1P CHARACTERISATION OF THE BINDING OF [^{3}H]Ro 25-6981 TO RECOMBINANT N-METHYL-D-ASPARTATE RECEPTORS

L. M. Hawkins, P. L. Chazot, and F. A. Stephenson
Dept. Pharmaceutical and Biological Chemistry, School of Pharmacy, 29/39 Brunswick Square, London, WC1N 1AX.

[R-(R,S)]-α-(4-Hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidine propanol (Ro 25-6981) is a high affinity, activity-dependent blocker of *N*-methyl-D-aspartate (NMDA) receptors. Functional studies on NMDA receptors expressed in *Xenopus* oocytes demonstrated that Ro 25-6981 is highly selectivity for receptors containing the NR2B subunit (Fischer *et al.*, 1997). Recently, the binding characteristics of [^{3}H] Ro 25-6981 have been described in rat forebrain (Mutel *et al.*, 1998). In this study, we describe the binding of [^{3}H] Ro 25-6981 to both membrane-bound and immunopurified recombinant NMDA receptors expressed in human embryonic kidney cells (HEK) 293 cells.

HEK 293 cells were transfected with NMDA receptor subunit clones using the Ca^{2+} phosphate method (Cik *et al.*, 1993). [^{3}H] Ro 25-6981 binding was carried out in 50 mM Tris citrate, pH 7.1, containing 5 mM EDTA and 5 mM EGTA. Cell homogenates were incubated with the radioligand for 2 h at 4°C, the reaction terminated by filtration, followed by five washes with phosphate buffer, pH 7.4, at 4°C. Non-specific binding was defined using 1 mM spermidine. Results were analysed by non-linear regression using Graph PAD Prism.

No specific binding was detected in cells transfected with either the NR1-1a or NR2B subunits alone or the NR1-1a/NR2A or NR1-1a/NR2C subunit combinations. Saturable [^{3}H] Ro 25-6981 binding was found to cells expressing the NR1-1a and NR2B subunits with an affinity of K_D = 7.2 ± 2.2 nM (n= 4). Based on this observed selectivity for the NR2B subunit, we therefore investigated whether NR1-1a/NR2A/NR2B receptors, which have been shown to exist *in vivo* albeit as a minor population (Chazot and Stephenson 1997), retained high affinity [^{3}H] Ro 25-6981 binding. HEK 293 cells transfected with the NR1-1a, NR2A and NR2B subunit clones bound [^{3}H] Ro 25-6981 with a K_D = 8.6 ± 0.1 nM (n=3). From previous studies we know that cells transfected with NR1-1a, NR2A and NR2B subunit clones yield at least NR1-1a/NR2A, NR1-1a/NR2B and NR1-1a/NR2A/NR2B receptors. Thus to investigate the binding of [^{3}H] Ro 25-6981 to NR1-1a/N2A/NR2B receptors, transfected cells were solubilised and subjected to anti-NR2A immunoaffinity chromatography (Chazot and Stephenson 1997) and the presence of anti-NR1, anti-NR2A and anti-NR2B antibody immunoreactivities confirmed by Western blotting. Specific [^{3}H] Ro 25-6981 binding was detected to the anti-NR2A-immunopurified material i.e. NR1-1a/NR2A and NR1-1a/NR2A/NR2B. Saturation studies showed that [^{3}H] Ro 25-6981 bound with high affinity, K_D = 10.9 ± 1.2 nM (n=2), to the NR2A subunit-containing material.

In conclusion, we have shown that [^{3}H] Ro 25-6981 selectively binds with high affinity to binary NR1-1a/NR2B receptors. In addition, high affinity [^{3}H] Ro 25-6981 binding is retained to NR1-1a/NR2A/NR2B receptors.

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2P HARMANE, NORHARMANE AND TETRAHYDRO β-CARBOLINE HAVE HIGH AFFINITY FOR RAT IMIDAZOLINE BINDING SITES

A.L. Hudson, R. Price, R.J. Tyacke, M. D. Lalies, C. A. Parker, & D.J. Nutt Psychopharmacology Unit, School of Medical Sciences, University of Bristol, Bristol BS8 1TD.

Imidazoline (I) binding sites are present in the brains and periphery of several species including rat and man. I_1 site-selective drugs such as moxonidine lower blood pressure while I_2 site selective compounds can interact with monoamine oxidase (Eglen *et al.*, 1998). Agmatine has been proposed as the endogenous neurotransmitter but has rather low affinity for I sites in binding studies (for review see Eglen *et al.*, 1998). We have now examined whether a group of compounds known as the β-carbolines have affinity for I_1 sites in rat kidney and I_2 sites in rat brain.

Brains and kidneys were removed from male Wistar rats (250g), homogenised (10 vol 50 mM Tris-HCl buffer, pH 7.4) and pelleted by centrifugation (32,000g). Aliquots of membrane (500 µg) were incubated in the above buffer with either 3nM [^{3}H]clonidine (in the presence of 10µM rauwolscine) or 1nM [^{3}H]2-BFI, to label I_1 and I_2 sites, respectively (45min, 25°C, final volume 0.5ml). The specific components of binding were defined by 10µM clonidine (I_1 sites) and BU224 (2-(4,5-dihydroimidaz-2-yl)quinoline, 10µM, I_2 sites). Bound ligand was separated by filtration and determined by scintillation counting (Lione *et al.*, 1998). Results were analysed by Prism (GraphPad Software).

Agmatine was found to be of low affinity for I sites (Table 1), this is in agreement with our previous findings (Lione *et al.*, 1998). The indoleamine tryptamine was of 16-fold higher affinity than agmatine for I_2 sites, having a K_i value of 27 µM in rat brain membranes (Table 1). This prompted us to examine the condensation products of tryptamine, the β-carbolines.

Norharmane and noreleagnine (tetrahydro β-carboline) were of moderate affinity for I_1 sites but of high affinity for I_2 sites. For the latter sites, binding curves were biphasic and yielded the high affinity K_i values shown in Table 1. Harmane was of high affinity for both I_1 and I_2 sites with an IC_{50} and K_i value in the low nM range (Table 1). The classical inverse agonist for the benzodiazepine receptor β-carboline-3-carboxylic acid ethyl ester (β-CCE), was of low affinity for I sites (Table 1).

Compound	I_1 site (IC_{50}, nM)	I_2 site (K_i, nM)
Agmatine	36,532±3,080	416,700±118,800
Tryptamine	nd	26,700±4,300
Norharmane	586.6±117.6	87.2
Noreleagnine	8,830±3,472	9.5±1.2
Harmane	30.1±6.8	49.4±17.4
β-CCE	25,077±14,241	>100,000

Table 1. Affinities of compounds for I_1 sites and I_2 sites in rat kidney and brain membranes respectively, data are means ± s.e.mean, from 4 - 6 experiments performed in triplicate (nd = not determined).

These data demonstrate that there are endogenous β-carbolines which demonstrate much higher affinity for I sites than the previously proposed transmitter agmatine. We suggest these β-carbolines may have a neuromodulatory role *via* I sites and are possibly endogenous ligands for these novel receptors.

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3P REGULATION OF NEUROPEPTIDE Y RELEASE FROM HYPOTHALAMIC SLICES BY Y1, Y2 AND Y5 NPY RECEPTOR LIGANDS

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

The potent orexigenic neuropeptide, neuropeptide Y, (NPY), which is found in high concentrations in mammalian hypothalamus, is thought to mediate the drive to locate and consume food during periods of starvation or a shift to negative energy balance (King and Williams, 1998). A dysregulation of hypothalamic NPY activity has been suggested to participate in numerous pathophysiological states, such as cancer cachexia and obesity leading to marked changes in energy balance (King and Williams, 1998). We have therefore investigated NPY release from hypothalamic slices from adult rat brain in order to understand factors regulating NPY activity. Male adult Wistar rats (250-300g) were killed by CO_2 inhalation. Hypothalamic blocks were dissected free and placed in cold gassed (95% O_2/5% CO_2) Krebs bicarbonate buffer (4°C; pH 7.4). Hypothalamic slices (approximately 0.1 mm^3) from each animal were prepared by cross-chopping with a razor blade and preincubating them in warm, gassed Krebs buffer (0.4 ml; 37°C) for 1 h with four changes of fresh buffer. Slices were then incubated for four periods of 15 min each in fresh Krebs buffer (0.4ml) and then stimulated for 15 min with 60 mM KCl in Krebs buffer followed by three 15 min recovery periods of normal Krebs buffer. NPY release was measured by radioimmunoassay using high-affinity polyclonal anti-NPY antibodies. NPY concentrations in the slices were also measured by tissue sonication at the end of the experiment. Basal and potassium-stimulated NPY release were significantly reduced in calcium-free Krebs buffer demonstrating calcium-dependent release. Potassium dependent release was approximately 3 times basal release. Basal and potassium-stimulated NPY release was reduced by the NPY Y5 agonist, human pancreatic polypeptide (hPP) (100nM and 1 μM) (stimulated release; control = 122 ± 47 pg/15 min; 1 μM hPP = 28 ± 2 pg/15 min **P<0.01; ANOVA and Bonferroni modified t-test, mean ± S.E.M.) and the Y2 agonists, NPY(13-36) (10nM – 1μM) and N-acetyl-[Leu^{28},Leu^{31}]NPY(24-36) (1nM – 10nM) (control = 115 ± 24 pg/15min, plus 10 nM N-acetyl-{Leu^{28},Leu^{31}]NPY(24-36) = 46 ± 3 pg/15 min **P<0.01). The NPY(13-36)-induced reduction in potassium-stimulated release was partially reversed by the weak Y2 antagonist, T4-[NPY(33-36)]$_4$ (Grouzmann *et al.* 1997) (stimulated release; control = 238 ± 19 pg/15 min; 100nM NPY(13-36) = 91 ± 18 pg/15min **P<0.01; 1μM Y2 antagonist = 240 ± 55 pg/15min; 100 nM NPY(13-36) plus 1 μM Y2 antagonist = 150 ± 31 pg/15min. The Y1 antagonists BIBP3226 or BIBO3304 (Wieland *et al.* 1998) and Y5 antagonist 2-[naphthalen-1-yl]-3-phenyl propane-1,2-diamine (1nM – 10 μM) did not significantly alter either basal or stimulated NPY release. In conclusion, we have demonstrated that NPY release from hypothalamic slices is calcium-dependent and inhibited by Y2 and Y5 agonists, which may act as autoreceptors.

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4P CHANGES IN OREXIN mRNA DURING ALTERED ENERGY BALANCE: INFLUENCES OF LEPTIN AND INSULIN

X. Cai, M. Tadayyon[1], R. Buckingham[1], S. Wilson[1], J.R.S. Arch[1], P.S. Widdowson, J.P.H. Wilding & G. Williams. Diabetes and Endocrinology Research Unit, Dept. Medicine, Liverpool University, L69 3GA, and [1]SmithKline Beecham Pharmaceuticals, Harlow, Essex CM19 5AD, U.K.

The orexins, orexin A and B, which are derived from a common precursor polypeptide, are new additions to the family of hypothalamic-derived neuropeptide transmitters that increase food intake when injected into rat brain (Sakuari *et al.* 1998). However the role of the orexins in regulating energy stores and metabolism is presently unknown. We have examined hypothalamic orexin mRNA concentrations in various physiological and pathophysiological states of altered energy balance to understand the regulatory mechanism of orexin synthesis. Male Wistar rats (approximately 200g) were either starved for 48 hours, made diabetic for 14 days via injection of 55mg/kg streptozocin in sterile saline via the tail vein (plasma glucose = 39.9 ± 2.6 versus 9.1 ± 0.6 mmol/l), made hypoglycaemic (plasma glucose = 5.6 ± 2.5 versus 15.4 ± 4.9 mmol/l) via daily injections of insulin (porcine insulatard; Novo Nordisk, 20-60U/kg/s.c.) or made obese via feeding a highly palatable diet rich in fat over 8 weeks (Widdowson *et al.* 1997). Control rats were allowed free access to standard laboratory chow, *ad libitum* and were injected with sterile saline, identical to experimental animals, where appropriate. Rats were killed by CO_2 inhalation and blood removed from the heart, for measurements of plasma leptin and insulin concentrations using radioimmunoassay kits. The hypothalami were dissected and snap frozen in liquid N_2. Orexin mRNA was measured in hypothalamic blocks via Northern blotting using as randomly-labelled digoxigenin cDNA probe and the blots analysed using computer densitometry following normalisation against 18S ribosomal RNA. Relative to freely-fed rats, 48h starved rats exhibited elevated hypothalamic orexin mRNA (189 % of controls; P<0.01, Student's t-test) and reduced plasma leptin (starved = 0.17 ± 0.05 versus 4.41 ± 0.60ng/ml; mean ± S.E.M. P<0.01; n=8) and insulin concentrations (starved = 12.1 ± 2.3 versus 30.5 ± 3.6 μU/ml; P<0.01), but normoglycaemia was maintained. Orexin mRNA was not significantly different in diabetic rats (115 %, n =10) relative to non-diabetic controls (n = 10) displaying a similar pattern of reduced plasma leptin and insulin concentrations. Furthermore, orexin mRNA (156 %; P = 0.08, n=8), leptin (hypoglycaemic = 8.01 ± 0.70 versus 2.61 ± 0.15 ng/ml; P<0.01) and insulin concentrations (hypoglycaemic = 463.5 ± 32.9 versus 25.8 ± 2.7 μU/ml; P<0.01) were elevated in insulin-induced hypoglycaemia versus controls. Dietary-obese rats were normoglycaemic, hyperleptinaemic (obese = 1.42 ± 0.29; obese = 5.05 ± 0.33ng/ml, P<0./01), but not hyperinsulinaemic (obese = 19.0 ± 2.4; controls = 15.1 ± 1.7 μU/ml) but did not show a significant change in orexin mRNA. In conclusion, we have demonstrated elevated hypothalamic orexin mRNA concentrations in response to altered energy balance. However, the changes in orexin mRNA do not appear to be closely regulated by changes in either leptin or insulin concentrations.

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5P ELECTROCONVULSIVE SHOCK INDUCES LOCALISED AND PERSISTENT INCREASES IN BDNF PROTEIN IN RAT BRAIN

J.M. Elliott, S.L. Phipps, Q. Pei, T. Zetterstrom and D.G. Grahame-Smith. School of Pharmacy, De Montfort University, Leicester LE1 9BH and Dept. of Clinical Pharmacology, Radcliffe Infirmary, University of Oxford, OX2 6HE.

In common with other antidepressant treatments, electroconvulsive therapy (ECT) must be administered repeatedly and there is a characteristic delay of several weeks before therapeutic efficacy is evident in depressed patients. In the rat, acute administration of electroconvulsive shock (ECS) induces a rapid but transient increase in the expression of mRNA for brain derived neurotrophic factor (BDNF) (Nibuya et al., 1995). Following chronic administration, however, the increase in BDNF mRNA is sustained for up to 48h after the last shock (Zetterstrom et al., 1998). We have therefore investigated the effects of chronic ECS on the distribution and persistence of expression of BDNF protein in rat brain.

Male SD rats (250g) were anaesthetised with halothane and ECS induced via earclip electrodes (150V for 1s). Sham control animals were anaesthetised and electrodes attached but no current was applied. ECS was administered 5 times over 10 days and animals were killed at varying times after the last shock. The brain was isolated and dissected on ice then immediately homogenised in 10 vol. of denaturing buffer containing protease inhibitors. Aliquots were stored at -70°C until assay. BDNF protein was quantified by Western blot analysis. Aliquots of brain tissue were thawed and diluted in Laemmli buffer then heat-denatured (94°C for 4 min). Gels containing 15% polyacrylamide were loaded with 20µl brain samples or BDNF standards and separated by electrphoresis (35V for 20 min then 100V for 100 min). Proteins were blotted onto PVDF membranes overnight (35V) and BDNF was identified using a polyclonal anti-BDNF antibody (Santa-Cruz Biotechnology) coupled to ECL Plus detection system (Amersham plc). Blots were visualised using Hyperfilm-ECL and the intensity of the bands quantified by digital analysis using MCID Image Analysis System with reference to internal BDNF standards.

BDNF protein expression in controls was higher in hippocampus (5.17 ± 0.38 ng/mg protein) compared to striatum (2.19 ± 0.35), fronto-parietal cortex (2.34 ± 0.25) and cerebellum (1.80 ± 0.09) (mean ± s.e.m., n=6). Following chronic administration of ECS, BDNF protein levels were significantly increased in hippocampus (+43 ± 6%; $p<0.001$) and fronto-parietal cortex (+31 ± 5%; $p<0.01$) but not in striatum (+11 ± 4%) or cerebellum (+1 ± 11%) when compared to sham controls assayed 24h after the last shock (n=6-11, Dunnett's t-test). Time-course studies carried out in the hippocampus indicated that this increase in BDNF protein expression was maintained at both 48h (+52 ± 8%; $p<0.001$) and 72h (+54 ± 14%; $p<0.01$) after the last ECS and even after 7 days (+12 ± 4%; $p<0.05$) but not after 14 days (+5 ± 3%) (n=7-8).

These data confirm the localised distribution of BDNF protein in rat brain and demonstrate that the discrete increases in BDNF mRNA observed after chronic ECS are translated into similar regional increases in BDNF protein. In the hippocampus, this increase is maintained for up to 7 days after the last ECS. Further work is needed to determine the consequences of such sustained increase in neurotrophin level and its possible implications for the antidepressant activity associated with ECT.

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6P DEPLETION OF STRIATAL GLUTATHIONE BY L-BUTHIONINE-[S,R]-SULPHOXAMINE DOES NOT ALTER BASAL OR K⁺-STIMULATED DOPAMINE EFFLUX IN THE RAT STRIATUM: AN *IN VIVO* MICRODIALYSIS STUDY

S. Rose, F. Aziz-Cooke, P. Jenner. Neurodegenerative Diseases Research Centre, Guy's, King's and St Thomas' School of Biological Sciences, Division of Pharmacology and Therapeutics, Manresa Rd, London UK.

Glutathione (GSH) protects against oxidative damage by removing hydrogen peroxide but is also involved in DNA synthesis and repair, protein synthesis, amino acid transport and enzyme activation (see Lomaestro & Malone, 1995). In additional, GSH may act as a carrier for nitric oxide (NO) (Gibson & Lilley, 1997). In the rat striatum, NO decreases dopamine release *in vivo* (Silva *et al.*, 1995). Therefore, depletion of GSH in the striatum would prevent this inhibitory action of NO on dopaminergic neurones. To test this hypothesis we investigated the effect of depletion of striatal GSH on basal and K⁺-stimulated dopamine efflux by *in vivo* microdialysis.

Male Wistar rats (280-320g) were injected with either L-buthionine-[S,R]-sulphoxamine (BSO; 3.2mg/20µl in 0.9% saline, n=6) or vehicle (0.9% saline, n=6) i.c.v. bilaterally using standard stereotaxic techniques under halothane anaesthesia. Following 18-21 hours recovery, rats were anaesthetised using chloral hydrate, and anaesthesia was maintained throughout the experimental period. Microdialysis probes were inserted into the striatum bilaterally using standard stereotaxic techniques. Probes were perfused with artificial extracellular fluid (aECF) at a flow rate of 2µl/min. One hour after implantation, dialysate samples were collected every 10 min throughout the experimental period and analysed for levels of DA, DOPAC and HVA by HPLC with electrochemical detection. Basal dopamine efflux was determined for 50 min. Probes were then perfused with aECF containing K^+ (40mM) for a period of 30 min followed by normal aECF for the remainder of the experiment (up to 240 minutes). At the end of the experiment brains were removed and levels of GSH were determined in the striatum using Bioxytech GSH-400 colorimetric assay.

Infusion of BSO bilaterally into the cerebral ventricles reduced GSH levels in the striatum compared to saline-treated controls (saline: left, 1.86±0.14; right, 1.52±0.06. BSO: left, 0.76±0.16*; right, 0.70±0.22*, nmol/mg tissue, * $p<0.05$ vs saline-treated control, Student's t-test). There was no difference between left and right sides within treatment groups.

Basal efflux of dopamine was unaffected by GSH depletion (saline: left, 49 ±3; right, 46±7. BSO: left, 41±7; right, 39±0.4; (pmol/20µl) $p>0.05$, 1-way ANOVA). Additionally, GSH depletion did not alter K^+-stimulated dopamine efflux (total K^+-stimulated dopamine efflux:- saline: left, 3573±1313; right, 4051±983. BSO: left, 3580±766; right, 4072±859, (pmol), $p>0.05$ 1-way ANOVA; Figure 1).

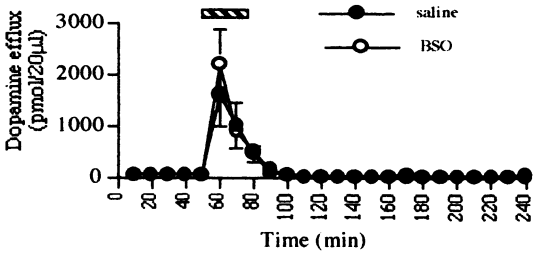

Figure 1 The effect of GSH depletion on basal and K^+-stimulated dopamine efflux in the left striatum. ▨ K^+-stimulation (40mM).

Acute depletion of GSH in the striatum did not alter basal or K^+-stimulated dopamine efflux. This suggests that GSH does not act as a carrier for nitric oxide (NO) in the striatum in a manner that alters its ability to effect its inhibitory actions on dopaminergic neurones (Silva *et al.*, 1995).

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7P NEUROSTEROID MODULATION OF $GABA_A$ RECEPTOR-MEDIATED MINIATURE INHIBITORY POSTSYNAPTIC CURRENTS IN THE RAT HIPPOCAMPUS

Sarah C. Harney, Bruno G. Frenguelli and Jeremy J. Lambert. Department of Pharmacology & Neuroscience, University of Dundee, Dundee DD1 9SY.

Neurosteroids such as 5β-pregnan-3α-ol-20 one (5β3α) are potent endogenous modulators of the $GABA_A$ receptor (Lambert *et al.*, 1995). In this study the modulation of $GABA_A$ receptor-mediated inhibitory synaptic transmission by neurosteroids was examined using the whole cell patch-clamp technique in the hippocampal slice preparation. Transverse slices were prepared from 18-22 day old rats of either sex (30-50g in weight) killed by cervical dislocation. Bicuculline-sensitive miniature inhibitory postsynaptic currents (mIPSCs) were recorded from visually identified CA1 pyramidal and dentate gyrus granule neurones at -60 mV, in the presence of 2 mM kynurenic acid and 0.5 μM tetrodotoxin, at 35 °C. Data are presented as the mean ± s.e.mean. The characteristics of mIPSCs from CA1 and dentate gyrus granule cells are summarised in Table 1. (Note for both neuronal cell types the decay of the averaged mIPSCs was best fit by the sum of two exponentials, described by two time constants, τ_1 and τ_2).

In CA1 pyramidal neurones physiological concentrations of 5β3α (10-30 nM) had no effect on mIPSC amplitude or 10-90% rise time, but prolonged mIPSC decay resulting in an increased net charge transfer, which was significant for 30 nM (control = 490 ± 48 fC; 30 nM 5β3α = 744 ± 97 fC; n = 4; paired t-test, $p < 0.05$). Dentate gyrus granule neurones were relatively less sensitive to the neurosteroid, with 10 and 30 nM 5β3α having little or no effect and 100 nM producing an increase in charge transfer in only 4 of 6 cells tested. Overall this effect was not significant (control = 684 ± 90 fC; 100 nM 5β3α = 890 ± 123 fC; n = 6, paired t-test, $p > 0.05$). This differential sensitivity to a neurosteroid in two regions of the hippocampus may reflect a difference in $GABA_A$ receptor subunit expression.

Table 1. Characteristics of mIPSCs recorded from CA1 pyramidal and dentate gyrus granule neurones.

	Peak amp. (pA)	Charge transfer (fC)	10-90% Rise time (μs)	τ_1, τ_2 (ms)	$\%\tau_1$
CA1 (n =10)	42 ± 3	616 ± 70	650 ± 20	4 ± 0.4 14 ± 1	38±3
Dentate gyrus (n = 11)	55 ± 5	688 ± 76	590 ± 60	3 ± 1 16 ± 2	39±7

Lambert JJ, Belelli D, Hill-Venning C *et al.* (1995) Trends Pharmacol. Sci. 16: 295-303.

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8P ACUTE MECHANICAL NOCICEPTIVE PROCESSING IN SHEEP IS MEDIATED BY ACTIVATION OF N-METHYL D-ASPARTATE RECEPTORS, NITRIC OXIDE SYNTHASE AND CYCLOOXYGENASE-2 ENZYMES

S. Dolan & A.M. Nolan, Department of Veterinary Preclinical Studies, University of Glasgow, Glasgow, G61 1QH

Activation of the excitatory amino acid (EAA) receptor subtype N-methyl D-aspartate (NMDA) contributes to spinal nociceptive processing following tissue damage. This process has been shown to be mediated by activation of nitric oxide synthase (NOS) (Meller *et al.*, 1994) and cyclooxygenase-2 (COX-2) enzymes (Malmberg & Yaksh, 1992). The present study examined the contribution of these enzymes and NMDA receptor activation in mediating acute mechanical nociceptive processing, in the absence of tissue inflammation or injury.

Five adult female sheep (60 - 70 kg) were implanted with intrathecal catheters (Portex, UK) at cervical level C3-C6 under halothane anaesthesia. Mechanical withdrawal thresholds were measured (in Newtons) as described previously (Nolan *et al.*, 1987). Forelimb lift was defined as the end response. The effects of intrathecal (*i.t.*) NMDA (200 nmol) plus 0.9 % saline or a selective antagonist/enzyme inhibitor on mechanical thresholds were assessed for a period of 40 mins. Drugs were administered in a crossover design. Values reported are means ± SEM. Data were analysed using one way ANOVA with post-hoc Dunnett's test.

NMDA *i.t.* significantly reduced mechanical withdrawal thresholds by 57.6 ± 5% (6.0 ± 1.5 N) ($p < 0.001$). The mean time of maximum effect was 10 ± 5 minutes. This reduction was significantly attenuated by pre-treatment with the NMDA receptor antagonist dizocilpine (MK 801) (100 nmol), the selective COX-2 inhibitor 5,5-dimethyl-3-(3-flourophenyl)-4-(4-methylsulphonyl) phenyl-2(5H)-furanone (DFU) (200 nmol) and the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) (2 μmol) (maximum decrease 11.2 ± 11%, 17.3 ± 6% and 17.0 ± 8%, respectively). In contrast, the non-NMDA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) (200 nmol - 1 μmol) and the metabotropic glutamate receptor antagonist (S)-α-methyl-4-carboxyphenylglycine (MCPG) (200 nmol - 2 μmol) failed to block NMDA-induced effects (maximum decrease 43.1 ± 6% and 38.7 ± 6%, respectively). Pre-administration of saline had no effect on NMDA-induced changes. Administration of MK801, DFU or L-NAME alone had no effect on mechanical withdrawal thresholds measured (n = 3).

In contrast to previous reports (Meller *et al.*, 1993), spinal administration of NMDA resulted in increased sensitivity to normally non-noxious mechanical stimuli. This alteration in sensory processing, attained without peripheral or central tissue damage, demonstrates that NMDA receptors are involved in mediating acute mechanical nociceptive input, through activation of NOS and COX-2 enzymes.

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9P ATYPICAL PHARMACOLOGY OF THE EXCITATION BY DOPAMINE OF RAT SUBTHALAMIC NUCLEUS NEURONES *IN VITRO*

Azita Tofighy[1] and Michael G. Lacey
Department of Pharmacology, Division of Neuroscience, The Medical School, University of Birmingham, BIRMINGHAM B15 2TT. [1]Department of Biosciences, School of Natural & Environmental Sciences, Coventry University, COVENTRY CV1 5FB.

The subthalamic nucleus (SThN) occupies a pivotal position in the circuitry of the basal ganglia, wherein it exerts a critical influence on the control of voluntary movement. Midbrain dopamine systems, which also play a crucial role in modulating basal ganglia circuitry, have been shown to the innervate the SThN, but the functional significance of this is presently unclear.

The effect of dopamine and other dopamine receptor ligands, applied by superfusion, was assessed on the firing rate of extracellularly recorded single SThN neurones in sagittal slices of rat ventral midbrain (Abbott *et al.*, 1997). In some cases, comparisons were drawn with the action of the same drugs on the firing of substantia nigra pars compacta (SNC) neurones, recorded in the same way in the same preparation. All cells studied fired action potentials spontaneously at a regular rate of 12.2 ± 0.5 Hz (n=28 SThN neurones) or 4.6 ± 0.6 Hz (n=8 SNC neurones).

Dopamine (30 μM) caused an increase in SThN cell firing of 3.6 ± 0.3 Hz in all 28 cells tested, which reversed completely on washout. The agonist of D1-like receptors, SKF 38393 (10 μM), was without effect (n=3), while the agonists of D2-like receptors, quinpirole (10 μM) and bromocriptine (30 μM), caused reversible increases in SThN cell firing of 3.5 ± 0.2 Hz (n=13) and 2.75 ± 0.57 Hz (n=6) respectively, also in all cells tested. However, contrary to expectation, the excitation of SThN neurones by dopamine (30 μM) was unaffected ($P>0.1$; 2-tailed paired *t*-test) by (-)-eticlopride (1 μM, n=5), (+)-butaclamol (1 μM, n=6), or ritanserin (10 μM, n=8), all of which are known antagonists of D2-like receptors. The excitation of SThN neurones by quinpirole (10 μM) was also unaffected by (-)-eticlopride (1 μM, n=5) and (+)-butaclamol (1 μM, n=6). In contrast, the inhibitory action of dopamine (30 μM) on the firing of SNC neurones, well established as being mediated by D2-like receptors, was completely abolished by (-)-eticlopride (1 μM, n=2) and (+)-butaclamol (1 μM, n=6).

Thus the excitation by dopamine of SThN neurones can be mimicked by known agonists of D2-like receptors. However, the ineffectiveness of several D2 antagonists suggests a novel pharmacology for this particular effect of dopamine.

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10P 6-HYDROXYDOPAMINE-INDUCED INCREASE IN FREE RADICAL PRODUCTION IN RAT STRIATUM IS ACCOMPANIED BY OXIDATIVE DNA DAMAGE: AN *IN VIVO* MICRODIALYSIS STUDY

B. Ferger*, S. Rose, A. Jenner, B. Halliwell, P. Jenner. Neurodegenerative Diseases Research Centre, Guy's, King's and St Thomas' School of Biological Sciences, Division of Pharmacology and Therapeutics, Manresa Rd, London UK. *Institute of Pharmacology and Toxicology, University of Marburg, Marburg, Germany

6-Hydroxydopamine (6-OHDA) produces lesions of dopaminergic neurones through production of oxygen free radicals (Chiueh *et al.*, 1993) and inhibition of the mitochondrial function (Glinka *et al.*, 1998; Youdim *et al.*, 1997). However, the time-course of radical formation and subsequent oxidative damage has not been studied. We now investigate the effect of acute 6-OHDA perfusion in to the rat striatum on hydroxyl radical production and DNA base damage.

Male Wistar rats (280-320g) were anaesthetised with chloral hydrate (400mg/kg i.p.) and anaesthesia was maintained throughout the procedure. Microdialysis probes were inserted bilaterally into the striatum using standard stereotaxic techniques. Probes were perfused at a flow rate of 2μl/min with artificial extracellular fluid (aECF). One hour after implantation, dialysate samples were collected every 10 min throughout the experimental period and analysed for levels of 2,3-dihydroxybenzoic acid (2,3-DHBA) by HPLC with electrochemical detection. At least 30 min after implantation, sodium salicylate (5mM) was included in the aECF. The hydroxylation product of sodium salicylate, 2,3-DHBA, was measured in the dialysate as a marker of hydroxyl radical formation. After determination of basal levels of 2,3-DHBA for 60 min, 6-OHDA (100μM) was incorporated in the aECF perfusing the right probe for 60 min followed by 60 min with normal aECF. The left side received normal aECF throughout. Immediately following the microdialysis, striata were dissected out, DNA was extracted, hydrolysed and derivatized for gas GC/MS measurement of DNA base alterations.

Perfusion with 6-OHDA resulted in a rapid and substantial increase in 2,3-DHBA formation which was not sustained following removal of 6-OHDA from the aECF (Figure 1). Oxidation products of most DNA bases showed a tendency to be increased following 6-OHDA administration. The increases in 5-hydroxycytosine, hypoxanthine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FAPy-guanine) reached statistical significance (control: 0.03±0.004., 11.8±2.6, 1.3±0.6, 6-OHDA: 0.07±0.02*, 26.9±7.3*, 3.3±0.6* nmol/mgDNA respectively ; *p<0.05 vs control, paired Student's t-test).

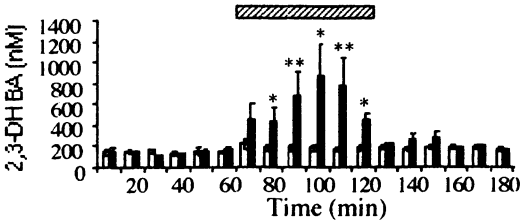

Figure1 The effect of 6-OHDA, ■ 100 μM and control, □ on 2,3-DHBA formation in the rat striatum. Data is expressed as mean ± s.e.mean, n=10. * p<0.05, ** p< 0.01 non-parametric ANOVA followed by Wilcoxon's test). ▨ period of perfusion with 6-OHDA

This is the first study which investigates the time-course of 6-OHDA-induced hydroxyl radical formation with the subsequent analysis of oxidative DNA base damage. These findings suggest that hydroxyl radical formation and oxidative DNA damage are an early mechanism involved in neuronal cell death. The rapid onset and reversible increase of 2,3-DHBA suggests a direct effect via hydroxyl radical formation rather than to an inhibition of the mitochondrial respiratory chain, however, further studies are required to establish the exact mechanism involved (Glinka *et al.*, 1998; Youdim *et al.*, 1997).

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11P CHARACTERISATION OF P2X RECEPTORS IN RAT TRIGEMINAL MESENCEPHALIC NUCLEUS

M. K. Patel, B. S. Khakh, and G. Henderson. Department of Pharmacology, University of Bristol, Bristol BS8 1TD.

In situ hybridisation experiments have detected mRNA for $P2X_4$ $P2X_5$ and $P2X_6$ purinoceptor subunits in trigeminal mesencephalic nucleus (MNV) neurones and we have previously demonstrated that these neurones express functional P2X receptors (Khakh *et al.*, 1997). In the present study we have sought to further characterise the type(s) of P2X receptor present using first αβmethylene adenosine diphosphate (αβMeADP), an agent which activates P2X receptors in locus coeruleus (LC) neurones (Sansum *et al.*, 1998), and second the potent antagonist 2'3'-O-(2,4,6-trinitrophenyl)-ATP (TNP-ATP) which shows selectivity for $P2X_1$, $P2X_3$ and $P2X_{2/3}$ over $P2X_2$, $P2X_4$ and $P2X_6$ receptors (Virginio *et al.*, 1998).

Pontine brain slices containing the MNV and LC were prepared from male Wistar rats (14-28 days postnatal). Slices were superfused (2.5mls. min^{-1}: 30^0C) with artificial cerebrospinal fluid solution comprising (mM) NaCl, 126; KCl, 2.5; NaH_2PO_4, 1.24; MgCl, 1.3; CaCl, 2.4; $NaHCO_3$, 26; and D-glucose, 10. Whole-cell patch clamp recordings were made from MNV neurones using electrodes filled with (mM) CsCl, 130; EGTA, 5; Hepes, 10; CaCl, 1; MgCl, 2; Na_2-GTP, 0.5; and Mg-ATP, 5. Extracellular recordings of LC neurone firing rate were made using electrodes filled with 2M NaCl. Data are presented as means ± SD.

ATP-γ-S (30μM) and αβMeATP (300μM) applied for 2 min evoked inward currents in MNV neurones of 558±298 pA (n=8) and 351±135pA (n=7) respectively whilst αβMeADP (300μM; n=4) had no effect. A similar result was obtained on MNV neurones from mature rats (weight 220-240g; n=5). In contrast in LC neurones all three agonists were effective in increasing ($P<0.05$) the spontaneous firing rate. The increase in firing rate being from 1.5±0.8Hz to 3.0±1.8Hz for ATP-γ-S (30μM; n=6), 1.4±0.8Hz to 2.8±1.2Hz for αβMeATP (300μM; n=6) and 1.6±0.9Hz to 3.3±1.8Hz for αβMeADP (300μM; n=7). The effect of TNP-ATP was tested on MNV neurones against the current evoked by ATP-γ-S (30μM). TNP-ATP (10nM) reduced ($P<0.05$) the inward current by 37±21% (n=10). Increasing the concentration of the antagonist to 100nM and 10μM did not produce any further antagonism, the evoked current being reduced by 44±9% (n=5) and 33±19% (n=9) respectively.

The lack of effect of αβMeADP in MNV neurones shows that the P2X receptors expressed in MNV neurones are different from those expressed in LC neurones. Whilst the reduction in the P2X current by a low concentration of TNP-ATP could suggest the presence of $P2X_1$, $P2X_3$ or $P2X_{2/3}$ receptors we cannot rule out the involvement of $P2X_5$ receptors.

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12P CHARACTERISATION OF P2 RECEPTORS ON CEREBRAL CORTICAL CELL CULTURES FROM EMBRYONIC RATS

G.C. Bennett, A.P.D.W. Ford & M.R. Boarder, Urogenital Pharmacology, Center for Biological Research, Roche Bioscience, Palo Alto, CA 94304 USA and Dept of Cell Physiology and Pharmacology, University of Leicester, Leicester LE1 9HN.

Two types of P2 receptor exist; the ionotropic P2X and metabotropic P2Y receptors (Fredholm *et al.*, 1997). It has been demonstrated that receptors of both subtypes are present in various regions of rat brain (Edwards *et al.*, 1992; von Kügelgen *et al.*, 1994). The aim of this study was to characterise the P2 receptors present on cerebral cortical cells from embryonic rats.

Pregnant Sprague Dawley rats were killed 21 days after mating. The embryos were excised and the embryonic cortices dissected. Cells were dispersed by enzymatic digestion and cultured in DMEM containing the neuronal survival supplement, B27 (Gibco). Cultures were maintained on poly-D-lysine coated, 96 well plates for 8 days. Cells were then loaded with Fluo-3AM and increases in $[Ca^{2+}]_i$ measured using a fluorescence imaging plate reader. The antagonist activity of Suramin, PPADS, RB2 (reactive blue 2) and adenosine bisphosphate was studied following preincubation for 15 minutes prior to agonist addition. Brain primary cell cultures will contain a heterologous population of cells. Staining with β-tubulin and glial fibrillary acid protein was performed to determine the proportions of cell types present. The neuronal population comprised 52% and the glia 48% of the overall population. The purines shown in Figure 1 produced dose dependant increases in $[Ca^{2+}]_i$ giving the pEC_{50} values in Table 1. With increasing concentration, antagonists caused rightward shift and collapse of all the agonist concentration-effect curves with a potency which was not significantly different between agonists. Table 2 gives the mean pA_2 values for each antagonist.

Figure 1. Agonist concentration-effect curves showing increases in fluorescence ($[Ca^{2+}]_i$) with increasing [Agonist] (mean ± s.e., n=6).

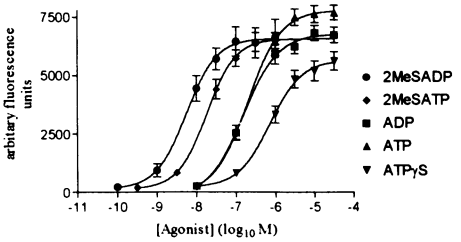

Table 1. pEC_{50} values for active agonists (mean ± s.e., n=6)

Agonist	2MeSADP	2MeSATP	ADP	ATP	ATPγS
pEC_{50}	8.20±0.05	7.41±0.07	6.87±0.	6.74±0.	6.18±0.

Table 2. Mean antagonist pA_2 values against 2MeSATP (n=5)

Antagonist	Suramin	PPADS	RB2	ABP
pA_2	5.79±0.03	6.31±0.14	6.31±0.12	4.83±0.15

The results of this study indicate that the stimulation seen in response to purines was via activation of P2Y receptors. The rank order of agonist potencies and pA_2 values are comparable to those published for the cloned $P2Y_1$ receptor. ATP displays a significantly higher maximum than the other agonists ($p<0.05$, t-test) which may indicate that a mixed population of receptors, on one or more cells types, is present.

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13P DISTRIBUTION AND ABUNDANCE OF BINDING SITES FOR A POTENT, SELECTIVE AMPA RECEPTOR ANTAGONIST [^{3}H]Ro 48-8587 IN HUMAN BRAIN

J.G. Richards, J. Messer, Z. Bleuel, P. Malherbe, A. Klingelschmidt, R.L.M. Faull[1] & V. Mutel, Pharma Division, Preclinical CNS Research, F.Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland; and [1]Dept. of Anatomy with Radiology, Faculty of Medicine & Health Science, University of Auckland, New Zealand.

α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors are ligand-gated cation channels for L-glutamate. They are hetero-oligomeric proteins composed of the subunits Glu1-4 (flip and flop forms), the Glu2 subunit determining their Ca^{++} permeability. The receptors play key roles in brain physiology and pathology. Ro 48-8587 (9-imidazol-1-yl-8-nitro-2,3,5,6-tetrahydro-[1,2,4]triazolo-[1,5c]-quinazoline-2,5-dione) is, to-date, the most potent and selective AMPA antagonist. It has anticonvulsive properties *in vivo* and is moderately neuroprotective in a focal brain ischemia model. The binding characteristics of [^{3}H]Ro 48-8587 in rat whole brain homogenates and brain sections have been recently reported (Mutel et al., 1998).

We have now studied its binding characteristics, as well as the cellular sites of synthesis of Glu 1-4 subunits, in human postmortem brain. Primary visual cortex, caudate putamen, hippocampal formation and cerebellum were obtained from the New Zealand Neurological Foundation Human Brain Bank. The study was performed under ethical approval by the University of Auckland Human Subjects Ethics Committee. The brain tissues were from healthy subjects with no known history of neurological disease or drug treatment. They were rapidly frozen in dry-ice and stored at -80°C until used. Cryostat sections (12μm) were prepared and pre-incubated (2x 10min) in Tris HCl buffer pH 7.0 at 22°C (final volume 130ml) then incubated in 1nM [^{3}H]Ro 48-8587 (TRK 1058, Amersham) in the same volume of buffer for 60min at 4°C. This was followed by three washes at 4°C (2x 30sec. + 1min) in 130ml buffer alone.; non-specific binding was determined in the presence of 100μM quisqualate. Sections were exposed, together with tritium microscales, to tritium-sensitive imaging plates (BAS-TR2025) for 4 days and subsequently to Hyperfilm Tritium^R (Amersham) for 3 weeks at 4°C. The plates were scanned in a Fujifilm BAS-5000 high resolution phosphor imager and measured with a MCID M2 image analysis system (Imaging Research Inc., St. Catherines, Ontario, Canada). The distribution of human Glu 1-4 mRNAs was investigated using a hybridization histochemistry technique previously described (Saura et al., 1998).

A high density of high-affinity specific binding was observed in human cerebral cortex (layers I-III >V,VI >> IV), caudate putamen, Dendritic layers of the hippocampal formation and cerebellar molecular layer; non-specific binding was <10% of total binding. Specific binding values ranged from 1600 to 200 fmol/mg protein in hippocampal CA3 and cerebellar granule cell layers, respectively. There was a good correlation between the distribution of binding sites and Glu 1-4 transcripts, revealed by hybridization histochemistry .

We conclude that [^{3}H]Ro 48-8587, due to its potency, high specificity and pharmacological selectivitiy, is currently the radioligand of choice for future studies on the regulation of AMPA receptors in neurological or psychiatric diseases.

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14P 5-CT STIMULATION OF ADENYLYL CYCLASE ACTIVITY IN GUINEA-PIG HIPPOCAMPUS: EVIDENCE FOR INVOLVEMENT OF 5-HT$_7$ AND 5-HT$_{1A}$ RECEPTORS

D.R. Thomas, A.M. Brown, S. G. Taylor, and D.N. Middlemiss, Dept. of Neuroscience Research, SmithKline Beecham Pharmaceuticals, Harlow, CM19 5AW, U.K.

A number of 5-HT receptors have been reported to mediate stimulation of adenylyl cyclase (AC) activity in guinea pig hippocampus, including 5-HT$_4$, 5-HT$_{1A}$-like (Shenker *et al.*, 1987) and 5-HT$_7$-like receptors (Tsou *et al.*, 1994). However, definitive evidence for 5-HT$_7$ functional coupling in brain is absent, partly due to a lack of selective ligands. Furthermore, it has been suggested that the 5-HT$_{1A}$-like response reported in guinea pig hippocampus may actually reflect 5-HT$_7$ receptor stimulation (Hoyer *et al.*, 1994). We have therefore investigated 5-HT$_7$ receptor function in this tissue using the selective 5-HT$_7$ antagonist SB-258719 (Thomas *et al.*, 1998) and a number of other 5-HT receptor antagonists.

AC activity was measured in well-washed and incubated (37°C, 20 min) guinea pig hippocampal membranes by measuring the conversion of [α-^{33}P]-ATP to [^{33}P]-cAMP (Thomas *et al.*, 1998), in the presence of 1μM GR-113808 to prevent 5-HT$_4$ receptor stimulation by 5-HT.

