or from other factors remains to be determined.

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Daly, S.A. & Waddington, J.L. (1993) Psychopharmacology **113**, 45-50.
Darney, J.R.Jr., Lewis, M.H., Brewster, W.K., Nichols, D.E. & Mailman, R.B. (1991) Neuropsychopharmacology **5**, 187-195.
DeNinno, M.P. *et al.* (1991) Eur. J. Pharmacol. **199**, 209-219.
Deveney, A.M. & Waddington, J.L. Br. J. Pharmacol. (in press).
Deveney, A.M. & Waddington, J.L. Psychopharmacology (in press).
Mottola, D.M., Brewster, W.K., Cook, L.L., Nichols, D.E. & Mailman, R.B. (1992) J. Pharmacol. Exp. Ther. **262**, 383-393.

335P BRAIN LEVELS OF MHPG-SO_4 ARE NOT RELIABLE INDICATORS OF CENTRAL NORADRENALINE TURNOVER IN RATS TREATED WITH THE α_2-ANTAGONIST, ATIPAMEZOLE

Ewen MacDonald & Kirsti Laitinen, Department of Pharmacology & Toxicology, University of Kuopio, PL 1627, FIN-70211 Kuopio, Finland.

After treatment for 10 days with the specific α_2-adrenoceptor antagonist, atipamezole (ATI) (3 mg/kg, twice daily, gavage) we did not observe any tolerance in terms of receptor blockade (assessed by the ability to antagonize sedative and hypothermic effects of α_2-agonist (MacDonald et al. 1991). However, tolerance did develop to many of the neurochemical changes seen with α_2-antagonists, notably the elevation in the level of the noradrenaline (NA) metabolite, 3-methoxy-4-hydroxyphenylglycol sulphate (MHPG-SO_4).

We measured NA turnover in brain from female Wistar rats (weight range 160 - 200 g) by i) the ability of ATI to accelerate the depletion of whole brain NA concentrations 4 h after α-methyl-p-tyrosine (α-MPT, 250 mg/kg) and ii) overflow of hypothalamic NA measured by in vivo microdialysis. In rats treated with α-MPT alone, brain NA levels were reduced to 52% of control and ATI accelerated this decline by a further 9% ($P<0.05$). In rats treated with ATI for 10 days, a similar ATI-induced acceleration (10%; $P<0.01$) was observed. There was no difference in the ATI-induced NA overflow in hypothalamus between rats receiving ATI for the first time (dose 50 µM infused for 1 h via microdialysis probe) and those accustomed to its effects twice daily treatment for 9 days. The time course and peak effect were similar in naive rats (max. 251 ± 54% 30 min after end of infusion) and accustomed rats (max. 313 ± 78% 30 min after infusion).

Rats were accustomed to the injection schedule by intubating them twice daily for 9 days with water. On the 10th day they received a single dose of ATI (3 mg/kg, gavage). Brain MHPG-SO_4 levels in these rats rose only to the same extent as in rats treated with ATI for 10 days and significantly less than in rats whose first injection was ATI (table 1). Brain NA levels were only decreased in the rats receiving a single dose of ATI, 10 days' treatment or simply accustoming the rats to handling and injection prevented this effect.

Table 1: Effect of single or mutiple doses (10 days, bid) of atipamezole (3 mg/kg) on brain levels of noradrenaline and its metabolite, MHPG-SO_4, four h after oral injection

Treatment	NA (nmol/g)	MHPG-SO_4 (nmol/g)
H_20 1 dose	2.61 ± 0.05	0.45 ± 0.01
ATI 1 dose	2.26 ± 0.03*	0.78 ± 0.03*
H_20 10 days (bid)	2.72 ± 0.05	0.43 ± 0.01
ATI 10 days (bid)	2.50 ± 0.07	0.64 ± 0.03*
H_20 9 days /ATI 1 dose	2.54 ± 0.06	0.63 ± 0.01*

Means ± SEM (n = 5) (*; $P<0.01$, ANOVA + Scheffe test)

In conclusion, the increase in brain MHPG-SO_4 occurring after ATI is only partly due to increased brain NA turnover. The α_2-adrenoceptor antagonist potentiates the stresses involved in the injections etc. and these contribute both directly and indirectly to the neurochemical changes seen in brain 4 h after drug treatment.

MacDonald, E., Laitinen, K. Konstandi M. et al. *Br. J. Pharmacol* (1991) **104**, 58P.

336P AN ELECTROPHYSIOLOGICAL STUDY OF THE SELECTIVITY OF ANTAGONIST ACTION OF CIPROFLOXACIN AND BIPHENYLACETIC ACID ON THE RAT ISOLATED VAGUS NERVE

M. A. Green, A.Markham and R.F. Halliwell, Dept. of Pharmacology, School of Health Sciences, University of Sunderland, SR1 3SD.

We have previously demonstrated that biphenylacetic acid (BPAA) potentiates the antagonist effects of the 4-quinolone, ciprofloxacin, at the $GABA_A$ receptor by more than 3000 times (Green and Halliwell, 1994). In the present study, we have investigated the selectivity of the antagonist actions of ciprofloxacin alone, and in combination with BPAA. To this end, the effects of ciprofloxacin and BPAA were determined on the $5\text{-}HT_3$, nicotinic acetylcholine (nACh), P_{2x} purinoceptor and $GABA_A$ receptor, of the rat isolated vagus nerve and were compared with those of their selective antagonists, MDL 72222 (3-tropanyl-3-5-dichlorobenzoate), hexamethonium, pyridoxal phosphate-6-azophenyl-2'-4'-disulphonic acid (PPADS) and bicuculline, respectively.

The vagus nerves were isolated from freshly killed male Wistar rats (100 - 300g). A conventional 'grease-gap' technique was used to record, extracellularly, agonist-evoked changes in membrane polarisation. Drugs were applied to the nerve *via* a perfusion system. All recordings were made at ambient room temperature (18-23°C). Agonist-evoked responses were measured at their peak. Responses in the presence of drugs are expressed as a percentage of those in the absence of drugs (control).

GABA (3-3000μM), 5-HT (10nM-10μM), Dimethylphenyl piperazinium (DMPP, 3-300μM) and α,β-methylene adenosine 5' triphosphate (α,β-MeATP, 3-300μM) concentration-dependently depolarised the vagus nerve with geometric mean EC_{50} (and 95% confidence limits) of 66μM (53-79, n=21), 0.78μM (0.37-1.19, n=7), 16μM (9-23, n=6) and 29μM (12-46, n=4), respectively. Concentrations approximating the EC_{50} values of 50μM GABA, 0.5 μM 5-HT, 10μM DMPP and 30μM α,β-MeATP were used to assess the selectivity of action of ciprofloxacin and BPAA.

In control pharmacological experiments, responses to GABA, 5-HT, DMPP and α,β-MeATP were selectively antagonised by 10μM bicuculline (17±6%, n=5), 1μM MDL 72222 (10±4%, n=5), 300μM hexamethonium (12±5%, n=4) and 10μM PPADS (21±5%, n=4), respectively.

Ciprofloxacin (10μM) and BPAA (10μM) had little or no effect on responses to GABA, 5-HT, DMPP or α,β-MeATP. However, in combination these drugs antagonised the GABA-evoked response to 29 ±3% of control (n=19). Responses to 5-HT, DMPP and α,β-MeATP were little or not inhibited by this combination, the responses being 128±11% (n=6), 127±26% (n=6) and 81±8% (n=9) of control, respectively. Ciprofloxacin (100μM) and BPAA (100μM) reduced the GABA response to 64±3% (n=12) and 75±1% (n=21) of control, respectively. These drugs alone caused little or no reduction in responses to the other three agonists. The combination of ciprofloxacin plus BPAA (both at 100μM) abolished the GABA response (n=14) whereas the 5-HT, DMPP and α,β-MeATP responses were 76±8% (n=12), 102±23% (n=5) and 78±10% (n=5) of control, respectively.