5-HT stimulated AC activity by (mean ± s.e.mean) 20 ± 1.5% (pEC_{50} 7.7 ± 0.1, n=4). 5-CT (pEC_{50} 8.4 ± 0.2, n=8) was a full agonist, whereas 8-OH-DPAT (pEC_{50} 7.5 ± 0.4, n=4) displayed partial agonism compared to 5-HT (intrinsic efficacy 0.4 ± 0.1). SB-258719 (5μM) surmountably antagonised the response to 5-CT with a pK_B (7.2 ± 0.1, n=5) consistent with its potency at the human 5-HT$_7$ receptor (pA_2 7.2 ± 0.2, Thomas *et al.*, 1998). The slope of the 5-CT concentration-response curve was similar in the absence and presence of SB-258719 (0.78 ± 0.12 and 0.79 ± 0.12 respectively). Methiothepin (pK_B 7.4 ± 0.2, n=3) displayed a similar antagonist profile to SB-258719.

WAY-100635 (100nM) and cyanopindolol (1μM), at concentrations which antagonise 5-HT$_{1A}$ but not 5-HT$_7$ receptors, did not inhibit basal AC activity but induced a biphasic 5-CT concentration-response curve (with WAY-100635, 2 site fit RMSq = 14.48 vs 15.37 for 1 site fit (n=6); with cyanopindolol, 2 site fit RMSq 3.29 vs 3.17 for 1 site fit (n=3), Bates and Watts, 1988), consistent with antagonism of the response to higher (100nM to 10μM) 5-CT concentrations. The calculated pK_Bs for WAY-100635 and cyanopindolol (9.6 and 8.4 respectively) were consistent with their reported 5-HT$_{1A}$ receptor affinities (eg. Hoyer et al., 1994).

The above data are consistent with the presence of 5-HT$_7$ receptors in guinea pig hippocampus which are positively coupled to AC. In addition, the data suggest that 5-HT$_7$ receptor-mediated responses in this tissue may be augmented by a mechanism involving 5-HT$_{1A}$ receptor activation.

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15P INTERACTION OF OESTRONE AND 17β-OESTRADIOL WITH THE 5-HT$_3$ RECEPTOR

Joy A. Steele and *Ian L. Martin, Department of Pharmacology, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2H7. *Present address: Department of Pharmaceutical and Biological Sciences, Aston University, Birmingham, UK.

The 5-HT3 receptor is a member of the ligand-gated ion channel family of proteins and is composed of five subunits assembled in a pseudo-symmetric array around a central cation channel. It is widely distributed in the mammalian peripheral and central nervous systems but the concentration in the CNS is low. Antagonists of the receptor have proved to be efficacious in the treatment of radiation- and chemotherapeutic-induced emesis.

We have used NCB-20 cells to explore the interaction of steroids with the 5-HT3 receptor expressed by these cells (Maricq et al., 1991) using the whole cell configuration of the patch clamp technique. Two application protocols were used to differentiate between interaction of the steroids with the open and resting state of the channel. Co-application with a non-desensitising concentration of 5-HT (0.5μM) showed that estrone and 17ß-estradiol exhibited a higher affinity for the open conformation than the closed conformation which was probed with a two minute pre-pulse of the steroid, followed by co-application with 5-HT (10μM). Estriol, showed no significant preference for either the open or closed state (Table 1).

The block was rapid, reversible and complete. It did not show any voltage dependence i.e. the magnitude of the block did not depend on the voltage and the reversal potential did not change. The block with estrone was not competitive with 5-HT.

The block was steroid specific and only seen at concentrations above 10 μM for testosterone, progesterone and several metabolites (testosterone, estriol, diethylstilbesterol, progesterone, dexamethasone, corticosterone, deoxycorticosterone, alphaxalone, pregnanalone, allopregnanalone, allotetrahydrocorticosterone, pregnenalone sulphate and dehydroepianndrosterone).

It is interesting to speculate about the consequences of this block. Morning sickness commonly occurs in early pregnancy but abates after about 12 weeks. At this time, previously published data shows that this is precisely the time at which the concentrations of estrone increase significantly (Tulchinsky et al., 1972) and thus may provide relief to this condition.

Table 1

	Resting conformation IC50 (95% CI) (n = slope; N = cell no.)	Open conformation IC50 (95% CI) (n = slope; N = cell no.)
estrone	347 nM (316 - 398) (n = 1.1; N = 15)	1.5 nM (1.2 - 2.1) (n = 0.8; N = 11)
17ß-estradiol	3.1 μM (2.7 - 3.5) (n = 1.3; N = 8)	81 nM (60 - 112) (n = 0.8; N = 10)
estriol	33 μM (23 - 46) (n = 0.8; N = 6)	21 μM (10 - 43) (n = 0.5; N = 5)

This work was supported by the MRC (Canada)

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16P AGONIST-INDUCED UP-REGULATION OF AN EPITOPE-TAGGED HUMAN $D_{2(SHORT)}$ DOPAMINE RECEPTOR

J. L. Franklin & P. G. Strange. School of Animal and Microbial Sciences. University of Reading, P. O. Box 228, Whitekinghts, Reading, RG6 6AJ.

The aim of this study was to investigate the regulation of a FLAG epitope-tagged D_{2s} dopamine receptor stably expressed in Chinese hamster ovary (CHO) cells, through ligand binding experiments and immunocytochemical techniques.

The FLAG epitope tag was engineered onto the N-terminus of the human (h) D_{2s} dopamine receptor and the cDNA encoding this FLAGhD_{2s} dopamine receptor was stably expressed in CHO cells. The pharmacological and functional profile of this FLAGhD_{2s} receptor was determined using [^{3}H]spiperone saturation and competition binding assays and through the [^{35}S]GTPγS binding assay and was shown to be analogous to that of a recombinant wild type D_{2s} dopamine receptor expressed in CHO cells.

Pre-treatment of the FLAGhD_{2s} expressing cells with 1 mM dopamine resulted in a significant increase in total receptor number when compared with untreated cells (104 ± 12.4% (mean ± s. e. mean); $p< 0.001$), as assessed by [^{3}H]spiperone saturation analysis. A significant increase in receptor number was also observed after pre-treatment with 1 μM dopamine (47 ± 6.4% (mean ± s. e. mean); $p< 0.05$), whilst 1 nM dopamine had no significant effect on receptor number ($p> 0.05$). The observed K_D values of the FLAGhD_{2s} receptor for [^{3}H]spiperone were unaffected by preincubation with agonist. This agonist-induced up-regulation was time-dependent with a maximum increase in receptor number observed after 14-16 hours ($t_{½}$ of 8-9 hours) following pre-treatment with 1 mM dopamine.

The responsiveness of the FLAGhD_{2s} dopamine receptors after pre-treatment with 1 mM dopamine for 16 hours was investigated using the [^{35}S]GTPγS binding assay. A significant decrease (32.7 ± 4.4% (mean ± s. e. mean); $p<0.05$) in the maximal dopamine-stimulated GDP/GTP exchange was observed. This reduction in efficacy was accompanied by a three-fold decrease in the potency of dopamine to stimulate [^{35}S]GTPγS binding (EC_{50} = 973 ± 37 nM in dopamine treated cells compared with 331 ± 18 nM in untreated cells).

The effect of the protein synthesis inhibitor, cycloheximide, on dopamine-induced up-regulation was assessed. Cycloheximide at a concentration of 5 μg/ml was added to the cells simultaneously with the addition of 1 mM dopamine. After treatment for 16 hours, the dopamine-induced increase in receptor number was reduced by about 90% indicating that increased protein synthesis is required for agonist-induced FLAGhD_{2s} dopamine receptor up-regulation. Basal levels of FLAGhD_{2s} dopamine receptors were not affected by cycloheximide.

The m2 anti-FLAG antibody was used to visualise FLAGhD_{2s} dopamine receptors expressed in CHO cells. Fixed and immunofluorescently labelled CHO cells were imaged using confocal microscopy and a comparison of the amount of receptors present in untreated (basal) CHO cells and in cells which had been preincubated with 1 mM dopamine for 16 hours was made. In both basal and treated cells the receptors were seen to be distributed throughout the cell and a brighter punctate intracellular staining pattern was also observed, indicating that there were intracellular pools of FLAGhD_{2s} dopamine receptors.

The density of receptors present in basal and treated cells was determined through image analysis. A binary overlay was constructed which compared with the observed visual fluorescence and it was assumed that the intensity of fluorescence correlated to the amount of labelled FLAGhD_{2s} dopamine receptors. In dopamine treated cells there was an apparent increase in the intracellular receptor pools and these pools were seen to be predominantly in a perinuclear location. It was determined that there were approximately twice the density of fluorescently labelled FLAGhD_{2s} dopamine receptors in agonist treated cells compared with basal cells, a value in good agreement with the data obtained through radioligand binding studies.

This work was supported by a BBSRC studentship.

17P EVIDENCE FOR INTERACTIONS BETWEEN D_1 AND D_{2s} DOPAMINE RECEPTORS CO-EXPRESSED IN BHK CELLS

D.T. Page[1], N. O'Conner[1], M. Rogers[2], F. Martin[2], K.M. O'Boyle[1].
[1]Department of Pharmacology or [2]National Agricultural and Veterinary Biotechnology Centre, University College, Dublin, Belfield, Dublin 4, Ireland.

The neurotransmitter dopamine (DA) mediates its effects by actions on two classes of G-protein coupled receptors, D_1-like and D_2-like. A substantial body of *in vitro* and *in vivo* evidence suggests that these two receptor families interact with each other (Seeman et al., 1989; Waddington et al., 1994). The aim of this study was to establish a cultured cell line co-expressing both D_1-like and D_2-like receptors which could be used to investigate intracellular receptor interactions. Baby Hamster Kidney (BHK) cells stably expressing the human D_1 DA receptor were stably transfected with cDNA encoding the human D_{2S} DA receptor subtype using the expression vector pCEP4. ^{3}H-SCH23390 (0.06-4.0 nM) and ^{3}H-spiperone (0.06-2.0 nM) were used in saturation binding experiments on membrane preparations prepared from co-transfected cells to determine levels of expression of D_1 and D_{2S} receptors, respectively. Non-specific binding was determined in the presence of 100 nM piflutixol (D_1) or 1 μM domperidone (D_2). Agonist-induced changes in cAMP levels were measured in intact co-transfected cells using a cAMP-protein binding method.

The density (Bmax) of D_1 receptors in co-transfected cell membranes was 1618±204 fmol/mg and the apparent dissociation constant (Kd) for ^{3}H-SCH23390 binding was 1.029±0.085 nM (mean±s.e.m., n=4). ^{3}H-Spiperone binding to D_{2S} receptors occurred with a Bmax of 1611±44 fmol/mg and a Kd of 0.372±0.124 nM (mean±s.e.m., n=5). The basal level of cAMP generated by these cells was 72 ± 7 pmol/mg (n=3). DA, a mixed D_1/D_2 agonist, caused a concentration-dependent increase in cAMP levels in co-transfected cells which was enhanced in the presence of the D_2 antagonist eticlopride (1 μM) (Emax DA: 1233±85 pmol/mg, Emax DA+eticlopride: 1727 ± 72 pmol/mg; $p<0.05$, t-test; mean ± s.e.m., n=3). The D_2 agonist, quinpirole, reduced levels of forskolin-stimulated cAMP in co-transfected cells by 51.6%. When D_1 saturation binding experiments were conducted in the presence of DA (500 nM) there was a significant reduction in the Bmax of ^{3}H-SCH23390 binding. This effect of DA on D_1 receptor density was reversed by the D_2 selective antagonist, eticlopride (100 nM) (Table 1).

Table 1. ^{3}H-SCH23390 binding to D_1/D_{2S}/BHK membranes

	Bmax (fmol/mg)	Kd (nM)
control	1618±204	1.029±0.085
control + DA	1401±182*	1.614±0.051
control + DA + etic	1660±298	2.594±0.378*

mean±s.e.m., n=4. *$p<0.05$ vs control, paired t-test

In conclusion, this study describes a novel recombinant cell system stably co-expressing D_1 receptors which are positively coupled to adenylyl cyclase and D_{2s} receptors which are negatively coupled to this enyme. These cells can be used as a model system for analysis of intracellular receptor interactions. The present data indicate that such interactions do occur within D_1/D_{2S}/BHK cells at the ligand binding level.

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18P GENOMIC STRUCTURE OF THE HUMAN $P2X_2$ RECEPTOR: ALTERNATIVE SPLICING GENERATES MULTIPLE TRANSCRIPTS

R.A. McMahon[1], P.T. Hurley[1], T.M.Egan[2], A. Nelson[1], M. Rogers[1] and F. Martin[1].
[1]National Agricultural and Veterinary Biotechnology Centre, University College Dublin. [2]Department of Pharmacological and Physiological Sciences, St. Louis University Health Science Center.

ATP functions as a neurotransmitter by binding to and activating two classes of membrane receptors, P2X which forms a ligand gated ion channel, and P2Y, which is a G-protein coupled receptor. To date, receptors $P2X_{1-7}$ have been cloned in the rat. The corresponding human homologues have all been described, with the exception of the $P2X_2$. In the rat, only two of the multiple splice variants reported for the $P2X_2$ receptor were functional (Simon *et al.*,1997; Koshimizu *et al.*, 1998). The aim of this project was to clone and characterise the human $P2X_2$ receptor. A fragment of the $P2X_2$ receptor was obtained from infected tonsil cDNA by designing degenerate oligonucleotides based on alignments of rat P2X receptors. A number of different tissue sources were investigated using $P2X_2$ specific primers including brain, heart, placenta, spinal cord, pituitary, and tonsil. Pituitary and placenta sources showed significant expression and were therefore used as a template for RACE. A modified method using a random hexamer with the sequence NNNATG was used to generate second strand cDNA. This template was used in inverse PCR to isolate the start site. Primers corresponding to the translation start and stop sites were used to obtain $P2X_2$ open reading frames. This generated a number of bands which corresponded to multiple transcripts. Sequencing verified the isolation of fragments corresponding to the rat $P2X_{2A}$ and $P2X_{2B}$ receptor cDNAs with homologies of 82% and 85% respectively. Interestingly, the difference in % homologies is as a result of the lower homology (61%) between the sequence which generates the $P2X_{2A}$, thus supporting the theory that the $P2X_{2B}$ cDNA is in fact the fully spliced receptor. Other variants isolated included transcripts with a 76bp insert in TM2 of both the $P2X_{2A}$ and $P2X_{2B}$ isoforms which corresponds to the splice variant first described by Housley *et al.*, (1995). A 5th variant differed from the others in that it contained an 88 amino acid deletion from the extracellular loop which includes 6 of the 10 conserved cysteine residues. In order to further understand the multiple splice variants obtained, it was decided to isolate the genomic clone. Primers corresponding to the start and stop translation sites were used to amplify out a fragment of 2.8kb. Sequencing verified isolation of the human $P2X_2$ gene. Bioinformatic analysis confirmed that all insertions corresponded to introns, and that deletions were either as a result of complete exons being removed, or due to the use of cryptic donor sites within exons (the 88 amino acid deletion). The expressed human $P2X_{2A}$ and $P2X_{2B}$ receptors are being assessed using patch clamp techniques.

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Accession numbers $P2X_{2A}$:AF109387
$P2X_{2B}$:AF109388

19P DETERMINANTS OF HUMAN P2X$_7$ LARGE PORE FORMATION

I.P. Chessell, A.D. Michel, & P.P.A. Humphrey. Glaxo Institute of Applied Pharmacology, University of Cambridge, Tennis Court Rd, Cambridge, CB2 1QJ

The cytolytic P2X$_7$ (P2Z) receptor is the seventh member of the P2X family of ligand-gated channels (Surprenant *et al.*, 1996). This bifunctional receptor has two phenotypic states. First, it functions as a ligand-gated non-selective cation channel, and second, in certain circumstances, application of agonist causes formation of large ligand-gated "pores" in the cell membrane, permeable to molecules with molecular weights of up to ~ 800Da.

In this study we investigated the properties of formation of this large pore in HEK-293 cells transfected stably with human recombinant P2X$_7$ receptors. Whole-cell recordings were made using Cs-aspartate containing electrodes (2.5-4 MΩ), at 22°C, in a HEPES-buffered low divalent extracellular medium (zero added Mg^{2+}, 0.5mM Ca^{2+}). At a holding potential of –90mV, application of 100 μM benzoylbenzoyl ATP (BzATP) produced inward currents with a short initial plateau (after 2s) of 1741±210 pA, followed by a progressive increase in inward current, reaching a final plateau of 4234±250 pA (P<0.01, Student's paired t-test) after 87.2±6.3s (n=32). The increase in inward current in the continued presence of BzATP was associated with formation of the large pore, as in the presence of 2 μM extracellular YO-PRO-1, intracellular fluorescence developed with the same timecourse as development of the large inward current. After large pore formation EC_{50} values calculated for BzATP (16.7 [6.1-46.0] μM) were smaller than those determined at the "channel" form of the receptor (58.6 [29.9-114.9] μM, P<0.05, Student's unpaired t-test).

The speed of formation of the large pore was dependent on the magnitude of the initial current, and thus, presumably, on P2X$_7$ channel density. Thus, there was a correlation between initial inward current magnitude and speed of pore formation (i.e. time to reach large current plateau; P<0.02, Pearson's rank), and between initial (channel) current magnitude and the subsequent magnitude of the large pore current (P<0.02). When cells were voltage clamped at +50mV, the time taken for formation of the large pore was not significantly affected (94.9±9.1s), and the ratio between the initial (channel) and final (pore) current plateau was not changed when holding potentials were –90mV (pore current 296±20% of channel current) or +50mV (pore current 260±29% of channel current; n=9).

Further investigation of channel to pore transition was made using a monoclonal antibody, directed towards the extracellular loop of the receptor, which we have demonstrated to be a functional antagonist (Buell *et al.*, 1998). In naïve cells, with brief (1s) agonist exposure, application of the antibody for 5min caused a significant reduction of BzATP (100μM) induced inward currents to 26.6±4.2% of control (n=9; P<0.05, Student's paired t-test). However, in cells where pore formation was induced by long exposure to BzATP, the antibody was almost ineffective at blocking inward currents (reduction to 80.6±10.5% of control). However, the antibody significantly slowed channel to pore transition, extending the time taken to reach the pore plateau to 479.8±66.9s (n=12).

On the basis of these data, we speculate that pore formation involves interaction between P2X$_7$ receptor subunits, but is not dependent on directional ion flux through the receptor. Given that the agonist potency of BzATP changes when the receptor is in the large pore form, and that the monoclonal antibody is unable to block responses mediated by receptors in the pore form, we also propose that channel to pore transition involves a conformational change at the level of the receptor.

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20P SPECIES DIFFERENCES BETWEEN P2X$_7$ RECEPTORS

A.D.Hibell, J. Simon, A.D. Michel, I.P. Chessell & P.P.A. Humphrey, Glaxo Institute of Applied Pharmacology, Dept of Pharmacology, University of Cambridge, Cambridge CB2 1QJ

The P2X$_7$ receptor is an ATP-gated cation channel that is thought to represent the previously described P2Z receptor (Surprenant et al., 1996). This receptor can function as a ligand-gated, non-selective, cation channel and, following prolonged activation, as a ligand-gated pore enabling solutes with MW < 900 to enter cells (Surprenant *et al.*, 1996). Previously, the compound KN62 has been shown to antagonise the human, but not the rat, P2X$_7$ receptor (Humphreys *et al.*, 1998). In the present study we demonstrate further species differences between the P2X$_7$ receptors.

Studies were performed using HEK293 cells transfected with the human, rat or mouse recombinant P2X$_7$ receptors. The cellular accumulation of the fluorescent, DNA binding dye, YO-PRO-1 (Hickman *et al.*, 1994; Michel *et al.*, 1998), was measured at 37°C in a 96 well plate fluorimeter (excitation and emission wavelengths of 480 and 530 nm, respectively) in a buffer comprising (mM) :- 10 Hepes, 10 glucose, 5 KCl, 1 $CaCl_2$, 280 sucrose (pH 7.4). Data are the mean±s.e.mean of 3-5 experiments. Antagonists were pre-incubated for 20mins.

In HEK293 cells expressing the rat, human or mouse P2X$_7$ receptor, 2'&3'-O-benzoylbenzoyl-ATP (DbATP) increased cellular YO-PRO-1 accumulation. The pEC_{50} values for DbATP were 6.6±0.1 (rat), 6.1±0.1 (human) and 4.7±0.2 (mouse). The times (min) taken for 50% of maximal DbATP-stimulated YO-PRO-1 accumulation to occur were 12±1 (rat), 25±4 (human) and 109±5 (mouse). In addition to the kinetic differences, the antagonists oxidised ATP (OxATP), pyridoxal 5-phosphate (P5P) and pyridoxalphosphate-6-azophenyl-2',4'–disulphonic acid (PPADS) differentiated between the species homologues.

Thus, at the rat and human P2X$_7$ receptor, PPADS (10nM-1μM), P5P (0.3-10 μM) and OxATP (3-100μM) decreased the maximum response to DbATP. Antagonist effects on the mouse P2X$_7$ receptor were only observed at higher concentrations of PPADS (3-30μM) and P5P (3-30μM) whereas OxATP was only active at 100μM. Since the compounds were not competitive antagonists, direct estimates of potency could not be determined Instead, pIC_{50} values to inhibit responses to either an EC_{50} or supra-maximal concentration of DbATP were calculated (See Table). Using this approach PPADS, P5P and OxATP were more potent antagonists of human than mouse P2X$_7$ receptors. KN62 (0.1–3μM) was not an antagonist of the rat P2X$_7$ receptor but blocked the human and mouse receptors (pIC_{50} values of 8.2±0.2 and 6.8±0.5 using an EC_{50} concentration of DbATP).

	PPADS		P5P		OxATP	
	(a)	(b)	(a)	(b)	(a)	(b)
Rat	7.0 (0.2)	6.2 (0.1)	6.5 (0.1)	6.2 (0.2)	5.0 (0.1)	4.5 (0.2)
Human	8.1 (0.1)	7.7 (0.1)	7.0 (0.2)	5.9 (0.2)	5.9 (0.2)	5.4 (0.1)
Mouse	5.1 (0.1)	nd	5.3 (0.1)	nd	< 4	nd

pIC_{50} (mean±s.e.mean) determined using DbATP concentrations (a) equal to or (b) 30 times the EC_{50}. nd = not determined.

This study has identified marked species differences in the potency of DbATP as a P2X$_7$ agonist, and in the rates of agonist-stimulated YO-PRO-1 accumulation. The interaction of antagonists with the P2X$_7$ receptor is complex, however, potency estimates for PPADS, P5P and OxATP appear to vary markedly between the three species studied.

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21P DIFFERING PHARMACOLOGICAL PROPERTIES OF THE HUMAN AND RAT P2Y4 RECEPTORS

C. Kennedy, C.L. Herold[1], A. Qi[1], R.A. Nicholas[1] & T.K. Harden[1], Dept. of Physiology and Pharmacology, University of Strathclyde, Glasgow G4 0NR, [1]Dept. of Pharmacology, University of North Carolina at Chapel Hill, NC27599.

P2Y receptors are heptahelical receptors linked to activation of phospholipase C, generation of inositol phosphates (IP) and release of Ca^{2+} stores. At the human P2Y4 (hP2Y4) receptor, uridine 5'-triphosphate (UTP) is a potent, full agonist, but adenosine 5'-triphosphate (ATP) has been reported to be a full agonist, a partial agonist or inactive. In contrast, UTP and ATP are full agonists at the rat P2Y4 (rP2Y4) receptor (Bogdanov *et al.*, 1998; Webb *et al.*, 1998). Here, we have compared the activity of several nucleotides at hP2Y4 and rP2Y4 receptors.

P2Y4 receptors were stably expressed in 1321N1 cells as described previously (Nicholas *et al.*, 1996). To measure intracellular [Ca^{2+}] with Fura 2, cells were plated at low density on glass coverslips, which were then placed in a chamber of 200 µl volume and superfused at 1.4 ml/min. Agonists were applied for 30 s in the superfusate and the peak response measured. To measure IP production, cells were incubated overnight in 24 well plates with 0.4 µCi of *myo*-[^{3}H]-inositol/ well. Agonists were subsequently added together with 10 mM LiCl for 5 min at 37°C. The reactions were stopped by aspirating the media and adding 0.5 ml boiling 10 mM EDTA, pH 8.0. [^{3}H]-IPs were isolated using Dowex AG1-X8 anion-exchange columns.

In cells expressing the hP2Y4 receptor, UTP, guanosine 5'-triphosphate (GTP) and inosine 5'-triphosphate (ITP) all evoked a rapid, reversible rise in intracellular [Ca^{2+}] with similar maximum responses and EC50 values of 489 nM, 6.8 µM and 7.6 µM respectively. ATP, cytidine 5'-triphosphate (CTP) and xanthine 5'-triphosphate (XTP) were all ineffective at 100 µM. Coapplication of ATP (10 µM) shifted the concentration-response curve for UTP ~12-fold to the right. In contrast, all nucleotides were agonists in rP2Y4 receptor-expressing cells; EC50 = UTP, 100 nM; ATP, 300 nM; ITP, 1.9 µM; GTP, 2.6 µM; CTP, 6.6 µM and XTP, 28.9 µM.

In cells expressing hP2Y4 receptors, ATP evoked IP synthesis with lower potency (EC50 = 288 nM; 95% cl = 214-389 nM) and efficacy (max = 52±4%) than UTP (EC50 = 35 nM; 95% cl = 23-54 nM). In cells expressing rP2Y4 receptors ATP also raised IP levels (EC50 = 219 nM; 95% cl =155-319 nM) less potently than UTP (EC50 = 14 nM; 95% cl = 10-19 nM), but with a similar maximum effect. UDPglucose pyrophosphorylase removes the γ-phosphate group from UTP, but not ATP in the presence of glucose-1-phosphate. When included in the bathing media (10 µg/ml), it inhibited responses to both UTP and ATP at hP2Y4 receptors, but only those to UTP at rP2Y4 receptors.

Thus, ATP is a weak antagonist at hP2Y4 receptors, but a potent agonist at rP2Y4 receptors. The apparent agonism by ATP at the hP2Y4 receptor observed in measurements of IPs, depends upon production of UTP in the bathing media.

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22P DIFFERENTIAL COUPLING OF THE HUMAN P2Y11 RECEPTOR TO PHOSPHOLIPASE C AND ADENYLYL CYCLASE

C. Kennedy, A. Qi[1], R.A. Nicholas[1] & T.K. Harden[1], Dept. of Physiology and Pharmacology, University of Strathclyde, Glasgow G4 0NR, [1]Dept. of Pharmacology, University of North Carolina at Chapel Hill, NC27599.

Five human P2Y (hP2Y) receptors have been cloned to date and all are linked to activation of phospholipase C, generation of inositol phosphates (IP) and release of Ca^{2+} stores. The hP2Y11 receptor is unusual as it is also reported to be linked to activation of adenylyl cyclase over the same concentration range of adenosine 5'-triphosphate (ATP) (EC50 ≈ 40 µM for both actions, Communi *et al.*, 1997). Here, we have further characterised the interaction of the hP2Y11 receptor with these two second messenger pathways.

The hP2Y11 receptor was amplified by PCR from genomic DNA and stably expressed in 1321N1 cells using a retroviral expression system as described previously (Nicholas *et al.*, 1996). To measure IP production, cells were incubated overnight with 0.4µCi of *myo*-[^{3}H]-inositol. Agonists were then added along with 10 mM LiCl for 5 min at 37°C. The reactions were stopped by aspirating the media and adding 0.5 ml boiling 10 mM EDTA, pH 8.0. [^{3}H]-IPs were isolated using Dowex AG1-X8 anion-exchange columns. To monitor cAMP, cells were incubated for 2 hr with 2 µCi of [^{3}H]-adenine. Agonists were added in the presence of IBMX (200 µM) for 5 min. The reactions were terminated by aspirating the media and adding 1 ml ice cold trichloroacetic acid. [^{3}H]-cAMP was isolated using Dowex and alumina columns.

In cells expressing the hP2Y11 receptor, ATP evoked IP hydrolysis (EC50 = 2.3 µM; 95% cl = 1.0-5.0 µM). However, ATP increased cAMP levels only at 30 µM and above and a clear maximum to its concentration-response curve was not reached. Indomethacin (10 µM) had no effect on the increase in cAMP levels. Adenosine 5'-diphosphate (100 µM) increased IP levels by 60% of the maximum response to ATP, but did not change cAMP levels. Uridine 5'-triphosphate, uridine 5'-diphosphate, adenosine 5'-monophosphate and adenosine (all 100 µM) were inactive and did not inhibit responses to ATP.

This differential coupling of hP2Y11 receptors to phospholipase C and adenylyl cyclase differs from the data of Communi *et al.*, (1997). Several possible explanations were examined. The ability of ATP to induce IP hydrolysis was unaffected by including IBMX (200 µM) in the bathing media. Likewise, the rise in cAMP levels was similar when Ro20-1724 (300 µM) rather than IBMX was used to inhibit phosphodiesterase. When the hP2Y11 receptor was expressed in CHO-K1 cells, ATP again evoked IP synthesis, with similar potency to that seen in 1321N1 cells (EC50 = 5.2 µM; 95% cl = 2.9-9.5 µM). However, the potency of ATP to evoke cAMP synthesis was greatly increased (EC50 = 35.4 µM; 95% cl = 17.4-72.4 µM).

These studies confirm that ATP acts at the hP2Y11 receptor to increase IP levels. However, ATP is less potent in increasing cAMP synthesis, and this latter effect is dependent upon the cell type in which the hP2Y11 receptor is expressed.

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23P P2X RECEPTORS ON MOUSE AND GUINEA-PIG PELVIC GANGLION NEURONS EXHIBIT DIFFERENT Zn^{2+} AND pH SENSITIVITIES

Y. Zhong, P.M. Dunn & G. Burnstock. Autonomic Neuroscience Institute, Department of Anatomy and Developmental Biology, Royal Free and University College Medical School, Rowland Hill Street, London, NW3 2PF, UK

Adenosine 5'-triphosphate (ATP) acts as a fast excitatory neurotransmitter in the central and peripheral nervous system, where it activates a class of ligand gated ion channels, the P2X receptors (Burnstock, 1997). To date, seven P2X receptor subunits have been cloned, with different though overlapping pharmacological properties. The P2X receptors on rat pelvic ganglion neurons are of the $P2X_2$ subtype (Zhong *et al.*, 1998). The potency of ATP on these neurons is increased by both Zn^{2+} and H^+. In this study, we compared the effects of Zn^{2+} and pH on ATP responses on the pelvic ganglion neurons of mouse and guinea-pig.

Single neurons of the pelvic ganglia from adult mice and guinea-pigs were enzymatically isolated and maintained in tissue culture for up to 48 hrs. Whole cell voltage-clamp recording was carried out at a holding membrane potential of -70mV. All results are expressed as mean ± s.e.mean.

Rapid application of ATP 100μM on to isolated pelvic ganglion neurons of mouse and guinea-pig induced inward currents, which desensitised slowly with time constants of 21.0±3.1s (n=4) and 12.2±0.7s (n=6), respectively. The dose-response relationships of ATP on mouse and guinea-pig pelvic ganglion neurons yielded EC_{50} values of 150μM ($logEC_{50}$=-3.82±0.08, data from 15 cells), and 35μM ($logEC_{50}$=-4.46±0.09, data from 29 cells), respectively.

On mouse pelvic ganglion neurons, co-application of Zn^{2+} 10μM greatly enhanced the responses to ATP 100μM (to 217±14% of control, n=5), and decreased the EC_{50} of ATP to 42μM (log EC_{50}=-4.38±0.07, n=5). In contrast, on guinea-pig pelvic ganglion neurons, co-application of Zn^{2+} 100μM inhibited responses to ATP 30μM (to 65±0.1% of control, n=7). This inhibition appeared to be non-competitive.

On mouse pelvic ganglion neurons, lowering the pH from 7.4 to 6.8 markedly potentiated of the responses to 100μM ATP (to 201±8.7% of control, n=7), and shifted the ATP dose-response curve to the left by 0.41±0.03 log units (n=6). Raising the pH to 8.0 attenuated the responses to ATP 100μM (to 25±2.4% of control, n=7), and shifted the ATP dose-response curve to the right by 0.54±0.06 log units (n=6). On guinea-pig pelvic ganglion neurons, however, lowering the pH from 7.4 to 6.8 produced only a small enhancement of the responses to 30μM ATP (to 119±5.5% of control, n=5), and the ATP dose-response curve was only shifted to the left by 0.13±0.04 log units (n=6). Raising the pH to 8.0 produced a slight attenuation of the responses to ATP 30μM (to 81±3.5% of control, n=16), and the ATP dose-response curve was shifted to the right by 0.14±0.04 log units (n=11).

To conclude, while the pharmacology of the P2X receptors on mouse pelvic ganglion neurons is very similar to that of the rat, the receptors on guinea-pig pelvic ganglion neurons are distinct, being inhibited by Zn^{2+} and are much less sensitive to changes in extracellular pH.

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24P DIADENOSINE POLYPHOSPHATES AS PHARMACOLOGICAL TOOLS TO IDENTIFY $P2X_{1,2,3,4}$ SUBUNITS

S.G. Brown, S.S. Wildman, B.F. King & G. Burnstock, Autonomic Neuroscience Institute, Royal Free Hospital Medical School, Hampstead, London NW3 2PF.

Diadenosine polyphosphates (Ap_nA, n=2-6) are naturally-occurring adenine dinucleotides, in which two adenine molecules are linked at the 5′ position of the ribose moiety by a chain of phosphates varying from 2 to 6 in length. We have studied the pharmacological activity of these naturally-occurring dinucleotides at rat $P2X_1$, $P2X_3$ and $P2X_4$ receptors with a view to finding selective agonists for these P2X subunits.

Under voltage-clamp conditions (V_h= -60mV to -90mV), agonist-evoked responses (inward cationic currents) were recorded from defolliculated *Xenopus* oocytes expressing either $rP2X_1$, $rP2X_3$ or $rP2X_4$ receptors. At $rP2X_1$ receptors, only Ap_6A was a full agonist (EC_{50}, 720±80nM, n=4) (*mean± s.e.mean*), although 2-fold less potent than ATP (EC_{50}, 300±10nM, n=4). On the other hand, Ap_4A (EC_{50}, 38±11nM, n=4) was 8-fold more potent than ATP but a partial agonist (40% of ATP maximum). Ap_5A (EC_{50}, 900±100nM, n=4) also was a partial agonist (45% of ATP maximum) as was Ap_3A (EC_{50}, >100μM; 15% of ATP maximum). Ap_2A was inactive as an agonist and modulator of ATP-responses at $rP2X_1$.

At $rP2X_3$ receptors, three dinucleotides were full agonists: Ap_4A (EC_{50}, 803±119nM, n=4), Ap_5A (EC_{50}, 1.3±0.3μM, n=4), Ap_6A (1.6±0.4μM, n=4). Each compound was as potent as ATP (EC_{50}, 1.8±0.3μM, n=4) or more so. Ap_2A (100μM) acted solely as a potentiator (175±26%) of control responses (taken as 100%) to submaximal ATP (0.3μM, approx. EC_{25}). The EC_{50} value for potentiation of ATP-responses was 8.3±0.7μM (n=4). This potentiating effect was reversed on washout.

At $rP2X_4$ receptors, Ap_4A (EC_{50}, 3.0±0.4μM, n=4) was as potent as ATP (EC_{50}, 4.1±1.0μM, n=4), although a partial agonist (30% of ATP maximum). Ap_6A (1-300μM) was a weak agonist (EC_{50}, >100μM), evoking responses 10% of the ATP maximum. Ap_2A, Ap_3A and Ap_5A (0.1-300μM) were inactive as agonists. However, Ap_2A (EC_{50}, 1.6±0.8μM, n=4) and Ap_3A (EC_{50}, 926±116nM, n=4) potentiated ATP-responses (to 3μM) by 146±7% and 154±13%, respectively. This potentiating effect was reversed on washout.

The above data for $rP2X_1$, $rP2X_3$ and $rP2X_4$ receptors contrast with like data for $rP2X_2$ receptors (Pintor *et al.* 1996), where only Ap_4A was full agonist (EC_{50}, 15.2±1μM, n=4) yet 4-fold less potent than ATP (EC_{50}, 3.7±0.7mM, n=4), Ap_5A (EC_{50}, 2.9±0.7nM, n=4) was a potentiator of ATP-responses and other dinucleotides were inactive. A picture is emerging that there is selectivity in the actions of diadenosine polyphosphates at the above recombinant P2X receptors. These compounds might prove to be useful tools in identifying specific P2X subunits in endogenous P2X receptor in whole tissues when, currently, there is a lack of selective agonists and antagonists for ATP-gated ion-channels.

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25P CHARACTERISATION OF THE CLONED FISH SOMATOSTATIN RECEPTOR sst_3, A MEMBER OF THE $SRIF_1$-RECEPTOR FAMILY: ATYPICAL PHARMACOLOGICAL FEATURES

S.S. Siehler, G.K.H. Zupanc (1), K. Seuwen & D. Hoyer
Preclinical Research, Novartis Pharma AG, S-386/745, 4002 Basel, Switzerland and (1) School of Biological Sciences, University of Manchester, Manchester M13 9PT,UK

A somatostatin receptor cloned from the teleostean fish *Apteronotus albifrons* was characterised pharmacologically in CCL39 Chinese hamster lung fibroblast cells. Due to sequence homology with the human sst_3 receptor, the fish receptor was called sst_3 receptor. Fish sst_3 receptors expressed in CCL39 cells bound [Leu^8, D-Trp^{22}, ^{125}I -Tyr^{25}]$SRIF_{28}$ ([^{125}I]LTT-$SRIF_{28}$) with high affinity (pK_d = 10.47 ± 0.12; B_{max}= 4470 ± 240 fmol/mg protein). The pharmacological profile of fish sst_3 binding sites was established in radioligand competition studies using established SRIF analogues (for abbreviations see Hoyer et al., 1994): (pK_d-values) seglitide (9.15 ± 0.08), $SRIF_{25}$ (9.00 ± 0.07), $SRIF_{14}$ (8.93 ± 0.19), $SRIF_{28}$ (8.84 ± 0.15), cortistatin 14 (8.82 ± 0.10) and BIM 23014 (8.06 ± 0.08) bound with high affinity, while the fish-specific $SRIF_{22}$ peptide revealed only low affinity (5.33 ± 0.08). The pharmacological profile of [^{125}I]LTT-$SRIF_{28}$- binding to fish sst_3 receptors is typical for the $SRIF_1$ receptor family and showed a high correlation with the human sst_5 receptor profile (correlation coefficient r = 0.920) > sst_2 (r = 0.832) > sst_3 (r = 0.689), and no correlation with sst_1 and sst_4 receptors expressed in CCL39 cells.
Forskolin-stimulated adenylate cyclase activity in CCL39 cells expressing fish sst_3 receptors was inhibited by $SRIF_{14}$ (pEC_{50} = 9.52 ± 0.01) and $SRIF_{28}$ (pEC_{50} = 9.19 ± 0.01); by 87% and 91%, respectively. Inhibition of adenylate cyclase activity was blocked by pertussis toxin, compatible with coupling to $G_{i\alpha}$ and/or $G_{o\alpha}$ in CCL39 cells. This is similar to mammalian $sst_{1\text{-}5}$ receptors (Hoyer et al, 1995), and therefore might be conserved within the vertebrate phylum.
[^{125}I]LTT-$SRIF_{28}$ binding in different tissues of *Apteronotus albifrons* suggests SRIF receptor sites to be present in brain, liver, heart, spleen and stomach, but not in gut. RT-PCR revealed low expression of the fish sst_3 receptor in brain, but not in other tissues. The pharmacological profile of fish sst_3 receptors, correlated well with brain (r = 0.942), but less with liver [^{125}I]LTT-$SRIF_{28}$ binding (r = 0.826).
A comparison of [^{125}I]LTT-$SRIF_{28}$, [^{125}I]Tyr^{10}-cortistatin (Siehler et al, 1998), [^{125}I]CGP 23996 and [^{125}I]Tyr^3-octreotide binding to $fsst_3$ receptors showed high affinity binding (pK_d values: 10.47, 10.87, 9.59 and 9.57) but revealed different Bmax values: 4500, 4000, 3400 and 1500 fmoles/mg, respectively. In addition, although pharmacological profiles defined by these radioligands were similar, up to 300 fold differences in apparent affinity were observed for several SRIF analogues depending on the radioligand used. The data suggest that the different radioligands used label and/or induce different conformational states of the $fsst_3$ receptor.

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26P SYSTEM- AND AGONIST-DEPENDENT LIGAND-RECEPTOR INTERACTIONS AT HUMAN SOMATOSTATIN $hsst_5$ RECEPTORS: RADIOLIGAND AND [^{35}S]GTPγS BINDING AND ADENYLATE CYCLASE INHIBITION STUDIES

S.S. Siehler, K. Seuwen & D. Hoyer Preclinical Research, Novartis Pharma AG, S-386/745, 4002 Basel, Switzerland

Agonist radioligand binding, [^{35}S]GTPγS binding and inhibition of forskolin-stimulated adenylate cyclase activity were investigated at human somatostatin $hsst_5$ receptors stably transfected into CCL39 Chinese hamster lung fibroblast cells. [^{125}I]LTT-$SRIF_{28}$([Leu^8, D-Trp^{22}, ^{125}I-Tyr^{25}]$SRIF_{28}$),[^{125}I][Tyr^{10}]CST_{14} ([^{125}I][Tyr^{10}]cortistatin$_{14}$, Sihler et al, 1998) and [^{125}I][Tyr^3]octreotide bound with high affinity to $hsst_5$ receptors (pK_d = 10.48, 10.33 and 9.64, respectively), but labelled different receptor densities (B_{max} = 6950, 5630 and 920 fmol/mg, respectively).
The non-hydrolysable GTP-analogue GppNHp (guanylylimido-diphosphate) inhibited binding of [^{125}I][Tyr^3]octreotide at $hsst_5$ receptors by 88 % (pEC_{50} = 7.77), whereas binding of [^{125}I]LTT-$SRIF_{28}$ and[^{125}I][Tyr^{10}]CST_{14} was only reduced by 47 % and 31 %, respectively (pEC_{50} = 5.87 and 4.81).
Depending on the radioligand used, agonist affinities varied significantly at $hsst_5$ receptors. Affinity profiles of SRIF ligands (abbreviations: see Hoyer et al., 1994) at $hsst_5$ receptors were: (a) [^{125}I]LTT-$SRIF_{28}$ and [^{125}I][Tyr^{10}]CST_{14}: $SRIF_{14}$ (pK_d = 9.53, 9.01) ≈ $SRIF_{28}$ (9.39, 9.18) > seglitide (8.70, 9.14) > octreotide (7.17, 7.31) > cyclo SA (6.38, 6.02); (b) [^{125}I][Tyr^3]octreotide: $SRIF_{28}$ (10.30) ≈ seglitide (10.18) > $SRIF_{14}$ (9.87) > octreotide (9.48) > cyclo SA (8.25).
G-protein activation via $hsst_5$ receptors was studied in [^{35}S]GTPγS binding experiments and correlated significantly, although to various degrees with the affinity profiles determined using [^{125}I]LTT-$SRIF_{28}$, [^{125}I][Tyr^{10}]CST_{14} and [^{125}I][Tyr^3] octreotide: correlation coefficient r = 0.874, 0.882, and 0.814, respectively. pEC_{50}-values of SRIF agonists to stimulate [^{35}S]GTPγS binding were lower than affinities determined in radioligand binding and displayed a different rank order of potency: $SRIF_{14}$ (pEC_{50} = 8.39) > seglitide (7.89) > $SRIF_{28}$ (7.65) > octreotide (6.89) > cyclo SA (5.82).
The inhibition of forskolin-stimulated adenylate cyclase (FSAC) activity mediated by $hsst_5$ receptors correlated significantly although moderately, with affinity profiles defined with [^{125}I][Tyr^{10}]CST_{14}, [^{125}I][Tyr^3]octreotide and [^{125}I]LTT-$SRIF_{28}$ (r = 0.861, 0.867 and 0.768, respectively). The rank order of agonists inhibiting FSAC activity was comparable to that of [^{35}S]GTPγS binding, but with higher potencies: seglitide (pEC_{50} = 9.33) > $SRIF_{28}$ (8.38) > octreotide (7.68) > cyclo SA (5.69).
Taken together, the results suggest that: (1) multiple agonist-receptor conformations can be achieved at $hsst_5$ receptors, (2) the nature of agonist-induced receptor/G-protein/effector interactions might be more complex than initially suggested by the ternary complex model, and (3) different rank orders of apparent potency can be obtained at recombinant receptors depending on the nature of the agonist and the type of ligand/receptor interaction studied: radioligand binding, [^{35}S]GTPγS binding or adenylate cyclase activity.

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27P INCREASE IN PROTEASOME ACTIVITY 6 h AFTER A SINGLE BOLUS ADMINISTRATION OF THE β_2-ADRENOCEPTOR AGONIST CLENBUTEROL IN CONSCIOUS RATS

P. Rajab, D. Constantin-Teodosiu, R. J. Mayer, S M. Gardiner, T. Bennett & P. L. Greenhaff. School of Biomedical Sciences, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

It has previously been demonstrated that protein catabolism in skeletal muscle occurs through a mechanism that is both ATP- and ubiquitin- dependent and involves an enzyme complex known as the 26S proteasome (Fagan *et al.*, 1987). 26S proteasome activation has been shown to occur in advance of protein degradation (Dawson *et al.*, 1995). Previously we have shown that 4 days treatment with clenbuterol (250 μg $kg^{-1}day^{-1}$, s.c.) in rats induces an increase in proteasome activity and an increase in the expression of the MSS1, TBP1 and p42 ATPase subunits in soleus, a slow contracting muscle. There were no effects on the fast contracting muscle extensor digitorum longus (EDL) or the heart (Rajab *et al.*, 1998). The aims of the present study were to investigate (a) if the proteasome pathway is sensitive to administration of a single dose of clenbuterol and (b) if so, whether or not the response is accompanied by a change in proteasome ATPase subunit expression.

Under anaesthesia (sodium methohexitone, 40-60 mg kg^{-1} i.p., supplemented) male Sprague-Dawley rats (330-371g) had s.c. and i.v. catheters implanted. Twenty four h after surgery a single dose of clenbuterol (250 μg kg^{-1}; n=6), or saline (0.1 ml; n=7), was administered s.c. Six h later, the animals were terminally anaesthetised (sodium methohexitone, 20-30 mg kg^{-1} i.v.) and the soleus muscle removed and snap frozen in liquid nitrogen. Frozen muscle samples (~30-40mg) were homogenised in 20 volumes of ice-cold homogenising buffer (20mM TRIS-HCl pH 7.5, 2mM ATP, 5mM $MgCl_2$, 1mM DTT). The homogenate was centrifuged at 8,000 x *g* for 20 min at 4°C, and the supernatant was collected (soluble muscle extract). The chymotrypsin-like activity of the 20S and 26S proteasomes (Orlowski *et al.*, 1990) was measured using the fluorogenic substrate, Suc-LLVY-MCA (Sigma, UK; see Dawson *et al.*, 1995). Specific activity in a sample was obtained by correcting the chymotrypsin-like activity for protein concentration measured according to the method of Bradford (1976). Data below are expressed as mean ± s.e.mean. Comparison of differences in proteasome activity between the groups was by unpaired Student's t-test. A P-value of 0.05 was taken as significant. Individual muscle extracts were also incubated for 20 min at 37°C with the proteasomal inhibitor, lactacystin (1 μm; Calbiochem-Novabiochem Corporation, U.K.), prior to assaying for chymotrypsin-like activity. Changes in proteasomal ATPase subunit (MSS1, TBP1 and p42) expression were investigated by densitometry on representative Western blots (Dawson *et al.*, 1995).

Following clenbuterol, there was a 34% ($P<0.01$) difference in proteasome activity between the two groups (controls, 264±19; treatment, 355±25 fluorescence units (hr^{-1} μg $prot^{-1}$)). However, there were no changes in the content of MSS1, TBP1 or p42 ATPase subunits. These results demonstrate that a single bolus s.c. administration of clenbuterol in rats increases proteasome activity but, in contrast to 4 days treatment (Rajab *et al.*, 1998), does not affect ATPase subunit expression.

P. Rajab held an MRC studentship during this work.

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28P EFFECTS OF PYRETHROIDS ON VOLUME-SENSITIVE CHLORIDE CHANNELS

Culliford, S.J. and Kozlowski, R.Z
University Department of Pharmacology, Oxford University, Mansfield Rd, Oxford OX1 3QT.

Pyrethroids are well-established insecticides with Na^+ channel blockade being their primary mode of action (Narashi 1996). There are two classes of pyrethroids. Type I possess a cyano group in the α-position while in type II this group is lacking (Narashi, 1996). Type II pyrethroids have also been shown to inhibit GABA-induced Cl currents in cultured hippocampal neurones (Frey *et al* 1989) and voltage-gated Cl^- channels in mammalian neuroblastoma cells (Forshaw *et al* 1993). However, the effects of pyrethroids on other classes of Cl^- channel have not been examined. Volume-activated Cl^- channels are permeable to the organic osmolyte taurine (Strange *et al* 1996) and are ubiquitously distributed in mammalian tissue. We therefore felt it important to determine whether pyrethroid insecticides can affect such channels in mammalian cells. Consequently, we examined the effects of three type I (cypermethrin, fenpropathrin and cyfluthrin) and four type II (bioallethrin, bifenthrin, tetramethrin and tefluthrin) pyrethroids on volume-sensitive Cl^- channel activity in an epithelial (HeLa) and neuronal (C6 glioma) cell-line. ^{14}C-taurine efflux was used to assay channel activity as previously described (Hall *et al* 1996).

In HeLa cells exposure to a hypotonic medium (KCl (95 mM), $CaCl_2$ (1.3.mM), $MgCl_2$ (0.5 mM), HEPES (10 mM), pH 7.4 with NaOH) significantly ($p<0.001$; Student *t* test; throughout) increased the rate constant for ^{14}C-taurine efflux from 0.026 ± 0.011 min^{-1} to 0.306 ± 0.028 min^{-1} (mean ± SEM, throughout; n=6) compared with efflux in an isotonic medium (150 mM KCl). In C6 glioma the rate constant of ^{14}C-taurine increased from 0.027 ± 0.004 min^{-1} to 0.157 ± 0.019 min^{-1} (n=6; $p<0.001$) in hypotonic conditions. In HeLa cells only the type II pyrethroid, tetramethrin, showed any inhibitory activity at 100μM, reducing volume-sensitive taurine efflux by 53.9 ± 2.2 % (n=3; $p<0.001$). In contrast in C6 glioma cells both types or pyrethroid inhibited volume-sensitive taurine efflux. The type I pyrethroid fenpropathrin (100μM) inhibited swelling-activated taurine efflux by 54.1 ± 6.3 % (n=3; $p<0.005$), while the type II pyrethroids tetramethrin and bioallethrin inhibited efflux of the osmolyte by 75.9 ± 2.3 % (n=3; $p<0.001$) and 51.0 ± 14.1 % (n=3; $p<0.05$), respectively at 100μM.

The results indicate that type I and type II pyrethroids inhibit volume-sensitive Cl^- channels and highlight a novel site of action for these compounds. Furthermore, it appears that these compounds exhibit differential effects in different mammalian cell types. The consequences of such effects *in vivo*, however, remain to be elucidated.

R Z Kozlowski is a British Heart Foundation Lecturer.

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29P MORPHINE ATTENUATES THE RESPONSE TO CARDIOPULMONARY RECEPTOR STIMULATION IN THE ANAESTHETIZED RAT

M. Ohnishi, *H.W. Marshall, E. Kirkman, & *R.A. Little, Department of Biological Sciences, Science Laboratories, University of Durham, Durham DH1 3LE & *NWIRC, University of Manchester, Manchester M13 9PT

Right atrial injection of the $5HT_3$ receptor agonist phenylbiguanide (PBG) activaties pulmonary and cardiac afferent C-fibres leading to bradycardia, hypotension and apnoea (Daly et al. 1988). The afferent pathways of these reflexes are carried in the vagus, as are the efferent pathways mediating the bradycardia (Daly et al. 1988). The cardio-respiratory response to activation of the cardiopulmonary afferent C-fibres is remarkably similar to that elicited by primary thoracic blast injury (Ohnishi et al. 1998), casualties of which may be treated with morphine. It is known that morphine can attenuate another vagal reflex, the response to severe haemorrhage (Ohnishi et al. 1997), via an action in the central nervous system. The aim of the current study was to determine the effect of morphine on the bradyacrdia and hypotension induced by PBG.

The study was conducted on male Wistar rats (240-252 g body weight). Stainless steel guide cannulae were implanted into the lateral cerebral ventricle (icv) under pentobarbitone anaesthesia (60 mg. kg^{-1} ip) 7-15 days before the study. On the day of the study surgical anaesthesia was induced and maintained with isoflurane (3.0-3.5% in O_2/N_2O, FIO_2=0.5). On completion of surgery anaesthesia was maintained with alphadolone/ alphaxalone (19-21 $mg.kg^{-1}.h^{-1}$ iv). Injections of PBG was made via a cannula inserted into the right jugular vein and advanced so that its tip lay close to the right atrium (iv). Heart period (HP) was measured from the electrocardiogram, arterial blood pressure via the ventral tail artery and body temperature via a rectal probe. Body temperature was maintained at 38.0°C using external heating. At the end of the study the animals were killed with an overdose of anaesthetic.

A submaximal cardiovascular response to PBG was determined in each rat by injecting PBG within the range 1-32 μg iv. Once the submaximal dose had been established in each rat this was used for the remainder of the study. The range of submaximal doses was 2-16 μg PBG. The animals were allocated randomly to two groups. Group I (n=9) received 0.9% saline (20 μl icv) while Group II (n=9) received morphine (10 μg in 20 μl saline icv). 5 min after the respective icv injection, the response to PBG was determined again. In Group I, before saline icv, PBG produced a significant ($P<0.05$, 2 way ANOVA) increase in HP from 146±4 to 484±31 ms, and a significant fall in mean arterial blood pressure (MAP) from 101±3 to 60±2 mmHg (mean±s.e.m). The response to PBG was not significantly different after saline (icv). In Group II PBG initially produced a significant increase in HP from 144±3 to 435±22 ms and a fall in MAP from 105±3 to 64±3 mmHg. Morphine significantly attenuated the PBG-induced bradycardia in Group II, compared with the saline-treated Group I. In Group II after morphine, PBG increased HP from 156±4 ms to 220±13 ms. The PBG-induced fall in blood pressure was not significantly different to that seen at the same time point in Group I.

These results indicate that morphine, administered centrally, can attenuate the bradycardia but not the hypotension induced by PBG.

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30P EFFECTS OF MORPHINE ON THE RESPONSE TO PRIMARY THORACIC BLAST INJURY IN THE ANAESTHETIZED RAT

E. Kirkman, M. Ohnishi, & *P. Watkins, Department of Biological Sciences, Science Laboratories, University of Durham, Durham DH1 3LE & *DERA, Medical Countermeasures, CBD, Porton Down, Salisbury, SP4 0JQ

Primary blast injury to the thorax produces a bradycardia, hypotension and apnoea (Krohn *et al.* 1942). This response is a reflex with vagal afferent and efferent pathways (Ohnishi *et al.*, 1998). Blast casualties may be given morphine as part of their clinical treatment. Morphine modifies other vagal reflexes, e.g. the response to haemorrhage (Ohnishi *et al.*, 1997) and that to pharmacological activation of the pulmonary/cardiac afferent C-fibres (Ohnishi *et al.*, this meeting). This study determined whether morphine could modify the response to thoracic blast.

Male Wistar rats (244-268 g body weight) were used in four groups. Surgical anaesthesia was induced and maintained with isoflurane (3.0-3.5% in O_2/N_2O, FIO_2=0.5). On completion of surgery anaesthesia was maintained with alphadolone/ alphaxalone (19-21 $mg.kg^{-1}.h^{-1}$ iv). Heart period (HP) was measured from the electrocardiogram, arterial blood pressure via the ventral tail artery and body temperature via a rectal probe. Body temperature was maintained at 38.0°C using external heating. At the end of the study the animals were killed with an overdose of anaesthetic.

Group I (n=8) and IV (n=8) received 0.9% saline (1 $ml.kg^{-1}$ iv) while Group II (n=7) and III (n=5) received morphine (0.5 $mg.kg^{-1}$ iv). 10-15 min later baseline measurements were made and the animals subjected to a blast wave focused on the ventral thorax (Group I and II, Tatic *et al.*, 1996), or the sound of blast (Group III and IV, sham blast). In Group I HP increased significantly from 135±2 ms at baseline to 523±79 ms (mean±s.e.m) after blast ($P<0.05$, repeated measures ANOVA, latency 3.4±0.6 sec), and was still elevated 30 min after blast when HP was 159±4 ms. Mean arterial blood pressure (MBP) fell significantly from 122±3 mmHg at baseline to 35±4 mmHg after blast (latency 2.3±0.1 sec) and 104±3 mmHg 30 min later. There was apnoea with a mean duration of 24±2 sec following blast. In Group II blast produced a significant increase in HP above baseline (HP 153±8, 374±72 and 171±10 ms at baseline, after blast and 30 min later, respectively). This was not significantly different to that seen in Group I. MBP fell significantly in Group II from a baseline of 106±4 mmHg to 29±3 mmHg after blast and 96±4 mmHg 30 min later. There were no significant differences in the nadir of blood pressure seen immediately after blast in Groups I and II but subsequent recovery was slower in Group II. Morphine significantly prolonged the apnoea after blast (68±17 s in Group II *vs* 24±2 s in Group I; Mann Whitney U test). Sham blast did not produce any cardiovascular effects or apnoea in Groups III and IV.

These results indicate that morphine does not significantly attenuate the bradycardia associated with thoracic blast, unlike its effects on the bradycardia associated with haemorrhage and stimulation of pulmonary/cardiac afferent C-fibres. Morphine does, however, augment the apnoea associated with blast and delay the recovery of blood pressure.

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31P PRETREATMENT OF PORCINE VEIN GRAFTS WITH THAPSIGARGIN REDUCES NEOINTIMAL THICKNESS

J.Y. Jeremy, C.T. Lloyd, D. Mehta, J.L. Johnson, S.J. George, S. Birkett, P. Gadsdon and G.D. Angelini, Bristol Heart Institute, University of Bristol, UK.

Neointima (NI) formation is a principal cause of vein graft failure following coronary artery bypass grafting (CABG) (Jeremy *et al.*, 1997). NI formation involves the proliferation and migration of vascular smooth muscle cells (VSMCs) of medial origin, to the intimal surface where they continue to proliferate and secrete matrix proteins (Jeremy *et al.*, 1997).We have previously shown that short term exposure to thapsigargin (TG), through depletion of intracellular calcium pools, inhibits both the migration and proliferation of VSMCs and NI formation in human saphenous vein *in vitro* (George *et al.*, 1997; Birkett *et al.*, 1997; Shukla *et al.*, 1997). We therefore proposed that pre-exposure of saphenous veins to TG may constitute a therapeutic approach to the treatment of vein graft failure. In order to explore this proposal, we investigated the effect of pre-exposure of saphenous veins to TG on NI formation *in vivo* using a pig model of bypass vein graft surgery (Mehta *et al.*, 1998).

Landrace pigs (25 kg) were anaesthetised and 2 cm lengths of saphenous veins excised and incubated in isotonic phosphate buffered saline (PBS; pH, 7.4) containing 10 nM or 100 nM TG for one hour at 37°C. Controls comprised of veins incubated in PBS alone. Sections (2 cm) of the carotid artery were excised and the vein then anastomosed (end to end) into the carotid artery (Mehta *et al.*, 1998). One month after surgery vein grafts were fixed *in situ* with 10 % formalin in PBS at 100 mm Hg and excised. Sections were prepared from vein grafts using a microtome and stained with haematoxylin & eosin. Medial and intimal thickness and luminal area was then assessed by computer assisted planimetry (Mehta *et al.*, 1998).

Neointimal thickness was significantly diminished in porcine vein grafts treated with TG compared to untreated grafts (table 1). Medial thickness was unaltered and luminal area increased in TG-treated grafts compared to untreated grafts (table 1). Histology also demonstrated that VSMCs were viable, indicating that the inhibitory effect of TG is not mediated through cytotoxicity.

	untreated graft	+10 nM TG	+100 nM TG
medial thickness (mm)	0.50 ± 0.12	0.42 ± 0.10	0.38 ± 0.08
intimal thickness (mm)	0.40 ± 0.11	0.26 ± 0.05*	0.12 ± 0.03*
luminal area (mm^2)	8.60 ± 1.2	10.6 ± 1.60	13.6 ± 1.04*

Table 1. Effect of pretreatment with TG on medial and neointimal thickness and luminal area in porcine saphenous vein grafts one month after surgery. (Mean ± S.E.M; n = 5). * $p < 0.01$ compared to untreated grafts (ANOVA).

These data demonstrate that pre-exposure of saphenous veins to TG reduces NI formation and thickening in vein grafts, *in vivo*. The lack of effect of TG on medial thickening may be of importance to the adaptation of vein grafts to arterial conditions. These data consolidate that TG exerts a long term effect on VSMC proliferation and migration, key events in NI formation (Jeremy *et al.*, 1997). The study also demonstrates that intracellular calcium pools in VSMC constitute possible targets for the therapeutic intervention of vein graft failure.

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32P NIFEDIPINE ATTENUATES NEUROPEPTIDE Y EFFECTS *IN VIVO*

A. Bischoff & M. C. Michel. Dept of Medicine, University of Essen, 45122 Essen, Germany

Organic Ca^{2+} entry blockers can inhibit numerous cardiovascular responses to neuropeptide Y (NPY) *in vitro*, but little is known about their inhibition of NPY effects *in vivo*. Moreover, it is unclear whether they also inhibit responses occuring via more recently discovered subtypes such as the Y_5 receptor. Therefore, we have determined how nifedipine alters the cardiovascular and renal responses to systemic NPY administration in two studies.

In the first study male thiobarbital-anaesthetized Wistar rats (260-370 g) were systemically infused with vehicle or 1.7 μg kg^{-1} min^{-1} nifedipine. NPY was given by consecutive intravenous bolus injections of 0.3-30 μg kg^{-1} every 30 min. Mean arterial pressure (MAP) was measured via a catheter in the femoral artery connected to a Statham transducer, renal blood flow (RBF) was determined by an electromagnetic flow sensor (Skalar) placed on the renal artery. Maximal changes of MAP and RBF occur within 30 s after NPY bolus injection and are given as mean ± s. e. mean of 7-8 animals. Statistical significance was determined by two-tailed t-tests with $P < 0.05$ considered significant. Nifedipine treatment lowered basal MAP from 101± 4 to 88 ± 5 mm Hg ($P < 0.05$) but did not alter basal RBF (6.5 ± 0.5 and 6.6 ± 0.6 ml min^{-1}). NPY bolus injections dose-dependently elevated MAP and decreased RBF, and nifedipine infusion attenuated these effects. For example the MAP elevation by 10 μg kg^{-1} NPY was reduced from 26 ± 5 to 7 ± 3 mm Hg and the RBF reduction from 4.3 ± 0.3 to 3.4 ± 0.3 ml min^{-1} (both $P < 0.05$).

In the second study animals were prepared as previously described (Bischoff *et al.* 1996): Briefly, renal perfusion pressure was fixed at 100 mm Hg by an adjustable clamp on the abdominal aorta of unilaterally nephrectomized, thiobarbital-anaesthetized rats (n = 9-10). NPY (2 μg kg^{-1} min^{-1}) or vehicle concomitant with nifedipine (1.7 μg kg^{-1} min^{-1} starting 1 h before NPY) or its vehicle were infused for 1 h during which urine and sodium excretion were determined in 15 min collection periods via a catheter in the ureter. Nifedipine infusion did not significantly change basal urine flow rate (104 ± 12 vs. 94 ± 8 μl/15 min) and sodium excretion (8.3 ± 1.3 vs. 7.4 ± 1.3 μmol/15 min) or endogenous creatinine clearance (1.1 ± 0.1 vs. 0.9± 0.1 ml min^{-1}). However, nifedipine treatment significantly attenuated NPY-induced increases of urine flow rate and sodium excretion (137 ± 22 vs. 74 ± 21 μl/15 min and 8.7 ± 2.1 vs. 2.4 ± 1.4 μmol/15 min, respectively; both $P < 0.05$). Endogenous creatinine clearance was not changed by NPY infusion whether nifedipine was present or not.

Our data demonstrate that the dihydropyridine-type Ca^{2+} entry blocker nifedipine inhibits vascular and tubular NPY effects in anaesthetized rats *in vivo*. Since vascular and tubular NPY effects occur via Y_1 and Y_5 receptor subtypes, respectively (Bischoff & Michel 1998), we propose that both subtypes couple to influx of extracellular Ca^{2+} via nifedipine-sensitive channels.

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33P INVESTIGATION OF THE EFFECTS OF MDMA AT α-ADRENOCEPTORS IN THE RAT

Aisling Lavelle, Valerie Honner & Jim Docherty, Department of Physiology, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2.

The recreational abuse of methylenedioxymethamphetamine (MDMA, ecstasy) is associated with serious cardiovascular side effects (Bedford Russell et al., 1992). In an effort to elucidate these actions, we have examined the effects of MDMA on cardiovascular and neuronal responses in the rat.

Rat atrial slices were pre-incubated with 3H-noradrenaline, and stimulation-evoked (2Hz 360 pulses, 0.5 msec, supramaximal voltage) release of tritium was taken to indicate release of neurotransmitter. Responses obtained after test drug or vehicle were expressed as a percentage of the control response. Prostatic and epididymal portions of rat vas deferens were set up in organ baths for recording of isometric tension responses to single pulse electrical field stimulation (0.5 msec pulses, supramaximal voltage). Responses in epididymal portions were obtained in the presence of nifedipine (10 μM) to block the postjunctional actions of α-adrenoceptor agonists. Drugs or vehicle were added cumulatively at intervals of 5 min and a response to a single electrical stimulus was obtained 5 min after the drug administration.

MDMA (1-10 μM) significantly increased basal release of noradrenaline, and this effect was reduced or abolished by the re-uptake inhibitors cocaine (30 μM) or desipramine (1 μM), suggesting that this action of MDMA is due to uptake into the nerve terminals and displacement of noradrenaline. In the presence of desipramine (1 μM), MDMA (3-30 μM) inhibited the stimulation-evoked release of noradrenaline (e.g. MDMA 10 μM reduced the stimulation-evoked response to 67.6±9.3% of control; vehicle response was 101.8±7.5% of control; $P<0.05$). In prostatic portions of rat vas deferens, MDMA produced a concentration-dependent inhibition of nerve-evoked contractions, and produced direct contractions. In the presence of cocaine (3 μM), direct contractions to MDMA were absent but MDMA still produced a concentration-dependent inhibition of the stimulation-evoked contractions with a pD2 (-logIC50) of 5.12±0.21 (n=4). In epididymal portions of rat vas deferens, MDMA produced a concentration-dependent inhibition of nerve-evoked contractions with a pD2 of 5.88±0.16 (n=4). In radioligand binding studies employing 3H-yohimbine, MDMA showed similar affinities for α2B, α2C & α2D-adrenoceptor sites, with Ki values in the same range as its potency prejunctionally in rat atrium (e.g. at rat submandibular α2D-adrenoceptors, a Ki of 5.31±0.14, n=4,was obtained, -logM).

It is concluded that MDMA has significant α2- adrenoceptor agonist actions which may contribute to its cardiovascular side effects.

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34P MELATONIN CONSTRICTS RAT CEREBRAL ARTERIOLES *IN VIVO* BY BLOCKING Ca^{2+}-ACTIVATED K^+ (BK_{Ca}) CHANNNELS

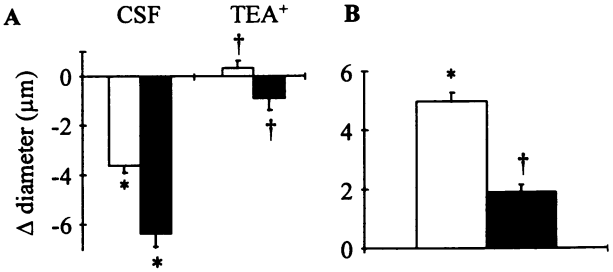
J.M. Chillon, O. Régrigny, I. Lartaud-Idjouadiene, P. Delagrange[1], E. Scalbert[1], & J. Atkinson. Dept. of Cardiovascular Pharmacology, P.O. Box 403, Faculty of Pharmacy, Nancy and [1]IRIS, Courbevoie, France.

Based on our finding that melatonin decreased the lower limit of cerebral blood flow autoregulation in rat, we previously suggested that melatonin constricts small diameter cerebral arterioles (Régrigny *et al*, 1998). Melatonin is known to constrict large-diameter cerebral arteries *in vitro*, following G protein-dependent inhibition of BK_{Ca} (Geary *et al*, 1997). The goal of this study was to investigate the contractile effects and the mechanism of action of melatonin on small diameter cerebral arterioles *in vivo*.

We measured the internal diameter of first order cerebral arterioles in male Wistar rats (weight = 588±31g, n = 6) using an open skull preparation. Animals were anesthetized with sodium pentobarbitone (60 $mg.kg^{-1}$, i.p.), paralyzed with gallamine triethiodide (20 $mg.kg^{-1}$, i.v.) and mechanically ventilated with room air (60 $strokes.min^{-1}$; 10 $ml.kg^{-1}$). Cerebral arterioles were exposed to 2 cumulative doses of melatonin prior to and after 20 min of superfusion with artificial cerebrospinal fluid (CSF) + tetraethylammonium (TEA^+; BK_{Ca} channel blocker). Cerebral arterioles were exposed to NS1619 (BK_{Ca} channel activator, Geary *et al*, 1997) prior to and 15 min after addition of melatonin. All drugs were added to the CSF at their final concentration and superfused in the cranial window until a stable response was obtained.

Melatonin constricted cerebral arterioles (Fig 1A). TEA^+ induced a constriction (5 ± 1μm) similar in amplitude to that induced by melatonin. There was no additional vasoconstriction when melatonin was added in the presence of TEA^+ (Fig 1A). Melatonin diminished dilatation induced by NS1619 (Fig 1B). The vasoconstrictor effect of melatonin on cerebral arterioles appear to be mediated by inhibition of BK_{Ca} channels. This arteriolar constrictor effect may explain the decrease of the lower limit of cerebral blood flow autoregulation (Régrigny *et al*, 1998).

Figure 1: A: Effect of melatonin (3.10^{-9}M, open bars and 3.10^{-8}M, filled bars) prior to (CSF) and after TEA^+ (TEA^+; 10^{-4}M). **B:** Vasodilatory response induced by NS1619. (10^{-5}M) in the absence (open bar) and presence (filled bar) of melatonin (3.10^{-8}M). Values are means ± SEM. * $P<0.05$ *vs.* baseline diameter. † $P<0.05$ *vs.* responses induced by melatonin (A) or *vs.* responses induced by NS1619 (B).



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35P THE HAEMODYNAMIC PROFILE OF THE POTASSIUM CHANNEL OPENER, ZD6169, IN THE ANAESTHETISED DOG

S. J. G. Loxham, S.M. Poucher and C.G. Heapy Cardiovascular, Musculoskeletal and Metabolism Research Department; Zeneca Pharmaceuticals, Alderley Park, Macclesfield, UK.

ZD6169 ((S)-N-(4-benzoylphenyl)-3,3,3-trifluoro-2-hydroxy-2-methyl-propionamide) is a potassium channel opener indicated for the treatment of urge incontinence (Heppner, *et al*, 1996). This study examined the vascular effects of ZD6169 on the cardiac and sketelal muscle beds.

Six male beagle dogs (12 to 15 kg) were pre-medicated with morphine (10 mg, im), anaesthetised with sodium pentobarbitone (49.1 ± 1.1 mg kg^{-1}, iv,) and artificially ventilated. The chest was opened and the heart surgically denervated by ligation of the left and right cardiac sympathetic nerves and left and right vagii (sectioned in the neck). The left hind limb was surgically denervated by section of the sciatic and femoral nerves. Left circumflex coronary artery (LCX) and femoral artery (FA) blood flow was measured with a doppler flow probe. Myocardial blood flow was measured using coloured microspheres. LCX and FA vascular resistance (Mean arterial blood pressure (MABP) / flow)responses to ZD6169 (1 - 3000 ug kg^{-1}, iv) were measured at the peak response and 5 min after administration. Myocardial blood flow was measured 5 min after ZD6169 (30 and 1000 ug kg^{-1}, iv).

MABP, heart rate, FA and LCX vascular resistance prior to ZD6169 administration were 102.0±3.88 mmHg, 126.5±5.64 beats min^{-1}, 3.47 ± 0.45 mmHg ml^{-1} min and 4.64 ± 1.47 mmHg ml^{-1} min respectively). ZD6169 caused dose dependant reductions in blood pressure. Vascular resistance was decreased in both the LCX and FA resistance beds by ZD6169, with the FA bed ($ED_{50\%}$ 44 (95% c.l. 11 - 178) μg kg^{-1}) being more sensitive to ZD6169 than the LCX bed ($ED_{50\%}$ 127 (95% c.l. 79 - 203) μg kg^{-1}). However, the maximum response to ZD6169 was greater in the LCX bed (-4.66 ± 1.5 vs -1.15 ± 0.37 mmHg ml^{-1} min). The magnitude of all response to ZD6169 was reduced at 5 min compared to the peak response but these were still significant (Table 1). Although epicardial blood flow was elevated in all tissue samples following ZD6169 (1000 μg kg^{-1}, iv) this failed to reach statistical significance (0.56 ± 0.24 vs 1.05 ± 0.25 ml min^{-1} g muscle $mass^{-1}$). The current study shows, that the hind-limb vascular bed was more sensitive than the coronary vascular bed to low doses of ZD6169, but that the magnitude of response to higher doses was greater in the coronary circulation in the anaesthetised areflexic beagle dog.

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Table 1. LCX and FA vascular resistance responses to ZD6169 in the anaesthetised dog.

	Femoral vascular resistance (mmHg ml^{-1} min)		Circumflex vascular resistance (mmHg ml^{-1} min)	
ZD6169 (μg kg^{-1}, iv)	Peak	5 min	Peak	5 min
1	-0.34 ± 0.15*	-0.37 ± 0.17*	0.04 ± 0.21	0.06 ± 0.18
3	-0.43 ± 0.14*	-0.37 ± 0.13*	0.22 ± 0.39	0.20 ± 0.32
30	-0.61 ± 0.10*	-0.37 ± 0.11*	-0.28 ± 0.16	0.14 ± 0.27
300	-1.61 ± 0.41*	-0.62 ± 0.12**	-3.27 ± 0.55**	-0.30 ± 0.18*
1000	-1.78 ± 0.55	-0.95 ± 0.15	-4.48 ± 1.35*	-2.92 ± 1.1

(mean ± se; Statistical analysis by ANOVA followed by Students t-test: P<0.05, *; P<0.005** compared with vehicle control group.).

36P A ROLE FOR THE ENDOTHELIUM IN K^+ CHANNEL MODULATION OF VASOCONSTRICTION IN RAT MESENTERIC ARTERIES

K.A. Dora and C.J. Garland

Department of Pharmacology, University of Bristol, University Walk, Bristol BS8 1TD, UK.

Endothelial cells release hyperpolarizing factor (EDHF) and nitric oxide (NO), which both cause vasodilation. To study EDHF responses, a constrictor agonist such as phenylephrine (PE) is used to prestimulate the smooth muscle cells in the presence of NO synthase inhibitors. Under these conditions, the potency of PE is considerably increased, an effect attributed to an inhibition of 'basal' NO release (Plane *et al.*, 1996). However, in pressure myographs basal NO release is insufficient to affect myogenic tone, whereas in the presence of PE, NO release is stimulated as a result of hetero-cellular calcium signaling (Dora *et al.*, 1997). The resultant increase in endothelial cell calcium may also stimulate the release EDHF, so inhibition of one or both of these factors may underlie increased contraction. We tested this possibility by comparing contraction to PE in endothelium intact and denuded preparations, and after sequentially blocking the release of NO and EDHF. The latter was blocked with apamin and charybdotoxin (ChTX) (Plane *et al.*, 1997).

Small arterial segments (D_{100}=100-200 μm) from male Wistar rat mesenteric arcades were suspended in a Mulvany-Halpern myograph containing modified Krebs-Ringer bicarbonate solution (37°C, aerated with a 95% O_2:5% CO_2 gas mixture) and indomethacin (2.8 μM). After normalization and equilibration, all arteries used maximally relaxed to ACh (1 μM, in pre-contracted vessels). In these studies L-NAME (100 μM), apamin (50 nM) or ChTX (50 nM) were added to the organ bath at least 20 min before addition of PE, and had no effect on basal tension. In each experiment, a concentration of PE (1-3 μM) was chosen to evoke only a small increase in tension under control conditions, and the same concentration was then used throughout the remainder of the experiment. In some arteries, a hair was used to destroy the endothelium, assessed as a loss of relaxation to ACh (1-3 μM).

Data were obtained after 5 min stimulation with PE and are means ± SEM (expressed as % of maximum contraction).

The magnitude of the control response (14.6 ± 3.2 %, n=17) did not vary with time. L-NAME (32.7 ± 5.3 %, n=17), L-NAME + apamin (50.3 ± 5.2 %, n=11), and L-NAME + ChTX (63.9 ± 4.3 %, n=4) each caused significant increases in PE-mediated contraction. The combination of L-NAME with apamin plus ChTX caused the greatest augmentation (80.1 ± 3.9 %, n=12).

In denuded arteries, control responses (37.9 ± 12.2 %, n=6) did not vary with time. Neither L-NAME (39.7 ± 8.5 %, n=6) nor apamin (46.9 ± 20.2 %, n=3) caused significant increases in PE contraction, whereas ChTX (66.0 ± 7.2 %, n=3) did.

These observations provide further evidence supporting the presence of apamin- and ChTX-sensitive K^+ channels in endothelial cells (Edwards *et al.*, 1998), as well as ChTX-sensitive K^+ channels in smooth muscle cells.

In summary, stimulation of smooth muscle cells with PE activates endothelial cell processes including NO synthesis and K^+ channel opening, the latter causing the most pronounced modulation of contraction. This observation provides further evidence supporting an important role for endothelial cell hyperpolarization in controlling small artery diameter.

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37P EFFECTS OF THE $I_{K,ATP}$ BLOCKERS GLIBENCLAMIDE AND HMR 1883 ON CARDIAC ELECTROPHYSIOLOGY DURING REGIONAL ISCHAEMIA AND REPERFUSION

S. Dhein, K. Krüsemann* & P. Pejman*, (O.-E. Brodde), Institute of Pharmacology, University of Halle, Magdeburger Str.4, 06097 Halle (Saale); *Institute of Pharmacology, University of Cologne, Gleueler Str. 24, 50931 Köln, Germany

Clinical and experimental evidence indicates an antiarrhythmic effect of sulfonylureas (Billman *et al.* 1993; Lomuscio *et al.* 1994), which might be blunted by their vascular action. The aim of our study was to investigate the effect of glibenclamide and the new sulfonthiourea compound HMR1883 (1-[[5-[2-(5-chloro-o-anisamido)ethyl]-2-methoxyphenyl]-sulfonyl]-3-methyl-thiourea) on cardiac electrophysiology in the course of regional ischemia and reperfusion. For that purpose, isolated hearts from male White New Zealand rabbits perfused with Tyrode's solution at constant pressure (70cm H_2O, 37°C) according to the Langendorff-technique were pretreated with either vehicle (n=7), 3 µM glibenclamide (n=7) or 3 µM HMR1883 (n=7) before regional ischemia was induced by ligation of the descending branch of the left coronary artery for 45 min followed by 45 min reperfusion. Unipolar epicardial electrocardiograms were recorded simultaneously from 256 AgCl electrodes as described (Dhein *et al.*, 1998) on the ventricular surface revealing the epicardial activation pattern, velocity, the repolarisation pattern, the epicardial activation-recovery interval (ARI), the dispersion of ARI, the degree of ST-elevation and the area in which ST-elevation was observed. Coronary ligation resulted in a decrease in coronary flow by 35% and in left ventricular pressure by 40% in all series. The area at risk was 23±3% in all series. However in glibenclamide but not in HMR1883 treated hearts recovery of CF upon reperfusion was significantly depressed (control: 25.5±4; HMR1883: 23±2.5; glibenclamide: 16±1 ml/min, values at 2 min reperfusion). The elevation of the ST-segments in early ischemia was fully prevented by both treatments (control: 140±43; HMR1883: 75±34; glibenclamide: 58±8 a.u.). Both drugs inhibited the shortening of the activation-recovery-intervals during ischemia treatments (control: -53±5; HMR1883: -22±11; glibenclamide: -37±6%). Ischemia led to an increase in dispersion of ARI (from 9.5±2 to 40±6ms). This increase during late ischemia and reperfusion was significantly inhibited by both substances treatments (control: 21±3; HMR1883: 14±3; glibenclamide: 14.4±3ms after 45min reperfusion). Ventricular fibrillation was observed in 3/7 (control series), 0/7 (glibenclamide series) and 0/7 (HMR1883 series).

We conclude hat both glibenclamide and HMR1883 exert an antiarrhythmic effect in this model. This may be related to the prevention of both ARI-shortening and of the increase in dispersion. These results indicate that HMR1883 does not interfere with postischemic hyperemia in contrast to glibenclamide.

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38P CANNABINOID RECEPTOR-MEDIATED INHIBITION OF A METHOXAMINE-INDUCED Cl^- CURRENT IN RAT MESENTERIC ARTERY

R. White[1], R.D. Pinnock[2], K. Lee[2] & C.R. Hiley[1]. [1]Department of Pharmacology, University of Cambridge, Cambridge CB2 1QJ and [2]Parke-Davis Neuroscience Research Centre, Cambridge CB2 2QB

There is increasing evidence that agonist-induced vasoconstriction is mediated in part by opening of Cl^- channels which leads to membrane depolarisation and subsequent activation of voltage-activated Ca^{2+} channels (Large & Wang, 1996). We have recently investigated the mechanisms by which cannabinoid agonists relax methoxamine-precontracted rat small mesenteric arteries (White & Hiley, 1998a), and postulated that these agents might cause relaxation by modulating the Cl^- channel associated with α-adrenoceptor-mediated depolarisation, as may be the case for nitric oxide (Lamb & Barna, 1998a,b)

Male Wistar rats (250-350 g; Tucks, Rayleigh, Essex) were killed with an overdose of sodium pentobarbitone. Mesenteric arteries were removed and pinned to the silicone rubber base of an organ chamber (volume 0.5 ml) for intracellular microelectrode recording as described by White & Hiley (1998b). Transmembrane potential was recorded with 1 M filled KCl-glass micro-electrodes (tip resistance 30-60 MΩ).

The resting membrane potential of rat mesenteric artery smooth muscle cells was –57.5±0.3 mV (n = 58). Methoxamine (0.3 - 10 µM) induced concentration-dependent depolarisation of the mesenteric artery smooth muscle cells in the presence of 30 µM verapamil; a sub-maximal concentration of 1 µM was chosen for further study. Depolarisation to methoxamine (6.6±0.6 mV; n = 8) was inhibited by the stable GDP analogue, GDP-β-S (1 mM in the electrode solution; 2.3±0.9 mV; n = 4), the phospholipase C inhibitor U 73122 (10 µM applied extracellularly), and the Ca^{2+}-chelating agent, BAPTA (10 mM in the electrode solution; 3.0±0.7 mV; n = 4). U 73343, the inactive analogue of U 73122, had no effect. The effect of methoxamine was sensitive to the non-selective Cl^- channel blocker, DIDS (100 µM) and niflumic acid (10 µM), an inhibitor of Ca^{2+}-activated Cl^- channels, but was not affected by tamoxifen (10 µM), which inhibits volume-regulated Cl^- channels. Further evidence that methoxamine activated a Cl^- current was obtained by substitution of NaCl in the Krebs-Henseleit solution. Methoxamine-induced depolarisations (6.4±1.0 mV; n = 5) were potentiated by Na^+ methane-sulphonate (10.0±1.1 mV; n = 4), and reduced by NaBr (3.8±0.6 mV; n = 4), consistent with previous findings (Lamb & Barna, 1998a,b).

Depolarisation to methoxamine was inhibited in a concentration-dependent manner by the endogenous cannabinoid, anandamide; at 1 µM, it reduced the depolarisation to methoxamine by 73%. This inhibition was reduced by 52% in the presence of the cannabinoid receptor antagonist SR 141716A (3 µM) while, alone, SR 141716A had no significant effect on the response to methoxamine. The synthetic cannabinoid receptor agonist, CP 55,940 (10 µM) also inhibited the depolarisation induced by methoxamine (by 79%).

These results show that α_1-adrenoceptor-mediated depolarisation of intact resistance arteries occurs through G-protein-mediated activation of phospholipase C, with the subsequent release of BAPTA-sensitive intracellular Ca^{2+} stores activating niflumic acid-sensitive Ca^{2+}-activated Cl^- channels. Furthermore, vaso-relaxation to cannabinoids may result from receptor-mediated modulation of the activity of agonist-stimulated, Ca^{2+}-activated Cl^- channels, although the mechanism remains to be elucidated.

RW is an AJ Clark Student of the British Pharmacological Society.

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39P ENHANCED ACTIVITY OF SEROTONIN RECEPTORS IN THE COLLARED CAROTID ARTERY OF THE RABBIT

I.S. Geerts, K.E. Matthys, A.G. Herman and H. Bult

Division of Pharmacology, University of Antwerp (UIA), B-2610 Wilrijk, Belgium

Patients with atherosclerosis are prone to the development of coronary vasospasm to serotonin (5-hydroxytryptamine, 5-HT). A similar hyperactivity develops when a non-occlusive silicone collar is placed around the rabbit carotid artery. This study aimed to investigate possible alterations of the 5-HT receptor subtype(s) in this model using different agonists and antagonists.

Collars were placed around both carotid arteries of anaesthetised (sodium pentobarbital, 30mg/kg, IV) male New Zealand White rabbits (2.2-3.5 kg). One week later the rabbits were anaesthetised again, the arteries were removed and the rabbits were killed (overdose sodium pentobarbital). Rings (2mm) from inside (= collar) and outside (= control) the collar were mounted in organ baths (10ml) to measure isometric force at 6g loading tension. pA_2 values were calculated according to Schild (1947). If slopes were different from unity, pK_b values were estimated by non-linear regression (Peeters, 1998). The results are shown as mean ± s.e. mean, n = number of rabbits.

Cumulative dose response curves (DRC) confirmed the increased sensitivity to 5-HT in collared arteries ($-\log EC_{50}$ = pD_2 = 6.50±0.06 control; 7.38±0.07 collar, n=12, p<0.01 paired Student's t-test). The involvement of 5-HT_3 and 5-HT_4 receptors was ruled out as 5-HT_3 (tropanyl-3,5-dichlorobenzoate) and 5-HT_4 (GR113808A, Kaumann, 1993) antagonists did not influence the 5-HT DRC in control and collared rings. The mixed $5\text{-HT}_{2A}/5\text{-HT}_1$ antagonists spiperone and methysergide shifted the 5-HT DRCs parallel to the right, indicating a competitive surmountable antagonism (slope of Schild plot not different from unity). Collar placement did not significantly alter slope or pA_2 (Table 1). Ketanserin and ritanserin (5-HT_{2A} antagonists) displaced the 5-HT DRCs to the right, but the slope of the Schild plot deviated from unity, pointing to a mixed antagonism. Their apparent pK_b values were not influenced by the collar (Table 1). In contrast, the non-surmountable antagonism of methiothepin (preferential 5-HT_{1B}) in control rings became surmountable with a lower apparent pK_b in collared rings (Table 1). Moreover sumatriptan ($5\text{-HT}_{1B/1D}$ agonist) was virtually ineffective in control rings, but constricted collared rings (60±11% of the response to 50mM KCl at 0.1mM sumatriptan, p<0.01 paired Student's t-test). The collared rings were significantly more sensitive to 5-carboxamidotryptamine (5-HT_1 agonist, control pD_2 = 5.80 ± 0.14; collar 7.29±0.08, n=8, p<0.01 paired Student's t-test).

In conclusion, the results suggest that up-regulation of 5-HT_{1B} or 5-HT_{1D} receptor activity explains the hypersensitivity to serotonin in collared arteries.

Table 1. Effect of collar on pA_2 and apparent pK_b values.

	Control		collar	
	pA_2	**slope**	**pA_2**	**Slope**
Spiperone	8.87±0.07	0.97±0.06	9.07±0.13	0.88±0.09
Methysergide	8.05±0.08	1.04±0.06	7.96±0.09	0.94±0.07
	pK_b		**pK_b**	
Ketanserin	8.39±0.05		8.61±0.15	
Ritanserin	8.72±0.18		8.37±0.13	
Methiothepin	10.11±0.07		9.03±0.09*	

Mean ± s.e. mean, n = 7 - 8. * rings with collar different from control rings, p < 0.01, unpaired Student's t-test.

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40P PURINE RECEPTORS IN RABBIT THORACIC AORTA ARE MORE VULNERABLE TO DENATURATION ON STORAGE AT 4 °C IN UNIVERSITY OF WISCONSIN SOLUTION THAN CHOLINERGIC RECEPTORS

B.Alexander, JVD Gryf-Lowczowski, J Salisbury, D Sherlock, IS Benjamin. Academic Department of Surgery, The Rayne Institute, King's College School of Medicine & Dentistry, London, SE5 9NU.