In conclusion, these data indicate a selective antagonism of the $GABA_A$ receptor by ciprofloxacin alone, and in combination with BPAA, which may contribute to the convulsant effects associated with the combined use of these compounds. The precise mechanism of the synergistic antagonism of the $GABA_A$ receptor by ciprofloxacin and BPAA is at present under further investigation.

Green, M.A. and Halliwell, R.F. (1994) Br.J.Pharmacol., 113, 35P.

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337P STUDIES ON THE MECHANISMS UNDERLYING SENSITISATION OF THE MESOACCUMBENS DOPAMINE RESPONSE TO NICOTINE IN THE RAT

C.E. Birrell & D.J.K. Balfour, Department of Pharmacology and Clinical Pharmacology, University of Dundee Medical School, Ninewells Hospital, Dundee DD1 9SY.

In vivo microdialysis studies have shown that the prior administration of nicotine in the form of daily injections sensitises rats to the effects of the drug on dopamine (DA) overflow in the nucleus accumbens (NAc). The principal objective of this study was to identify the mechanisms underlying this sensitisation.

Groups of male Sprague Dawley rats (~350g at the beginning of the experiment) were given 5 daily subcutaneous (sc) injections of nicotine (0.4mg/kg) or saline (controls). Dialysis studies were performed on day 6 using the procedure described by Benwell and Balfour (1992). The pretreatment protocol was examined for its effects on the mesoaccumbens DA responses to sc saline (control responses), nicotine (0.4mg/kg) and d-amphetamine (0.2mg/kg) and intraperitoneal (ip) injections of saline (control responses) and raclopride (0.05, and 0.1mg/kg). The putative influence of pretreatment on DA secretion evoked by the local depolarisation of the terminals in the NAc was studied by investigating the effects of increasing the concentration of KCl in the medium used to perfuse the probe from 4mM to 50 or 100mM. DA overflow was measured using HPLC with electrochemical detection and is expressed as percentages of the mean baseline levels ± the sem. The data were analysed statistically using an ANOVA for repeated measures.In agreement with previous results (Benwell and Balfour 1992), nicotine pretreatment increased ($p<0.05$) the mean mesolimbic DA response to sc nicotine, measured over 60 minutes after the injection, from 123 ± 12% (n = 4) to 187 ± 15 % (n=6). The administration of amphetamine to saline-pretreated rats increased the mean DA overflow to 154 ± 26% (n=5). Nicotine pretreatment had no significant effect on this response. Changing the solution used to perfuse the probe to one containing a depolarising concentration of KCl evoked a dose-dependent increase in DA overflow to 230 ± 68% (50mM KCl; n=4) and 327 ± 87% (100mM; n=4). These responses to KCl were also unaffected by pretreatment with nicotine. Injections of raclopride (0.05mg/kg) to saline-pretreated rats caused a significant increase ($F(1,6)=8.0$; $p<0.05$) in DA overflow from a mean value of 95 ± 13% to 210 ± 39 % (n=4 for both groups). This response was not further increased by the higher dose tested (0.1mg/kg). The responses to raclopride were abolished ($F(1,6)=6.3$; $p<0.05$) in the rats which had been pretreated with nicotine.

The data do not support the hypothesis that the sensitisation of the mesolimbic DA response to nicotine, observed in nicotine-pretreated rats, is associated with significant changes in the pools of DA available for release from the nerve terminals in the NAc but that sensitisation may be related to reduced inhibitory tone mediated by DA autoreceptors. The mechanism underlying this loss of inhibitory tone remains to be established.

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Benwell, M.E.M. & Balfour, D.J.K. (1992) Br. J. Pharmacol. **105**, 849-856.

338P CHARACTERISATION AND AUTORADIOGRAPHICAL LOCALIZATION OF IMIDAZOLINE-2 (I_2) SITES LABELLED BY [^{3}H]2-(-2-BENZOFURANYL)-2-IMIDAZOLINE IN RAT BRAIN

L.A.Lione, D.J.Nutt, and A.L.Hudson. Psychopharmacology Unit, School of Medical Sciences, University of Bristol, BS8 1TD.