Arteries, used as conduits for liver transplantation, are frequently stored in University of Wisconsin solution (UW) because this has been shown to retain optimal conditions for liver preservation. We have previously shown that storage of rabbit thoracic aorta in UW may reduce endothelium-independent vasodilatation, possibly by degradation, yet preserves endothelium-dependent vasodilatation (Gryf-Lowczowski *et al.*, 1997). The present study determined whether storage in UW preserved endothelium-dependent vasodilatation to acetylcholine (ACh) and adenosine-5'-triphosphate (ATP), since purines are important regulators of hepatic vascular tone. Thoracic aortae from 18 male New Zealand White rabbits were excised, following a lethal dose of sodium pentobarbitone, and stored as cut rings in solutions of UW, Krebs'-Büllbring buffer (KB) and saline (n=6 per solution) at 4^0C. Freshly-harvested rings and those subjected to 8 days cold-storage were mounted upon isometric fine-wire myographs at 2g tension and allowed to equilibrate for 1 hour in KB at 37^0C, gassed with 95% O_2 / 5% CO_2 , before experiments proceeded. The tone of the preparations was raised by the addition of 2 X 10^{-6} M noradrenaline to the organ baths which produced 75.3 ± 2.4% of the available maximum contraction. Concentration-dependent vasodilatations were then obtained to ACh and ATP. Endothelium-dependent, NO-mediated responses to ACh were not significantly altered after 8 days storage in UW but were reduced after storage in KB at -log (M) concentrations of 7.0 (47.05 ± 4.2 vs. 13.20 ± 7.22*); 6.5 (66.82±4.83 vs. 26.60 ± 9.48*) and 6.0 (83.68 ± 5.26 vs. 31.00 ± 9.83**), UW vs. KB respectively. These were totally abolished after removal of the endothelium, saline storage or after testing in incubation with 100μM N^G-nitro-L-arginine methyl ester (L-NAME). In contrast, endothelium-dependent vasodilatations to ATP were not significantly changed following storage in KB compared to freshly harvested vessels, but were significantly reduced after 8 days storage in UW at -log (M) concentrations of 5.0 (25.09 ± 14.10 vs. 8.88 ± 6.64*); 4.5 (39.19 ± 7.72 vs. 19.41 ± 10.46* and 4.0 (58.56 ± 25.35 vs. 36.79 ± 11.78**), KB vs. UW respectively. L-NAME significantly attenuated vasodilator responses to ATP in fresh rings at -log (M) concentrations of 4.5 (45.83 ± 10.65 vs. 21.74 ± 2.06†) and 4.0 (69.63 ± 9.02 vs. 15.74 ± 7.41†††) and in rings stored in KB at -log (M) concentrations of 5.5 (10.99 ± 3.24 vs. 2.81 ± 1.06†); 5.0 (25.09 ± 5.76 vs. 4.93 ± 2.65††); 4.5 (39.19 ± 7.72 vs. 9.40 ± 3.27††); 4.0 (58.56 ± 10.35 vs. 15.74 ± 7.41††) and 3.5 (66.94 ±11.27 vs. 32.36 ± 9.45†) before vs. after L-NAME. but not in UW. De-endothelialisation still permitted a maximum of 25% vasodilatation to ATP which was not attenuated by 8-phenyltheophylline. Thus the residual vasodilatation remaining in KB-stored rings was endothelium-independent and not due to P_1A_2 purine receptor activation. Therefore storage of rabbit thoracic aorta in UW appears detrimental to preservation of endothelium-dependent vasodilatation to ATP but not ACh. *P<0.05, **P<0.01, ANOVAR, Bonferroni adjusted. †P<0.05, ††P<0.01, †††P<0.001, Student's paired t-test.

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41P INCREASED APOPTOSIS IN CELLS OVEREXPRESSING LIPOCORTIN 1

Silvia Canaider, Egle Solito#, Nicolas J Goulding, Roderick J Flower & Mauro Perretti.
Dpt. Biochem. Pharmacol., The William Harvey Research Institute, London, UK, and # Istitut Cochin de Génétique Moleculaire, Unit 332 Inserm, Paris, France.

U937 cells (a human myelomonocytic cell line) transfected with antisense or sense DNA for the protein lipocortin 1 (LC1) [Solito *et al.*, 1998] were used to determine whether endogenous LC1 plays a role in the regulation of apoptosis.

U937 control cells, antisense (36.4AS clone) or sense (16S clone) were cultured in RPMI-1640 medium supplemented with 10% FCS, as previously described [Solito *et al.*, 1998]. Cells were incubated with tumour necrosis factor-α (TNF- α; 0.05-5 $ngml^{-1}$) or etoposide (20 μgml^{-1}) for a period spanning 12-48 h or 6-24 h, respectively. At the end of the incubation period cells were washed and prepared for further analysis. Initially, the percentage of cells expressing apoptotic nuclei was determined following staining of cytospin preparations (10 min at 1,200 r.p.m.) with May-Grunwald and Giemsa, counting at least 200 cells per slide. Apoptosis was also assessed by using a commercially available kit based upon the binding of annexin V (R&D, Abingdon, UK). Cells were incubated with fluorescein-conjugated annexin V and propidium iodide (PI; Sigma Chemical Co., Poole, UK) for 15 min at 20°C, prior to flow cytometric (FACS) analysis. The percentage of positive cells in the green (FL1; annexin V positive) and red channel (FL2; PI positive) were then determined. Caspase-3 activity was measured by a commercial kit (Insigth Biotechnology Ltd., Wembley, UK), whilst bcl-2, bax and LC1 levels were measured either by Western blotting or by FACS analyses using specific antibodies (Santa Cruz Biotechnology). Cell associated fluorescence was expressed in median fluorescence intensity (MFI) units. Data are reported as mean ± s.e.mean of n experiments.

TNF-α caused a reproducible and time-dependent degree of apoptosis only at the highest concentration tested of 5 ng/ml. There was no difference between the response of control and 36.4AS cells, whereas 16S U937 cells were more apoptotic (P <0.05) (e.g., % of positive cells at 24 h: 9.5 ± 2.5, 5 ± 1 and 29 ± 9 for control, 36.4AS and 16S cells, respectively) (n=3 experiments in duplicate). This was confirmed by annexin V binding, where an annexin V positivity of 23 ± 3% and 34 ± 8% was found in control and 36.4AS cells respectively, *vs.* a values of 50 ± 8% (P <0.05) in 16S cells (n=4 experiments). Increased apoptosis in LC1 DNA sense transfected cells was also seen after cell treatment with etoposide, e.g. 14 ± 9%, 13 ± 8% and 49 ± 16% of annexin V positivity for control, 36.AS and 16S cells, respectively (n=4 experiments, P <0.05 for 16S clone). One h after etoposide treatment, U937 16S cells had higher caspase-3 activity than normal cells, with 68 ± 4 and 24 ± 5 arbitrary units, respectively (n=3 experiments; P <0.05). A similar increase was also detected 1 h post-TNF- α, with 69 ± 9 and 46 ± 2 arbitrary units in 16S and normal U937 cells (n=4; P <0.05). In a preliminary experiment using TNF- α to induce apoptosis, bax levels were increased with respect to basal values, with a more marked change in 16S cells (537 MFI units) than in control U937 cells (358 MFI units) 3 h post-etoposide treatment. Such a difference was also confirmed by western blotting. Finally, treatment of control U937 cells with TNF- α augmented intracellular LC1 levels from by almost 30% within 6 h post-treatment. In conclusion, enhanced intracellular expression of LC1 by sense DNA transfection results in an increased degree of apoptosis with concomitant changes in recognised apoptotic markers.

Solito *et al.*, Br. J. Pharmacol. 124: 1675-1683, 1998

MP is an ARC Post-doctoral fellow whereas RJF is a Principal Fellow of the Wellcome Trust

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42P EVIDENCE FOR A ROLE OF PROSTACYCLIN IN COX-2 SUPPRESSION OF GM-CSF RELEASE BY HUMAN VASCULAR CELLS

S. J. Stanford, J.R. Pepper and J.A. Mitchell
Imperial College of Science, Technology and Medicine, Unit of Critical Care Medicine, Royal Brompton Campus, Sydney Street, London SW3 6NP

Neutrophil recruitment and activation are primary events in the development of a number of vascular diseases. Once present in the vessel wall neutrophils do not differentiate and rapidly die. Neutrophil survival can be promoted by the cytokine granulocyte macrophage-colony stimulating factor (GM-CSF). GM-CSF is released from a variety of cell types. We have recently shown that human venous smooth muscle cells can be induced to release GM-CSF and to express cyclo-oxygenase-2 (COX-2) when stimulated with inflammatory cytokines such as IL-1β and TNFα (Mitchell *et al.*, 1998). Furthermore, we have shown that GM-CSF release is further increased when indomethacin or a range of other NSAIDs are included together with the cytokines (Stanford *et al.*, 1998). In this study we have investigated the mechanism by which inhibition of COX increases GM-CSF release from these cells.

Samples of saphenous vein (SV) were dissected clean, cut into small pieces and placed in supplemented culture medium as described previously (Bishop-Bailey *et al.*, 1997). Following explantation, cultured venous cells were plated onto 96 well plates. When cells reached confluence culture medium was replaced with new medium. In some experiments cells were pretreated with indomethacin (10μM) and/or IL-1β (1ng/ml). In others, increasing concentrations of prostaglandin (PG) E_2 ($1x10^{-8}$-$1x10^{-5}$M), the prostacyclin (PGI_2) mimetic Cicaprost ($1x10^{-10}$-$1x10^{-7}$M) or dibutyryl cAMP ($1x10^{-8}$-$1x10^{-4}$M) were added to venous cells pretreated with indomethacin in the presence of IL-1β. After 24-hours the medium was removed and GM-CSF release was measured by ELISA (Saunders *et al.*, 1997).

Basal release of GM-CSF from human cultured venous smooth muscle cells was very low or undetectable (*Figure 1*). As reported previously (Mitchell *et al*, 1998: Stanford *et al*, 1998), GM-CSF release was increased in the presence of IL-1β and further increased in the presence of IL-1β and indomethacin. This increase was inhibited in a concentration-dependent manner by PGE_2 (*Figure 1a*) and Cicaprost (*Figure 1b*), (P<0.05: one way ANOVA).

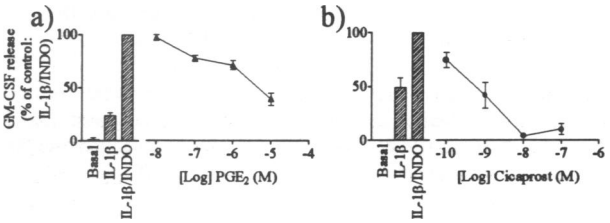

Figure 1. Effect of increasing concentrations of a) PGE_2 and b) Cicaprost on GM-CSF release from human cultured venous smooth muscle cells pretreated with indomethacin (INDO: 10μM) and stimulated with IL-1β (1ng/ml). Data is shown as mean±s.e.m where n=9 from 3 patients.

Similarly, at the highest concentration tested, dibutyryl cAMP ($1x10^{-4}$M) inhibited GM-CSF release to 10.0±1.2% (mean±s.e.m: n=6) of the control.

Thus, COX-2 activity suppresses GM-CSF release from human cultured venous smooth muscle cells. This effect was mimicked by Cicaprost, PGE_2 and dibutyryl cAMP. We found that Cicaprost was approximately 10,000x more potent than PGE_2 as an inhibitor of GM-CSF release. These observations suggest that PGI_2 is the principle metabolite involved in the COX-2 induced suppression of GM-CSF release. These results suggest that the therapeutic benefits of PGI_2 in some vascular diseases may be related to suppression of GM-CSF release.

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43P EFFECTS OF HEPARINASES AND HEPARIN UPON THE ADHESIVE FUNCTIONS AND SURFACE CHARACTERISTICS OF HUMAN UMBILICAL; VEIN ENDOTHELIAL CELLS

R. Lever, [1]S. Haq, [2]M.M. Grundy, [3]N.V. Richardson & C.P. Page. Department of Pharmacology, King's College London. SW3 6LX. [1]Surface Science Research Centre, University of Liverpool, L69 3BX. [2]Scottish Association for Marine Science, Oban, PA34 4AD. [3]Department of Chemistry, University of St. Andrews, Fife, KY16 9ST.

Heparin, in addition to its role as an anticoagulant, is known to possess anti-inflammatory characteristics. We have demonstrated previously that heparin inhibits the adhesion of PMNs to HUVECs *in vitro*, when either cell type is stimulated (Lever & Page, (1998); Lever & Page, (1997)) and it is thought that the anti-adhesive actions of heparin are related to the fact that the molecule is extensively sulphated. It is well established that the glycosaminoglycan (GAG) heparan sulphate exists on endothelial cells and may be involved in modulation of the adhesion process. Indeed, it has been demonstrated that certain inflammatory cells and tumour cells can release heparan-degrading enzymes, facilitating their extravasation into tissue sites (Vlodavsky *et al.* (1992)). In the present study, we have employed a Fourier-transform infrared spectroscopy (FTIR) technique to examine the chemical composition of the surface of endothelial cells and to investigate the effects of treatment with heparinase enzymes and heparin upon the characteristics of this surface. In addition, we looked at the effects of treating HUVECs with heparinase enzymes upon the adhesion of polymorphonuclear leucocytes (PMNs) to these cells.

For adhesion studies, monolayers of HUVECs were grown to confluency in 96-well plates and some wells were treated with heparinases I, II or III ($0.5Uml^{-1}$). Cells were washed and some wells were treated with heparin ($100Uml^{-1}$) for 20 minutes before washing again. ^{51}Cr-labelled PMNs from healthy, human donors (n=6) were applied to HUVEC monolayers for 30 minutes at 37° C, following which non-adherent cells were removed by washing and adherent cells were lysed with detergent. Adhesion was quantified by counting the radioactivity in lysates on a γ-counter. For FTIR experiments, HUVECs were grown in 6-well plates and were treated with heparinase enzymes and/or heparin as for adhesion experiments. Cells were manually scraped, smeared onto a zinc-selenide crystal and dried under nitrogen. A 60 second scan was then performed on a commercial FTIR machine (Mattson Instruments). Treatment with a combination of heparinases I, II and III was found to modify the HUVEC spectra (compared to untreated cells) in the region which corresponds to the presence of sulphate groups. When heparinase treated cells were exposed to unfractionated heparin for 20 minutes the sulphate peak was restored. Additional sulphate bands were found to be present on these cells which correspond to those found on the IR spectrum of heparin itself. However, HUVECs treated with heparinase enzymes ± heparin supported PMN adhesion to no greater or lesser extent than untreated cells (basal adhesion 7 ± 4%; minimum adhesion to treated cells 5 ± 4%; maximum adhesion to treated cells 10 ± 6%; $P > 0.05$). Heparin itself had no effect upon basal PMN adhesion to unstimulated HUVECs (3 ± 14% inhibition, $P > 0.05$).

The results of this study indicate that heparin can restore sulphation patterns upon endothelial cells which have been enzymatically modified by heparinases, possibly a mechanism by which heparin may affect inflammatory cell trafficking. Given that heparin reduces PMN-HUVEC adhesion under model conditions of inflammation but not basally, it is possible that removal of endothelial heparan sulphate affects adhesion only in the event of cellular activation.

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44P ROLE OF NEUROKININ 1 (NK_1) RECEPTORS IN THE VASCULAR AND CELLULAR PHASES OF INFLAMMATION INDUCED BY THERMAL INJURY AND MUSTARD OIL

E. Pintér, B. Brown, J.R.S. Hoult & S.D. Brain, Pharmacology Group & Vascular Biology Research Centre, Biomedical Sciences Division, King's College, Manresa Road, London, SW3 6LX, U.K.

A role for the tachykinin substance P in the immediate oedema formation observed after thermal injury has been suggested (Saria, 1984; Siney & Brain, 1996). There are also studies which indicate that substance P can stimulate neutrophil accumulation (Brain, 1997 for review).

The effect of the non-peptide selective NK_1 receptor antagonist SR140333 has been investigated on oedema formation and neutrophil accumulation induced by thermal injury (50 °C for 5 min), mustard oil, substance P and the NK_1 agonist GR73632 in the abdominal skin of rats anaesthetised with thiopentone sodium (100 mg/kg i.p.). Skin oedema was measured as the accumulation of i.v. ^{125}I-albumin (Siney & Brain, 1996). Neutrophil accumulation in the skin sites was determined by assay of myeloperoxidase (Waller et al., 1997).

SR140333 significantly inhibited the early oedema formation induced by thermal injury. However, a dosing strategy of SR140333 which blocked NK_1 receptors for 5 h failed to influence neutrophil accumulation after thermal injury. In separate experiments topical application of mustard oil (2 % dissolved in paraffin oil), a neurogenic inflammation stimulant, caused NK_1 receptor-mediated early neurogenic plasma extravasation, but did not induce cutaneous neutrophil accumulation over 5 h (see Table 1). Intradermal substance P and GR73632 at high doses (1 nmol/site) also failed to elicit neutrophil accumulation (results not shown).

In conclusion, despite an early tachykinin NK_1 receptor-dependent oedema after thermal injury, the results suggest that subsequent neutrophil accumulation is not mediated by neurogenic inflammatory mechanisms. Furthermore, we have not obtained any evidence to suggest that either endogenous or exogenous tachykinins can directly induce neutrophil accumulation in the rat cutaneous microvasculature.

Table 1. Effect of SR140333 pretreatment on plasma extravasation (0-10 min) and neutrophil accumulation (0-300 min) induced by thermal injury and mustard oil. Results are expressed as mean ± s.e.mean, n=5-6, **P<0.01 and ***P<0.001 vs. vehicle treated groups. ##P<0.01 and ###P<0.001 vs. unheated or paraffin oil treated control groups (ANOVA followed by Tukey-Kramer's test).

Oedema (μl plasma /site)	unheated	heated	paraffin	mustard
vehicle	9.3 ± 0.8	71.58 ± 5.2 ###	28.2 ± 2.1	80.1 ± 5.3 ##
SR140333 i.v. 240 nmol/kg	7.25 ± 0.9	19.4 ± 2.5 ***	22.5 ± 1.9	23.1 ± 0.8 **

Neutrophils (x 10^6 cells /site)	unheated	heated	paraffin	mustard
vehicle	0.5 ± 0.1	1.34 ± 0.08 ###	0.17 ± 0.05	0.12 ± 0.04
SR140333 i.v.+s.c. 240+240 nmol/kg	0.56 ± 0.1	1.3 ± 0.1 ###		

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45P ACTH 4-10 ACTIVATES MELANOCORTIN TYPE 3 RECEPTOR TO INHIBIT NEUTROPHIL MIGRATION

Stephen J Getting, Roderick J Flower & Mauro Perretti.
Dept. Biochemical Pharmacology, William Harvey Research Institute, Charterhouse Square, London EC1M 6BQ.

The efficacy that adrenocorticotrophic hormone (ACTH1-39) displays in the clinical management of human gouty arthritis (Ritter *et al.*, 1994) has prompted this study, in which we have tested the effect of the heptapeptide ACTH4-10 (MEHFRWG) as well as that of the melanocortin type 3 and 4 receptor (MC3-R and MC4-R) mixed antagonist SHU9119 (Fan *et al.*, 1997) in a murine model of crystal-induced inflammation (Getting *et al.*, 1997).

Male Swiss Albino mice (28-32 g) were treated i.v. with ACTH (20 ng) or ACTH4-10 (100 µg) 2 h prior to blood collection and corticosterone (CCS) levels in the plasma were measured by radioimmunoassay (ICN Flow). In other cases ACTH4-10 (10-200 µg), or 100 µg of a scrambled peptide (MGREWFH), were given s.c. 30 min before injection of 3 mg i.p. urate crystals. Some mice were also injected with SHU9119 (10 µg i.p.). Peritoneal cavities were washed with 3 ml of phosphate-buffered saline supplemented with 25 Uml^{-1} heparin 2 h or 6 h later, polymorphonuclear cells (PMN) were counted in a Neubauer hæmatocytometer and KC was measured in cell-free aliquots of lavage fluids by ELISA (R&D,UK). *In vitro*, ACTH (1-100 $ngml^{-1}$) or ACTH4-10 (1-200 $\mu g\ m^{-1}$) were added alone or together with SHU9119 (10 μgml^{-1}) to peritoneal macrophage (MØ) cultures enriched by adhesion to plastic (2 h, 37°C), and intracellular accumulation of cAMP was measured by enzyme immunoassay after a further 30 min of incubation (Amersham, UK). Finally, the presence of MC3-R and MC4-R mRNA expression in MØ was monitored by PCR analysis. Data were analysed by ANOVA taking a P value less than 0.05 as significant.

Whereas ACTH induced an intense release of CCS in the mouse plasma (460 ± 24 $ngml^{-1}$ *vs.* 42 ± 8 $ngml^{-1}$ in untreated mice; n=6; P <0.05), no increase was seen with ACTH4-10. Injection of crystals caused PMN accumulation almost maximal by the 6 h time-point (8.3 ± 0.4 $x10^6$ cells, n=27). Treatment of mice with ACTH4-10 inhibited PMN accumulation in a dose-dependent manner, with an approximate ED_{50} of 100 µg per mouse (n=27, P <0.01) and a maximal inhibition of 60% at the 200 µg dose. Scrambled ACTH4-10 did not modify crystal-induced PMN migration (n=6). Once animals were injected with 10 µg i.p. SHU9119, ACTH4-10 (100 µg s.c.) was no longer effective, with a calculated inhibition of PMN influx being reduced from 51% to 17% in the absence or presence of the mixed antagonist, respectively (n=10). The CXC chemokine KC was detected in the lavage fluids both at 2 h (5100 ± 400 pg per cavity) and 6 h (767 ± 85 pg per cavity) post-crystal injection. ACTH4-10 reduced KC release by 33% and 73%, respectively (n=10-18, P <0.01). The latter effect was reduced by SHU9119 to 33% of inhibition. ACTH (1-100 $ngml^{-1}$) and ACTH4-10 (1-200 μgml^{-1}) promoted cAMP formation in adherent MØ with 940 ± 80 and 650 ± 40 fmol per well being detected at the maximal concentrations tested, respectively (n=9, P<0.05); this effect was abrogated by co-addition of SHU9119 (n=6). Finally, MC3-R but not MC4-R mRNA was detected in mouse peritoneal MØ by PCR analysis (giving a product of 820 base pairs). In conclusion, we report a CCS-independent anti-inflammatory effect of an ACTH-derived peptide. MC3-R and ACTH4-10 may be useful targets for a novel pharmacological approach to anti-inflammatory therapy.

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46P DEATH OF RAT AORTIC SMOOTH MUSCLE CELLS IS REDUCED BY HEME OXYGENASE-1 INDUCTION AND EXOGENOUS BILIVERDIN

Lorna C. Hamilton and Timothy D. Warner. *Vascular Inflammation, The William Harvey Research Institute, Charterhouse Square, London EC1M 6BQ.*

All aerobically metabolising cells generate oxygen-derived free radicals and hence cells are equipped with abundant antioxidant defences to prevent oxidative damage. However, during pathological states, the generation of free radicals exceeds the cell's antioxidant capacity resulting in oxidative stress and potentially cell death. Heme oxygenase (HO) metabolises the pro-oxidant heme into the antioxidant biliverdin and is thus considered to be an antioxidant enzyme. HO exists in two distinct isoforms, inducible HO-1 and constitutive HO-2 (Maines *et al.*, 1982). Significantly all of the stimuli that induce HO-1 create oxidative stress within the cell. Nitric oxide (NO) donors are widely reported to generate oxidative stress resulting in cell death by apoptosis (Sandau and Brüne, 1996). Here we have investigated whether the prior induction of HO-1 within rat aortic smooth muscle cells (RASM) will protect them against NO-induced death.

RASM, grown to confluence in 6-well plates, were treated with either methemoglobin (Hb; 0.3 µM - 30 µM) or xanthine oxidase (XOD; 0.1 U – 5 U) for 12 h. Western blot analysis for HO-1 protein was then carried out. RASM were also grown to confluence in 96-well plates and were treated with Hb (30 µM) or XOD (1 U) for 12 h in serum-free medium. Cells were then washed with sterile phosphate buffered saline (PBS) and fresh serum-free medium replaced. For a further 16 h cells were treated with increasing concentrations of the NO donors sodium nitroprusside (SNP; 10 µM - 1 mM), S-nitroso-N-acetylpenicillamine (SNAP; 10 µM - 300 µM) or 3-morpholinosydnonimine (SIN-1, 10 µM - 1 mM). The medium was then removed and the release of lactate dehydrogenase (LDH) determined. In a separate series of experiments, RASM were treated with biliverdin (10 µM) for 2 h but medium was not replaced before treatment with SNP, SIN-1 or SNAP as above. LDH release was also measured in the medium removed from these cells.

There was a concentration-dependent increase in HO-1 expression following treatment of RASM with Hb or XOD, as determined by western blot analysis. Optimal HO-1 expression was achieved using 30 µM Hb or 1 U XOD for 12 h. At these concentrations, the amount of LDH released by Hb or XOD-treated cells was not significantly different to control. Exposure of untreated RASM to SNP, SIN-1 or SNAP produced concentration-dependent increases in cell death (% cytotoxicity; 100 % determined as LDH release following exposure to distilled water). At the highest concentrations SNP (1 mM), SIN-1 (1 mM) and SNAP (300 µM) caused, 75 ± 18, 70 ± 4 and 98 ± 15 % cytotoxicity respectively (n = 6-7). Pretreatment of RASM with Hb significantly ($p < 0.05$, ANOVA plus Dunnett's test) reduced cell death at the highest concentrations of SNP, SIN-1 and SNAP to 30 ± 5, 18 ± 2 and 16 ± 2 % cytotoxicity respectively (n = 6-7). However, HO-1 expression induced by XOD treatment only significantly ($p < 0.05$) reduced cell death following exposure to the highest concentration of SNAP (300 µM) (98 ± 15 % to 43 ± 18%, n = 4). Similarly, pretreatment of cells with biliverdin, the product of HO activity, significantly ($p < 0.05$) reduced cell death caused by the highest concentrations of SNP, SIN-1 and SNAP to 26 ± 0.7, 18 ± 3 and 66 ± 3 % cytotoxicity respectively (n = 5 for each).

In conclusion, HO-1 expression in RASM and exogenously applied biliverdin to RASM reduced oxidative stress and thus cell death induced by the NO donors SNP, SIN-1 and SNAP. Hence, increased HO-1 expression within the vascular smooth muscle may be important in cardiovascular diseases which are initiated or accelerated by overproduction of free radicals.

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47P ANGIOTENSIN AT_2 RECEPTOR STIMULATION EXACERBATES MYOCARDIAL ISCHAEMIA-REPERFUSION INJURY

W.R. Ford & C.R. Hiley. Department of Pharmacology, University of Cambridge, Cambridge CB2 1QJ

PD 123,319 (1-[[4-(dimethylamino)-3-methylphenyl]-methyl]-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5c]pyridine-6-carboxylic acid), an angiotensin AT_2 receptor antagonist has been shown to protect rat isolated working hearts from ischaemia-reperfusion (IR) injury (Ford *et al.*, 1996); however such hearts have a maximally dilated circulation such that AT_1 receptor-mediated coronary vasoconstriction is not observed (Ford *et al.* 1998). Since AT_2 receptor stimulation may inhibit responses to AT_1 receptor activation (Hein *et al.*, 1995), AT_2 receptor antagonism might augment coronary vasoconstriction to angiotensin II (Ang II). In conditions in which there is AT_1 receptor-mediated vasoconstriction, the loss of AT_2 receptor-mediated modulation may counteract the direct protective effect against IR injury afforded by antagonism of AT_2 receptors.

We have therefore examined the effect of Ang II (1 nM), in the presence and absence of PD 123,319 (1 μM), on the recovery of isolated hearts from male Wistar rats (250-350g) perfused by the Langendorff method at a constant pressure (75 mmHg). Left ventricular developed pressure (LVDP) was measured with a pressurised (5 - 10 mmHg) balloon in the left ventricle and connected to a pressure transducer. Heart rate (HR) was derived from the pressure signal. Rate pressure product (RPP) was calculated as LVDP x HR. Coronary flow (CF) was measured with flow probes, placed between the perfusate reservoir and the heart, connected to a Transonic T206 flow meter. Baseline mechanical function was recorded during an initial 30 min aerobic perfusion and then during 20 min of global, no-flow ischaemia followed by 30 min reperfusion. Infusions of vehicle (n = 6), Ang II (n = 9) or Ang II + PD 123,319 (n = 9) were started 5 min prior to ischaemia, stopped at the onset of ischaemia and recommenced upon reperfusion. Values, expressed as mean ± s.e.m, obtained at the end of reperfusion were compared with Ang II values by analysis of variance.

Mechanical function after 30 min of reperfusion in vehicle-treated hearts was depressed compared to baseline levels (LVDP to 50±7%, HR to 82±5%, RPP to 42±8% and CF to 57±5%). Ang II caused coronary vasoconstriction and reduced recovery of mechanical function below that of vehicle-treated hearts (Table 1). Compared with Ang II alone, Ang II + PD 123,319 increased post-ischaemic recovery of HR and RPP but had no significant effect on LVDP or CF. The presence of PD 123,319 did not alter Ang II-mediated coronary vasoconstriction (Table 1).

Table 1. Mechanical function after 30 min of reperfusion following 20 min of global ischaemia.

	n	LVDP (mmHg)	HR (beats min^{-1})	CF (ml min^{-1})	RPP ($x10^{-3}$)
Vehicle	6	65±8*	186±11*	6.1±0.4*	122±22*
Ang II	9	40±6	103±10	2.8±0.4	44±9
Ang II+ PD 123,319	9	56±7	163±19*	3.2±0.4	96±19*

Data are mean±s.e.mean; * $P < 0.05$ versus Ang II (analysis of variance followed by Dunnett's test).

In conclusion, there is no evidence that AT_2-receptor antagonism augments Ang II mediated coronary vasoconstriction in rat isolated heart. Furthermore, a component of the deleterious effect of Ang II on IR injury, which is unrelated to coronary vasoconstriction, is mediated to AT_2 receptor stimulation.

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48P EFFECTS OF sCR1sLe^x ON THE INFARCT SIZE FOLLOWING REGIONAL MYOCARDIAL ISCHAEMIA AND REPERFUSION IN THE ANAESTHETISED RAT

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

There is evidence that the activation of the complement system (Smith *et al.*, 1993) and the expression of adhesion molecules (Weyrich *et al.*, 1995) in response to myocardial ischaemia may initiate a number of pathophysiologic responses that contribute to the progression of myocardial ischaemia and reperfusion injury. This study investigated the effects of sCR1sLe^x, an agent which functions both as a complement inhibitor (e.g. inhibits the activation of C3 and C5) and a selectin antagonist (e.g. blocks E- and P-selectin mediated cell adhesion), on the infarct size caused by regional myocardial ischaemia and reperfusion in the rat (Bertino *et al.*, 1996).

Thirty-male Wistar rats (210-310 g) were anaesthetised with thiopentone sodium (120 mg·kg^{-1} i.p.). All animals were tracheotomised and ventilated (tidal volume: 10 ml·kg^{-1}, 70 strokes·min^{-1}, inspiratory oxygen-concentration: 30%, positive end-exspiratory pressure: 1-2 mmHg). The carotid artery was cannulated to measure mean arterial blood pressure (MAP) and the jugular vein was cannulated for the administration of drugs. The chest was opened by a left-sided thoracotomy, the pericardium incised and an atraumatic needle was placed around the left anterior descending coronary artery (LAD). The animals were allowed to recover for 30 min and subsequently the LAD was occluded for 30 min and then reperfused for 2 h. At the end of the experiment, the LAD was re-occluded, and 1 ml of Evans Blue dye (2% w/v) was injected into the jugular vein to determine the perfused and the non-perfused (area at risk, AR) myocardium. Infarct size (IS) was determined by incubation of the slices of the heart with p-nitro-blue tetrazolium (NBT, 0.5 mg·ml^{-1}).

The following groups were studied: **(1)** LAD-occlusion plus injection of vehicle (3.4 ml·kg^{-1} of PBS) at 5 min prior reperfusion of the LAD (n=10), **(2)** LAD-occlusion plus injection at 5 min prior to reperfusion of sCR1sLe^x (15 mg·kg^{-1} bolus injection over 30 s, n=10) or **(3)** sCR1sLe^x (5 mg·kg^{-1}, n=10).

Data are expressed as mean±s.e.mean. Statistical differences were analysed by ANOVA followed by a Bonferoni's test.

The mean values for the AR ranged from 51±3 % to 54±3 % and, hence, were similar in all animal groups studied ($p>0.05$). In rats which had received an infusion of the vehicle (PBS) for sCR1sLe^x, occlusion of the LAD (for 30 min) followed by reperfusion (for 2 h) resulted in an infarct size of 61±2% of the AR (control, n=10). When compared to vehicle, injection of sCR1sLe^x (15 mg·kg^{-1} i.v., n=10) caused a significant reduction in IS of approximately 50 % (Figure 1). Interestingly, the lower dose of sCR1sLe^x used (5 mg·kg^{-1} i.v., n=10) caused a similar reduction in IS (Figure 1). None of the drugs used had any haemodynamic effects.

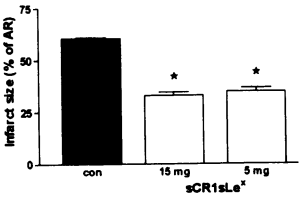

Figure 1. Effects of sCR1sLe^x on myocardial infarct size after LAD-occlusion and reperfusion.

Thus, sCR1sLe^x causes a substantial reduction in myocardial infarct size without causing any apparent side effects. The mechanism of the cardioprotective effects of this agent warrants further investigation but may involve inhibition of complement-activation and prevention of PMN-adhaesion.

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49P REDUCTION OF MYOCARDIAL INJURY BY THE EP_3 RECEPTOR AGONIST TEI-3356 IN THE RAT MAY INVOLVE THE ACTIVATION OF PKC AND OF K_{ATP}-CHANNELS IN THE RAT

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

We have recently reported that the EP_3-receptor agonist ONO-AE-248 reduces the infarct size caused by myocardial ischaemia and reperfusion in the rat (Zacharowski *et al.*, 1998). This study investigated the effects of the prostanoid EP_3-receptor agonist TEI-3356 (Negishi *et al.*, 1994) on the infarct size caused by regional myocardial ischaemia and reperfusion in the rat. In addition, we have also elucidated the role of protein kinase C (PKC) and ATP-sensitive potassium (K_{ATP}) channels in the cardioprotective effects of TEI-3356.

Sixty-four male Wistar rats (240-350 g) were anaesthetised with thiopentone sodium (120 $mg \cdot kg^{-1}$ i.p.). All animals were tracheotomised and ventilated (tidal volume: 10 $ml \cdot kg^{-1}$, 70 $strokes \cdot min^{-1}$, inspiratory oxygen-concentration: 30%, positive end-exspiratory pressure: 1-2 mmHg). The carotid artery was cannulated to measure mean arterial blood pressure (MAP) and the jugular vein was cannulated for the administration of drugs. The chest was opened by a left-sided thoracotomy, the pericardium incised and a needle was placed around the left anterior descending coronary artery (LAD). The animals were allowed to recover for 30 min and subsequently the LAD was occluded for 25 min and then reperfused for 2 h. At the end of the experiment, the LAD was re-occluded and 1 ml of Evans Blue dye (2% w/v) was injected into the jugular vein to determine the perfused and the non-perfused (area at risk, AR) myocardium. Infarct size (IS) was determined by incubation of the slices of the heart with p-nitro-blue tetrazolium (NBT, 0.5 $mg \cdot ml^{-1}$). The following groups were studied: No LAD-occlusion and infusion of **(1)** vehicle (saline, n=3), **(2)** TEI-3356 (TEI, 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$, n=3), **(3)** 5-hydroxydecanoate (5-HD, 5 $mg \cdot kg^{-1}$, n=3), **(4)** staurosporine (Stau, 1 $\mu g \cdot kg^{-1}$, n=3), **(5)** chelerythrine (Chel, 0.7 $mg \cdot kg^{-1}$, n=3), and LAD-occlusion and infusion of **(6)** vehicle (control, n=8), **(7)** TEI (n=8), **(8)** 5-HD (n=6), **(9)** 5-HD + TEI (n=6), **(10)** Stau (n=6), **(11)** Stau + TEI (n=6), **(12)** Chel (n=6) and **(13)** Chel + TEI (n=5). All infusions started 10 min prior LAD-occlusion. Data are expressed as mean±s.e.mean. Statistical differences were analysed by ANOVA followed by a Bonferoni's test.

Treatment of rats with TEI-3356 resulted in a significant reduction in IS from 60±3% (control) to 36±3% ($p<0.05$) of the AR. TEI-3356 did not cause a reduction in blood pressure or pressure-rate index. Pretreatment of rats with either 5-HD (inhibitor of K_{ATP}-channels, Garlid *et al.*, 1997), Stau or Chel (inhibitors of PKC, Brooks *et al.*, 1996) attenuated the cardioprotective effects of TEI-3356 (Tab. 1). However, neither 5-HD, Stau or Chel alone did affect IS (Tab. 1). In sham-operated animals, none of the drugs used had any affect on any of the parameters measured.

Table 1: Effects of TEI-3356 and inhibitors of PKC and of K_{ATP}-channels on myocardial infarct size.

	IS (%)	AR (%)		IS (%)	AR (%)
control	60±3	46±2	Stau	66±2	49±3
TEI	36±3*	54±3	Stau+TEI	53±7	48±4
5-HD	63±3	46±3	Chel	59±7	50±3
5-HD+TEI	56±3	51±2	Chel+TEI	59±10	53±3

Thus, TEI-3356 reduces myocardial infarct size in the rat by a mechanism(s) which may involve the activation of PKC and the opening of K_{ATP}-channels.

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50P STUDIES WITH CGS 26303, AN INHIBITOR OF ENDOTHELIN CONVERTING ENZYME, SUPPORTS THE PRESENCE OF INTRACELLULAR ENDOTHELIN-CONVERTING ENZYME IN HUMAN VASCULAR SMOOTH MUSCLE CELLS

Mandy Woods, Elizabeth G. Wood, [1]Jane A. Mitchell and Timothy D. Warner.

Vascular Inflammation. The William Harvey Research Institute, Charterhouse Square, London EC1M 6BQ and [1]Unit of Critical Care Medicine, Royal Brompton Hospital, London SW3 6NP.

Exposure of human vascular smooth muscle cells (HVSMCs) to cytokines and lipopolysaccharide markedly elevates release of endothelin-1 (ET-1) into the culture medium (Woods *et al.*, 1998a). This cytokine stimulated release of ET-1 is inhibited by phosphoramidon, an endothelin converting enzyme (ECE) inhibitor. However, phosphoramidon inhibits the conversion of exogenous big ET-1 more potently than it inhibits the endogenous production of ET-1. This implies that HVSMCs produce mature ET-1 via a pathway dependent upon intracellular ECE (Woods *et al.*, 1998b). Here, using other known inhibitors of ECE, namely CGS 26303 and CGS 26393 we have attempted to characterise further the synthetic pathway present within cytokine treated HSVMCs.

Saphenous vein (SV) and internal mammary artery (IMA) were obtained from patients undergoing coronary artery bypass graft surgery. Explants of HVSMCs were grown in DMEM supplemented with 2mM glutamine, streptomycin ($100 \mu g.ml^{-1}$), penicillin ($100U.ml^{-1}$) and 15% foetal calf serum (37°C; 5% CO_2; 95% air). HVSMCs were identified by α-actin staining. Cells in culture grown on 96 well plates were treated with a mixture of tumour necrosis factor-α ($10ng.ml^{-1}$) and interferon-γ ($1000U.ml^{-1}$) for 48h in the presence of CGS 26303, CGS 26393 (prodrug for CGS 26303) and CGS 24592 (1nM-1mM). Alternatively, HVSMCs were treated with cytokines as above for 24h, medium replaced and cells then incubated with big ET-1 (1μM) for 2h in the presence of CGS26303. ET-1 and big ET-1 levels were measured by specific sandwich ELISA (R&D Systems, Assay Designs, Inc.). CGS 26303 caused a concentration-dependent inhibition of the cytokine induced release of ET-1 from IMA and SV (Table 1). Similiarly, CGS 26393 inhibited endogenous ET-1 release with a log IC_{50} value of –4.4±0.09 in IMA and of –3.8±0.08 (n=5) in SV HVSMCs. This inhibition of endogenous ET-1 production was accompanied by matched increases in the accumulation of big ET-1. CGS 24592, a neutral endopeptidase inhibitor lacking ECE inhibitory activity had no effect on cytokine-stimulated ET-1 production. CGS 26303 inhibited the conversion of exogenous big ET-1 about 10 times more potently than the endogenous production of ET-1 (Table 1).

CGS 26303	Exogenous ET-1 (log IC_{50} values)	Endogenous ET-1 (log IC_{50} values)
IMA	-4.3±0.13	-3.5±0.09*
SV	-4.8±0.15	-3.6±0.08**

Table 1. Inhibitory effect of CGS 26303 on the formation of ET-1 by HVSMCs (*$p<0.001$, **$p<0.0001$ unpaired t test).

These results support our previous conclusion that HVSMCs contain an intracellular ECE responsible for the endogenous synthesis of ET-1. Therefore, under the influence of pro-inflammatory cytokines the vascular smooth muscle can become an important site of ET-1 production.

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51P THE POLY(ADP-RIBOSE) SYNTHETASE INHIBITOR 3-AMINOBENZAMIDE REDUCES ISCHAEMIA-REPERFUSION INJURY IN THE KIDNEY OF THE RAT *IN VIVO*

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

Generation of reactive oxygen species (ROS) has been implicated in the pathogenesis of renal ischaemia-reperfusion injury (Paller *et al.*, 1984). ROS produce DNA strand breaks which leads to the activation of the DNA-repair enzyme poly(ADP-ribose) synthetase (PARS) (Schraufstatter *et al.*, 1986). During episodes of oxidant stress, excessive PARS activation can result in depletion of its substrate NAD and subsequently of ATP, leading to cellular dysfunction and eventual cell death (Schraufstatter *et al.*, 1986). We have previously reported that 3-aminobenzamide (3-AB) inhibits ROS-mediated PARS activation in rat renal proximal tubular cells and protects them against ROS-mediated cellular injury and death (Chatterjee & Thiemermann, 1998). The aim of this study was to investigate the effect of 3-AB in a model of renal ischaemia-reperfusion injury in the anaesthetised rat.

Eighteen male Wistar rats (240-280 g) were anaesthetised with sodium thiopentone (120 $mg \cdot kg^{-1}$ i.p.) and tracheotomised to facilitate spontaneous respiration. The carotid artery was cannulated to monitor mean arterial blood pressure and the jugular vein was cannulated for administration of drugs. Following a midline laparotomy and isolation of the renal pedicles, the rats were allowed to recover for 30 min before being divided into three groups. Group 1 (sham rats, n=6) were maintained under anaesthesia for the duration of the experiment (45 min + 6 h). Group 2 (control rats, n=6) were subjected to bilateral renal pedicle clamping for 45 min followed by reperfusion for 6 h. Group 3 (3-AB treated rats, n=6) were manipulated as described for Group 2 but were given a bolus injection of 3-AB (10 $mg \cdot kg^{-1}$, in saline, i.v.) 1 min prior to beginning reperfusion followed by an infusion of 3-AB (5 $mg \cdot kg^{-1} \cdot h^{-1}$, in saline, i.v.). Ischaemia-reperfusion injury was assessed by measurement of the plasma levels of creatinine, aspartate aminotransferase (AST) and γ-glutamyl transferase (γ-GT).

	Creatinine ($\mu mol \cdot l^{-1}$)	AST ($iu \cdot l^{-1}$)	γ-GT ($iu \cdot l^{-1}$)
Group 1 (shams)	36.3 ± 2.4	166 ± 19	0.7 ± 0.4
Group 2 (controls)	255 ± 50 *	1379 ± 145 *	4.5 ± 0.9 *
Group 3 (3-AB)	142 ± 6.4 +	663 ± 129 +	1.8 ± 0.5 +

Table 1: Effect of 3-AB on biochemical markers of ischaemia-reperfusion injury (*p < 0.05 vs. shams, +p < 0.05 vs. controls, n=6). Data are expressed as mean ± s.e.mean and were analysed using one-way ANOVA followed by the Bonferroni's post significance test. A p value of less than 0.05 was considered to be significant.

Bilateral renal clamping followed by reperfusion of the kidneys produced increases in serum creatinine, AST and γ-GT; all of which are biochemical markers of ischaemia-reperfusion injury (table 1). Infusion of 3-AB prior to and during reperfusion of previously ischaemic kidneys reduced the degree of ischaemia-reperfusion injury (table 1). These results demonstrate that the PARS inhibitor 3-AB reduces the ischaemia-reperfusion injury in rat kidneys *in vivo* and suggest that activation of PARS may contribute to renal reperfusion-injury.

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52P ACUTE SYSTEMIC INFLAMMATION IMPAIRS ENDOTHELIUM-DEPENDENT DILATATION IN HUMANS

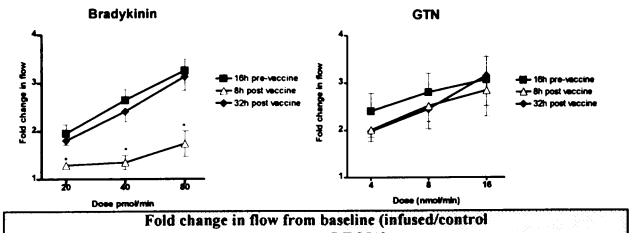
A.D Hingorani, K. Bhagat, M. Palacios, G.E. Griffin†, R.J. MacAllister, P. Vallance. Centre for Clinical Pharmacology, WIBR, UCL, London WC1E 6JJ, †Division of Infectious Diseases, School of Medicine, St.George's Hospital, London W12 ORE.

Chronic systemic infection and inflammation are implicated in the development of atherosclerosis and an acute infective or inflammatory episode transiently increases the risk of a cardiovascular event. However, the mechanisms of these associations are unclear. Since endothelial dysfunction underlies a variety of cardiovascular diseases, we examined the effect of a transient inflammatory stimulus on the function of the arterial endothelium in humans.

A mild, transient systemic inflammatory response was generated in 6 healthy male volunteers (age 30 to 37 years) by intramuscular injection of 0.025 mg *S. typhi* capsular polysaccharide vaccine. Venous occlusion plethysmography was used to measure the forearm blood flow during intra-arterial infusion of incremental doses of the "endothelium-dependent" dilators acetylcholine (ACh) and bradykinin (BK) and the "endothelium-independent" dilators glyceryl trinitrate (GTN) and verapamil (Ver). Blood flow measurements were made 16h before, and again 8h and 32h after vaccination. Pulse and blood pressure measurement and venous sampling for the measurement of cytokines was performed hourly for 8 hours after vaccination.

Following vaccination, white cell count rose from 4.0 (±0.3) $\times 10^9$/L to 6.1 (±0.5) $\times 10^9$/L at 8h ($p<0.01$), serum levels of interleukin (IL)-6 rose from 2.1 (±0.4) pg/ml pre-vaccine to 5.8 (±3.2) pg/ml 8h post vaccine ($p=0.07$), and the level of IL-1 receptor antagonist rose from 188.0 (±35.9) pg/ml to peak at 593.6 (±198.0) pg/ml after 3h, a 191% increase from baseline ($p<0.05$). There was no change in temperature, heart rate, blood pressure, the plasma levels of IL-1 or tumour necrosis factor α, or in resting forearm blood flow. Prior to vaccination, there was a dose-dependent increase in forearm blood flow to all the agonists studied. After vaccination, there was a profound, selective, but temporary blunting in the dilator response to endothelium-dependent dilators BK and ACh with no significant change in the reponse to GTN or Ver (Table and figure; (*$p<0.05$ by ANOVA of dose-response curves). In the absence of vaccination, there was no change in response to either endothelium-dependent or endothelium-independent dilators when studies were conducted 24h apart (n=3).

Forearm blood flow response to BK and GTN following vaccination



Fold change in flow from baseline (infused/control arm) [mean S.E.M.)]								
ACh								
Pre vaccine Dose (nmol/min)			8h post vaccine Dose (nmol/min)			16h post vaccine Dose (nmol/min)		
25	50	100	25	50	100	25	50	100
2.0 (0.4)	2.1 (0.5)	3.7 (0.5)	1.6* (0.4)	2.0* (0.6)	1.9* (0.5)	2.9 (0.8)	3.3 (0.8)	3.8 (0.5)
Verapamil								
Pre vaccine Dose (nmol/min)			8h post vaccine Dose (nmol/min)			16h post vaccine Dose (nmol/min)		
20	40	80	20	40	80	20	40	80
2.3 (0.6)	2.3 (0.5)	3.3 (1.2)	2.3 (0.5)	2.3 (0.5)	2.6 (0.6)	2.0 (0.2)	2.1 (0.2)	2.5 (0.2)

Vaccination with capsular polysaccharide typhoid vaccine generates a mild systemic inflammatory response which produces temporary but profound dysfunction of the arterial endothelium. If the inflammation which follows naturally-occuring infective or inflammatory episodes perturbs the normal function of the vascular endothelium in the same way, this might contribute to the observed association between infection and inflammation and the enhanced risk of an acute cardiovascular event.

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53P ENHANCEMENT OF ENDOTHELIUM-DEPENDENT VASODILATATION BY OESTRADIOL 17-β: ROLE OF THE PTERIN PATHWAY

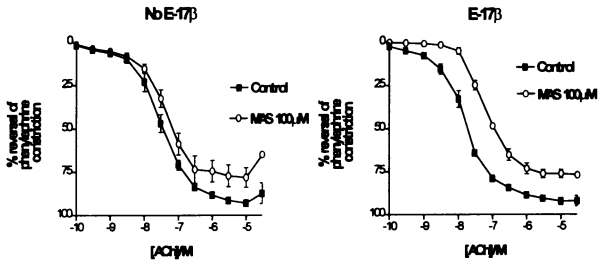
Z.Chen, A. Ahluwalia, D.L. Selwood[#], P Vallance, **A.D Hingorani**. Centre for Clinical Pharmacology, [#]Bioorganic and Medicinal Chemistry, WIBR, UCL, London WC1E 6JJ.

Oestradiol 17-β (E-17β) has vasodilator actions which may contribute both to atheroprotection in women and to the reduced vascular resistance of pregnancy. At physiological concentrations (nM), E-17β potentiates vasodilatation to endothelium-dependent agonists e.g. acetylcholine (ACh), possibly as a result of increased expression of endothelial nitric oxide synthase (eNOS). When inducible NOS expresssion is increased by lipopolysaccharide (LPS), there is also co-induction of GTP cyclohydrolase-1 (GTPCH-1) the first (and rate-limiting step) in the biosynthesis of tetrahydrobiopterin (BH_4) (an essential co-factor for NOSs). We hypothesised that, in an analagous manner, E 17-β might co-induce the activity of endothelial NO and pterin pathways.

Male Sprague-Dawley rats were killed by dislocation of the neck, the thoracic aorta removed, cleaned and cut into 4mm rings. Endothelium-intact rings were suspended at 1g resting tension in tissue baths containing oxygenated Krebs solution (±E-17β; 30nM) at 37°C. Tissues were precontracted with phenylephrine to 90% of the contractile response to KCl (48mM) and concentration response curves (CRC) to ACh (10^{-10}-10^{-6}M) [or the NO donor spermine-NO (10^{-10}-10^{-6}M)] were constructed. Relaxations were measured before and after incubation for 3h with methoxyacetylserotonin (MAS; 1, 10 or 100μM - an inhibitor of sepiapterin reductase the terminal enzyme in BH_4 biosynthesis), 2,4,diamino-6-hydroxypyrimidine (DAHP; 1.5 mM - an inhibitor of GTPCH-1), or vehicle. In separate experiments, 15mm segments of thoracic aorta were incubated for 3h with E-17β (30nM), lipopolysaccharide (1μg/ml), or vehicle, and expression of eNOS, iNOS and GTPCH-I was detected by Western blotting.

E-17β (30nM) caused a leftward shift of the ACh CRC with EC_{50}[95%CI] values of 32nM[25-41] and 16nM[14-19] in the absence and presence of E-17β respectively ($p<0.01$, 2-way ANOVA, n=5). In contrast, there was no change in spermine-NO-induced relaxation (n=3). E-17α (a non-functional isomer of E-17β) had no effect on ACh relaxation (EC_{50} values 56nM[36-86] in both treated and untreated vessels. MAS produced a greater inhibition of ACh-induced relaxation in E-17β-treated vessels compared to untreated controls (Table and Figure).

(Figure) MAS attenuates ACh-mediated relaxation



(Table) EC_{50} for ACh in control and MAS-treated tissues in presence or absence of E-17β(n=4-8)

	Control	MAS concentration (μM)			
		1	10	100	
Mean EC_{50} for ACh (nM)[CI]	32[25-41]	33[24-47]	38[30-50]	40[29-58]	- E-17β**
	16[14-19]	25[19-33]	36[29-45]	61[52-72]	+ E-17β**

**p<0.0001 for MAS effect by 2-way ANOVA

DAHP also attenuated ACh relaxation in E-17β-treated vessels (EC_{50} 36nM [21-63] in its absence and 65nM [48-88] in its presence; p=0.02, n=5) but not in untreated vessels (n=5). GTPCH-1 immunoreactivity was enhanced in tissues treated with LPS or E-17β, but not in tissues incubated with saline vehicle. iNOS immunoreactivity was detected only in LPS-treated tissues, and eNOS immunoreactivity in all tissues. These data are consistent with the hypothesis that or E-17β enhances ACh-mediated relaxation by co-enhancement of the activities of the pterin and endothelial NO pathways. **A.D.H. and A.A. are both BHF Intermediate Fellows.** We are grateful to Prof I Nagatsu for the kind gift of anti-GTPCH-1 antibody.

54P ATHEROSCLEROSIS-ASSOCIATED $G_{\alpha i-1}$ OVEREXPRESSION INDUCES CHANGES IN RECEPTOR COUPLING AND ENDOTHELIN-1 PRODUCTION IN HUMAN CULTURED CORONARY ENDOTHELIAL CELLS

A. Blanchette, T.-D. Nguyen, E. Thorin, Montreal Heart Institute, Research Center, 5000 Belanger Street, Montreal, Qc, H1T 1C8, Canada.

Patients with coronary artery disease show elevated plasma endothelin (ET) levels. Furthermore, intracoronary infusion of acetylcholine increases plasma ET concentration in these patients which is associated with a paradoxical constriction to acetylcholine. Such altered responses may be the consequence of a change in receptor coupling and/or G protein function. To confirm this hypothesis, we examined whether basal and stimulated immunoreactive-(ir) ET release was altered in cultured coronary endothelial cells from atherosclerotic patients and if a change in G protein function occured.

Rings of epicardial coronary arteries (external diameter ≤ 450 μm) free of atherosclerotic lesions were sampled from hearts of patients undergoing transplantation for ischemic/atherosclerotic (ICM, n=3) or non-ischemic/dilated (DCM, n=4) cardiomyopathies. Endothelial cells were isolated by cloning and characterized by positive immunostaining for factor VIII/von Willebrand factor (1/25 dilution) and negative staining for smooth muscle α-actin (1/400 dilution). Cell lines were used at passages 2 through 4. G protein expression in endothelial cell membranes was quantified by Western blot analysis. Basal and stimulated release of ir-ET in serum-free culture medium were measured in duplicate by radio-immunoassay. Results are expressed as mean±s.e.m. All experimental protocols have been performed in each cell lines in duplicate.

Expression of $G\alpha_{i-1}$ proteins was 5 fold increased ($P<0.05$) in coronary endothelial cells from ICM hearts without change in $G\alpha_{i-2}$ and $G\alpha_q$ protein expression compared to DCM hearts. Basal ir-ET release was greater ($P<0.05$) in cultured endothelial cells isolated from coronary arteries of ICM than DCM hearts (3.3±0.3 and 1.4±0.1 pg/μg protein/30 min, respectively). *Pertussis* toxin (PTX; 50 ng/mL, 4hrs) given to inactivate $G\alpha_{i/o}$ proteins, had no effect on basal ir-ET release in coronary endothelial cells from DCM hearts (1.5±0.1 pg/μg protein/30 min) but decreased it ($P<0.05$) in cells from ICM hearts (2.3±0.3 pg/μg protein/30 min). Oxymetazoline (OXY, 10 μmol/L), an α_2-adrenergic receptor agonist, increased ($P<0.05$) ir-ET release more ($P<0.05$) in coronary endothelial cells from ICM (+1.4±0.2 pg/μg protein/30 min) than in cells isolated from DCM hearts (+0.46±0.04 pg/μg protein/30 min). PTX augmented OXY-induced ir-ET release in coronary endothelial cell from DCM hearts (+2.1±0.1 pg/μg protein/30 min; $P<0.05$). In contrast, PTX suppressed ir-ET release triggered by OXY in cells isolated from ICM hearts (+0.2±0.1 pg/μg protein/30 min; $P<0.05$).

In summary, inactivation of $G_{i/o}$ proteins facilitates OXY-induced ir-ET release. In coronary endothelial cells from patients with coronary artery disease, inactivation of $G_{i/o}$ proteins decreases basal ir-ET release and inhibits OXY-mediated ir-ET release. These data demonstrate that $G_{i/o}$ proteins are constitutively active in cultured coronary endothelial cells from atherosclerotic patients. Secondly, they show that α_2-adrenergic receptor coupling is changed in tissues from patients with ischemic heart disease.

In conclusion, we suggest that $G\alpha_{i-1}$ protein over-expression is responsible for the change in G protein function and receptor coupling.

55P MODULATION OF ENDOTHELIN RECEPTOR DENSITIES FOLLOWING ENDOGENOUS OVEREXPRESSION OF ENDOTHELIN-1 IN THE RAT

[1,2] S. Télémaque-Potts, [2]M. Yanagisawa & [1]A.P. Davenport
[1]Clinical Pharmacology Unit, University of Cambridge, Box 110, Addenbrooke's Hospital, Cambridge, CB2 2QQ, UK
[2]Department of Molecular Genetics, H.H.M.I., UT Southwestern Medical Center, Dallas, TX, USA 75235.

Adenovirus-mediated hepatic overexpression of endothelin-1 (ET-1), to plasma levels observed in various vascular diseases, leads to systemic hypertension in rats (Niranjan *et al.*, 1996). Since ET-1 can activate both ET_A and ET_B receptors, we were interested in studying the modulation of these receptors under pathological conditions. Receptor binding studies were performed on three different tissues: the liver, where the genes are primarily overexpressed, the heart and the kidney, two tissues highly sensitive to the actions of ET-1.

Methods. Wistar rats (male, 250-350 g, n=14) were injected with $5x10^9$ pfu/ml of adenovirus containing either human prepro-endothelin-1 gene (Ad.CMV.ET-1, n=7) or β–galactosidase gene, used as control (Ad.CMV.β–gal, n=7). Ninety-six hours later, animals were anaesthetised (xylazine and ketamine: 15 and 90 mg/kg, i.m.) and blood pressure was monitored to confirm systemic hypertension. Tissues were collected, washed in cold PBS, frozen in liquid nitrogen and kept at -80 ^{0}C. Cryostat sections of tissue (10 μm) were incubated with a fixed concentration of [^{125}I]-ET-1 (0.1 nM) and increasing concentrations of unlabelled selective ET_A receptor antagonist, PD156707 (10 pM-100 μM), for 2 hr at 22 ^{0}C (Maguire *et al.*, 1997). Data were analysed using RADLIG 4.0 (Biosoft) and were compared using Student's two-tailed *t*-test. P values lower than 0.05 were considered significant.

Results. From the competition binding studies in the liver affinities for ET receptors in the Ad.CMV.ET-1 group (K_D ET_A: 0.15 nM; ET_B: 103 nM; n=6) were similar to those observed in the control group: (K_D ET_A: 0.40 nM, ET_B: 94 nM, n=6). However, there was a significant reduction of both ET receptor densities when compared to control (from 28 ± 5 to 12 ± 4 fmol/mg protein and 67 ± 7 to 33 ± 7 fmol/mg protein, $P<0.05$). In the heart, affinity values for ET receptors did not differ from the control group (K_D ET_A: 0.23 vs. 0.32 nM, ET_B: 43 vs. 47 nM, respectively, n = 6-7/group) However, the ET_A receptors density is significantly reduced (from 112 ± 8 to 82 ± 7 fmol/mg protein, $P<0.05$) in the Ad.CMV.ET-1 group. In the kidney, the ET receptor affinities were similar between Ad.CMV.ET-1 and Ad.CMV.β–gal animals (K_D ET_A: 0.24 vs. 0.87 nM, ET_B: 96 vs. 137 nM, respectively, n = 6/group). Receptor densities were not altered in the Ad.CMV.ET-1 group (B_{max} for ET_A: 15 ± 5 vs. 19 ± 5 fmol/mg protein, B_{max} for ET_B: 93 ± 11 vs. 82 ± 8 fmol/mg protein, respectively, n = 6/group).

These data show that elevated levels of plasma ET-1 associated with systemic hypertension lead to tissue-specific modulation of ET receptors. The compensatory downregulation of ET_A receptors in the heart supports the proposed role of endothelin system in the development of cardiovascular diseases.

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56P MODULATION OF CONTRACTION OF VASA VASORUM: MECHANISMS AND IMPLICATIONS FOR CONDUIT VESSEL PHYSIOLOGY

R.S. Scotland, P.J. Vallance & A. Ahluwalia. Centre for Clinical Pharmacology, UCL, London WC1E 6JJ.

The walls of certain large blood vessels are nourished by the vasa vasorum, a network of microvessels which penetrate the adventitia and media of the vessel wall. Recently we have demonstrated that isolated porcine aortic vasa contract in response to endothelin-1 (ET-1) but not to certain other vasoconstrictors (Scotland *et al.*,1997). We have now characterised the responses to ET-1 and investigated the possibility that changes in basal tension alters the profile of contractile reactivity of the vasa vasorum

Porcine aortas were collected from an abattoir. Small arteries penetrating the aorta were dissected out and mounted in a tension myograph. The internal diameter was determined (Mulvany & Halpern,1977) (142 ± 3 μm, n=130) and vessels repeatedly contracted with 125mM K^+ (KPSS) until the contraction was constant. Contractile concentration response curves were then constructed to ET-1 (0.01-300nM) or ET_B agonist sarafotoxin 6c (S6c, 0.01-100nM) in the absence and presence of ET_A antagonist, BQ123 (10μM), ET_B antagonist, BQ788 (1μM) or a combination of both. In a separate study concentration-response curves were constructed to noradrenaline (NA) or thromboxane A_2 -mimetic (U44069) in the absence and then presence of ET-1 (1-3 nM) or K^+ (10-20 mM) to raise basal tone by approximately 10%. The results are expressed as % of the initial response to KPSS.

To determine the source of Ca^{2+} involved in contraction of vasa in contractile response curves were constructed in Ca^{2+}-free Krebs (+ 2mM EGTA) or in the presence of cyclopiazonic acid (CPA;10 μM,30 min) an inhibitor of sarcoplasmic reticulum Ca-ATPase, nifedipine (10μM,30 min) an L-type Ca^{2+} channel blocker, or SKF 96365 (30μM,30 min) a purported inhibitor of receptor-operated Ca^{2+} channels or Ni^{2+} (1mM,60 min.), an inhibitor of non-selective cation entry.

ET-1 (n=32) and S6c (n=7) produced concentration-dependent contractions giving EC_{50} values of 15.3 ± 0.8 nM and 3.6 ± 0.2 nM respectively. BQ123 caused a rightward shift of ET-1 response curve which became biphasic. BQ788 caused a 3.5-fold and 80-fold shift of curves to ET-1 and S6c respectively. Pretreatment with BQ123+ BQ788 caused a monophasic 17-fold shift of ET-1 curves. ET-1 responses were abolished in Ca^{2+}-free Krebs and attenuated in the presence of nifedipine (44%), SKF 96365 (61%), nifedipine + SKF 96365 (75%) or Ni^{2+} (55%). Partial precontraction with ET-1 significantly ($P<0.01$) potentiated responses to NA (n=7) but not to U44069 (n=4) whilst raised K^+ enhanced responses to both constrictors. Max responses to NA were 9.7 ± 1.5% and 33.0 ± 4.8% in the absence and presence of ET-1 respectively; these responses were abolished by nifedipine but unaffected by pretreatment with CPA (n=4). In the absence and presence of K^+ max responses were 7.9 ± 2.1% and 46.5 ± 9.4% for NA and 2.8 ± 1.8% and 83.5 ± 6.6 % for U44069; the latter being unaffected by CPA (n=7)

In conclusion, contraction to ET-1 of porcine vasa is mediated by ET_A and ET_B receptors and involves influx of Ca^{2+} through L-type and non-L-type channels. Furthermore, elevation of basal tone markedly enhances the reactivity to constrictors which otherwise have little or no effect. Potentiated NA responses are mediated by influx of Ca^{2+} through L-type Ca^{2+} channels. Together our findings suggest that ET-1 may play an important role in the regulation blood flow through vasa vasorum.

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57P EFFECT OF MIBEFRADIL AND VERAPAMIL ON ELECTRICAL FIELD STIMULATION-INDUCED CONTRACTIONS IN THE RAT TAIL ARTERY

R. van der Lee, M. Pfaffendorf & P.A. van Zwieten. Dept. Pharmacotherapy, Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

In 1997 Göthert and Molderings showed that mibefradil could block the ω-conotoxin GVIA-sensitive sympathetic noradrenaline release in human right atrial appendages with an IC_{50}-value of 1 μM. Earlier, in 1995, Bezprozvanny and Tsien already showed that mibefradil is capable of blocking the N-type calcium channel, in addition to the L- and T-type calcium channels. Calcium influx through the N-type calcium channel triggers noradrenaline release from sympathetic nerve terminals. The aim of our study was to test whether mibefradil would be able to exert a stronger effect than the L-type calcium antagonist verapamil on endogenous noradrenaline release provoked by electrical field stimulation (EFS) in the rat tail artery. For comparison the effect of both CA on contractions caused by exogenous noradrenaline was observed. Male Wistar rats (weight 243 ± 7 g) were stunned by a blow on the head and then decapitated. The tail was cut off by the base and the tail artery exposed. From each rat, four segments (diameter 662 ± 12 μm) of approximately 2mm length (n=4-6 for each concentration) were isolated and a 40 μM stainless steel wire was inserted into the lumen of the vessel. These preparations were transferred to the organ chambers of an isometric wire myograph. The experimental protocol consisted of an initial stimulation with frequencies ranging from 0.25 to 4 Hz. After 15 min rest the preparations were stimulated with a frequency of 4 Hz for 30 s which served as the reference contraction. The vessels were incubated with either mibefradil or verapamil (concentration range 0.3-10 μM) for 15 min. With intervals of 15 min the vessels were stimulated three times with 4 Hz (30 s) in the presence of either CA. For the calculation of the log IC_{50}-values and the E_{max} the third EFS-induced contraction was used. In control experiments with tetrodotoxin (1 μM), guanethidine (1 μM) and prazosin (1 μM) it was established that the contractions induced by EFS were nerve-mediated and resulted from at least 80% α_1-receptor activation. Repeated EFS-induced contractions remained stable for at least six consecutive stimulations. The experiments with exogenous noradrenaline started with a priming procedure of two contractions with 1 μM noradrenaline with an interval of 15 min. Then a baseline concentration-response curve (CRC) was constructed (0.03-3 μM). The vessels were incubated with either mibefradil or verapamil (concentration range 0.3-10 μM) for 15 min. With intervals of 15 min three CRC's were constructed. The third CRC was used for further analysis. The effect on the EFS-induced contraction of both CA was concentration-dependent. Log IC_{50}values could be calculated for both CA: -6.43 ± 0.06 and -5.75 ± 0.10 for verapamil (n=4) and mibefradil (n=6), respectively. These values differ statistically significant from each other (p=0.0006). The E_{max}-values are expressed as percentage inhibition from the control EFS-induced contraction. Verapamil at the maximum concentration of 10 μM inhibited the EFS-induced contraction by 55± 2 % (n=4). Mibefradil in the same concentration, however, was able to block the EFS-induced contraction almost completely: 98 ± 1% (n=6) ($p<0.0001$). There was no significant difference in the inhibition of the effects of exogenous noradrenaline between mibefradil and verapamil (70 ± 6% and 75 ± 5%, respectively with the CA at 10 μM and noradrenaline at 1 μM) In conclusion, mibefradil appears to inhibit presynaptic N-type calcium channels, which may explain its high efficacy in inhibiting EFS-induced contractions.

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58P "CLASSIC" AS WELL AS "REMOTE" CARDIAC PRECONDITIONING IS PREVENTED BY Hoe 140

C.L. van Heijningen, P.R. Saxena & R.G. Schoemaker, Department of Pharmacology, Erasmus University Rotterdam, The Netherlands.

Cardiac tissue can be protected against infarction by a brief period of cardiac ischemia, classic preconditioning, as well as by mesenteric ischemia, remote preconditioning (Gho *et al.*, 1996). Whereas the mechanism underlying classic preconditioning has been under extensive investigation, for remote preconditioning this is virtually unknown. Since hexamethonium blocks and reperfusion of the mesenteric artery is necessary, a neurogenic pathway, activated during reperfusion seemed to be involved. In the present study, the role of bradykinin is investigated in remote as well as classic preconditioning, using the B_2 receptor antagonist Hoe 140. Under pentobarbital anaesthesia and artificial respiration, rats were subjected to 60 min coronary artery occlusion (CAO), followed by 3 h reperfusion. This was preceded by either no preconditioning, classic preconditioning by CAO, or remote preconditioning by mesenteric artery occlusion (MAO), in the absence or presence of B_2 receptor blockade with Hoe 140 (see Table 1). Heart rate and blood pressure were recorded continuously and body temperature was kept between 36.5 and 37.5 °C. At the end of the protocol, the heart was excised and perfused with cold saline. The coronary artery was reoccluded and the heart was perfused with trypan blue to distinguish between perfused and non-perfused (=risk area, RA) tissue. The RA was stained with nitro blue tetrazolium to identify the irreversibly damaged tissue (=infarct area, IA). Tissue was cut and dried, and RA and IA were expressed as percentage of left ventricular dry weight. Administration of Hoe 140 had no effect on blood pressure or heart rate. Average RA were similar in all groups (ranging from 49±7 to 57±6 %). Results are expressed as IA/RA ratios and summarised in Table 1. Hoe 140 had no effect on non-preconditioned hearts. The cardiac protection by classic preconditioning was completely abolished by Hoe 140. Similarly, cardiac protection by remote preconditioning through brief MAO was blocked by Hoe 140. To verify whether local bradykinin in the mesenteric vascular bed could cause cardiac preconditioning, rats were infused intramesenterically for 5 min with either saline or bradykinin (1 μg/min) (Janssen *et al.*, 1989). Five min later, 60 min CAO and 3 h reperfusion was performed. Intramesenteric bradykinin infusion caused significant cardiac preconditioning (IA/RA ratio: 25±4, n=5 versus saline: 63±7, n=3). In conclusion, our data indicate that in addition to its role in classic preconditioning, bradykinin plays a major role in remote preconditioning.

Table 1: Infarct (IA)/ risk (RA) area ratios in anaesthetised rats.

Preconditioning	Control (n)	Hoe 140 (n)
None	66±3 (8)	58±6 (6)
Classic	37±3* (6)	73±5# (7)
Remote	37±4* (6)	65±7# (7)

Classic: 15 min CAO, 10 min reperfusion; Remote: 15 min MAO, 10 min reperfusion; Hoe 140: 100 μg Hoe 140, i.v. 10 min before preconditioning; *: significant effect of preconditioning; #: significant effect of Hoe 140.

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59P RENAL REPERFUSION INJURY IS AMELIORATED BY ALLOPURINOL

D. Hestin & E.J. Johns, Dept of Physiology, The Medical School, Birmingham B15 2TT

Ischaemia of the kidney causes renal vasoconstriction, tubular obstruction and back leakage of glomerular filtrate, resulting in a decrease in glomerular filtration rate (GFR) and severe tubular damage. During ischaemia, ATP is degraded to hypoxanthine, the substrate for xanthine oxydase (XO). After reperfusion, XO reacts with oxygen to generate free radicals, which are toxic to cellular components (McCord, 1985). Allopurinol (AL) is a potent inhibitor of XO and thus can protect against post-ischaemic injury in many organs (Leff & Repine, 1990).

To determine whether AL could prevent post-ischaemic renal injury, we compared the renal haemodynamic and excretory function [GFR, fractional (%) Na^+ and K^+ excretion (FeNa, FeK)] in differents groups of rats, receiving either 50 or 100 $mg.kg^{-1}$ AL, or pH adjusted saline in equal volume. Male Wistar rats (250-350g) were anaesthetised [halothane in O_2-NO_2 followed by chloralose -urethane]. A femoral artery and vein were canulated for infusion of inulin and arterial pressure measurements. A flank incision exposed the left kidney, whose ureter was canulated and renal artery cleared to place a flowmeter probe for measuring renal blood flow (RBF). After a 30 min stabilisation period, two 15 min clearance periods (CP) were taken to establish control levels.The left renal artery was clamped for 30 min, or left untouched in sham experiments, and function followed for 2h or 6h after reperfusion. Additional CP were taken, two 15 min CP plus two 30 min CP in the 2h study, and two 30 min CP 1h and 5h after reperfusion in the 6h experiments. AL was dissolved with sodium chloride 0.1 N and brought up to a final volume of 2 ml. AL was given i.v. as a bolus, 50% of the dose 15 min before occlusion and 50% 5 min before reperfusion. The following groups were studied: (a) 8 rats were subjected to renal ischaemia and given saline and followed over 2 h; (b) 8 rats underwent the same procedure as (a) and were followed over 6h; (c) and (d) 6 rats in each group were subjected to renal ischaemia and given AL 50 mg kg^{-1}, and followed for (c) 2h and (d) 6h; groups (e) and (f) were similar to groups (c) and (d) but given AL 100 mg kg^{-1}. Values are expressed as mean±s.e. ANOVA with post hoc tests was used as a statistical test to compare changes over time between the groups (a), (c) and (e), and (b), (d) and (f).

Reperfusion caused a marked fall in GFR, from basal levels of 4.9±0.3 ml min^{-1} kg^{-1} (group a), which was significantly less in the AL groups ($P<0.001$). The recovery in GFR 2 and 6h was faster in groups (c), (d), (e) and (f), all $P<0.001$, which received AL. FeNa, at a basal level of 2±0.2% (group a), was markedly elevated following reperfusion, but in the presence of AL the increase was smaller, and the rate of recovery faster than in untreated rats ($P<0.02$). AL had no significant effect on basal mean arterial pressure (101±5 in group a), RBF (29±3 ml min^{-1} kg^{-1}), or FeK.

table1 Mean values of GFR (ml min^{-1} kg^{-1}) and FeNa (%) at the end of experiments

	Untreated		AL 50 mg kg^{-1}		AL 100 mg kg^{-1}	
	(a) n=8	(b) n=8	(c) n=6	(d) n=6	(e) n=6	(f) n=6
GFR	0.8±0.1	1±0.1	2.4±0.3	2.9±0.3	2.8±0.5	2.8±0.3
FeNa, %	28±6	12±4	14±3	7±2	5±1	5±1

In conclusion, AL exerts a protective effect against ischaemic damage at both the vascular level, as reflected by the preservation of GFR, as well as at tubular level, as seen by the faster recovery in FeNa.

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60P LOCOMOTOR EFFECTS FOLLOWING INJECTION OF THE GROUP II mGluR AGONIST, DCG-IV, INTO THE SUBSTANTIA NIGRA PARS RETICULATA OF THE RESERPINE-TREATED RAT MODEL OF PARKINSON'S DISEASE

L. Dawson, A. Chadha & S. Duty. Neurodegenerative Disease Research Centre, Kings College London, London SW3 6LX.

Group II metabotropic glutamate receptors (mGluRs) function as autoreceptors in some brain regions, thereby controlling the release of glutamate (Shigemoto et al., 1997). In Parkinson's disease (PD), overactivity of the subthalamic nucleus (STN) results in excess glutamate release in the basal ganglia output regions, the substantia nigra pars reticulata (SNr) and medial globus pallidus (Mitchell et al., 1989). We hypothesised that, if Gp II mGluRs are present on STN terminals, their activation would oppose this excess glutamate release thus providing symptomatic relief in PD. The present study examines this hypothesis in the reserpine-treated rat model of PD.

Under general anaesthesia (60 mg / kg i.p. Sagatal), male Sprague Dawley rats (250-270g) were stereotaxically implanted with 23-gauge guide cannulae positioned directly above the SNr at co-ordinates: 3.7 mm anterior to and 3.6 mm above the interaural line, 2 mm lateral to the midline. Approximately 1 week later, rats were treated with reserpine (5 mg / kg s.c.). After 18 hours, when a stable parkinsonian state was attained, animals were placed in hemispherical bowls and baseline activity videotaped for 30 min. Rats then received a unilateral injection of (2S,1R,2R,3R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) (0.125, 0.25, 0.5, 0.75 or 1 nmole in 0.1 µl PBS, pH 7.4), via a 30-gauge injection needle inserted through the guide cannulae, and were videotaped for 90 min. After a minimum 6 hour interval, animals received a second dose of DCG-IV and were filmed for a further 90 min. Net contralateral rotations (measured as an index of antiparkinsonian potential) were counted in 5 min time bins over this period. Time to peak, magnitude of peak and total duration of the locomotor responses were also evaluated. Statistical significance between the effects of doses was assessed using a 1-way ANOVA with a Student Newman Keuls post-hoc analysis (significance level $p<0.05$). To confirm the specificity of DCG-IV, the effects of the Gp II-selective antagonist, (2S)-α-ethylglutamic acid (EGLU) were examined. Six hours following an initial dose of DCG-IV (0.25 nmole), rats were treated with either EGLU (100 nmole / 0.15 µl) or vehicle (0.3 mM NaOH in PBS; pH 7.4). After a 30 min equilibration period, rats received a second dose of DCG-IV (0.25 nmole). Net contralateral rotations / 90 min were compared pre- and post- EGLU/vehicle using a 2-tailed Student's t-test ($p<0.05$).

DCG-IV produced a dose-dependent increase in net contralateral rotations over the 90 min period. Parallel, dose-dependent increases in the peak response, time to peak and total duration of response were also observed (Table 1). At the highest dose tested (1 nmole) all animals produced convulsions. EGLU, but not vehicle, significantly inhibited DCG-IV-induced contralateral rotations / 90 min by 68.7 ± 10 % (mean ± sem , n=6), confirming specificity.

DCG-IV (nmoles)	0.125 (6)	0.25 (8)	0.5 (8)	0.75 (6)
Rotations / 90 min	11 ± 2	125 ± 20 *	264 ± 51*	417 ± 58*
Peak response / 5 min	2 ± 1	26 ± 4*	37 ± 5	67 ± 11*
Time to peak (min)	9.0 ± 1.5	12.5 ± 1.6	20.0 ± 3.9*	28.0 ± 1.1*
Duration (min)	14.0 ± 2.0	32.5 ± 2.5*	45.6 ± 5.1*	50.0 ± 3.5

Table 1. Effect of DCG-IV on locomotor parameters in the reserpine treated rat. Values are mean ± sem. Numbers in parentheses = number of animals. * Denotes significant differences between this and previous dose.

In conclusion, these data indicate that DCG-IV displays antiparkinsonian potential in the reserpine-treated rodent model of PD via activation of Gp II mGluRs. The cellular mechanism underlying this response remains to be determined, but probably reflects stimulation of autoreceptors on STN terminals in the SNr.

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61P α-SYNUCLEIN mRNA EXPRESSION IN THE NUCLEUS ACCUMBENS, STRIATUM AND SUBSTANTIA NIGRA OF RAT IS DIFERENTIALLY AFFECTED BY 6-HYDROXYDOPAMINE LESIONING

B-Y. Zeng, B. Dass, A. Owen, S. Rose, C. Cannizzaro, B.C. Tel, P. Jenner, Neurodegenerative Disease Research Centre, Division of Pharmacology & Therapeutics, Guy's, King's and St Thomas' School of Biomedical Sciences, King's College, Manresa Road, London SW3 6LX, UK

The point mutations in the gene encoding for α-synuclein occur in autosomal dominant forms of Parkinson's disease (PD) (Polymeropoulos *et al.*, 1997; Kruger *et al.*, 1998). However, α-synuclein protein immunostaining is also found in Lewy bodies in sporadic case of PD (Spillantini *et al.*, 1997). Little is known about the effect of damage to the nigro-striatal pathway on α-synuclein localization. We now report on the effect of 6-hydroxydopamine (6-OHDA) lesioning of the rat nigro-striatal pathway and subsequent L-DOPA administration on the gene expression of α-synuclein.

Unilateral or sham lesions of the nigro-striatal pathway in male Wistar rats were produced by stereotaxic injection of 8 μg of 6-OHDA or 0.9% saline into the left medial forebrain bundle (AP-2.2, L+1.5, V-8.0; Paxinos & Watson, 1986). L-DOPA (50 mg/kg/day) plus carbidopa (25 mg/kg/day) or carbidopa (25 mg/kg/day) alone was given by intraperitoneal injection into sham- and 6-OHDA-lesioned rats for 3 weeks beginning 4 weeks after the lesion. Subsequently, cryostat sections (20 μm) through the nucleus accumbens (AP 1.7 mm from bregma, the striatum (AP 0.2 mm from bregma) and substantia nigra (AP -5.3 from bregma) were incubated with ^{35}S-labelled oligodeoxyribonucleotide probes hybridizing with α-synuclein and adjacent nigral section for tyrosine hydroxylase (TH) mRNA. Quantitative evaluation of autoradiograms of the hybridization signal was undertaken by computerized densitometry.

Sham lesions had no effect on α-synuclein or TH mRNA expression (data not shown). 6-OHDA lesioning markedly decreased both α-synuclein and TH mRNA levels in the substantia nigra of the lesioned side compared to control animals (Table 1). α-synuclein mRNA levels in the denervated nucleus accumbens and striatum were not altered by a 6-OHDA lesion. L-DOPA plus carbidopa administration had no effect on α-synuclein mRNA expression.

These results suggests the colocalization of α-synuclein and TH mRNA in nigral dopaminenergic neurones. In the striatum and nucleus accumbens, dopamine terminals do not appear to regulate α-synuclein mRNA expression.

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Table 1: α-synuclein mRNA expression (nCi/mg) in the lesioned side of sham- and 6-OHDA-lesioned rats

	TH mRNA		α-synuclein mRNA	
	Substantia nigra	Substantia nigra	Striatum	Nucleus accumbens
Sham (n=7)	15.1±2.3	7.0±0.5	9.1±0.4	8.1±0.7
Sham+L-DOPA (n=6)	17.9±1.7	9.3±1.1	8.3±0.5	8.7±0.4
6-OHDA (n=7)	2.2±0.4*	0.8±0.3*	8.4±0.3	7.1±0.5
6-OHDA+L-DOPA (n=6)	2.6±0.4*	1.2±0.3*	10.4±0.4	8.2±0.2

Statistics by two-way ANOVA followed by post hoc Newman-Keuls test, *P<0.01 vs Sham-lesioned groups

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62P TOPOGRAPHICAL EVALUATION OF BEHAVIOURAL PHENOTYPE IN A LINE OF MICE WITH TARGETED GENE DELETION OF THE D_2 DOPAMINE RECEPTOR

J.J. Clifford[1], A. Usiello[2], D. Vallone[2], A. Kinsella[3], E. Borrelli[2] & J.L. Waddington[1]. [1]Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin 2, Ireland; [2]IGBMC, Strasbourg, France; [3]DIT, Dublin 8, Ireland

Behavioural phenotype in mice with targeted gene deletion of dopamine (DA) receptor subtypes can provide important clues to their functional roles in the absence of drugs with appropriate subtype selectivity. However, this has yet to be assessed at an ethological level, in terms of the topography of each individual element of behaviour within the natural repertoire of the mouse vis-a-vis all other elements of behaviour. Following our recent examination of such phenotype in D_{1A} 'knockouts' (Clifford et al., 1997, 1998), we describe the topographical evaluation of spontaneous behaviour and D_2-like agonist-induced responsivity in a line of mice with D_2 'knock-out'.

D_2 'knockout' mice were constructed, bred and genotyped as described previously (Baik et al., 1995). At approximately 12 weeks, mice were assessed using an ethologically-based rapid time-sampling behavioural checklist approach (Clifford et al., 1997, 1998) over initial exploration and subsequently following challenge with 0.1 - 12.5 mg/kg s.c. RU 24213, a D_2-like agonist (Deveney & Waddington, 1996). Statistical analysis was by analysis of variance (ANOVA) followed by Student's t-test or Mann-Whitney U-test.

Over a 1 hr period of initial exploration, D_2-null mice [$D_2^{-/-}$; n=33] evidenced relative to wildtypes [$D_2^{+/+}$; n=32] modest but significant (P<0.05) reductions in locomotion [-23%], rearing free [-37%], rearing to wall [-47%] and grooming [-27%]; sniffing, sifting and stillness were not altered, while rearing seated was increased [+35%]. Individual elements of behaviour habituated similarly over a 6 hr period for both genotypes. These topographical shifts between behaviours in D_2-null mice were distinct from those evident in D_{1A}-null mice (Clifford et al., 1998); however, both $D_2^{-/-}$ and $D_{1A}^{-/-}$ profiles were characterised overall by the substantial preservation of behavioural topography relative to its profound suppression by acute administration of D_2-like and D_1-like antagonists, respectively, to wildtypes. The dose-dependent induction of stereotyped sniffing and ponderous locomotion by RU 24213 in wildtypes was essentially absent in D_2-null mice.

This abolition of D_2-like agonist responsivity in D_2-null mice vis-a-vis relative preservation of spontaneous behavioural topography suggests compensatory processes subsequent to the developmental absence of D_2 receptors that are able to sustain function under naturalistic, tonic conditions but not during phasic challenge.

These studies were supported by RCSI and Forbairt. We thank Hoechst-Marion-Roussel for RU 24213.

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63P THE EFFECTS OF CENTRAL DOPA DECARBOXYLASE INHIBITION ON THE MOTOR ACTIONS OF A86929 AND QUINPIROLE ADMINISTERED IN COMBINATION TO MPTP-LESIONED MARMOSETS

S.A. Treseder, M. Jackson, S. Rose, P. Jenner, C.D. Marsden.
Neurodegenerative Diseases Research Centre, Division of Pharmacology and Therapeutics, Guy's, King's and St Thomas' School of Biomedical Sciences, Manresa Rd, London UK.

L-DOPA may play a neuromodulatory role in motor function since accumulation of endogenous L-DOPA, following inhibition of central dopa decarboxylase (DDC) activity, potentiates quinpirole-induced locomotor activity in rats (Yue *et al.*, 1994). However, the effect of endogenous L-DOPA on combined D-1 and D-2 receptor function in MPTP-treated primates is not known. We now report the effect of NSD-1015 (3-hydroxybenzyl hydrazine) on locomotor activity and reversal of disability induced by the combined administration of the D-1 agonist A86929 and the D-2 agonist quinpirole.

Adult marmosets (n=14) were treated with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 10mg/kg sc over 5 days) until stable motor deficits developed. NSD-1015 (10-50mg/kg ip) or vehicle was administered to animals (n=4) and locomotor activity and disability were monitored. In a separate experiment, animals (n=6) were treated with NSD-1015 (3.125-25mg/kg) and 45min later L-DOPA methyl ester (ME) (15.625mg/kg) was administered. After 90min, blood samples were taken and plasma analysed for L-DOPA, 3-O-methyl-DOPA (3OMD) and DA using HPLC with electrochemical detection (method previously described by Rose *et al.*, 1991). One way ANOVA followed by Dunnet's test were used to analyse statistical differences. In a further study, NSD-1015 (25mg/kg) or vehicle was administered (n=4) 90 min prior to combinations of quinpirole (0.05-0.1mg/kg ip) and A86929 (0.04-0.08mg/kg ip). Locomotor activity was measured automatically and behavioural disability assessed simultaneously using a rating scale of 0 (normal) to 18 (marked motor deficits). Data (expressed mean ±s.e.mean) was analysed by one-way ANOVA followed by Mann Whitney test.

NSD-1015 induced severe akinesia and rigidity with a gross loss of balance. Locomotor counts were dose dependently attenuated ($p<0.05$ at 50mg/kg vs vehicle) while disability scores were increased ($p<0.05$ for 25 and 50mg/kg vs vehicle). NSD-1015, dose dependently increased plasma L-DOPA and 3OMD and decreased dopamine and DOPAC levels following the administration of L-DOPA ME (table). From these results, 25mg/kg dose of NSD-1015 was chosen for further experiments.

NSD-1015 (mg/kg)	Plasma concentration (μM)			
	L-DOPA	3OMD	dopamine	DOPAC
vehicle	11.11(±0.93)	4.40(±0.33)	0.12(±0.03)	0.65(±0.14)
3.125	19.87(±1.59)*	8.98(±0.80)*	0.06(±0.03)	0.27(±0.05)**
6.25	20.54(±1.66)*	9.62(±1.31)*	0.12(±0.04)	0.12(±0.04)**
25	23.03(±2.60)**	11.37(±1.13)**	0.01(±0.01)*	0.07(±0.02)**

Table: Effects of NSD-1015 on plasma concentrations of L-DOPA, 3-OMD, DA and DOPAC following administration of L-DOPA ME. * $p<0.05$; ** $p<0.01$ vs. vehicle

The increase in locomotor activity and improvement in disability produced by low doses of A-86929 and quinpirole were abolished by NSD-1015 ($p<0.05$). By contrast, NSD-1015 had no effect on the improvement in locomotor activity and disability produced by the high dose combination (figure).

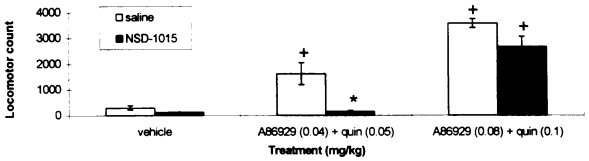

Figure: The effect of saline or NSD-1015 pre-treatment on mean total locomotor counts for 5h. *p<0.05 compared to saline. +p<0.05 compared to vehicle.

These results do not support a neuromodulatory role for endogenous L-DOPA in spontaneous or drug induced locomotor activity. Rather, they strengthen the argument for the importance of endogenous dopaminergic tone in the actions of DA agonists (Gomez-Mancilla *et al.*, 1993; Treseder *et al.*, 1998). A critical level of stimulation may be required at both D-1 and D-2 receptors for the reversal of motor deficits.

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64P THE ABILITY OF SELECTIVE MONOAMINE REUPTAKE INHIBITORS TO REVERSE THE MOTOR AND BEHAVIOURAL DEFICITS IN MPTP-TREATED COMMON MARMOSETS

M.J. Hansard, L.A. Smith, M.J. Jackson, S.C. Cheetham[Δ] & P. Jenner. Neurodegenerative Disease Research Centre, Division of Pharmacology & Therapeutics, Guy's, King's and St. Thomas' School of Biomedical Sciences, London, SW3 6LX U.K. & [Δ]Knoll Pharmaceuticals Research & Development, Nottingham, NG1 1GF U.K.