For many years [^{3}H]idazoxan has been widely used as a radioligand for the study of both α_2-adrenoceptors and I_2 sites (Michel & Insel, 1989). As yet no clear physiological function(s) for I_2 sites have emerged due, in part, to the lack of available selective ligands, although agmatine has been proposed to be the endogenous ligand for imidazoline sites (Li *et al*, 1994). Recently, we have described 2-(-2-benzofuranyl)2-imidazoline (2-BFI) as a highly selective ligand for I_2-sites over α_2-adrenoceptors in mammalian brain and to be a superior radioligand to idazoxan for the characterisation of I_2 sites in rabbit brain (Lione *et al*, 1995). This present study now describes the binding characteristics and autoradiography of [^{3}H]2-BFI in rat brain.

Radioligand binding and autoradiographic studies were performed on male Wistar (220-250g) rat whole brain membranes and 12μm thick sections based on the methods of Mallard *et al* (1992). Non-specific binding in all experiments was defined with 10μM 2-(4,5-dihydroimidaz-2-yl)-quinoline (BU224) or 6-fluoro-idazoxan.

Saturation binding studies yielded Bmax values of 144 ± 4.8 and 128 ± 12 fmoles mg^{-1} protein and K_D values of 1.74 ± 0.14 and 10.4 ± 2.68 nM for [^{3}H]2-BFI and [^{3}H]idazoxan binding respectively, revealing that both ligands bound to a similar number of sites to which [^{3}H]2-BFI displayed a higher affinity. [^{3}H]2-BFI also bound to an apparant second population of sites with lower affinity. In competition binding studies the imidazoline compounds idazoxan, 2-BFI, 6-fluoro-idazoxan, guanabenz , naphazoline and BU224 had high affinity for 70-80% of the [^{3}H]2-BFI binding at 1nM (Ki = 9.01, 1.83, 3.88, 11, 1.78, and 2.5 nM respectively) and moderate affinity for the remaining 20-30% (Ki >300 nM). In contrast the α_2-adrenoceptor agonists noradrenaline and clonidine, the α_2-adrenoceptor antagonists efaroxan and rauwolscine, the I_1-site selective drug moxonidine, the MAO_A inhibitor clorgyline, agmatine, histamine and dopamine had low affinity (Ki >10μM) for [^{3}H]2-BFI binding.
The regional distribution of 0.5nM [^{3}H]2-BFI binding was consistent with 5nM [^{3}H]idazoxan binding (Table 1). The overall distribution for both ligands is comparable to that described for I_2-sites labelled with [^{3}H]idazoxan (Mallard *et al*, 1992).

Table 1. Autoradiographical distribution of I_2-sites in rat brain (fmol mg^{-1} tissue, mean ± s.e.mean, n=3)

Region	[^{3}H]2-BFI	[^{3}H]idazoxan
interpeduncular nuc.	96.2 ± 2.9	107.1 ± 7.2
mammilary peduncle	116.6 ± 10.7	125.3 ± 7.7
arcuate nuc.	116.5 ± 18.5	136.8 ± 9.4
area postrema	89.0 + 5.5	105.9 + 18.5
pineal gland	66.0 ± 4.7	58.4 ± 5.1
ependyma	34.6 ± 1.5	41.3 ± 5.0

These results indicate that [^{3}H]2-BFI recognises I_2 sites on rat brain with high affinity and selectivity and support previous suggestions that [^{3}H]2-BFI represents a superior radioligand to [^{3}H]idazoxan for the study of I_2-sites.