We have previously shown that the non-selective monoamine reuptake inhibitors NS 2214 and BTS 74 398 reverse the motor and behavioural deficits in MPTP treated common marmosets (Pearce, *et al.*, 1995; Smith *et al.*, 1998). However, the neuronal basis of this effect remains unknown. We now compare the ability of selective dopamine (DA), noradrenaline (NA) and serotonin (5-HT) reuptake inhibitors to alter motor and disability function in this model in an attempt to elucidate the therapeutic role of reuptake inhibition of these three monoamines.

Adult common marmosets (348-489g, n=8) were treated with MPTP (10 mg/kg over 5 days, s.c.) to induce motor and behavioural deficits. Animals were treated orally with L-DOPA (12.5 mg/kg after 12.5 mg/kg carbidopa), GBR 12909 (2.5-10 mg/kg; (1-(2-[bis-(4-fluorophenyl)-methoxy]ethyl)-4-(3-phenyl propyl) piperazine) dihydrochloride), nisoxetine (1-5 mg/kg), sertraline (1-10 mg/kg) or 10% sucrose vehicle. Behavioural disability/10 min was scored blind on a scale 0-18 for 6h (normal-markedly impaired) and locomotor activity/10 min was automatically recorded for 6h. The Mann Whitney U Test was used for statistical analysis and all data is given as mean±sem of n=4.

Over the 6 hour period of testing, L-DOPA reversed both the motor and behavioural deficits (Table 1). The DA reuptake inhibitor GBR 12909 also reversed the motor and behavioural impairment, but only at the highest dose (10 mg/kg). In contrast, the selective NA reuptake inhibitor, nisoxetine, did not reverse either the motor impairments or behavioural disability. Additionally, the selective 5-HT reuptake inhibitor, sertraline, did not reverse the motor and behavioural abnormalities.

Table 1. The effect of selective monoamine reuptake inhibitors in MPTP treated common marmosets. *, $P<0.05$ relative to vehicle. #, Animals administered with GBR 12909 were administered with nisoxetine 4 weeks later.

Drug	Dose (mg/kg)	Locomotor Counts / 6 Hours	Disability Scores / 6 hours
L-DOPA	12.5	6673±997 *	109±4 *
Vehicle (GBR)		1184±248	239±7
GBR 12909	10	3905±1374 *	135±9 *
Vehicle (nisox)		2132±543	229±7
Nisoxetine#	5	1389±567	225±14
L-DOPA	12.5	5723±726 *	119±5 *
Vehicle (sert)		979±557	251±17
Sertraline	10	402±121	251±8

Selective DA reuptake inhibition can reverse the motor and behavioural deficits in MPTP treated common marmosets although not to the extent observed with NS 2214 and BTS 74 398 that also interact with NA and/or 5-HT reuptake sites. Neither selective NA or 5-HT reuptake inhibition alone reversed the motor deficits. A further conclusion is that although DA reuptake inhibition is a prerequisite for reversing the motor and behavioural deficits, interactions with other monoamine reuptake sites may facilitate this effect.

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65P 5-HT AGONISTS ATTENUATE OPERANT RESPONDING FOR AN ETHANOL PAIRED CONDITIONED STIMULUS

Neill, J.C.[1], Wilson, A.W.[2], Costall, B.[1]. 1. Postgraduate Studies in Pharmacology, University of Bradford, Bradford BD7 1DP, West Yorkshire, U.K. 2. SmithKline Beecham Pharmaceuticals, Harlow, CM19 5AW, UK.

There is considerable evidence to show that 5-HT is involved in alcohol drinking behaviour (see Sellers et al., 1992 for review). The aim of the present study was to investigate the role of 5-HT in mediation of the secondary, or conditioned reinforcing effects of ethanol. Thus, the effect of compounds which attenuated oral ethanol self-administration in an operant paradigm (Wilson et al., 1998) were examined on responding for an ethanol paired conditioned stimulus (CS).

Female Sprague-Dawley rats food-deprived to 85% free-feeding body weight (250-300g) were trained to associate a compound CS with the delivery of 8% ethanol (v/v) in daily 30 min sessions while fed prior to testing in the absence of water. Rats ingesting at least 1ml of 8% ethanol were given access to 8% ethanol without prior feeding under these conditions. Subsequently, responding on a lever giving access to the CS (CR-conditioned reinforcement lever) in the absence of ethanol was assessed following administration of 5-HT agonists and the dopamine receptor antagonist haloperidol, or vehicle (0.9% saline). Responding on the inactive lever-NCR (non-conditioned reinforcement lever) was recorded but had no programmed consequence. Drugs were given 30 min prior to testing by the intraperitoneal (i.p.) or subcutaneous (s.c.) route (1ml/kg). Data are shown as square root of CR and NCR responses and analysed by a two way ANOVA with two within subjects factors; lever and drug followed by post-hoc Dunnett's t-test.

Eleven out of 23 rats were trained successfully to respond for an ethanol paired CS (6.7±1.5 CR responses; 3.2±0.9 NCR responses; $F(1,10)=146$, $p<0.001$). The 5-HT releasing agent, d-fenfluramine (0.3-1.0mg/kg), the 5-HT_{1A} receptor agonist 8-OH-DPAT (0.1mg/kg) the 5-$HT_{1B/2C}$ receptor agonist, TFMPP (0.1-0.3mg/kg) and the non-selective dopamine receptor antagonist, haloperidol (0.25mg/kg) all had a significant and selective effect to reduce CR responding (Table 1). The 5-HT re-uptake inhibitor, fluoxetine (1.0-10.0mg/kg i.p.) significantly reduced CR responding ($p<0.05$-$p<0.001$) but also had a significant effect to reduce NCR responding at the highest dose tested ($p<0.05$; 10mg/kg). In marked contrast, the 5-HT_2 receptor agonist DOI (0.5-1.0mg/kg s.c.) was without effect in this paradigm (CR responses 4.8±0.8 at 1.0mg/kg; vehicle 6.2±1.0).

Table 1 The effect of 5-HT agonists and haloperidol on responding for an ethanol paired CS.

Drug	Dose (mg/kg)	CR lever	NCR lever
Vehicle		6±1.0	2.9±0.3
d-fenfluramine	1.0 (i.p.)	3±0.7**	3.1±1.2
8-OH-DPAT	0.1 (s.c.)	3.1±0.9**	2.6±0.7
TFMPP	0.3 (i.p.)	3.0±0.6**	3.2±0.4
haloperidol	0.25 (s.c.)	2.6±0.7***	2.0±1.0

Data are shown as mean±sem square root of lever responses on CR or NCR (n=11). **p<0.01; ***p<0.001, Dunnett's t-test compared with vehicle (minimal effective dose shown).

These results show that, under the present experimental conditions, rats acquired a new response for presentation of an ethanol paired CS. In agreement with a previous study, the dopaminergic system was found to influence the conditioned reinforcing properties of ethanol (Slawecki et al., 1997). It has further been shown that 5-HT plays an important role in mediating the secondary reinforcing properties of ethanol. In particular, activation of 5-HT_{1A} and 5-HT_{1B}, but not 5-HT_2 receptors reduced responding for an ethanol paired CS.

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66P EFFECT OF THE 5-HT_6 ANTAGONIST, Ro 04-6790 ON FOOD CONSUMPTION IN RATS TRAINED TO A FIXED FEEDING REGIME

J. C Bentley, C. A. Marsden, †A. J. Sleight and K. C. F. Fone.
School of Biomedical Sciences, Nottingham University, NG7 2UH,
†F. Hoffmann-La Roche, 4070-Basel, Switzerland.

To meet the criteria for 5-hydroxytryptamine (5-HT) receptor classification, definitive evidence of 5-ht_6 receptor function is required. Experiments with an antisense oligonucleotide (A.O.) directed to the 5-ht_6 receptor suggested that down-regulation produced a stretching, yawning and chewing behaviour (Bourson *et al.*, 1995) and may reduce food intake (Bentley *et al.*, 1997). The stretching behaviour is also observed in rats treated with the selective 5-ht_6 receptor antagonist, 4-amino-N-(2,6 bis-methylamino-pyrimidin-4-yl)-benzene (Ro 04-6790, Sleight *et al.*, 1998). The current study examines the effect of the 5-ht_6 receptor antagonist, Ro 04-6790 on a fixed feeding regime.

Lister hooded rats (230-330g) were singly housed on a 12 h light-dark cycle and allowed water *ad libitum*. Standard laboratory chow was provided in the home cage, daily between 10.00-14.00h for 7-10 days prior to testing. In experiment 1, Ro 04-6790 (3, 10, 30 mg kg^{-1}, i.p.) or saline (1ml kg^{-1}, i.p) was given 30 min prior to food and intake recorded every 30 min for 4 h. The next day, with no further drug administration, food intake was measured for the 4h feeding period. Experiment 2, investigated the mechanism of 5-ht_6 antagonist-induced hypophagia. Brain 5-HT levels were depleted by pre-treatment with para-chlorophenylalanine (pCPA, 150mg kg^{-1}, i.p) or saline (1ml kg^{-1}, i.p) daily x 3. Sixteen hours after the last injection, Ro 04-6790 (30 mg kg^{-1}, i.p) or saline (1ml kg^{-1}, i.p) was administered to all animals 30 min prior to food and measurements taken as in experiment 1. Once completed, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels were determined using high performance liquid chromatography with electrochemical detection.

Ro 04-6790 caused a dose-dependent decrease in food consumption (Table 1) in the first hour. On both the day of administration and the following day, 30 mg kg^{-1} Ro 04-6790 significantly reduced food intake compared with saline controls. pCPA pre-treatment significantly reduced 5-HT levels in the raphe nuclei, hypothalamus, hippocampus and striatum by between 82% and 93% compared with saline controls ($p<0.01$, mean ± s. e. mean, Scheffe's S test following ANOVA) and caused a similar reduction in 5-HIAA levels. Although pCPA treatment alone significantly reduced feeding compared with saline/saline controls, it did not affect the Ro 04-6790-induced hypophagia on the test day.

These data suggest that 5-ht_6 receptor antagonism reduces food consumption, as previously found following 5-ht_6 A.O. treatment (Bentley et al., 1997). The inability of extensive depletion of 5-HT to prevent the 5-ht_6 antagonist-induced hypophagia could be due to constitutive activity but also questions whether 5-HT is the endogenous neurotransmitter mediating this effect.

The MRC and F. Hoffmann-La Roche, Switzerland sponsor JCB.

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	Experiment 1, (n=8 each)				**Experiment 2,** (n=6 each)		
Treatment	Food intake (1h, g)	Food intake (4h, g)	Food intake, next day(4h, g)	Treatment	Food intake (1h, g)	Food intake (4h, g)	Food intake, next day(4h, g)
Saline	9.9 ± 0.3	19.9 ± 0.5	17.6 ± 0.8	Saline/Saline	10.9± 0.5	21.4 ± 0.7	20.3 ± 1.0
3 mg/kg	10.8± 0.6	21.9 ± 0.4	17.8 ± 0.5	pCPA/Saline	8.9 ± 0.7*	15.0 ± 1.3**	15.0 ± 1.1**
10 mg/kg	7.3 ± 0.9**	19.1 ± 1.3	17.3 ± 0.4	Saline/Ro 04-6790	2.6 ± 0.3**++	10.1 ± 1.0**++	14.2 ± 1.9**
30 mg/kg	3.0 ± 0.5**	10.5 ± 0.9**	11.4 ± 1.4**	pCPA/Ro 04-6790	4.4 ± 0.9**++	8.9 ± 0.9**++	14.7 ± 0.6**

Table 1. Total food consumption (mean ± s. e. mean). *p<0.05 and **p<0.01 compared with saline, ++p<0.01 compared with pCPA/saline (Duncan's New Multiple Range following ANOVA).

67P PROSOCIAL EFFECTS OF CHRONIC FLUOXETINE IN PAIR-DISRUPTED FEMALE GERBILS

C.A. Hendrie & N.J Starkey[1] (introduced by J.C. Neill), Psychology, University of Leeds LS2 9JT and [1]Psychology, University of Central Lancashire, Preston PR1 2HE

Disruption of established male/female pairs of Mongolian gerbils (*Meriones unguicalatus*) has been shown to have reliable effects on subsequent spontaneous social behaviour in females (Hendrie & Starkey, 1998). The present study was conducted to determine whether these effects could be attenuated by fluoxetine, a clinically active antidepressant.

Seventy female gerbils were housed in pairs with vasectomised male gerbils for 5 weeks. Half these females were then individually housed for a further week (pairs disrupted, PD) whilst the rest remained in their pairs (pairs maintained, PM). Animals were then treated intraperitoneally with 0, 5 or 10mg/kg fluoxetine for the next 14 days (n's = 10-15 per group) whilst kept in these same housing conditions. 1 h after final injection, the spontaneous social responses of each animal were assessed in the presence of a naive (stimulus) male using detailed behavioural analysis described in Hendrie & Starkey, 1998. Data are presented as means ± s.e. mean and were analysed by Analysis of Variance and orthogonal contrasts as follow-up. * = $p<0.05$ from vehicle control.

In agreement with previous findings (Hendrie & Starkey, 1998), there was a clear increase in freezing upon the social approach of the stimulus animal in vehicle treated PD females [PM = 23.6 ± 5.5; PD = 61.0 ± 14.7, $p<0.05$].

Effects of fluoxetine were (i) non-specific, seen in both housing conditions and (ii) specific, seen in one housing condition only.

Non-specific effects were increased locomotion in pair maintained [vehicle =194.9±16.5; 5mg/kg =231.6±22.6; 10mg/kg =273.3±12.3*] and pair disrupted females [vehicle =166.6±21.9, 5mg/kg =208.1±33.4; 10mg/kg =246.1±20.3*] and also decreased immobility [PM; vehicle =100.4±29.1; 5mg/kg =59.7±24.3; 10mg/kg =16.5±5.1* : PD; vehicle =103.4±25.3, 5mg/kg =74.0±32.4; 10mg/kg =47.8 ± 20.7*].

Specific effects were increased social approach [vehicle =14.3±2.5, 5mg/kg =16.7± 2.9, 10mg/kg =24.4±3.3*] and social investigation [vehicle =21.5±2.3; 5mg/kg =22.6±2.4; 10mg/kg = 29.1±2.0*] seen in PD females only. No specific effects were seen in PD females treated with 5mg/kg or PM females treated with either dose of fluoxetine.

Together these data show that whilst fluoxetine increases locomotor behaviour in PM females, in PD females it produces this effect and increases social behaviour. These findings confirm that the effects of fluoxetine are dependent on previous social history (Starkey & Hendrie, this meeting) and give further indication that the prosocial effects of this antidepressant can only seen against a background of social impairment.

Hendrie CA, Starkey NJ. (1998) Physiol Behav 63: 895-901

68P THE EFFECTS OF S-METHYLISOTHIOUREA (SMT), AN INDUCIBLE NITRIC OXIDE SYNTHASE INHIBITOR, ON HEPATIC VASCULAR RESPONSIVENESS IN RATS WITH SECONDARY BILIARY CIRRHOSIS

W Yang, I S Benjamin, B Alexander.
Academic Department of Surgery, King's College School of Medicine and Dentistry, Denmark Hill, London SE5 9NU.

Nitric oxide (NO) has been shown to be an important modulator of hepatic vascular tone in the rat. Enhancement of inducible nitric oxide synthase (iNOS) by bacterial lipopolysaccaride (LPS) is responsible for overproduction of NO and circulatory collapse in secondary biliary cirrhosis. SMT may reverse the hypotension and vascular hyporeactivity to vasoconstrictors caused by LPS and here we studied its influence on hepatic vascular reactivity to vasoconstrictors during biliary cirrhosis in rats. Male Wistar rats (200-250g) were anaesthetised with sodium pentobarbitone ((3mg $100g^{-1}$ i.p.) and biliary cirrhosis induced by bile duct ligation; control animals were sham-operated and both groups were allowed to recover. 4-5 weeks later, both groups were given SMT (10mg kg-1, i.p.) or saline one hour prior to anaesthesia and liver perfusion. Following anaesthesia, the livers were excised and perfused *in vitro* via the hepatic artery (HA) and portal vein (PV) cannulae, at constant flows, according to our previous protocols (Yang *et al* 1995). Concentration-dependent dose-response curves to noradrenaline (10^{-6}-10^{-2} M) and ATP (10^{-6}-10^{-1} M) were constructed by HA and PV bolus injections. These were repeated in the prsence of an infusion of L-arginine (5×10^{-4} M L^{-1}).

Cirrhotic rats without SMT showed lower HA responsiveness to noradrenaline and ATP compared to sham-operated rats; E_{max} = 73.9 ± 8.8 vs 190.6 ± 9.8** and 44.4± 4.9 vs. 177.2 ± 10.2** mmHg, noradrenaline and ATP, cirrhotic vs. sham rats respectively. There were no significant differences in HA responses to noradrenaline and ATP between cirrhotic rats with and without SMT. Interestingly, significant reductions were observed in sham-operated rats in HA responses to noradrenaline and ATP, pretreated with SMT. -Log (M) ED_{50} = 4.10 ± 0.09 vs. 5.01± 0.08†† and 2.18 ± 0.27 vs. 4.39 ± 0.18††, noradrenaline and ATP with vs. without SMT, respectively; E_{max} = 148.3 ± 21.1 vs. 190.6 ± 9.8† and 85.8 ± 18.5 vs. 177.2 ± 10.2††mmHg, noradrenaline and ATP with vs. without SMT, respectively. PV responses were enhanced to noradrenaline and attenuated to ATP in cirrhotic rats without SMT; -Log ED_{50} =.4.70 ± 0.08 vs. 3.90 ± 0.06*. and 2.30 ± 0.04 vs. 2.16 ± 0.03, noradrenaline and ATP, cirrhotic vs. sham-operated rats. There were no significant differences between PV responses to noradrenaline in rats with SMT and without SMT or between PV responses to ATP in sham-operated and cirrhotic rats pretreated with SMT. Infusion of L-arginine produced no significant effects on either HA or PV responses in cirrhotic rats. However, HA and PV responses to noradrenaline and ATP were signifcantly increased after infusion of L-arginine in sham-operated rats. HA responses:- E_{max} 148.3 ± 21.1 vs. 200.3 ± 25.9 mmHg† and 85.8 ± 18.5 vs. 121.8 ± 10.8 mmHg,† noradrenaline and ATP before vs. after L-arginine, respectively. PV responses:- E_{max} 8.5 ± 0.7 vs. 11.8 ± 1.2 mmHg † and 6.4 ± 0.7 vs. 8.2 ± 0.7 mmHg,† noradrenaline and ATP before vs. after L-arginine, respectively. These data therefore suggest that iNOS is not involved in the modulation of hepatic vascular tone in secondary biliary cirrhosis. †P<0.05, ††P<0.01, paired t-test; *P<0.05,**P<0.001, unpaired t test. Mean ± SE., n = 6 rats per group.

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69P NITRIC OXIDE-INDEPENDENT RELAXATION OF HUMAN ISOLATED CORONARY ARTERIES TO ACETYLCHOLINE: EFFECT OF CORONARY HEART DISEASE

E. Thorin, Montreal Heart Institute, Research Center, 5000 Belanger Street, Montréal, Qc, H1T 1C8, Canada.

Endothelium-derived nitric oxide (NO) regulates basal arterial tone and plays a significant role in acetylcholine (ACH)-induced relaxation of isolated vessels. NO also interacts with endothelin-1 (ET-1) and endothelium-derived hyperpolarizing factor(s) by decreasing their endothelial production and/or smooth muscle effects. We investigated the involvement of NO in the regulation of human isolated coronary arterial tone in the absence and in the presence of ACH.

Epicardial right coronary arteries were isolated from explanted hearts of patients undergoing transplantation for non-ischemic/idiopathic (DCM, n=4) or ischemic/atherosclerotic (ICM, n=4) cardiomyopathies. Segments (2 mm in length; external diameter = 450±25µm, n=52 rings) were mounted on a wire myograph to record changes in isometric tension. Contractions are expressed as % of the maximal response (E_{max}) to 127mM KCl-physiological salt solution (K-PSS). Relaxations are expressed as % inhibition of the preaddition tone. In some experiments, the endothelium was removed by gentle scraping of the lumen with a human hair. All experiments were performed in the presence of indomethacin (10µM). Results are given as mean±s.e.m. n=4 per group of experiments.

ACH (1 µmol/L) induced an endothelium-dependent relaxation of rings from non-ischemic (78±13% relaxation) and a contraction (38±4%E_{max}) of rings from ischemic hearts precontracted by angiotensin II (0.1 µmol/L). Substance P (0.1 µmol/L), however, induced an endothelium-dependent relaxation in both groups of vessels (88±9 and 100±1% relaxation in DCM and ICM, respectively). In arterial rings from DCM hearts, N^{ω}-nitro-L-arginine (L-NOARG, 100 µmol/L), a NO synthase inhibitor, decreased (P<0.05) substance P-induced relaxation (52±13% relaxation) of angiotensin II-precontracted vessels but did not affect ACH-induced relaxation (97±3% relaxation). In vessels from ischemic hearts, ACH-induced contraction was not affected by L-NOARG (30±3%E_{max}); the relaxation induced by substance P, however, was abolished (P<0.05) by the NO synthase inhibitor (3±3%E_{max}). When precontracted by a depolarizing solution (40 mmol/L KCl solution), ACH contracted vessels from DCM (39±9%E_{max}) and ICM (30±8%E_{max}) hearts, whereas substance P-induced relaxation (79±7 and 70±9% relaxation in DCM and ICM hearts, respectively) was abolished (P<0.05) by L-NOARG. In the absence of precontracting tone, ACH (0.001 - 30 µmol/L) induced a small contractile response (P<0.05) of vessels from non-ischemic hearts. This response was highly potentiated by L-NOARG (P<0.05). Rings from ischemic hearts responded more to ACH (P<0.05) than those from non-ischemic hearts and L-NOARG had no significant effect. BQ123 (1 µmol/L), an ET_A receptor antagonist, abolished (P<0.05) the facilitatory effect of L-NOARG on ACH-induced contraction of vessels from non-ischemic hearts but had no effects in segments of coronary arteries isolated from ischemic hearts.

In conclusion, our results suggest that 1) ACH-induced relaxation of human epicardial coronary arteries is independent of NO formation but sensitive to high K^+ and coronary artery disease. 2) Substance P mediates relaxation by stimulating the release of both endothelium-derived NO and a factor which relaxant effect is blocked by high K^+. In vessels isolated from ischemic hearts, substance P-induced relaxation is solely mediated by endothelium-derived NO. 3) Finally, endothelium-derived ET contributes to ACH-induced contraction after inhibition of NO synthase; this ET-mediated effect is absent in coronary arteries from ischemic hearts.

70P THE ACTION OF DIASPIRIN CROSS-LINKED HAEMOGLOBIN ON THE REACTIVITY OF HUMAN ISOLATED LEFT INTERNAL MAMMARY AND RADIAL ARTERIAL RINGS

B.A. Callingham, S. Hartshorn, A.E. Crosbie[1], C.R. Hiley, A. Vuylsteke[1], A.J. Ritchie[2] and R.D. Latimer[1] Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QJ and Departments of [1]Anaesthesia and [2]Surgery, Papworth Hospital, Cambridge, CB3 8RE

Risks in transfusion of whole blood have led to renewed interest in the use of blood substitutes based on haemoglobin. One is diaspirin cross-linked haemoglobin (DCLHb), which has a high oxygen carrying capacity (Snyder *et al.*, 1987). However, this compound raises resting blood pressure in experimental animals (Hamilton *et al.*, 1992) and contracts isolated rings of porcine pulmonary artery and vein (Freas *et al.*, 1995). Hypertension has also been seen in human subjects leading to the suggestion that DCLHb could sequester nitric oxide (NO). In order to test this suggestion, the effects of DCLHb have been studied on segments of human vessels.

Segments of left internal mammary artery (LIMA) and radial artery were obtained from patients during coronary artery by-pass surgery. After removal of surrounding connective tissue, the vessels were cut into 3 mm rings, mounted in individual organ baths containing aerated (95% O_2/5% CO_2) Krebs-Henseleit solution at 37°C and attached to isometric strain gauges for measurement of tension. Eicosanoid production was prevented by the addition of indomethacin (10 µM). After equilibration (LIMA, 1 h; radial artery, 2 h), all rings were tested for the presence of endothelium by addition of carbachol (10 µM) to rings precontracted with phenylephrine (3 µM). Cumulative concentration response curves were obtained for carbachol and sodium nitroprusside (SNP) in the presence and absence of DCLHb in phenylephrine-precontracted rings. Following preliminary observations that DCLHb could contract artery rings, cumulative concentration-response curves were obtained. Except when otherwise stated, statistical evaluation was by unpaired t-test.

Carbachol relaxed phenylephrine-contracted LIMA and radial artery rings, by 72.3±1.7% and 96.7±0.7%, respectively. EC_{50} values were 0.12 ± 0.02 and 0.26 ± 0.01 µM, respectively (n = 4 for LIMA and radial artery). SNP caused complete relaxation of rings whether they had endothelium or not. EC_{50} values were 20±1 nM (LIMA, n = 7) and 19 nM (radial, n = 5). When LIMA rings were examined in the presence of DCLHb (0.1 µM), maximal carbachol-induced relaxation was significantly reduced (to 46.3±0.7%, P < 0.01) and the EC_{50} increased to 1.2±0.1 µM (P < 0.01; n = 4). Addition of DCLHb after carbachol to phenylephrine-contracted LIMA rings resulted in an immediate reversal of the relaxation and an increase in tone to above the original phenylephrine-constricted level. DCLHb had no effect on the maximum relaxation to SNP in either LIMA or radial artery rings but EC_{50} values were, respectively, 60±2 nM (n = 3; P < 0.05 relative to control; Student's paired t-test) and 45 nM (n = 5). DCLHb caused rings to contract in the absence of phenylephrine with EC_{50} values of 0.16±0.01 µM (LIMA; n = 6) and 0.18 µM ± 0.01 µM (radial artery; n = 4). The maximal contractions were 38.7% and 42.9% of the phenylephrine maximum. When these experiments were repeated in the presence of L-NAME (300 µM), the contractions caused by DCLHb were unaltered.

Much of the reduction, caused by DCLHb, in the relaxation of pre-contracted LIMA and radial artery rings by carbachol and SNP could be explained by an interaction with NO. However, the fact that L-NAME does not antagonise the contraction caused by DCLHb suggests that another mechanism of action is also involved.

We thank Baxter Blood Substitutes for providing the DCLHb.

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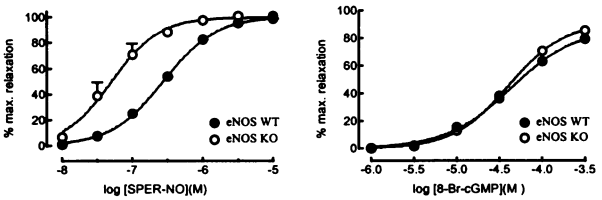
71P ENHANCED SENSITIVITY OF THE SOLUBLE GUANYLATE CYCLASE/CYCLIC GMP SYSTEM IN THE eNOS KNOCKOUT MOUSE

Monira B. Hussain, Adrian J. Hobbs & Raymond J. MacAllister, Wolfson Institute for Biomedical Research, University College London, London WC1E 6JJ, UK.

Soluble guanylate cyclase (sGC) is an intracellular receptor for NO (Hobbs 1997). Like many receptor-mediated transduction pathways, the sGC/cGMP system is down-regulated by its endogenous ligand, NO; this is exemplified by the development of tolerance to NO donors *in vitro* and *in vivo*. Such a process might be fundamental to the regulation of the NO pathway. The present study has attempted to characterise functionally these regulatory aspects of the sGC/cGMP system using tissues from animals in which genes encoding different isoforms of NO synthase (NOS) have been deleted.

Studies were carried out using aortic rings (2 mm wide) from male wild type (WT) and eNOS, nNOS and iNOS knockout (KO) mice. Endothelium-intact rings were mounted in Krebs solution under 0.3g tension and gassed with 95%O_2/5%CO_2 at 37°C. All tissues were contracted to 75-90 % of the maximum phenylephrine (PE) response and concentration-response curves to spermine-NONOate (SPER-NO; 10^{-9}M - 10^{-5}M), 8-Br-cGMP (10^{-6}M - $3x10^{-4}$M) or forskolin ($3x10^{-9}$M - 10^{-6}M) were obtained. To investigate the functional activity of phosphodiesterase type V (PDE V), concentration-response curves to SPER-NO was obtained before and after a 30 min incubation with the PDE V inhibitor zaprinast (10^{-5}M). Data were presented as % relaxation of the PE response. pEC_{50} values are used to compare the relaxant effects of the drugs.

SPER-NO was more potent on the eNOS WT than on the KO aorta (pEC_{50} 6.56±0.04 and 7.30±0.06 respectively; n=6; P<0.05; left panel figure) but had similar potency on nNOS WT and KO (pEC_{50} 6.87±0.04 and 6.83±0.04, respectively; n=4; P>0.05) and iNOS WT and KO (pEC_{50} 6.76±0.05 and 6.69±0.04, respectively; n=6; P>0.05). Forskolin (pEC_{50} 7.24±0.02 and 7.18±0.04, respectively; n=8; P>0.05) and 8-Br-cGMP (pEC_{50} 4.38±0.04 and 4.40±0.05, respectively; n=5; P>0.05; right panel figure) had similar effects on both eNOS WT and KO mice. Zaprinast had no effect on the responses to SPER-NO (pEC_{50} 6.56±0.06 and 6.43±0.12 on WT (n=6; P>0.05) and pEC_{50} 7.21±0.09 and 7.31±0.06 on KO (n=4; P>0.05) in the absence and presence of zaprinast, respectively).



The greater potency of SPER-NO on vessels from eNOS KO mice is consistent with up-regulation of sGC-mediated responses. This effect is specific to the eNOS KO animals because SPER-NO was equipotent on the nNOS and iNOS WT and KO mice. Further, the results with the adenylate cyclase activator forskolin suggest that the effect seen is specific to the sGC/cGMP system. 8-Br-cGMP, a G-kinase activator, had similar effects on eNOS WT and KO mice suggesting that G-kinase is not up-regulated functionally. Zaprinast did not alter the responses to SPER-NO consistent with low PDE V activity in these tissues. Therefore, these results suggest that up-regulation of the sGC/cGMP system occurs in chronic NO deficiency states and most likely involves increased sGC activity.

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72P THE ROLE OF NITRIC OXIDE IN HEPATIC ARTERIAL AND PORTAL VENOUS RESPONSES TO NORADRENALINE AND ATP IN RATS WITH SECONDARY BILIARY CIRRHOSIS

W Yang, I S Benjamin, B Alexander.
Academic Department of Surgery, King's College School of Medicine & Dentistry, Denmark Hill, London, SE5 9NU.

Nitric oxide (NO) is believed to be an important modulator of hepatic vascular tone under normal and diseased conditions but little is known regarding its role in the control of hepatic arterial (HA) and portal venous (PV) vascular tone during secondary biliary cirrhosis. The present study examined the changes of HA and PV vascular responses to noradrenaline and ATP with and without the presence of the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) in rat livers with secondary biliary cirrhosis.

Male Wistar rats (200-250g) were anaesthetised with sodium pentobarbitone (3mg $100g^{-1}$ i.p.) and secondary biliary cirrhosis was induced by bile duct ligation in one group of rats (CBDL); control animals were sham-operated and both groups were allowed to recover. Four weeks later both groups were anaesthetised, the common bile duct, common HA and PV cannulated and the livers excised and perfused *in vitro* with Krebs-Bülbring buffer (pH 7.4, 37°C) saturated with 95% O_2/5% CO_2 via the HA and PV cannulae at constant flow rates of 0.53±0.03 and 1.47±0.09 ml min^{-1} g $liver^{-1}$ respectively, according to our previous protocols (Yang *et al* 1995). Concentration-dependent dose-response curves were constructed to HA and PV (0.05ml) bolus injections noradrenaline (10^{-6}-10^{-2} M) and ATP (10^{-6}-10^{-1} M) with and without the addition of 100μM L-NAME. HA responses to noradrenaline and ATP were significantly reduced in CBDL livers compared to shams, E_{max} =73.9 ± 8.77 vs. 190.6 ± 9.8** mmHg and 44.4 ± 4.9 vs. 177.2 ± 10.2** mmHg, noradrenaline and ATP, CBDL vs. sham-operated rats. ATP responses were almost totally attenuated at most doses. HA responses to noradrenaline were restored in the presence of L-NAME in CBDL livers and were significantly different from the responses before the infusion of L-NAME (-Log ED_{50} = 4.92 ± 0.09 vs. 5.29 ± 0.06††, before vs. after L-NAME, respectively). Interestingly, PV responses to noradrenaline in CBDL livers were increased compared to sham-operated rat livers (-log ED_{50} = 4.69 ± 0.09 vs. 3.99 ± 0.07***, CBDL vs. shams, respectively). Conversely, PV responses to ATP were significantly reduced in CBDL livers compared to sham-operated rat livers (E_{max} = 4.6 ± 0.3 vs. 6.6 ± 0.5** mmHg, CBDL vs. shams, respectively). In sham-operated rat livers, neither HA nor PV responses to noradrenaline showed significant changes before and after infusion of L-NAME. HA responses to ATP in CBDL livers were significantly increased in the presence of L-NAME (E_{max} = 44.4 ± 4.9 vs. 87.8 ± 6.4††, before vs. after L-NAME, respectively. No significant differences were observed in PV responses to ATP between CBDL and sham-operated livers either in the presence or absence of L-NAME. The HA vasculature showed decreased responsiveness to noradrenaline and ATP in CBDL livers, in which the loss of vasomotor tone to noradrenaline, but not ATP, may be the result of increased production of NO. Vascular responses of the PV vasculature were enhanced to noradrenaline and attenuated to ATP in CBDL livers, and seem not related to increased NO production. ††P<0.01, Student's paired t-test; **P<0.01, ***P<0.001, Student's unpaired t-test. Mean ± SE, n = 8 rats per group.

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73P REPRODUCIBILITY OF BILATERAL FOREARM PLETHYSMOGRAPHY TO ENDOTHELIAL-DEPENDENT VASODILATOR

H. Walker, G. Jackson, J.M. Ritter & P.J. Chowienczyk
Department of Clinical Pharmacology, GKT, St Thomas' Hospital, London SE1 7EH

Forearm vasodilator responses, measured using bilateral strain gauge plethysmography, to brachial artery infusion of endothelium-dependent vasodilators such as acetylcholine are used to assess effects of interventions on endothelial function. The power of such studies depends on the within subject reproducibility of vasodilator responses. This has not been assessed formally and neither has the optimal method for expressing forearm blood flow vasodilator responses been established. Such responses are variably expressed as absolute values of forearm blood flow (FBF) or as the % change in blood flow ratio (FBFr) in the infused:non-infused arm. FBFr may allow for variation in blood flow resulting from changes in state of arousal and has been found to be the most reproducible method of expressing results for vasoconstrictor responses (Petrie *et al.*, 1998). Acetylcholine is rapidly metabolised in blood and therefore responses to brachial artery infusion may be influenced by strain gauge position. We measured forearm blood flow responses to brachial artery infusions of acetylcholine (7.5, 15 and 30 μg min^{-1}, each dose for 6 min) in 6 healthy normotensive men (age 22-30 years) on 3 occasions separated by at least one week. To assess the influence of strain gauge position we placed additional gauges on the infused arm 4 cm proximal and 4 cm distal to a middle gauge, which was placed at the conventional position of maximal forearm circumference. To examine the influence of agonist stability we also measured responses to salbutamol (0.3, 1 and 3 μg min^{-1}). Electrical calibration of the strain gauges remained stable with all variations less than 0.4%. Mechanical calibration with a purpose built precision device revealed the variation between visits of each gauge to be <4%. Within-subject coefficients of variation (CV) for FBF responses to acetylcholine derived from the middle strain gauge were 19.1±5.4, 20.4±4.6 and 18.9±6.2% (mean±sem) for doses 7.5, 15 and 30 μg min^{-1} respectively. These values were less than corresponding values of CV for FBFr (35.9±5.4, 41.3±7.3 and 29.2±5.8%, P<0.002 by ANOVA). Mean within-subject CV for the area under the dose response curve (AUC) calculated from FBF responses to acetylcholine was also lower than that for the AUC derived from FBFr (14.6±3.6 *vs.* 30.8±4.2%, P<0.002). There was a similar trend in the responses to salbutamol although statistical significance was not reached (FBF: 18.1±2.4, 16.1±1.2 and 14.8±2.2% *vs.* FBFr: 71.2±28.3, 33.1±11.2 and 31.1±8.7%, for doses 0.3, 1 and 10 μg min^{-1} respectively, P=0.1). FBF responses to acetylcholine and salbutamol determined from the proximal and middle gauges were similar. The corresponding values derived from the distal gauge to both agonists were lower in comparison with the results calculated from the middle gauge (P<0.01, by ANOVA for all doses). The difference between FBF responses derived from the middle and distal gauges was significantly greater for acetylcholine than for salbutamol (P<0.05). This study demonstrates that vasodilator responses to acetylcholine show least intra-subject variability when expressed as absolute values of flow in the infused arm rather than as a % change in forearm blood flow ratio. These results may in part reflect the large increase in blood flow to vasodilators (up to 800%) with consequent numerical amplification of small variations in baseline blood flow inherent in the formula for FBFr. This problem is reduced with vasoconstrictor responses since changes in blood flow are of the order of 50% Both agonists are dependent on strain gauge position. The dependence is greater for acetylcholine than salbutamol.

Petrie JR, Ueda S, Morris AD *et al.* Br J Clin Pharmacol 1998; 45: 131-139

74P GENISTEIN AND DAIDZEIN ACTIVATE THE L-ARGININE/NITRIC OXIDE SYSTEM IN HUMAN ENDOTHELIAL CELLS

L.R. Queen & A. Ferro. Department of Clinical Pharmacology, King's College London, St Thomas' Hospital, London SE1 7EH

Pre-menopausal women are protected from ischaemic heart disease as compared with men of a similar age and this is thought to be due largely to the effects of oestrogens. Following the menopause, cardiovascular risk rapidly approaches that of men. Our group has demonstrated decreased noradrenaline constriction (Kneale *et al*, 1997) and increased salbutamol dilatation (Kneale *et al*, 1998) in the forearm arterial bed of pre-menopausal women at mid-cycle when compared to men. These differences are abolished by nitric oxide synthase (NOS) inhibition, suggesting a greater basal nitric oxide (NO) release in these women. In accordance with these findings, oestrogen stimulates endothelial NO production (Hayashi *et al*, 1995).

Genistein and daidzein are naturally occurring isoflavonic phytoestrogens, found in soy and certain other plants. In view of the known effects of oestrogen on NO generation we wished to investigate the effects of these compounds on the L-arginine / NO system in human endothelium.

Human umbilical vein endothelial cells (HUVEC) were isolated from umbilical cords, obtained following uncomplicated pregnancies, by a standard collagenase digestion method.

Arginine transport: Confluent HUVEC at passage 3 were equilibrated for 30min with 100μM unlabelled L-arginine at 37°C. They were then exposed to either 10μM genistein, 10μM daidzein or vehicle for 20 min. After washing and exposure to [^{3}H] L-arginine (2.0μCi/ml) for 30 sec, cells were washed with excess unlabelled L-arginine (10mM). [^{3}H] was measured in formic acid cell digests by liquid scintillation counting.

NOS activity: Confluent HUVEC (passage 2) were harvested and 1ml cell suspension was incubated with [^{3}H] L-arginine (1μCi/ml) and either 10μM genistein, 10μM daidzein or vehicle for 40 min. For comparison the effects of 10nM 17-β-oestradiol (similar to concentrations found at mid-cycle) were also examined. The reaction was stopped by addition of ice-cold buffer containing the NOS inhibitor N^{ω}-nitro-L-arginine methyl ester (0.5mM). Cells were pelleted and lysed, [^{3}H] L-arginine was adsorbed by an excess of Dowex resin (Na^+ form) and [^{3}H] L-citrulline in the supernatant was measured by liquid scintillation counting.

All experiments were performed in triplicate and results corrected for amount of protein. Mean values for each experiment were used for further statistical analysis. Data were analysed by repeated measures ANOVA with statistical significance taken as $P < 0.05$ (two-sided). Results are presented as mean ± SEM.

Transport of L-arginine in control HUVEC was 2.3 ±0.3 pmol/μg protein/min (n=14). Genistein and daidzein each increased the rate of transport significantly, to 3.4 ±0.5 and 3.2 ±0.5 pmol/μg protein/min respectively.

[^{3}H] L-citrulline production in control cells was 8.2 ± 2.5 dpm/μg protein over 40 min (n=10). Genistein, daidzein and 17-β-oestrodiol stimulated [^{3}H] citrulline production significantly, by 56.0 ±11.3 %, 75.6 ±24.0% and 92.8 ±35.7% respectively.

We conclude that phytoestrogens can activate both L-arginine transport and NOS activity in human endothelial cells. Further studies are needed to determine the clinical implications of these findings.

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75P MECHANISM OF NITRIC OXIDE SYNTHASE ACTIVATION BY β-ADRENOCEPTORS IN HUMAN VASCULAR ENDOTHELIUM

L.R. Queen & A. Ferro. Department of Clinical Pharmacology, King's College London, St Thomas' Hospital, London SE1 7EH

Stimulation of β-adrenoceptors (β-AR) on vascular endothelial cells activates adenylyl cyclase and hence cyclic adenosine-3',5'-monophosphate (cAMP) formation (Ferro *et al*, 1997). In addition, β-AR stimulation or cAMP elevation activates the L-arginine / nitric oxide synthase (NOS) system in human vascular endothelium (Queen *et al*, 1998). The mechanism by which this occurs has not been established.

cAMP activates protein kinase A (PK-A), which causes phosphorylation of a variety of proteins. In addition, in vascular smooth muscle cells, cAMP can stimulate nitric oxide (NO) production by breakdown to adenosine which then stimulates extracellular A_2 purinoceptors (Dubey *et al*, 1998). In endothelial cells adenosine can also stimulate NO synthesis through A_2 purinoceptor activation (Sobrevia *et al*, 1997), and this effect is abolished by tyrosine kinase inhibition (Wyatt *et al*, 1998).

The aim of this work was to determine the involvement of A_2 purinoceptors, tyrosine kinase and PK-A in β-AR mediated NOS activation.

Human umbilical vein endothelial cells (HUVEC) were isolated from umbilical cords by a standard collagenase digestion method. Confluent cells (passage 2) were harvested and 1ml cell suspension was incubated with [^{3}H] L-arginine (1μCi/ml) and either 50μM SQ 22536 (an adenylyl cyclase inhibitor; Goldsmith & Abrams, 1991), 100nM H-89 (a PK-A inhibitor), 100μM tyrphostin 23 (T23, a tyrosine kinase inhibitor) or vehicle for 15 min at 37°C. In a separate set of experiments, cells were incubated with 100nM 3,7-dimethyl-1-propargylxanthine (DPMX, A_2 selective purinoceptor antagonist) or vehicle under the same conditions. Incubation was then performed with 1μM Isoprenaline (Iso) or vehicle for a further 25 min. Cells were pelleted and lysed, [^{3}H] L-arginine was adsorbed by an excess of Dowex resin (Na^+ form) and [^{3}H] L-citrulline in the supernatant was measured by liquid scintillation counting.

All experiments were performed in triplicate and results corrected for protein. Mean values for each experiment were used for statistical analysis. Data were analysed by repeated measures ANOVA. Results are presented as mean ± SEM.

In the first set of experiments, [^{3}H] L-citrulline production in control cells was 18.7 ± 5.7 dpm/μg protein (n=8). SQ22536, H89 and T23 did not affect basal [^{3}H] L-citrulline production. Iso increased [^{3}H] L-citrulline formation by 72.6 ± 20.8% ($P < 0.05$ vs control). This increase was significantly inhibited by SQ22536, H89 and T23. Percentage increases above control were 32.8 ± 14.4%, 0.25 ± 7.9% and 16.4 ± 17.6% respectively ($P < 0.05$ vs Iso alone, for each inhibitor).

In the second series of experiments, basal [^{3}H] L-citrulline production was 34.8 ± 8.2 dpm/μg protein (n=8). Iso increased [^{3}H] L-citrulline levels by 81.5 ± 14.5% ($P < 0.01$ vs control). DMPX caused a significant reduction in Iso stimulated [^{3}H] L-citrulline formation (33.6 ± 12.2% above control, $P < 0.01$ vs Iso alone).

We conclude that β-AR mediated NOS activation involves stimulation of adenylyl cyclase, PK-A, A_2 purinoceptors and tyrosine kinase pathways. Our results suggest a dual mechanism of NOS activation by β-AR, both involving cAMP: 1) PK-A activation, and 2) adenosine formation with subsequent stimulation of A_2 purinoceptors and tyrosine kinase pathways.

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76P INCREASED β-ADRENERGIC VASORELAXANT RESPONSES IN RAT CAROTID ARTERIES WITH NEOINTIMA ARE NOT RELATED TO ALTERATION IN THE NO SYSTEM

F.J. Heijenbrok, M-J. Mathy, M. Pfaffendorf and P.A. van Zwieten, Dept. of Pharmacotherapy, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

In a previous study[1] it has been shown that β-adrenoceptor-mediated vasodilation was transiently enhanced in rat carotid arteries with neointima. This effect was only apparent in arteries without endothelium: with reappearance of functionally active endothelial cells, this β-adrenergic effect had returned to control values. It was speculated that the increased β-adrenergic response in arteries with neointima but without endothelium may be related to the absence of the endothelial NO-system. This hypothesis was further investigated in this study.

Male Wistar rats (200-220g) were put either on a L-NAME (containing 15.0 mg L-NAME/kg/day) or on a normal diet for six weeks. Prior to the experiments, the body weight, mean arterial blood pressure and heart rate for both groups were measured, and the animals were sacrificed. The wet heart and wet lung weights were measured. Furthermore, the contractile responses of the isolated left common carotid artery from both groups to potassium chloride (K^+; 100mM), phenylephrine (PhE; 3μM), and to the TxA_2 agonist (U46619; 30nM), as well as the vasorelaxation induced by methacholine (MCh; 10μM, after precontraction with PhE, 3μM) were evaluated. To study both maximal response (E_{max}) and affinity (pD_2) for isoprenaline (ISO; 0.1 nM-10μM), a concentration-response curve was made after precontraction with U46619 (30nM). These experiments were performed in an isometric wire myograph (PSS, 37°C, pH7.4, gassed with 95% O_2/5% CO_2).

The body weight (375.3±11.1g vs. 384.6±10.0g, n=5) and heart rate (232.4±5,2bpm vs. 212.7±10,0bpm, n=5) were not significantly different between the L-NAME and the control group, respectively. However, mean arterial blood pressure was significantly elevated in the L-NAME rats: 128.4±3.4mmHg vs. 100.0±2.9mmHg (control), n=5, p<0,05. The heart and lungs of the L-NAME rats were not enlarged due to the diet, when compared to the control rat: wet heart weight = 0.356±0.030% vs. 0.327±0.012, n=5, and wet lung weight = 0.440±0.022% vs. 0.462±0.022%, n=5.

L-NAME treatment did not influence the contractile properties of the isolated common carotid artery. The responses to the different agonists were comparable between the L-NAME and control group: high potassium (K^+; 100mM)-induced contractions measured: 5.0±0.4N/m vs. 4.9±0.2, n=5; for phenylephrine (3μM): 2.9±0.4N/m vs. 2.8±0.3N/m, n=5; and for U46619 (30nM): 3.7±0.4N/m vs. 3.2±0.1N/m, n=5.

Conversely, the relaxant responses of the common carotid artery appeared significantly impaired by L-NAME treatment. Methacholine-induced relaxation was decreased (10μM): 19.8±9.6% vs. 63.2±3.1%, L-NAME vs. control, n=5, p<0.05. Also, the carotid arteries of the L-NAME group showed decreased vasorelaxation to isoprenaline (E_{max}): 36.1±9.4% vs. 65.9±6.0%, L-NAME vs. control, n=5, p<0.05. In addition, the sensitivity (pD_2) to isoprenaline was decreased in the L-NAME-treated arteries: 6.7±0.2 vs. 7.4±0.1, L-NAME vs. control, n=5, p<0.05.

In conclusion, L-NAME treatment suppresses the endothelium-dependent synthesis of nitric oxide and thus leads to significant elevation of blood pressure. The contractile responses of the different agonists appeared not to be influenced by L-NAME treatment. However, the relaxant responses to methacholine and to isoprenaline were significantly decreased due to NO-synthase inhibition, indicating that the isoprenaline-induced vasorelaxation may at least partially be NO-dependent in the rat carotid artery. From these data, it is concluded that the increase in β-adrenergic response in rat carotid artery with neointima is not due to the absence of NO-synthesis.

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77P ATRIAL DYSFUNCTION INDUCED BY ENDOTOXIN IN RATS IS MODULATED BY L-ARGININE: ROLE OF NITRIC OXIDE

S. Price, T.W. Evans, J.A. Mitchell
Imperial College of Science, Technology and Medicine, Department of Critical Care, Royal Brompton Campus, Sydney Street, London SW3 6NP

Nitric oxide (NO) has been implicated in the control of myocardial function under physiological conditions (Brutsaert *et al*, 1988). In sepsis, large amounts of NO are produced as a result of induction of NO synthase (iNOS), and are thought to result in the decreased vascular tone and vascular hyporeactivity seen. Controversy exists as to whether excessive production of NO by iNOS plays a role in the pathogenesis of myocardial dysfunction in septic shock (Keller et al 1995, Herbertson *et al* 1996). In this study, we have assessed the effects of endotoxaemia and NOS inhibition on atrial nitrite release and left atrial contractility in response to the beta agonist isoprenaline.

Control and endotoxaemic (LPS 20mg^{-1}kg^{-1} i.p 4h) male Wistar rats (250-300g) were anaesthetised with sodium pentabarbitone (100mg^{-1}kg i.p) and killed by cervical dislocation. Hearts were removed, the atria dissected into equal pieces, placed into culture medium and incubated at 37°C for one hour. Medium was then replaced with fresh medium and atrial tissue incubated at 37°C in the presence or absence of the NOS inhibitor L-NAME (10^{-3}M), and the specific iNOS inhibitor, 1400W (10^{-5}M), (Garvey *et al*, 1997). Medium was assayed at 24 and 48 hours for nitrite by the Greiss method. In assessment of left atrial contractility, hearts were removed as described above, and left atria were attached to a force-transducer in gassed (95%O_2: 5%CO_2) Krebs' buffer. Preparations were maintained at 37°C and paced at 0.5Hz, 100% above the threshold voltage. Peak-generated tension was measured in the presence and absence of L-NAME (10^{-3}M) and L-arginine (10^{-3}M), and concentration-response curves in response to isoprenaline (10^{-10} to $3x10^{-6}$M) were performed.

Atrial nitrite release is given in nmolmg^{-1}ml^{-1}, n=5 in each group shown, and data is shown as mean±S.E.M unless otherwise stated. Left atria from control rats released high levels of nitrite (2.56±0.67), which was inhibited with L-NAME (0.29±0.08) and 1400W (0.70±0.25). *In vivo* treatment with LPS increased release of nitrite (6.88±1.92), which was also inhibited by both L-NAME (0.82±0.3) and 1400W (1.65±0.1). There was no difference in generated left atrial tension (g) in atria from control and LPS-treated rats (control 0.19±0.04; n=6: LPS 0.18±0.05; n=6). Endotoxaemia depressed the inotropic response to isoprenaline, shown as percentage increase in peak-generated tension (% PGT) (Figure 1). L-NAME did not alter the inotropic responses to isoprenaline in atria from control (Emax, 23.66±2.5; n=4) or endotoxaemic (Emax, 16.26±3.5; n=5) rats. L-arginine had no effect on response to isoprenaline in atria from control rats, but in atria from endotoxaemic rats, L-arginine restored the response to isoprenaline (Figure 2). In a separate group of experiments, the effect of L-arginine on the response to isoprenaline in atria from LPS-treated rats was blocked in the presence of L-NAME (Emax, 7.7±1.9; n=6).

Figure 1; Krebs' buffer

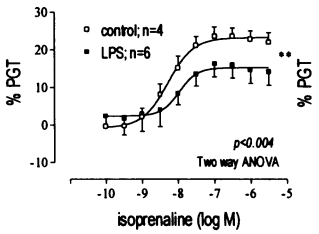
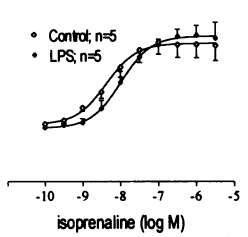


Figure 2; Krebs' buffer + L-arginine



This data shows that atrial tissue releases elevated levels of NO, and displays hyporeactivity to isoprenaline *ex vivo* after *in vivo* administration of endotoxin. The dysfunction seen in atrial contractility was reversed by L-arginine, suggesting that (i) the substrate for NOS is rate limiting and (ii) that NO from iNOS is cardioprotective in this model. These observations suggest that inhibitors of iNOS may have detrimental effects on cardiac function in some patients with septic shock.

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78P S-NITROSOTHIOLS ARE NITRIC OXIDE DONOR DRUGS THAT DO NOT ENGENDER VASCULAR TOLERANCE AND REMAIN EFFECTIVE IN GLYCERYL TRINITRATE-TOLERANT RAT FEMORAL ARTERIES

MEGSON IL,[1] ROSEBERRY MJ,[1] MILLER MR,[1] MAZZEI FA,[2] BUTLER AR[2] & WEBB DJ[1]
[1]Clinical Pharmacology Unit, University of Edinburgh, Western General Hospital, Edinburgh, EH4 2LH. [2]School of Chemistry, University of St Andrews, St Andrews, Fife, KY16 9ST.

Organic nitrates (e.g. glyceryl trinitrate; GTN) have long been used in the treatment of angina. The beneficial actions of nitrates are thought to involve nitric oxide (NO)-mediated dilatation of affected coronary arteries, and systemic venodilatation, resulting in reduced venous return and a reduction in both preload and afterload. NO release from nitrates is thought to be dependent on thiol-mediated intracellular metabolism, and thiol depletion is a possible cause of tolerance to these drugs with long-term use. S-nitrosothiols are NO donor drugs that do not require intracellular metabolism to generate NO, and previous results suggest that they might not engender tolerance (Bauer & Fung, 1991).This study set out to determine whether isolated rat femoral arteries become tolerant to S-nitrosothiols (S-nitroso-N-valerylpenicillamine; SNVP, and S-nitrosoglutathione; GSNO), and to establish whether these compounds are capable of eliciting vasodilatation in GTN-tolerant vessels.

Our model for tolerance involved precontracting (2-8 μM phenylephrine; 97.5±2.4 mmHg; n=36) perfused isolated rat femoral arteries from adult male Wistar rats (350-450 g). Vessels were treated throughout with supramaximal concentrations of the NO synthase inhibitor, N$^{\omega}$-nitro-L-arginine (L-NAME; 200 μM) to exclude involvement of endogenous NO. Perfusion with supramaximal concentrations of GTN (10^{-5} M), initially caused 36.5±4.1% vasodilatation (n=10). Pressure gradually recovered throughout the period of GTN perfusion and pre-treatment pressure was fully restored after an overnight incubation with GTN (t=20 h; n=10; Fig 1). Bolus injections of GTN (10 μl; 10^{-8}–10^{-3} M) did not cause vasodilatation at this time, whereas equivalent injections of GSNO and SNVP produced responses that were indistinguishable from those in parallel control vessels not perfused with GTN (P>0.05; 2-factor, repeated measures ANOVA). Similar experiments with overnight incubations of GSNO and SNVP showed that perfusion pressure at t=20 h had not recovered significantly over the incubation period (Fig 1). Pressure was restored to that of untreated controls (P<0.01; unpaired t-test) by blocking the later stages of the NO pathway with the guanylate cyclase inhibitor, [1H-[1,2,4] oxadiazole [4,3-a]quinoxalin-1-one (ODQ; 20 μM).

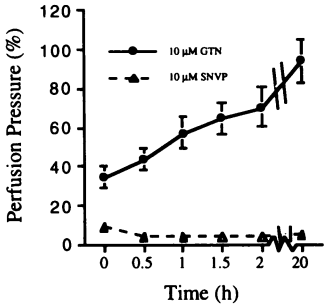

Figure 1. Effect of perfused concentrations of GTN and SNVP (both 10^{-5} M; n=10) on perfusion pressure in precontracted rat femoral arteries. Drug perfusions started at t=0 h and perfusion pressure immediately before drug treatment was 100%. P<0.001, 2-factor, repeated measures ANOVA.

Our experiments indicate that S-nitrosothiols are not cross-tolerant with GTN. Furthermore, unlike GTN, S-nitrosothiols did not engender vascular tolerance over a 20 h treatment period. Given that tolerance is a major limitation of nitrate therapy, S-nitrosothiols might present a viable alternative in the treatment of cardiovascular disease, particularly where long-term and high dose administration is required.

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79P THE PROLONGED PHASE OF ATP-INDUCED VASODILATATION IN RAT MESENTERIC VESSELS IS MEDIATED BY A CANNABINOID-TYPE LIGAND

S.J.Stanford and J.A.Mitchell
Imperial College of Science, Technology and Medicine, Unit of Critical Care, Royal Brompton Campus, Sydney Street, London SW3 6NP

In the rat isolated mesenteric bed, ATP produces vasodilatation which is endothelium-dependent and via P_{2y}-purinoceptor activation (Burnstock & Kennedy, 1986). Dilator actions of ATP, at doses of up to $1x10^{-8}$ moles, are inhibited by the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) (Rubino *et al.*, 1995: Stanford & Mitchell, 1998). We have recently identified two components of the dilator response to ATP, which become apparent at doses of more than $1x10^{-8}$ moles. The transient first phase is inhibited by both L-NAME and the guanylyl cyclase inhibitor ODQ, the second prolonged phase is not affected by either of these agents (Stanford & Mitchell, 1998). The mesenteric vessels are very sensitive to the effects of 'hyperpolarizing' factors which may include cannabinoids (Randall et al, 1996). Therefore we have assessed the effect of the selective CB_1 receptor antagonist, SR141716A (Showalter *et al*, 1996) on the two phases of ATP-induced dilator response in the rat isolated mesenteric bed.

Male Wistar rats (250-300g) were anaesthetised with sodium pentobarbitone ($100mg.kg^{-1}$; ip) and killed by cervical dislocation. The mesenteric artery was cannulated and the mesentery excised. The bed was perfused at a constant rate with Krebs' buffer, warmed to 37°C and gassed (95% O_2: 5% CO_2). Perfusion pressure, recorded via an arterial cannula, was raised to approximately 120mmHg by titration of methoxamine ($1x10^{-6}$ to $1.2x10^{-5}$M) added to the perfusate. The effects of 1-3µl volume injections of ATP ($1x10^{-7}$ and $3x10^{-7}$ moles) or the endogenous cannabinoid, anandamide ($1x10^{-8}$-$1x10^{-7}$ moles) were recorded. In some experiments SR141716A ($1x10^{-5}$M) was added to the perfusate.

As reported previously, at doses of $1x10^{-7}$ and $1x10^{-3}$ moles, ATP induced biphasic reductions in perfusion pressure. The first, transient phase of the relaxant response to ATP was unaffected by SR141716A (*Figure 1a*). In contrast, SR141716A inhibited the second, prolonged phase of the ATP induced relaxant response at both doses tested (*Figure 1b*). Anandamide induced a dose-dependent reduction in perfusion pressure which was also inhibited by SR141716A at all doses tested ($1x10^{-8}$-$1x10^{-7}$ moles) (representative dose of $1x10^{-7}$ moles given; mean±SEM), (control 50.3±8.4%; n=5: SR141716A 16.9±1.5%; n=4).

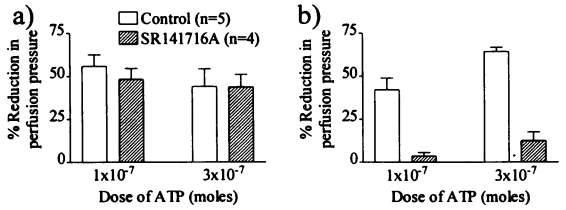

Figure 1. Effect of SR141716A ($1x10^{-5}$M) on a) the first phase and b) the second phase of the biphasic, ATP-induced reduction in perfusion pressure in the rat isolated mesenteric bed. Data represents mean±SEM.

In the rat isolated mesenteric bed ATP induces a biphasic reduction in perfusion pressure. The transient first phase was inhibited by both L-NAME and ODQ (Stanford & Mitchell; 1998). The second prolonged phase was inhibited by SR141716A. This compound also inhibited anandamide-induced vasodilatation in this bed. This data suggests that ATP acts by two distinct mechanisms to produce relaxation of the rat isolated mesenteric bed, NO and an endogenous cannabinoid being the mediators involved.

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80P EVIDENCE THAT TWO PATHWAYS OF Ca^{2+} ENTRY ARE INVOLVED IN THE INHIBITORY ACTION OF THAPSIGARGIN ON DRUG-INDUCED CYCLIC AMP ACCUMULATION IN HUMAN U373 MG ASTROCYTOMA CELLS

M.-P.M. Wong & J.M. Young, Department of Pharmacology, Univ. of Cambridge, Tennis Court Road, Cambridge, CB2 1QJ,

Agents which stimulate Ca^{2+} entry into human U373 MG astrocytoma cells inhibit drug-induced cyclic AMP accumulation (Wong *et al.*, 1998), presumably via an action on one of the Ca^{2+}-inhibitable isoforms of adenylyl cyclase (Cooper *et al.*, 1995). We present evidence here that at least two Ca^{2+} entry components are involved in the inhibitory action of thapsigargin.

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

Thapsigargin inhibited cyclic AMP accumulation induced by 10 µM forskolin in a concentration-dependent manner with an IC_{50} of 6.0 ± 0.3 nM and a maximum inhibition of 72 ± 1%. Isoprenaline-stimulated cyclic AMP accumulation was also inhibited by 100 nM thapsigargin. The % inhibition increased from 36 ± 3% after 1 min incubation to 75 ± 1% after 4 min. The increase in the % inhibition between 1 and 4 min with 5 µM thapsigargin was less marked (49 ± 4% to 69 ± 2%).

La^{3+} (1 µM) had no significant effect on basal cyclic AMP accumulation, but produced a small, but consistent, stimulation of the response to forskolin alone (10 ± 2%, n=7), although this was statistically significant in only 2 experiments. La^{3+} (1 µM) largely reversed the inhibitory effect of 100 nM thapsigargin on the response to forskolin over a 4 min incubation period (88 ± 2% of the response to forskolin alone, n=6), but was significantly more effective in reversing the inhibition by 5 µM thapsigargin (112 ± 2% of the response to forskolin alone, n=5). The inhibition by 5 µM thapsigargin of the response to isoprenaline was also reversed by 1 µM La^{3+} (108 ± 2% of the response to isoprenaline alone, n=4). However, when the incubation period with forskolin or isoprenaline was reduced to 1 min the extent of the reversal by 1 µM La^{3+} of the inhibitory action of 5 µM thapsigargin was notably less (55 ± 6%, n=4, and 33 ± 11%, n=3, reversal of the inhibition of cyclic AMP accumulation stimulated by isoprenaline and forskolin, respectively).

The lesser effect of 1 µM La^{3+} over a 1 min incubation was apparently not due to a slow equilibration with a Ca^{2+} entry site, since the increased level of $[Ca^{2+}]_i$ after thapsigargin was rapidly returned to near basal levels after addition of 1 µM La^{3+}. There was also no indication that Ca^{2+} released from intracellular stores makes a significant contribution to the inhibition of cyclic AMP accumulation at early times, since in the absence of added extracellular Ca^{2+} and in the presence of 0.2 mM EGTA, the mean inhibition by 5 µM thapsigargin of the response to 1 µM isoprenaline over a 1 min incubation was only 12 ± 2% (n=4).

These results suggest that the inhibition of cyclic AMP accumulation by thapsigargin is mediated by activation of two Ca^{2+} entry pathways. One is blocked by 1 µM La^{3+} and does not inactivate, whereas the other is relatively insensitive to La^{3+}, but appears to inactivate within approximately 1 min.

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81P ONTOGENIC INFLUENCES ON THE REGULATION OF RAT INTESTINAL Na^+ K^+ ATPase BY G PROTEINS

M.A. Vieira-Coelho, P. Serrão & P. Soares-da-Silva. Inst. Pharmacol. & Therap., Fac. Medicine, 4200 Porto, Portugal.

Spontaneously hypertensive and senescent rats have a defective regulation of renal tubular Na^+,K^+-ATPase by dopamine due to abnormal G protein activation (Gurich & Beach, 1994; Kansra et al., 1997). The present study was aimed to evaluate differences in the regulation of Na^+,K^+-ATPase by G proteins in epithelial intestinal cells from young (20-day old) and adult (60-day old) Wistar rats, since the latter ones have a defective inhibition of Na^+,K^+-ATPase by dopamine (Vieira-Coelho et al., 1998). Isolation of jejunal epithelial cells and Na^+,K^+-ATPase assay were performed as previously described (Vieira-Coelho et al., 1998; Kansra et al., 1997). Results are given as arithmetic means ± s.e. mean, n=5. Statistical analysis were performed by one-way analysis of variance (ANOVA) followed by Newman-Keuls test for multiple comparisons or Student's "t" test. A P-value less than 0.05 was assumed to denote a significant difference. Basal Na^+,K^+-ATPase activity (in nmol Pi mg protein^{-1} min^{-1}) in young and adult animals was 52±4 and 123±3, respectively. The presence of GTPγS (15, 50 and 150 μM), a nonhydrolyzable analogue of GTP, that irreversibly activates G proteins, resulted in a concentration dependent inhibition of intestinal Na^+,K^+-ATPase activity in young animals (24.5±6.8 % reduction, at maximal effect) with no effect in adult animals. Cholera toxin 0.1 μM (CTX) inhibited intestinal Na^+,K^+-ATPase activity by 20.1±4.1 % in young animals, but had no effect on intestinal Na^+,K^+-ATPase activity in adult animals. In young animals, pretreatment of intestinal cells with pertussis toxin (PTX) 0.1 nM potentiated the inhibitory effect of dopamine (1 μM) upon Na^+,K^+-ATPase (40.4±5.8 % reduction). In adult animals, although dopamine (1 μM) alone did not inhibit intestinal Na^+,K^+-ATPase, the addition of PTX (0.1 nM) resulted in a significant ($P<0.05$) inhibition of Na^+,K^+-ATPase activity (15.2±3.9 % reduction). When 18-day old rats were separated from their female progenitor and were given ordinary rat chow (the same used to feed adult animals) for two days marked changes were observed upon intestinal Na^+,K^+-ATPase activity in comparison with that from animals with the same age but kept on breast-feeding. Sodium pump activity increased two-fold (from 52±4 to 127±8 nmol Pi mg protein^{-1} min^{-1}) and became insensitive to inhibition by 1 μM dopamine. By contrast, intestinal Na^+,K^+-ATPase activity in 60-day old rats which have been submitted to unilateral nephrectomy two weeks before was significantly ($P<0.05$) lower than that in sham-operated rats (114±10 vs. 153±10 nmol Pi mg protein^{-1} min^{-1}) and was sensitive ($P<0.05$) to inhibition by 1 μM dopamine (18.2±3.7 % reduction). The inhibitory effect of dopamine was antagonised by D_1 (SKF 83566 1 μM) but not by D_2 (S-sulpiride 1 μM) selective antagonists. The results presented here indicate that intestinal Na^+,K^+-ATPase activity in young rats is regulated by both a PTX- and CTX-sensitive G proteins, whereas adult animals have a defective regulation of the enzyme by CTX-sensitive G proteins. Such difference may explain the failure of dopamine to inhibit intestinal Na^+,K^+-ATPase activity in adult rats. However, the sensitivity of the intestinal sodium pump to dopamine may also depend on general homoeostatic mechanisms responsible for water and electrolyte handling or due to protein and salt intake.

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82P DIFFERENTIAL REGULATION OF MITOGENESIS BY TRANSFECTED P2Y RECEPTORS

J.A. Roberts & M.R. Boarder, Dept of Cell Physiology and Pharmacology, University of Leicester, Medical Sciences Building, University Road, Leicester LE1 9HN

P2Y receptors responding to nucleotides play an important role in vascular function (Boarder and Hourani 1998). There is evidence that this involves stimulation of vascular smooth muscle cell mitogenesis by more than one subtype of P2Y receptor (Erlinge, 1998; Harper et al. 1998). In this study we set out to directly compare modulation of proliferation between the different transfected P2Y receptors in the same host cell.

1321N1 human astrocytoma cells showing no native responses to purinergic agonists were transfected with turkey $P2Y_1$, human $P2Y_2$, human $P2Y_4$ and rat $P2Y_6$ receptors. Phospholipase C (PLC) was measured by accumulation of [^{3}H]inositol phosphates ([^{3}H]InsP_x) in the presence of lithium, p42 and p44 MAPK phosphorylation by western blot with antibody specific for the tyrosine phosphorylated MAPK proteins, and p42/p44 MAPK activity by kinase assay with [^{32}P]ATP and a nonapeptide substrate. [^{3}H]-Thymidine incorporation into DNA was used as a marker for proliferation.

1321N1 cells transfected with the different P2Y receptors all showed increased accumulation of [^{3}H]InsP_x on addition of appropriate agonists (Charlton *et al.* 1996). $P2Y_1$ and $P2Y_2$ transfectants also show increased MAPK activity on agonist addition. In 1321N1 cells however $P2Y_4$ and $P2Y_6$ transfectants did not show a detectable increase in MAPK activation. Using [^{3}H]thymidine to study mitogenesis $P2Y_1$ transfectants gave a 42±8.9% (mean ± s.e., n=4, $p<0.05$, t-test) rise above basal in response to 30μM 2MeSATP (100%= response to 1nM PDGF) and showed an additive effect when used in addition to 1nM PDGF (157 ± 13.9% mean ± s.e.). $P2Y_2$ transfectants exhibited no increase in thymidine incorporation above basal when stimulated with 100μM UTP and no additional effect was seen with 1nM PDGF stimulation (n=3). $P2Y_4$ transfectants showed no significant difference to basal levels on addition of 100μM UTP, however addition of UTP with 1nM PDGF showed a 67.75±10.4% (mean ± s.e., n=5, $p<0.05$, t-test) decrease compared to 1nM PDGF alone. $P2Y_6$ transfectants on addition of 100μM UDP gave no significant increase above basal levels of thymidine incorporation and no additional effect on 1nM PDGF stimulation (n=3). All cell lines showed a significant increase on addition of 10% foetal calf serum.

These data indicate that the different P2Y receptor subtypes are able to differentially regulate mitogenesis, even when transfected into the same host cell and when giving a similar level of stimulation of PLC.

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83P ESTIMATION OF 'FULL' INVERSE AGONISM AT G-PROTEIN-COUPLED RECEPTORS BY [^{35}S]-GTPγS BINDING AT h5-HT$_{1D}$ RECEPTORS EXPRESSED IN CHO CELLS

V. Audinot, A. Newman-Tancredi, M.J. Millan.
Psychopharmacology Dept., IdR Servier,
125 Chemin de Ronde, Croissy-s-Seine, 78290, France.

Despite extensive study of inverse agonists *in vitro*, there is no consensus on how to define a 'full' inverse agonist. We addressed this issue by investigating the G-protein coupling of recombinant human (h) serotonin (5-HT) 5-HT$_{1D}$ receptors stably expressed in Chinese hamster ovary (CHO) cells. Receptor saturation binding was carried out with [^{3}H]-GR 125,743, yielding a B_{max} of 1569 ± 246 fmol/mg and a K_D of 0.67 ± 0.03 nM (n = 3). In competition studies with [^{3}H]-GR 125,743, the agonist, 5-HT, and the inverse agonist, methiothepin (Thomas et al., 1995), yielded K_i values of 0.90 ± 0.05 nM (n = 9) and 22.8 ± 6.5 nM (n = 3) respectively. [^{35}S]-GTPγS binding was carried out as described previously in Newman-Tancredi et al., 1997. Inhibition of [^{35}S]-GTPγS binding with GTPγS yielded biphasic isotherms in the absence of ligands (basal conditions). The number of high affinity (HA) [^{35}S]-GTPγS binding sites was increased by 5-HT (10 μM) but decreased by methiothepin (1 μM; Table 1). Drug treatment did not significantly alter low affinity (LA) binding sites ($p>0.05$, 2-tailed t-test; Table 1).

In untransfected CHO cell membranes, neither 5-HT nor methiothepin altered [^{35}S]-GTPγS binding from basal levels (277 ± 54, 229 ± 51 and 225 ± 48 fmol mg^{-1} nM^{-1} respectively; $p>0.05$, 2-tailed t-test; Table 1).

These data suggest that HA [^{35}S]-GTPγS binding reflects receptor-induced constitutive activity which can be modulated by ligands (whether agonists or inverse agonists). In contrast, LA [^{35}S]-GTPγS binding may be receptor-independent and therefore neither increased by agonists or reduced by inverse agonists. Thus, maximal negative efficacy cannot be greater than the inhibition of HA [^{35}S]-GTPγS binding present under basal conditions and the fractional negative efficacy of inverse agonists can be calculated by:

[(basal HA sites) - (inv. agonist HA sites)] * (basal HA sites)$^{-1}$

Under these conditions (as defined by the number of HA [^{35}S]-GTPγS binding sites), methiothepin exerts a negative efficacy of 0.79 relative to 'full' (= 1.0) inverse agonist efficacy at h5-HT$_{1D}$ receptors.

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Table 1: Inhibition of [^{35}S]-GTPγS binding to CHO-h5-HT$_{1D}$ membranes by GTPγS: actions of 5-HT and methiothepin.

Treatment (n)	[Drug] (μM)	HA sites (fmol/mg/nM)	pIC_{50} (high)	LA sites (fmol/mg/nM)	pIC_{50} (low)
Basal (7)	None	1252 ± 110	8.72 ± 0.02	299 ± 29	6.56 ± 0.10
+ 5-HT (4)	10	1739 ± 190[a]	8.89 ± 0.04[a]	247 ± 22	6.68 ± 0.15
+ Methiothepin (3)	1	261 ± 35[a]	8.42 ± 0.20[a]	339 ± 24	6.41 ± 0.14

The number of binding sites are expressed as fmol of [^{35}S]-GTPγS bound per mg of membrane protein per nM of total [^{35}S]-GTPγS in the assay. Asterisks indicate significance compared to respective basal values: [a]$p<0.05$, 2-tailed t-test.

84P EXTRACELLULAR MATRIX PROTECTION PROMOTES SURVIVAL OF HUMAN CULTURED AIRWAY SMOOTH MUSCLE CELLS

Charlotte K Billington, Timothy M Jobson & Ian P Hall.
Division of Therapeutics and Institute of Cell Signalling, University Hospital of Nottingham, Nottingham NG7 2UH

A cardinal feature of airway remodelling in chronic asthma is an increase in airway smooth muscle mass. Increased airway smooth muscle mass can result from increased cell proliferation, decreased cell death or the promotion of cell survival. We aimed to define factors which promote survival of airway smooth muscle cells by inhibition of apoptosis.

Human airway smooth muscle (HASM) cells were cultured as previously described (Widdop *et al.*, 1993). After seeding in serum free media, cells were incubated for 24h then fixed with 4% formaldehyde. Apoptosis was quantified by observer blinded counting of propidium iodide stained cells. Under these experimental conditions 8.3 ± 1.1 % (n=4) of HASM cells were apoptotic after 24h.

Addition of cycloheximide (50μM) at the time of seeding increased the level of apoptosis to 75.0 ± 4.8 % ($p<0.05$, n=4). This effect was reduced by 83.7 ± 5.7% ($p<0.05$, n=4) by the addition of 10% FCS suggesting that continued protein synthesis is required for cell survival under these experimental conditions. Fibronectin (10μg/ml) was also able to rescue the cells from cycloheximide induced apoptosis (97 ± 5.6% reduction cf cycloheximide alone, $p<0.05$, n=4) suggesting a role for extracellular matrix in promoting cell survival. Addition of collagen 4 (10μg/ml) resulted in a partial reduction (50.7 ± 5.9%, $p=0.05$,n=4) of apoptosis whereas vitronectin (10μg/ml) had no significant effect.

To define the integrin receptor involved in the interaction with matrix, we seeded cells in the presence of specific antibodies. Addition of the blocking antibody to the αV integrin subunit at seeding increased apoptosis by 2.9 ± 0.6 fold cf basal ($p<0.05$, n=4) as did addition of anti-β1 integrin antibody (2.0 ± 0.4 fold cf control, $p<0.05$, n=4).

30min preincubation with the calcium chelator BAPTA-AM or addition of SKF96365 (10μM) (Cabello & Schilling, 1993), an antagonist of receptor operated calcium channels (added prior to seeding), resulted in 94.8 ± 2.5% and 97.0 ± 0.9% apoptosis respectively.

These data suggest that interaction with extracellular matrix components can promote HASM cell survival by inhibition of apoptosis via interaction with αVβ1 integrin. This response is likely to play a critical role in controlling cell survival.

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85P IDENTIFICATION OF AN APPARENTLY NOVEL, NUCLEOTIDE-RECOGNISING RECEPTOR LINKED TO CHLORIDE SECRETION IN RABBIT AND HUMAN AIRWAY EPITHELIUM

C.E. Pollard, C.S. Davie, S.M. Roberts, P.D. Alexander, B. Springthorpe‡, M.P. Mortimore‡, S.D. Guile‡ & P.Leff. Departments of Pharmacology & ‡Medicinal Chemistry, Astra Charnwood, Loughborough, Leics., LE11 5RH.

There has been interest in the therapeutic potential of $P2Y_2$ receptor agonists to treat the pulmonary manifestations of cystic fibrosis (CF), a disease characterised by defective cAMP-dependent Cl^- secretion from epithelial tissues. This stems from the fact that $P2Y_2$ agonists like ATP activate Cl^- secretion from the airway epithelium of CF subjects via a Ca^{2+}-dependent Cl^- secretion pathway which remains functional (Knowles *et al.*, 1991). Whilst looking for better $P2Y_2$ receptor agonists, we have identified a receptor type distinct from $P2Y_2$ which recognises di-substituted adenine nucleotides such as AR-C68282KP. In the absence of a definitive classification based on structural and functional criteria, and for the sake of brevity, we have termed this receptor the '68282-receptor'.

The Ussing chamber technique was used to measure short circuit current (I_{sc}) in airway epithelium dissected from rabbit trachea or human nasal polyp. Experiments were performed at 37°C in Krebs buffer. The contribution of Na^+ absorption to I_{sc} was removed by adding 100μM amiloride to the apical surface of the tissue, leaving Cl^- secretion as the dominant active transport pathway. Cumulative concentration-effect (E/[A]) curves were constructed in 0.5 log unit increments and responses were expressed as a % of that to a marker concentration of $P2Y_2$ agonist. Unless stated otherwise, all compounds were added to the apical surface of the tissue and antagonists were added 5 minutes before starting E/[A] curves. Results quoted are $p[A]_{50}$ values except where the E/[A] curve asymptote (α) is indicated. Data sets (mean ± s.e.m. (n)) were compared using an unpaired, two-tailed, Student's *t*-test (* $p<0.05$ versus corresponding control).

In rabbit tissue, AR-C68282KP evoked a concentration-dependent increase in I_{sc} (7.5 ± 0.1 ; α=100 ± 4 (7)). Addition of the co-transport inhibitor bumetanide (100μM) to the basolateral side of the tissue caused a significant, non-competitive inhibition of E/[A] curves to AR-C68282KP, confirming Cl^- secretion as the ionic basis for this effect (control 7.6 ± 0.1; α=124 ± 6%; + bumetanide 7.3 ± 0.2; α=15 ± 1%* (4)). In the presence of 3μM AR-C69312KF, a close analogue of AR-C68282KP which showed little or no agonist activity, there was a significant, parallel, rightward shift of E/[A] curves to AR-C68282KP (control 7.6 ± 0.2; +AR-C69312KF 6.8 ± 0.2 (5)*; pA_2 6.3 ± 0.1). In contrast, E/[A] curves to ATP, acting at $P2Y_2$ receptors, were not affected by 3 μM AR-C69312KF (control 5.6 ± 0.1;+ AR-C69312KF 5.9 ± 0.1 (5)).

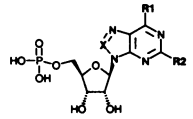
We investigated the possibility that AR-C68282KP or its potential nucleoside metabolites were activating Cl^- secretion via the P1 receptors in this tissue. However, although the P1 receptor antagonist 8-(p-sulfophenyl)theophylline (8SPT) (300 μM) caused a significant rightward shift of E/[A] curves to adenosine (control 5.7 ± 0.1; + 8SPT ~ 4 (5)) it did not affect E/[A] curves to AR-C68282KP (control 8.1 ± 0.2; + 8SPT 7.9 ± 0.2 (5)).

Testing of AR-C68282KP analogues yielded a potency order of: AR-C70148KP (8.3±0.1(5)) > AR-C68282KP (7.5±0.1(7)) = AR-C68238KP (7.5±0.1(4)) > AR-C67590KP (6.4±0.2(3)).

AR-C68282KP evoked Cl^- secretion in human nasal polyp epithelium from 5 different donors but its activity was variable, producing a family of curves whose $p[A]_{50}$ and α values changed in a manner compatible with variable receptor reserve or coupling efficiency ($p[A]_{50}$ range 6.4-7.2; α range 25-124%).

In conclusion, rabbit airway epithelium contains a receptor which recognises di-substituted adenine nucleotides, is linked to Cl^- secretion and is distinct from $P2Y_2$ or P1 receptors. There is preliminary evidence that this receptor is also present in human respiratory epithelium.

Structures



AR-C	R1	R2	X
70148KP	N-diethyl	S-pentyl	N
68282KP	N-diethyl	S-pentyl	C
68238KP	N-diethyl	N-diethyl	C
67590KP	N-diethyl	S-propyl	C
69312KF	N-dimethyl	S-pentyl	C

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86P MODULATION OF PITUITARY HORMONE RELEASE BY CENTRAL OR PERIPHERAL ADMINISTRATION OF ANTI-LIPOCORTIN 1 ANTISERA

J.G. Philip, P.O. Cover, [1]J.F. Morris, [2]R.J. Flower and J.C. Buckingham; Depts. of Neuroendocrinology, Imperial College School of Medicine, London W6 8RF, [1]Human Anatomy & Genetics, University of Oxford, OX1 3QX & [2]Biochem. Pharmacol., W.H.R.I., London EC1M 6BQ.

Our previous work suggests that lipocortin 1 (LC1) serves as a mediator of glucocorticoid action in the rat neuroendocrine system. In the present study we have attempted to delineate the central and peripheral influences of LC1 on pituitary hormone release by comparing the effects of central (i.c.v. via indwelling cannulae) and peripheral (s.c.) administration of a neutralising anti-LC1 polyclonal antibody (LC1 pAb) on the ability of corticosterone (CORT) to modulate the pituitary responses to rat interleukin 1ß (IL-1ß) given centrally (i.c.v.) or peripherally (i.p.). Adult male rats were treated with CORT (100μg/100g; i.p.) or vehicle (0.2ml/100g) and 75 min later with IL-1ß (10ng/rat; i.c.v. or 50μg/100g i.p.) or a corresponding volume of vehicle (3μl/rat i.c.v. or 0.2ml /100g, i.p.). They were killed 1h later and blood collected for immunoassay of ACTH, prolactin (PRL), GH, TSH and LH (data analysis by ANOVA plus Scheffés test). LC1 pAb was given either s.c. (400μl/rat) 24h before the steroid challenge or i.c.v. (3μl/rat) 15 min before the steroid; controls received equal volumes of non-immune sheep serum (NSS).

In rats treated centrally with NSS, IL-1ß (i.c.v.) produced significant ($p<0.01$, n=7-8) increases in plasma ACTH (28±2 *vs.* 0.9±0.4 pg/ml) and serum PRL (5.3±0.9 *vs.* 1.36±0.1 ng/ml) which were prevented by pretreatment with CORT. A similar response to IL-1ß was seen in rats pretreated with LC1 pAb i.c.v. (ACTH: 29±5.8 *vs.* 1.9±0.5 pg/ml; PRL: 6.7±0.87 *vs.* 1.59±0.14 ng/ml). However, LC1 pAb (i.c.v.) effectively quenched the inhibitory influence of CORT on IL-1ß (i.c.v.) induced ACTH release (IL-1ß+CORT+LC1 pAb: 19.3±3.4 *vs.* IL-1ß+CORT+NSS: 3.4±1.3 pg/ml) although it failed to reverse the inhibitory actions of CORT on the PRL response to IL-1ß (IL-1ß+CORT+LC1 pAb: 1.7±0.2 *vs.* IL-1ß+CORT +NSS: 1.5±0.2 ng/ml).

A similar pattern of results was obtained for ACTH when (a) LC1 pAb/NSS were given s.c. and IL-1ß was given i.c.v. and (b) LC1 pAb/NSS were given i.c.v. and IL-1ß was given i.p., i.e. the inhibitory effects of CORT on the increases in ACTH secretion induced by the cytokine were reversed specifically by treatment with LC1 pAb. By contrast, the profile of data obtained for PRL depended on the route of administration of both the antibody and the cytokine. Thus, when given s.c., LC1 pAb effectively reversed the CORT-induced suppression of PRL secretion induced by IL-1ß (i.c.v). By contrast and in accord with previous observations (Taylor *et al.*, 1995), i.p injection of IL-1ß had no effect on serum PRL; thus, when LC1 pAb was given i.c.v. and IL-1ß i.p., there were no significant differences between groups in serum PRL.

None of the treatments influenced the serum TSH or LH concentrations. However, CORT stimulated ($p<0.05$) GH release in rats treated with NSS i.c.v. (295±47 *vs.* 75±5.8 ng/ml) or s.c. (13.25±4.1 *vs.* 1.7±0.14 ng/ml). The responses to the steroid were attenuated ($p<0.05$) by central but not peripheral administration of LC1 pAb and also by central but not peripheral injection of IL-1ß.

These results add further support to our premise that LC1 serves as a mediator of glucocorticoid action in the neuroendocrine system. They also suggest that the regulatory actions of LC1 on ACTH secretion are effected at both the hypothalamic and pituitary levels; on the other hand, those on PRL appear to be exerted mainly on the pituitary gland while those on GH involve a central action.

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87P α,β-METHYLENE-ATP EVOKES EXCITATION OF MESENTERIC AFFERENT NERVES

A.J. Kirkup, D. Grundy, I.P. Chessell* and P.P.A. Humphrey*, Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN and *Glaxo Institute of Applied Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ.

The function of ATP as a neurotransmitter within the gastrointestinal tract is unequivocal (Burnstock, 1990). In addition, ATP may have a role in sensory transmission from the intestine since the cell bodies of sensory neurones, which project to the gut, are stimulated by the nucleotide *via* ionotropic P2X receptors (Khakh *et al.*, 1995). However, there is no direct evidence for the presence of P2X receptors on intestinal afferent nerve endings. We therefore examined the effects of the selective P2X receptor agonist, α,β-methylene-ATP (α,β-me-ATP), on afferent nerves supplying the jejunum in the anaesthetised rat. Furthermore, the effects of the antagonists, pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) or suramin, on responses to α,β-me-ATP, were evaluated.

Experiments were conducted with pentobarbitone-anaesthetised (60mg.kg^{-1}, i.p.) male Wistar rats (330-450g). A jugular vein and femoral artery were cannulated for systemic administration of drugs. A carotid artery was cannulated to enable measurement of haemodynamic parameters. Extracellular recordings were made from nerve bundles innervating a section of jejunum which was intubated to permit the recording of intraluminal pressure. Unless otherwise stated, data are shown as the mean±s.e.mean from 5-7 animals and analysed for statistical significance using the Student's *t*-test.

α,β-Me-ATP (1-300μg.kg^{-1}, i.a.) induced dose-dependent increases in mesenteric afferent nerve discharge (-log$_{10}$ ED$_{50}$ (g.kg^{-1}) = 4.50±0.16), blood pressure (-log$_{10}$ ED$_{50}$ (g.kg^{-1}) = 5.37±0.22), intraluminal pressure and a bradycardia but the effects on the latter two variables did not reach a maximum. On administration of the ED$_{50}$ dose of α,β-me-ATP (30 μg kg^{-1}, i.a.) the stimulation of afferent nerve activity was rapid, occurring 1.8±0.2s after injection, and consisted of a transient, intense burst which decayed after 3.8±1.0s. In contrast, the elevations in intraluminal pressure commenced 9.6±1.7s (P<0.05 *cf.* initiation of afferent discharge) after administration. No desensitisation of the responses to the ED$_{50}$ dose occurred when the agonist was administered every 15 min. Treatment with PPADS (20 mg kg^{-1}, i.v. 5 min) and suramin (80 mg kg^{-1}, i.v., 5 min) but not saline vehicle (0.9% *w/v* NaCl in distilled water) antagonised the peak effect of α,β-me-ATP (30 μg kg^{-1}, i.a.) on afferent nerve discharge, blood pressure and heart rate (Table 1). The antagonists did not affect afferent nerve activity evoked by 2-methyl-5-HT (10 μg, i.v.), by CCK (25 pmoles, i.v.) or by intraluminal distension with 0.5ml saline.

Table 1 Effect of PPADS and suramin on the responses produced by α,β-me-ATP on mean arterial pressure (MAP), heart rate (HR), intraluminal pressure (IP) and mesenteric nerve activity (MNA).

	% of response prior to antagonist treatment			
	MAP	**HR**	**IP**	**MNA**
PPADS	20±4**	25±26*	34±18	3±2*
Suramin	92±3*	66±10*	65±31	39±9*

*=P<0.05 & **=P<0.001

In conclusion, α,β-me-ATP evokes a PPADS- and suramin-sensitive excitation of mesenteric afferent nerves in the anaesthetised rat. We propose that this effect represents a direct action on the afferent nerve terminal and is mediated *via* P2X receptors.

A. J. Kirkup is a GlaxoWellcome Research Fellow.

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88P FASTING ENHANCES α_1-ADRENOCEPTOR-MEDIATED EFFECTS ON JEJUNAL EPITHELIAL TRANSPORT IN 20-DAY-OLD RATS

V.A. Lucas-Teixeira, M.A. Vieira-Coelho, M.P. Serrão & P. Soares-da-Silva. Inst. of Pharmacol. & Therap., Fac. of Medicine, 4200 Porto, Portugal.