Li, G., Regunathan, S., Barrow, C.J., *et al.*, (1994) *Science.*, **263**, 966-969.
Lione, L.A., Nutt, D.J., Lewis, J., *et al.*, (1995) *Br.J. Pharmacol.*, **114**, 412P.
Mallard, N.J., Hudson, A.L. & Nutt, D.J. (1992) *Br. J. Pharmacol.*, **106**, 1019-1027.
Michel, M. & Insel, P. (1989).*Trends.Pharmacol.Sci.*,**10**,342-344

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339P SELECTIVE INHIBITION OF GLUTAMATE UPTAKE DOES NOT POTENTIATE DEPOLARIZATIONS EVOKED BY APPLICATION OF GLUTAMATE TO THE RAT STRIATUM *IN VIVO*

J. Urenjak[1], E. Zilkha & T.P. Obrenovitch, Dept of Neurological Surgery, Institute of Neurology, Queen Square, London WC1N 3BG and [1]Dept of Discovery Biology, Pfizer Central Research, Sandwich CT13 9NG

Impaired glutamate (Glu) uptake is often postulated to play a major role in excitotoxic neuronal death (Szatkowski & Attwell, 1994). Here we examine the effects of L-*trans*-pyrrolidine-2,4-dicarboxylate (L-PDC), a selective inhibitor of Glu uptake, on depolarizations evoked by intracerebral application of Glu.

Microdialysis probes incorporating an electrode were implanted in the striatum of male Sprague-Dawley rats (250-350 g) and perfused at 1 μl min^{-1} with artificial CSF (ACSF) (Obrenovitch *et al.*, 1994). Under halothane anaesthesia, local, short depolarizations were produced by application of 5 or 20 mM Glu through the probe for 2 min, during basal conditions (i.e. in ACSF), with co-perfusion of 1 or 5 mM L-PDC (starting 10 min before application of 5 mM Glu), and again under basal conditions (Figure 1). Field (d.c.) potential was recorded between the microdialysis electrode and a reference electrode.

Microdialysis application of 5 and 20 mM Glu to the rat striatum evoked consistent depolarizations of 1.33 ± 0.07 and 3.83 ± 0.21 mV, respectively (mean ± s.e.mean, n = 7). These responses did not change significantly with time in control experiments. Perfusion of 1 and 5 mM L-PDC induced small shifts of the d.c. potential (0.51 ± 0.08 and 1.3 ± 0.16 mV, respectively) and failed to enhance Glu evoked responses (Figure 1). In fact, Glu induced depolarizations were slightly smaller with 5 mM L-PDC (0.97 ± 0.04 and 3.27 ± 0.13 mV for 5 and 20 mM Glu, respectively; $p < 0.05$, Student's paired t test) and decreased further during the subsequent recovery period to 0.47 ± 0.06 and 2.85 ± 0.08 mV, respectively.

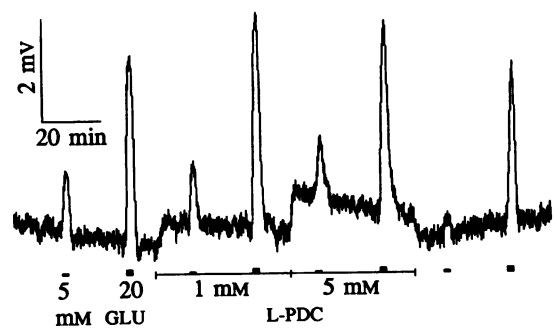

Figure 1. Representative effects of Glu uptake inhibition induced by L-PDC on the extracellular d.c. and Glu evoked responses. 5 or 20 mM Glu was perfused for 2 min through a microdialysis probe implanted in the rat striatum (solid, short horizontal bars). 1 or 5 mM of the Glu uptake inhibitor L-PDC was perfused starting 10 min before the application of 5 mM Glu.

These data confirm that very high extracellular concentrations of Glu must be applied to the brain in order to produce local depolarizations *in vivo* (Obrenovitch *et al.*, 1994), and suggest that this low potency of Glu does not result from efficient uptake. These results challenge the widely accepted view that high extracelluar Glu may underlie anoxic depolarization.

Szatkowski, M. & Attwell, D. (1994) *Trends Neurosci.* 17, 359-365.
Obrenovitch, T.P., Urenjak, J. & Zilkha, E. (1994) *Br J Pharmacol* 113, 1295-1302.