Intestinal mucosal structure and function may be regulated by systemic factors associated with oral feeding, as well as local responses initiated by contact of the mucosa with food. Fasting has been shown to change not only mucosal structure, but also its function, namely, its secretory capacity (Carey, H. V., et al, 1994). The pro-absorptive and secretory effects of catecholamines at the intestinal level are mediated mainly through the activation of α_2-adrenoceptors (Liu & Coupar, 1997; Vieira-Coelho & Soares-da-Silva, 1998) and these aspects have not yet been analysed in detail in young animals. The present study was intended to evaluate the effect of 24 hour fasting (with free access to water) on the ionic fluxes across the jejunal epithelium in young rats (20-day old) using short-circuit current (I_{sc}) measurements. Jejunal epithelial sheets were mounted in Ussing chambers (window area 0.28 cm^2) equipped with water-jacketed gas lifts bathed on both sides with Krebs-Hensleit solution. Transepithelial resistance (Ω.cm^2) was determined by altering the membrane potential stepwise (± 5 mV) and applying the Ohmic relationship. Changes in short circuit current (μA/cm^2) were continuously measured as an index of electrogenic ion transfer. Results are given as arithmetic means ± s.e. mean, n=4. Statistical analysis were performed by one-way analysis of variance (ANOVA) followed by Student's "t" test. Jejunal preparations from control and fasted rats exhibited a mean basal I_{sc} value (in μA/cm^2) of 15.9±6.1 and 12.0±2.1 (n=4), respectively. In control rats, UK 14,304 (3 to 3000 nM) when applied alone from the basolateral side produced a concentration dependent decrease in I_{sc} with a EC$_{50}$ value of 8.5 (5.5, 13.1) nM. In fasted rats, under the same experimental conditions, UK 14,304 produced a concentration dependent decrease in I_{sc} with a EC$_{50}$ value of 9.3 (8.0, 10.7) nM, but marked differences (P<0.05) were observed at the level of the maximal effect (E_{max}) obtained by UK 14,304 (control, 65±2 % reduction; fasted, 93±1 % reduction). The effect of frusemide (1 mM) in both control and fasted rats was a time-dependent decrease in I_{sc}, attaining its nadir 20 min after the addition of the Na^+,K^+,$2Cl^-$ (NKCC) co-transport inhibitor; control, 48±7 reduction; fasted 48±18 reduction). Ouabain (1 mM) produced a biphasic effect with an increase followed by a decrease in I_{sc}; the effect of ouabain in control and fasted rats presented a similar profile. In control rats, the decrease in I_{sc} produced by UK 14,304 was completely abolished by ouabain and frusemide. By contrast, in fasted rats the decrease in I_{sc} by UK 14,304 was not significantly affected by ouabain (1 mM) and only partially antagonised by frusemide (1 mM). In conclusion, the decrease in I_{sc} produced by UK 14,304 appears to be markedly potentiated in fasted animals, which may be related to an increase in the activities of Na^+-K^+ ATPase and NKCC co-transport or supersensitivity of intestinal α_2-adrenoceptors.

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89P THE AGE-DEPENDENT INHIBITORY EFFECTS OF DOPAMINE ON RAT JEJUNAL ELECTROLYTE TRANSPORT AND Na-K$^+$ ATPase ARE MEDIATED THROUGH D_1 RECEPTORS

M.A. Vieira-Coelho & P. Soares-da-Silva. Inst. Pharmacol. & Therap., Fac. Medicine, 4200 Porto, Portugal.

A high salt diet has been found to constitute an important stimulus for the production of dopamine in rat jejunal epithelial cells and this is accompanied, in 20-day old animals, by a decrease in sodium intestinal absorption (Finkel et al., 1994). This effect is accomplished, at the cellular level, by inhibition of Na^+,K^+-ATPase activity (Vieira-Coelho et al., 1998). The relative importance of this system in controlling sodium absorption assumes particular relevance in view of the findings that 40-day old rats submitted to a high salt diet have a fault in intestinal dopamine production during salt loading, in contrast to that occurring in 20-day old animals. The lack of changes in the jejunal function in response to high-salt diet coincides with the period in which the renal function has reached maturation (Robillard et al., 1992), suggesting the occurrence of complementary functions between the intestine and the kidney during development. The present study examined the effect of dopamine on rat jejunal electrolyte transport (rheogenic transport and Na^+,K^+-ATPase activity) in adult (60-day old) and young (20-day old) animals. Results are given as arithmetic means ± s.e. mean, n=4-6. Statistical analysis were performed by one-way analysis of variance (ANOVA) followed by Student's "t" test. Dopamine (0.1-100 μM) applied from the basolateral side produced a concentration dependent decrease in short circuit current (I_{sc}) with a EC_{50} values of 1.0 (0.4, 2.6) μM and 7.0 (3.3, 14.8) μM in adult and young rats, respectively. In the presence of phentolamine, the effect of dopamine (0.1-100 μM) in young rats was changed to a biphasic effect. Dopamine (0.1 to 3.0 μM) produced an increase in I_{sc} (maximal effect, 33.6±0.4 % increase) with a EC_{50} value of 0.1 (0.07, 2.5) μM. At higher concentrations (10 to 100 μM) the effect of dopamine was a concentration-dependent decrease in I_{sc} with a (EC_{50} = 35.5 [14.9, 84.5] μM). The increase in I_{sc} produced by low dopamine concentrations was completely abolished by SKF 83566 (1 μM), and not changed by S-sulpiride (1 μM). SKF 38393, but not quinerolane, increased I_{sc} (maximal effect, 30±6 % increase) with a EC_{50} value of 0.31 (0.23, 0.43) nM; this effect was abolished by SKF 83566 (1 μM) and ouabain (1 mM), but not by frusemide (1 mM). By contrast, in adult animals the effect of dopamine (0.1-100 μM) in the presence of phentolamine (0.2 μM) was a decrease in I_{sc} (EC_{50} = 12.9 [5.4, 30.7] μM). Na^+,K^+-ATPase activity (in nmol Pi mg protein^{-1} min^{-1}) in isolated jejunal epithelial cells from adult rats (123±3) was 2.4-fold that in young rats (52±4). In the presence of phentolamine (0.2 μM), both dopamine (1 μM) and SKF 38393 (10 nM), but not quinerolane (10 nM), significantly ($P<0.05$) decreased jejunal Na^+,K^+-ATPase activity in young animals but not in adult animals. In conclusion, in the presence of α-adrenoceptor blockade, dopamine produces a decrease in young rat jejunal electrolyte absorption. This effect, most likely due to inhibition of Na^+,K^+-ATPase, is mediated by D_1 receptors and is not present in adult rats.

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Supported by grant SAU 29/95.

90P SENSITIVITY TO UIC2 AND VERAPAMIL OF NATIVE AND HUMAN P-GLYCOPROTEIN IN LLC-PK_1 AND LLC-GA5 Col300 RENAL CELLS

P. Soares-da-Silva & P. Serrão. Inst. Pharmacol. & Therap., Fac. Medicine, 4200 Porto, Portugal.

The role of P-glycoprotein on the basal-to-apical uptake and flux of L-DOPA was studied in LLC-PK_1 cells and in LLC-GA5 Col300 cells, a renal cell line expressing the human P-glycoprotein in the apical membrane (Saeki et al., 1993). LLC-PK_1 cells (ATCC CRL 1392; passages 210-215) were grown in Medium 199 supplemented with 100 U ml^{-1} penicillin G, 0.25 μg ml^{-1} amphotericin B, 100 μg ml^{-1} streptomycin, 3% foetal bovine serum and 25 mM HEPES. The culture medium used to grow LLC-GA5 Col300 cells (passages 16-25) was similar to that described above with the exception that it contained 300 ng ml^{-1} colchicine. For transport studies, the cells were seeded onto collagen treated 0.2 μm polycarbonate filter supports (internal diameter 12 mm Transwell, Costar) at a density 13,000 cells per well (2.0 x 10^4 cells cm^2). For studies on P-glycoprotein activity, the cells were seeded in collagen treated glass cover slips (10 mm diameter) at a density of 30,000 cells per cover slip. For 24 hours prior to each experiment, the cell medium was free of foetal bovine serum. Results are arithmetic means with s.e.mean, n=4-8. P-glycoprotein activity was measured as the rate of accumulation of calcein (0.5 μM) in the presence and absence of verapamil (25 μM). In the absence of verapamil, LLC-GA5 Col300 cells accumulate less calcein (7.5±0.3 pmol mg protein^{-1} s^{-1}) than LLC-PK_1 cells (11.2±0.3 pmol mg protein^{-1} s^{-1}). In LLC-PK_1 cells, pre-treatment with verapamil (25 μM) for 30 min increased the rate of accumulation of calcein by 5-fold, whereas in LLC-GA5 Col300 cells no significant change in the rate of accumulation of calcein was observed. Exposure for 3 h to verapamil (25 μM) was found to increase the rate of accumulation of calcein by 2.5-fold in LLC-PK_1 cells and by 3.7-fold in LLC-GA5 Col300 cells. Thirty min exposure to UIC2 (3 μg ml^{-1}) or verapamil (25 μM) increased L-DOPA accumulation in LLC-PK_1 cells by 27±4% and 88±14% and reduced L-DOPA apical extrusion by 29±4% and 23±1%, respectively. By contrast, exposure to UIC2 (3 μg ml^{-1}) or verapamil (25 μM) for 3 h was found to decrease (45±1% and 38±2% reduction) the accumulation of L-DOPA in the cell monolayer and to produce a marked reduction (85±1% and 86±1% reduction) in apical extrusion of L-DOPA. In contrast to that observed in LLC-PK_1 cells, exposure of LLC-GA5 Col300 cells to UIC2 (3 μg ml^{-1}) or verapamil (25 μM) for 30 min produced no significant changes in cell accumulation and apical extrusion of L-DOPA. On the other hand, a more prolonged exposure (3 h) to UIC2 (3 μg ml^{-1}) or verapamil (25 μM) resulted in a marked increase in L-DOPA accumulation in the cell (105±13% and 146±24% increase) and a pronounced decrease (91±1% and 92±1% reduction) in the apical extrusion of L-DOPA. It is concluded, that apical extrusion of L-DOPA, in LLC-GA5 Col300 and LLC-PK_1 cells, is in part promoted through P-glycoprotein and, that native P-glycoprotein in LLC-PK_1 cells is more sensitive to inhibition by UIC2 and verapamil than cells expressing the human P-glycoprotein.

Saeki, T., et al. (1993). J. Biol. Chem., 268, 6077-6080.

Supported by grant SAU 123/96.

91P PROSTANOID EP RECEPTOR mRNA EXPRESSION IN HUMAN TISSUES: CORRELATION WITH FUNCTIONAL EXPRESSION

Coleman RA, Carey J, Murphy OE & Baxter GS. Pharmagene Laboratories Ltd, 2 Orchard Road, Royston, Herts, SG8 5HD.

We have previously reported the use of measurements of specific receptor mRNA as a surrogate marker of receptor expression (Baxter *et al*, 1998). In the present study, we have quantified the level of expression of specific mRNA for prostanoid EP_3-receptors in various human tissues, and compared the findings with the known distribution of functional EP_3-receptors in those same tissues from man and other animals (Coleman *et al*,1994; Jones *et al*, 1997).

Preparation of tissues, and extraction and quality control (QC) of RNA were essentially as described by Murphy *et al* (1998). Thus, total RNA was extracted from each tissue using TriZol™, and 1µg/µl solutions prepared in nuclease-free water containing RNase inhibitor. QC was established by integrity of ribosomal RNA, PCR amplification of actin, and A_{260} / A_{280} ratios ≥ 1.7. RNA was extracted from adrenal, breast, caudate, cerebellum, duodenum, endometrium, kidney, myometrium, oesophagus, omental adipose, placenta, pulmonary artery, spleen, stomach, substantia nigra, thyroid, tonsil, uterine cervix and vas deferens, n=3 donors for each (2 for myometrium and 1 for endometrium). Pairs of primers and a TaqMan probe were designed using Primer Express software (Perkin Elmer) to amplify: 1. an 80 base-pair fragment from the human EP_3 mRNA sequence (GenBank accession number L26976) ***Forward:*** *5' AAGGCCACGGCATCTCA GT 3'*; ***Reverse:*** *5' CACATGATCCCCATAAGCTGAA 3'*; ***Probe:*** *5'CG CATCACGACCGAGACGGC 3'*; and 2. a 78 base pair portion of the 'housekeeping' gene human glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ***Forward:*** *5' GAAGGTGAAGGTCGGAGTCAAC 3'*; ***Reverse:*** *5' CAGAGTTAAAAGCAGCCCTGGT 3'*; ***Probe:*** *5' TTTGG TCCGTATTGGGCGCCT 3'*. Expression of specific mRNA for both EP_3-receptor and GAPDH was quantified in 100ng total RNA, using the ABI Prism 7700 Sequence Detection System (Perkin Elmer) as described by Page *et al* (1998).

All tissue samples yielded total RNA of good yield and quality. The levels of mRNA for GAPDH were consistent within and between all tissues, except one example each of adipose, adrenal and tonsil,

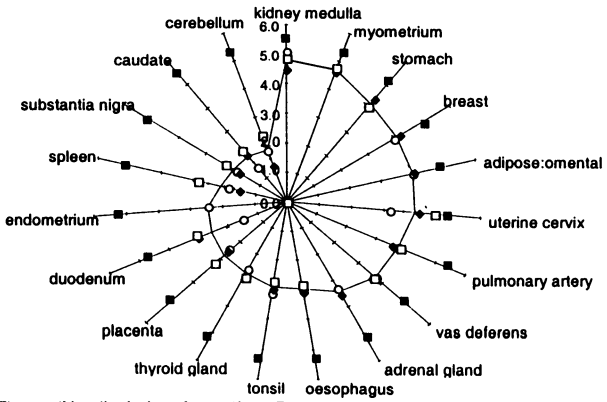

Figure: 'Nautilus' plot of specific mRNA for EP_3-receptor (individual levels ○, □ & ◆, mean:———) and GAPDH (mean: ■) in total RNA from 19 different tissues. Radii represent different tissues; concentric circles represent log copy number per 100ng total RNA.

where levels were low. Levels of EP_3 mRNA were generally consistent between examples of the same tissue (tissues with low GAPDH excluded), but differed by up to 1,000-fold between the different tissues. The nine highest expressing tissues have all been reported to express functional populations of EP_3-receptors in man (myometrium, stomach, uterine cervix, pulmonary vasculature) or other species (kidney medulla, adipose *omental and breast*, vas deferens and adrenal). Such functional expression has not been reported in any of the other ten tissues. These results support the use of specific mRNA as a surrogate marker of functional receptor expression.

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Coleman RA, Smith WL, Narumiya S (1994) *Pharmacol Rev.* **46**, 205-229.
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Murphy OE, Carey J, Miah A *et al.* (1998) *Br J Pharmacol.* 123, 114P
Page K, Day N, McKinnon H *et al.* (1998) *Cell Vision* (in press)

92P EXPRESSION OF CHOLINE ACETYLTRANSFERASE IN HUMAN GLIAL AND IMMUNE CELLS

I. Wessler, K. Racké*, F. Bittinger§, C.J. Kirkpatrick§, Dept. Pharmacol. and §Pathol., Univ. Mainz, Obere Zahlbacher Str. 67, D-55101 Mainz, Germany; *Dept. Pharmacol. Toxicol., Univ. Bonn, Germany.

Increasing experimental evidence has been amassed that acetylcholine represents a widely expressed "cytomolecule" in non-neuronal cells, for example epithelial cells (Sastry & Sadavongvivad, 1979; Grando, 1997; Wessler *et al.*, 1998). In the experiments described here we have further investigated the expression of choline acetyltransferase (ChAT) and/or the synthesis of acetylcholine in human glial and immune cells.

The expression of the non-neuronal cholinergic system was analysed i) by anti-ChAT immunohisto-/ cytochemistry applying mono- and polyclonal anti-ChAT antibodies (ab), ii) by immunofluorescence technique coupled with confocal laser microscopy, iii) by measuring ChAT enzyme activity, and finally iv) by measuring acetylcholine content using a sensitive and highly specific HPLC-method. Human tissue (neocortex; skin of the female breast) was obtained at surgery from patients with tumors. Human circulating blood cells (platelets, leukocytes) were obtained from volunteer donors and separated by standard protocols. Cultures of the human astrocytoma cell line U373 (American Type Culture Collection, ATCC HTB-17) or primary cultures of rat astrocytes were maintained in Minimum Essential Medium Eagle and Basal Medium Eagle supplemented with growth factors, respectively.

U373 cells or rat astrocytes were positively stained by applying poly- or monoclonal anti-ChAT ab. Cryosections of human neocortex were exposed to anti-ChAT ab and anti-GFAP ab (glial fibrillary acidic protein) to identify astrocytes. In these double labelling experiments (tissue from two patients) the expression of ChAT-like protein in human neocortical astrocytes *in situ* could be demonstrated.

Freshly isolated platelets, mononuclear cells and granulocytes were positively stained by anti-ChAT ab; ChAT enzyme activity was 0.4±0.1 (9),11±3 (7) and 430±90 (4) nmol mg protein^{-1} h^{-1}, respectively (means±s.e.m.). In granulocytes more than 85% of ChAT activity was found in the particulate cell fraction. Acetylcholine content did not correlate with ChAT activity, 75± 40 (4), 400±60 (9) and 3±2 (10) fmol acetylcholine 10^{-6} cells were found in platelets, mononuclear cells and granulocytes, respectively. In addition to circulating immune cells also alveolar macrophages and skin mast cells expressed ChAT protein.

In conclusion, the present experiments confirm the wide expression of ChAT and synthesis of acetylcholine in non-neuronal cells. It remains an important task to elucidate the biological role of non-neuronal acetylcholine in the brain and the immune system.

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Grando, S.A. (1997) *J. Invest. Dermatol. Symp. Proc.* **2**, 41-8.
Wessler I., Kirkpatrick, C.J. & Racké, K. (1998) *Pharmacol. Ther.* 1998; 77: 59-79.

93P PHARMACOLOGICAL CHARACTERISATION OF MUSCARINIC RECEPTORS IN DOG CILIARY SMOOTH MUSCLE

A. Choppin, S. S. Hegde & R. M. Eglen.
Center for Biological Research, Neurobiology Unit, Roche Bioscience, Palo Alto, CA 94304, USA.

The ciliary smooth muscle of the eye expresses muscarinic M_3 receptors (shown in cow (Honkanen *et al.*, 1990), dog (McIntyre and Quinn, 1995) and man (Woldemussie *et al.*, 1993)). Novel ligands including AQ-RA 741 (Doods *et al.*, 1991), darifenacin (M_3-selective) and PD 102807 (M_4-selective; Schwarz *et al.*, 1997) were used in the present study to re-examine the pharmacological characteristics of muscarinic receptors in dog ciliary muscle (DCM) and compared to those in the dog urinary bladder (DUB).

Using beagle or mongrel dogs, the methodology for contractile studies was similar to that described by Choppin *et al.* (1998). Cumulative concentration-response curves to (+)-cis-dioxolane (1nM - 300μM) were established in the absence and presence of antagonists (90 min equilibration). Antagonist affinities (pK_B) were determined using the Gaddum equation (Gaddum, 1943).
(+)-Cis-dioxolane induced concentration-dependent contractions of DCM and DUB (pEC_{50} = 7.18±0.07 and 6.55±0.07, respectively). The effects of (+)-cis-dioxolane were antagonized by a range of muscarinic receptor antagonists and the pK_B estimates are summarized in Table 1.

Table 1. pK_B estimates for muscarinic antagonists in DCM and DUB.

Antagonist (DCM/DUB)	pK_B (DCM)	pK_B(DUB)
Atropine (30-300/3-30nM)	8.25±0.14	9.21±0.09
Pirenzepine (1μM)	6.31±0.13	6.70±0.25
Zamifenacin (1.0/0.1μM)	6.46±0.19	7.69±0.11
Tolterodine (0.1-1.0/0.01-0.1μM)	7.97±0.14	8.68±0.12
Oxybutynin (0.1-1.0/0.01-0.1μM)	7.40±0.08	7.88±0.12
AQ-RA 741 (3μM)	6.16±0.15	7.08±0.23
PD 102807 (100nM)	<7.00	<7.00
Darifenacin (0.1-10.0/0.003μM)	NC	9.09±0.13

Values shown are means ± s.e.mean, n≥3. NC: not calculated.

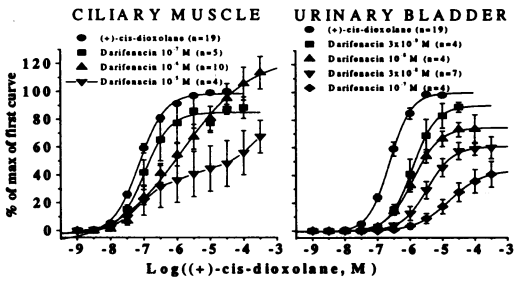

Figure 1: Effect of darifenacin on cumulative concentration-response curves to (+)-cis-dioxolane in dog ciliary muscle and urinary bladder smooth muscle. Contractile effects are normalized to the control maximum (Emax DCM = 452±64mg; DUB = 11±1g).The values shown are means±s.e.mean.

The antagonist potency profile in the DUB is consistent with the singular involvement of M_3 muscarinic receptors. In the DCM, the low affinity of pirenzepine, AQ-RA 741 and PD 102807 exclude the involvement of M_1, M_2 and M_4 muscarinic receptors. The antagonism produced by darifenacin (Figure 1) exhibited two phases of the contractile response: a high affinity, darifenacin-resistant (pK_B<6) component and a low affinity, darifenacin-sensitive (pK_B>8), possibly M_3-mediated, component. Collectively, the data suggest that contractile responses to (+)-cis-dioxolane in the DCM are mediated through more than one receptor, one of which resembles the M_3 muscarinic receptor. The potential involvement of a second atypical receptor possessing low affinity for darifenacin requires further investigation.

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McIntyre, P. and Quinn, P. (1995). *Br. J. Pharmacol.*, 115, 139P.
Schwarz *et al.* (1997). *Life Sci.*, 60, 1167.
Woldemussie *et al.* (1993). *Exp. Eye. Res.*, 56, 385-392.

94P IMPACT OF AGE ON THE INVOLVEMENT OF A PERTUSSIS TOXIN-SENSITIVE G_i-PROTEIN IN AGONIST-INDUCED CALCIUM-SENSITIVITY OF TENSION IN THE RAT TAIL ARTERY

E. Spitzbarth-Régrigny, C. Vandeputte, B. Corman[1], E. Tschirhart[2] & C. Capdeville-Atkinson. Cardiovascular Pharmacology Dept., Pharmacy Faculty, Henri Poincaré University, Nancy, [1]CEA/Saclay, Gif-sur-Yvette, France and [2]CRP-Santé, Luxembourg.

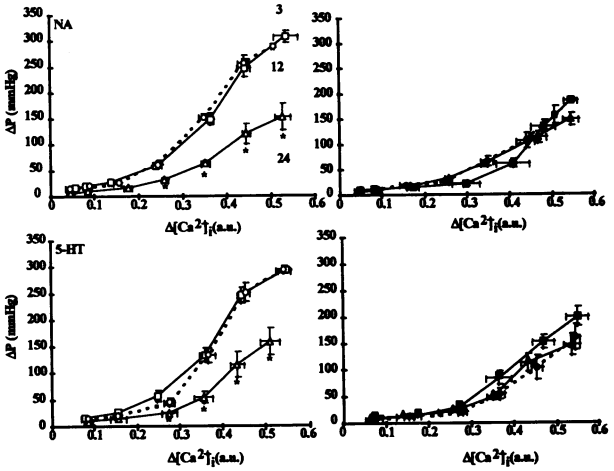
We have shown that in tail arteries from young - but not old - rats pertussis toxin (PTX) lowers the sensitivity of the contractile apparatus to intracellular calcium ($[Ca^{2+}]_i$) following stimulation with noradrenaline (Robert *et al.*, 1998). The aim of this study was to investigate whether PTX-sensitive G_i-proteins are involved in the amplification of $[Ca^{2+}]_i$-sensitivity following stimulation with another agonist, serotonin (5-HT).

Tail arteries were dissected out from WAG/Rij rats (3 month-old *squares*, 12 month-old *circles*, 24 month-old *triangles*, n = 6 per group) under sodium pentobarbitone anaesthesia (60 mg.kg^{-1}). De-endothelialised arteries were cannulated, mounted in a perfusion/cuvette-system placed in a dual wavelength spectrofluorometer and perfused with physiological salt solution. Arteries were loaded with fura 2/AM (5 μM) without (open symbols) or with PTX (1 μg.ml^{-1}, 2 h; full symbols). They were then stimulated with NA (0.01 to 10 μM, 2 min) and 5-HT (0.01 to 3 μM). NA- and 5-HT-elicited increases in $[Ca^{2+}]_i$ (arbitrary units, a.u.) and perfusion pressure (mmHg) were measured. Values are means ± s.e.m. Significant differences (P<0.05, * *vs* 3-month) were determined with 2-way ANOVA and the Bonferroni test.

Age and PTX had no effect on $[Ca^{2+}]_i$ mobilisation elicited by NA or 5-HT (Figure 1).

Agonist-induced vasoconstriction decreased with age. PTX lowered $[Ca^{2+}]_i$-sensitivity in young but not in old rats (Figure 1).

Figure 1 : Impact of age and PTX on agonist-induced $[Ca^{2+}]_i$ sensitivity of tension.



In conclusion, we suggest that agonist-induced vasoconstriction (both NA and 5-HT) in young rats has a PTX-sensitive G_i protein component which increases $[Ca^{2+}]_i$-sensitivity of tension. This is lost with age.

Robert, A., Tran, N.N.P., Giummelly, P. *et al.*, (1998) *Am. J. Physiol.*, 274 : R1604-R1612.

95P VASCULAR REACTIVITY OF ISOLATED SUBCUTANEOUS FAT RESISTANCE ARTERIES FROM OLDER PEOPLE

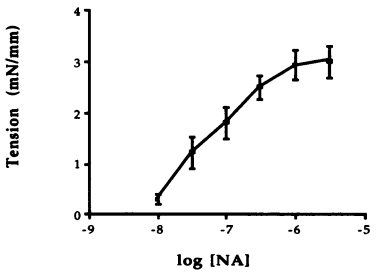
JE Graves[1], PD Taylor[1], TFT Antonios[1], R.J. MacAllister[2], DRJ. Singer[1]. [1]Department of Pharmacology and Clinical Pharmacology, St George's Hospital Medical School and [2]Centre for Clinical Pharmacology, UCL, London.

Ageing is associated with increased cardiovascular disease and in particular endothelial dysfunction. To investigate vascular contractile and endothelial function in older people, we examined the vasodilator/vasoconstrictor responses of isolated arteries from 15 healthy subjects >60 years of age (67.1 ± 1.3 years; diastolic blood pressure 82 ± 3 mmHG; systolic 139 ±5 mmHg).Arteries (i.d. 302 µM ± 27 µM) were dissected out and mounted on myograph and subjected to a standard normalisation procedure. Concentration responses were obtained to noradrenaline, the endothelium-dependent vasodilators acetylcholine and bradykinin and the endothelium-independent vasodilator sodium nitroprusside (SNP). Data are given as mean ± s.e.m.

The arteries exhibited a concentration-dependent constriction to noradrenaline with a maximum tension of 3.02 ± 0.43 mN/mm and a pEC_{50} of 7.20 ± 0.12 (Figure 1a). Arteries precontracted with noradrenaline relaxed to acetylcholine in a concentration-dependent manner with a maximum relaxation of 78% ± 9% and a pEC_{50} of 7.34 ± 0.12 (Figure 1b). Bradykinin also induced vasodilation with a pEC_{50} of 7.37 ± 0.05 and a maximum relaxation of 77% ± 7%. SNP caused relaxation with a pEC_{50} of 6.82 ± 0.06 and a maximum relaxation of 81% ± 6%.

These values are similar to reported values for subjects <60 years old and suggest that vasodilator and vasoconstrictor function in isolated subcutaneous fat resistance arteries from subjects >60 years old are not severely compromised. Our findings contrast with those of James et al (1997) who reported that the same arteries constricted less well to noradrenaline and relaxed less well to acetylcholine and bradykinin compared to reported values for younger subjects. The reasons for the disparate findings may lay in population or methodological differences.

1a



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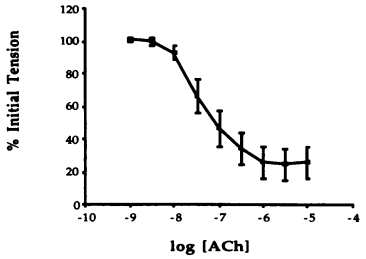

James M.A. et al (1997) Clin. Sci. 92, 139-145

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96P VITAMIN C DOES NOT ALTER ENDOTHELIUM-DEPENDENT DILATATION IN THE FOREARM OF ELDERLY HUMANS

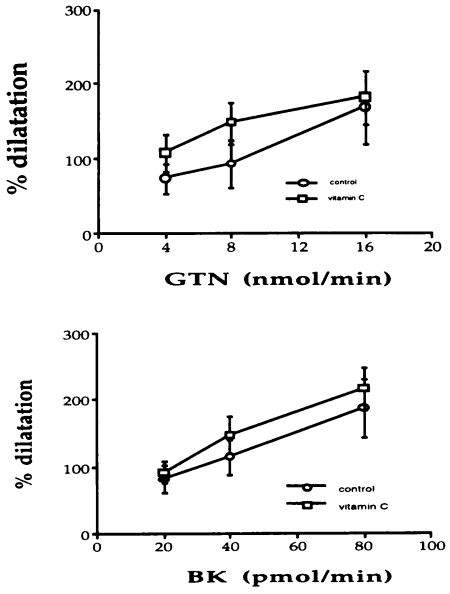
N. Singh[1], JE Graves[1], R.J. MacAllister[2], DRJ. Singer[1]. [1]Department of Pharmacology and Clinical Pharmacology, St George's Hospital Medical School and [2]Centre for Clinical Pharmacology, UCL.

The endothelium generates nitric oxide (NO) which dilates resistance vessels and is a determinant of blood pressure. NO has a short half-life *in vivo*, and under certain conditions is rapidly inactivated by oxidative free radicals. In cardiovascular diseases associated with increased oxidative stress, the anti-oxidant vitamin C has been used to augment NO-mediated dilatation *in vivo* Oxidative stress increases with age and raised concentrations of oxidative free radicals has been implicated as a determinant of vascular endothelial function in elderly subjects. The aim of this study was to assess the effect of vitamin C on endothelium-dependent dilatation in the forearm resistance bed of healthy elderly subjects.

Bilateral venous occlusion plethysmography was used to measure forearm blood flow (ml/min/100 ml forearm volume) during intra-arterial infusion of the endothelium-dependent dilator bradykinin (BK) and the NO donor glyceryl trinitrate (GTN). Studies were performed in 13 healthy elderly subjects (mean age 68 (range 61-80years); 6 male and 7 female, blood pressure 141/85 ±5/3 (SEM) mmHg). The response to cumulative doses of BK (20, 40 and 80 pmol/min; each dose for 3 min) or GTN (4, 8 and 16 nmol/min; each dose for 3 min) was determined alone and subsequently in the presence of vitamin C (25 mg/min). The effect of the infused drugs was calculated from the change in the ratio of blood flow in the infused to the control arm and expressed (mean ±SEM) as the percentage change in this ratio. Dose-response curves were compared by analysis of the area under the curve (AUC) using Students t test.

GTN (n=11) and bradykinin (n=13) caused dose-dependent dilatation of the forearm resistance bed (Figure). Vitamin C alone had no effect on basal blood flow. Vitamin C did not significantly alter the dilatation caused by GTN (AUC 631±185 before and 844±145 during vitamin C; P> 0.25) or BK (744±162 before and 892±129 during vitamin C; P> 0.33).

These results suggest that in healthy old age, vitamin C does not acutely increase the bio-availability of endogenous or exogenous NO and are consistent with functionally normal anti-oxidant defences in this group.



N.S. is a BHF Junior Research Fellow

97P AN INVESTIGATION OF THE PATHWAYS INVOLVED IN CHANGES IN BLADDER AND URETHRAL PRESSURES EVOKED BY DMPP, TYRAMINE AND BLADDER DISTENSION IN ANAESTHETISED RATS

A. Wibberley, *A.M. Naylor & A.G. Ramage
Department of Pharmacology, Royal Free Campus, University College London, Rowland Hill Street, London, NW3 2PF and *Discovery Biology, Pfizer Central Research, Pfizer Limited, Sandwich, Kent, CT13 9NJ

Micturition is dependent on a complex co-ordination of autonomic pathways organised both at peripheral and central levels. The exact nature and role of the transmitters involved in these pathway remains to be fully elucidated. Thus, this preliminary study examined the effects of atropine and prazosin on bladder and urethral responses to ganglionic stimulation with 1,1-dimethylphenylpiperazinium (DMPP), sympathetic nerve terminal stimulation with tyramine, and to the micturition reflex evoked by bladder distension with saline infusion.

Experiments were performed on spontaneously breathing female Sprague-Dawley rats (240-290g), anaesthetised with urethane (1.2g kg^{-1}, i.v.). Blood gases were monitored and maintained within the physiological range. Simultaneous recordings were made of urinary bladder (BLP) and urethral perfusion pressures, as previously described (Kakizaki *et al.*, 1997). Drug-evoked responses (in mmHg) were expressed as percentage changes before and after the administration of antagonists, and compared with vehicle controls by unpaired or paired Student's t-test. All values are means ± s.e.mean.

DMPP (0.5 mg kg^{-1}, i.v.) elicited an increase in BLP and was significantly ($P < 0.05$) attenuated by 44 ± 8% (n=7) by atropine sulphate (0.5 mg kg^{-1}, i.v.), and after subsequent administration of prazosin (0.2 mg kg^{-1}, i.v.) it was further attenuated by 33 ± 8% (n=6) of the initial contraction. Furthermore, DMPP evoked a transient increase in urethral perfusion pressure, which then decreased to below baseline levels. Atropine significantly inhibited 53 ± 12% (n=5) of the contractile response, which was subsequently abolished by prazosin (n=4). Both drugs significantly potentiated the relaxant effect. Tyramine (1 mg kg^{-1}, i.v.) evoked an increase in urethral perfusion pressure of which 4 ± 13% (n=6) was significantly inhibited by atropine,while prazosin caused an 86 ± 9% (n=6) inhibition. Reflex bladder contractions were reduced by 45 ± 6% after atropine (n=6), with no further change after prazosin (n=6). Reflex decreases in urethral perfusion pressure, were unaffected by either antagonists.

The present results indicate that DMPP and reflex-induced bladder contractions involve an atropine sensitive component, however DMPP-evoked contractions also involve a prazosin sensitive component. Further, these data also suggest that for both DMPP and the reflex-induced contractions there is a substantial NANC component which interestingly, seems to be larger in the latter. In addition, urethral relaxations evoked by DMPP and bladder distension are unaffected by either antagonist. However, the contractile responses of the urethra involve an atropine and a prazosin sensitive component.

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98P NON-ADRENERGIC NON-CHOLINERGIC RESPONSES IN THE RABBIT VAGINAL WALL ARE MEDIATED BY NITRERGIC NEUROTRANSMISSION

S. Cellek, T. Ziessen & S. Moncada,
The Wolfson Institute for Biomedical Research, University College London, 140 Tottenham Court Road, London W1P 9LN, UK

Nitrergic nerves have been shown by immunohistochemistry to be present in the human clitoral corpus cavernosum (Burnett *et al.*, 1997) and in the human vagina (Hoyle *et al.*, 1996). We have recently demonstrated that nitrergic neurotransmission is responsible for the non-adrenergic non-cholinergic (NANC) relaxation responses in the clitoral corpus cavernosum of the rabbit (Cellek & Moncada, 1998). Our present aim was to characterise the nitrergic neurotransmission in the vaginal wall of the rabbit.

The vaginal wall of the female New Zealand white rabbits (2.6-4.9kg) was dissected and mounted in horizontal superfusion chambers as described previously for penile and clitoral corpus cavernosum (Cellek & Moncada, 1997 & 1998). Electrical field stimulation (EFS; 50V, 0.3 ms pulse duration, 1-25 Hz, for 5 s, every 2 min) was applied via platinum electrodes on each side of the tissue. EFS elicited noradrenergic contractions which were enhanced in magnitude by a NO synthase inhibitor (300 μM N^G-nitro-L-arginine methylester (L-NAME) caused 24.5 ± 4.1 % enhancement; n=8) or a selective inhibitor of soluble guanylate cyclase (10 μM 1H-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ) caused 14.8 ± 4.6 % enhancement; n=8). A type V phosphodiesterase inhibitor, zaprinast (0.1-100 μM), inhibited the EFS-induced contractions in a concentration-dependent manner (IC_{50}=15.5 ± 2.6 μM; n=8). After inhibition of noradrenergic and cholinergic pathways with guanethidine (5.5 μM) and scopolamine (7 μM) respectively and elevation of the tone by phenylephrine (1 μM; EC_{80}), EFS of the tissue elicited reproducible relaxation responses which were blocked by L-NAME (IC_{50}=72.0 ± 25.4 μM; n=8), ODQ (IC_{50}=686 ± 262 nM; n=6) or TTX (1 μM). The effect of zaprinast on EFS-induced relaxations could not be evaluated since zaprinast (0.1-3 μM) caused loss of the tissue tone.

These results suggest that nitrergic neurotransmission mediates the NANC responses in the vaginal wall of the rabbit and that noradrenergic responses are modulated by the nitrergic system. Potentiation of nitrergic modulation of noradrenergic responses by zaprinast suggests that selective inhibitors of phosphodiesterase typeV may be used in the treatment of female sexual dysfunction where deficient clitoral tumescence or vaginal wall relaxation are responsible for the pathology (Park *et al.*, 1997).

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99P QUANTITATIVE ENZYME RADIOAUTOGRAPHY REVEALS AN UP-REGULATION OF MAO-A AND MAO-B IN DISCRETE BRAIN REGIONS OF PATIENTS WITH HUNTINGTON'S CHOREA

J.G.Richards, J. Messer, Z. Bleuel and R.L.M. Faull[1], Pharmaceuticals Division, Preclinical CNS Research, F. Hoffmann-La Roche Ltd, CH-4070 Basel, Switzerland; [1]Dept. of Anat.with Radiology, Faculty of Med. & Health Sci., Univ. of Auckland, NZ.

Huntington's disease is an autosomal dominant, inherited disorder that results in progressive degeneration of the basal ganglia and other forebrain structures and is associated with a clinical profile of movement, cognitive and psychiatric impairments for which there is at present no effective therapy (Huntington's Disease Collaborative Research Group, 1993). The disease is caused by expansion of CAG trinucleotide repeats within the coding region of a novel gene *huntingtin*. Neuropathological, neurochemical and behavioural features of the disease can be reproduced in animals by local injection of excitotoxic or metabolic toxins into the neostriatum (see Faull et al., 1995).

Monoamine oxidases (MAO. EC 1.4.3.4) are integral proteins of outer mitochondrial membranes. Isoenzymes (MAO-A and MAO-B) occur in various cells (both neuronal and non-neuronal in the CNS and peripheral organs) where they oxidatively deaminate biogenic and xenobiotic amines. In the CNS they play not only a physiological role in the metabolic inactivation of released monoamine transmitters (catecholamines, serotonin, histamine) and in the detoxification of xenobiotic amines but perhaps also a pathological role by indirectly generating cytotoxic free radicals during aging and in neurodegenerative diseases. Quantitative enzyme radioautography (using enzyme-selective reversible inhibitors of MAO-A and MAO-B, namely Ro 41-1049 and lazabemide, respectively) has been used to demonstrate, with high resolution, both age- and disease-related changes in enzyme activity in rat and human brain (see Richards et al., 1998). Thus, in Alzheimer brains increased binding of [^{3}H]lazabemide was shown to occur in plaque-associated astrocytes.

Samples of human brain (at the levels of caudate nucleus, globus pall-idus, substantia nigra and pons) from 5 non-diseased control individuals and 5 Huntington's disease cases were obtained at autopsy (5-11h post mortem) from The New Zealand Neurological Foundation Human Brain Bank (University of Auckland) and stored at -80^{0}C. Cryostat sections (16μm) of fresh-frozen tissue were prepared for *in vitro* binding with [^{3}H]Ro 41-1049 [TRK1062, Amersham] and [^{3}H]lazabemide [TRK1063, Amersham] (see Richards et al., 1998 for details); non-specific binding was determined in the presence of 1μM clorgyline or l-deprenyl, respectively. The binding was quantified radioautographically by exposing the radiolabelled sections, together with tritium microscales, to Hyperfilm Tritium (Amersham) for 4 weeks at 4^{0}C. The films were measured with a MCID M2 image analysis system (Imaging Res. Inc, St. Catherines, Ontario, Canada).

MAO-A was increased significantly (~50%; $p< 0.01$) in the putamen, central medial thalamic nucleus, substantia nigra pars compacta and in pons. Higher significant increases in MAO-B (75-200%) occurred in the putamen, ventral striatum, globus pallidus externus and internus, and insula cortex.

We conclude that MAO activities increase in regions of HC brains which are known to undergo neurodegeneration accompanied by glioses. Whether the increased enzyme activity is a cause or effect of the resulting loss of GABAergic neurons is yet to be clarified.

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100P STUDIES ON A NOVEL SELECTIVE β_3-ADRENOCEPTOR AGONIST IN HUMAN RIGHT ATRIAL APPENDAGE AND HUMAN WHITE ADIPOCYTES

J.R.S. Arch, A.J. Kaumann[1], P. Molenaar[1], M.V. Sennitt[2], J.M. Berge, H. Chapman & J. Kelly, SmithKline Beecham Pharmaceuticals, Welwyn, Herts, AL6 9AR, [1] Dept. Pharmacology, University of Melbourne, Victoria 3052, Australia and [2]St. George's Hospital Medical School, London, SW17 0RE

Most evidence for β_3-adrenoceptor-mediated responses in human tissue derives from studies that use CGP-12177 as the β_3-adrenoceptor agonist. However, recent work, especially in β_3-adrenoceptor knock-out mice, suggests that CGP-12177 can stimulate lipolysis via a putative β_4-adrenoceptor (Preitner *et al.*,1998). Moreover, CGP-12177 is far more potent as a β_1- or β_2-adrenoceptor antagonist than as a β_3- or "β_4"-adrenoceptor agonist. Here we describe a novel β_3-adrenoceptor agonist without the drawbacks of CGP-12177. Methods are described in detail in Sennitt *et al.*, (1998).

SB-251023 ((4-{1-[2-(S)-hydroxy-3-(4-hydroxyphenoxy)-propylamino]cyclopentylmethyl}phenoxymethyl)phenyl-phosphonic acid lithium salt) stimulated adenylyl cyclase activity ($pD_2 = 6.25 \pm 0.20$; intrinsic activity (IA) relative to isoprenaline = 0.66 ± 0.10, n=5) in Chinese hamster ovary cell membranes that expressed human cloned β_3-adrenoceptors (390 fmol/mg protein). It was more potent than, and of similar efficacy, to CGP-12177 in these experiments ($pD_2 = 5.61 \pm 0.11$; IA = 0.50 ± 0.07). SB-251023 had no significant agonist activity at human cloned β_1- or β_2-adrenoceptors and it had low affinities for β_1-adrenoceptors ($pK_i = 3.91 \pm 0.10$, n=6) and β_2-adrenoceptors ($pK_i = 4.37 \pm 0.05$, n=5) in binding studies using [^{125}I]-iodocyanopindolol.

SB-251023 stimulated human white adipocyte lipolysis, its IA (not maximal) at 10 μM being 0.45 ± 0.07 and its pD_2 relative to this response 6.19 ± 0.07, n=3. Maximum responses were achieved in the same experiments to isoprenaline ($pD_2 = 8.56 \pm 0.23$) and CGP-12177 ($pD_2 = 6.52 \pm 0.19$; IA = 0.38 ± 0.13). Nadolol (1μM) antagonised the response to isoprenaline ($pK_B = 7.94 \pm 0.11$), but not CGP-12177; it had little effect against SB-251023 (pK_B values <6, 6.3, 7.4).

SB-251023 did not stimulate atrial contractility nor (at 6μM) antagonise the "β_4"-adrenoceptor-mediated stimulant effect of CGP-12177 (1μM) in the presence of (-)-propranolol (200nM). In the presence of both nadolol (10μM) and SB-251023 (6μM) the effect of CGP-12177 was however blunted by (-)-bupranolol (1μM), consistent with the involvement of "β_4"-adrenoceptors. SB 251023 had a cardiodepressant effect ($pD_2 = 6.49 \pm 0.04$, n=7, 4 patients) that was not affected by (-)-propranolol (200 nM), nadolol (10 μM), or nadolol plus (-)-bupranolol (1μM), showing that it was not mediated by β_1- , β_2- , β_3- or putative β_4-adrenoceptors. In conclusion, SB-251023 is a selective human β_3-adrenoceptor agonist that lacks "β_4"-adrenoceptor agonist or antagonist activity, but has a non-β-adrenoceptor-mediated cardiodepressant effect. Its nadolol-insensitive lipolytic activity is consistent with data obtained using structurally and pharmacologically similar compounds in supporting a role for β_3-adrenoceptors in human white adipocyte lipolysis (Sennitt *et al.*, 1998).

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