340P EFFECTS OF L-701,324, A NOVEL ANTAGONIST AT THE GLYCINE SITE OF THE *N*-METHYL-D-ASPARTATE RECEPTOR, ON CORTICAL SPREADING DEPRESSION

T.P. Obrenovitch & E. Zilkha, Dept of Neurological Surgery, Institute of Neurology, Queen Square, London, WC1N 3BG

Spreading depression (SD) is a wave of cellular depolarization which may underlie the migraine aura and increase neuronal damage in stroke (Lauritzen, 1994; Obrenovitch, 1995). As SD elicitation requires *N*-methyl-D-aspartate (NMDA)-receptor activation, this study aimed to examine whether L-701,324 (7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2-(1H)-quinolone),a high affinity antagonist at the glycine site of the NMDA-receptor (Leeson & Iversen, 1994), inhibits the initiation and propagation of K^+-induced SD. Dizocilpine (MK-801), known to potently block SD, was used as reference compound.

Microdialysis probes incorporating an electrode were implanted in the cerebral cortex of male Sprague-Dawley rats (250-350 g), and perfused at 1 μl min^{-1} with artificial CSF (Obrenovitch *et al.*, 1994). Under halothane anaesthesia, 5 episodes of repetitive SD were elicited by switching to a medium containing 130 mM K^+ for 20 min, each separated by 40 min of perfusion with normal artificial CSF. SD initiation was recorded with the microdialysis electrode, and SD propagation with glass capillary electrodes inserted 3 mm posteriorly. L-701,324 (5 or 10 mg kg^{-1}), MK-801 (1 mg kg^{-1}) or vehicle was administered i.v. 10 min after the end of the second K^+-stimulus.

Initiation of SD by K^+ was inhibited by 10 mg kg^{-1} (but not by 5 mg kg^{-1}) of L-701,324 (Figure 1). The cumulative area of elicited SD peaks was significantly decreased 30 min after administration of L-701,324 (15.3 $\pm$ 2.1 mV min vs. 23.2 $\pm$ 1.1 mV min in controls, mean $\pm$ s.e.mean, $n = 6$, $P < 0.02$, Student's t test). The delay between application of 130 mM K^+ and occurrence of the first SD was also significantly increased. SD propagation was more sensitive than SD elicitation to L-701,324, as both 5 and 10 mg kg^{-1} produced an effective inhibition. MK-801, already at the dose of 1 mg kg^{-1}, virtually abolished SD initiation and completely blocked SD propagation.

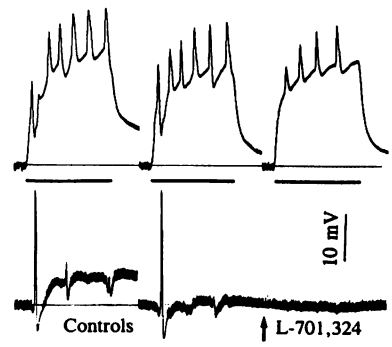

Figure 1. Typical effects of L-701,324 (10 mg kg^{-1}) on the initiation (top) and propagation (bottom) of SD induced by 20-min application of K^+ (horizontal bars).

These data demonstrate that L-701,324 has an inhibitory effect on SD initiation and propagation, an action likely to be beneficial in focal ischaemia, and possibly also against migraine. However, the efficacy of L-701,324 against SD is moderate in comparison to that of the NMDA-channel blocker MK-801.

Lauritzen, M. (1994) *Brain* 117, 199-210.
Leeson, P.D. & Iversen, L.L. (1994) *J. Med. Chem.* 37, 4053-4067.
Obrenovitch, T.P. (1995) *Cerebrovasc. Brain Metab. Rev.* 7, in press.
Obrenovitch, T.P., Urenjak, J. & Zilkha, E. (1994) *Br. J. Pharmacol.* 113, 1295-1302.

341P INHIBITION OF NITRIC OXIDE SYNTHASE REDUCES MPP^+-EVOKED HYDROXYL RADICAL FORMATION IN THE RAT STRIATUM *IN VIVO*

S. Rose, J.G. Hindmarsh, M.T Silva, S. Hasham, P. Jenner. Neurodegenerative Diseases Research Centre, Pharmacology Group, King's College London, UK.

Nitric oxide (NO), via peroxynitrite, may mediate neuronal degeneration following NMDA receptor stimulation (Dawson *et al*, 1991) which may in turn play a role in 1-methyl-4-phenyl pyridinium (MPP^+) toxicity (Turski *et al*, 1991). Indeed, MPP^+ causes increased hydroxyl radical ($OH^\cdot$) formation in the striatum (Chiueh *et al*, 1992) which might arise from the degradation of peroxynitrite. We now report the effect of inhibition of NO synthase (NOS) by L-nitroarginine methyl ester (L-NAME) and 7-nitroindazole sodium salt (7-NINA) on MPP^+-induced production of $OH^\cdot$ and dopamine efflux *in vivo*.

Microdialysis probes were inserted into the left striatum of male Wistar rats using standard stereotaxic techniques. Probes were perfused at a flow rate of 1μl/min with artificial extracellular fluid (aECF) containing sodium salicylate (5mM) to trap $OH^\cdot$, forming 2,3-dihydroxybenzoic acid (2,3-DHBA) (Chiueh *et. al*, 1992). In the first series of experiments, after a period of 2h, probes were perfused with MPP^+ (0.625-20mM) for 20 min followed by a return to aECF for 1h. Dialysate samples were collected over 20 min periods throughout the experimental period and analysed for levels of 2,3-DHBA and dopamine by HPLC with electrochemical detection. In a further series of experiments, after a period of 1h, probes were perfused with D-NAME (1mM, n=4), L-NAME (1mM, n=8), 7-NINA (1mM, n=5) or its control (aECF, n=5) for 1h. MPP^+ (10mM, in the presence of the drug/vehicle) was then perfused for a period of 20 min followed by a further 1h perfusion with drug or control. Dialysate samples were collected and analysed as above.

MPP^+ (0.625-20mM) increased 2,3-DHBA formation in a concentration-dependent manner, with concomitant concentration-dependent increases in dopamine efflux. L-NAME and 7-NINA, but not D-NAME, inhibited the MPP^+-induced increase in $OH^\cdot$ formation (Figure 1), with no effect on the increase in dopamine efflux.

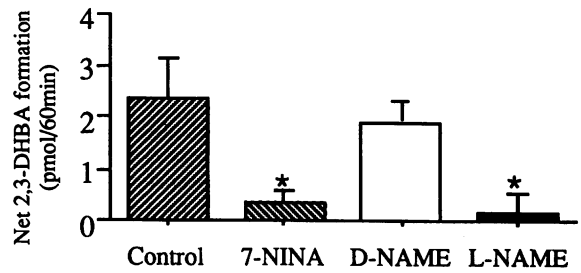

Figure 1 The effect of 7-NINA and L-NAME on MPP^+-evoked hydroxyl radical formation in the rat striatum. * $p < 0.05$ compared to control (Student's t-test).

Inhibition of NO synthase prevents MPP^+-evoked $OH^\cdot$ formation in the rat striatum *in vivo*. This may be the result of peroxynitrite production following dopamine autoxidation and subsequent formation of superoxide radicals, or the excess stimulation of NMDA receptors by glutamate following MPP^+ administration. In conclusion, NO contributes to oxidative stress seen following MPP^+ administration. Thus, inhibitors of NOS may have a neuroprotective role in the neurodegeneration underlying Parkinson's disease.

Chiueh CC, Krishna G, Tulsi T Obata T, Lang K, Huang S-J & Murphy DL (1992). *Free Radic. Biol. Med.* **13**: 581-583.
Dawson VL, Dawson TM, London ED, Bredt DS & Snyder SH (1991). *Proc Natl. Acad. Sci. USA.* **88**: 6368-6371.
Turski L, Bressler K, Rettig K-J, Löschmann P-A & Wachel H (1991) *Nature* **349**: 414-418.