285P THE EFFECT OF DIETARY SODIUM ON RENAL ADENOSINE A_1 RECEPTORS IN THE RAT

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Renal interstitial levels of adenosine are enhanced and supressed in sodium loaded and sodium deprived rats, respectively (Siragy & Linden, 1996). Such changes in intrarenal adenosine levels may alter the population and/or responsiveness of adenosine A_1 receptors within the kidney. The aim of this work was to investigate the effect of dietary sodium intake on the binding characteristics and mRNA levels of renal adenosine A_1 receptors in the rat.

Male Wistar rats (200 - 250g) were maintained on a normal (0.4%, w/w, NaCl), low (0.04%) or high (4%) sodium diet for 7 days. On day 8, the animals were killed, and both kidneys were removed and immediately freeze clamped in liquid nitrogen. Renal cell membranes, obtained from 24 rats on a normal sodium diet and 12 rats on either a low or high sodium diet, were diluted to 1 mg ml^{-1} protein and preincubated with adenosine deaminase (5U ml^{-1}) for 30 minutes at 37°C; followed by 5 hours incubation at 4°C with the selective A_1 antagonist, [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine (0.08 - 12nM). Total RNA was isolated from whole kidneys, with A_1 receptor mRNA quantitated by the reverse transcriptase polymerase chain reaction (Gould *et al.*, 1997).

Renal cell membranes obtained from sodium restricted animals showed statistically significant ($P < 0.01$) increases in both Bmax (46%) and Kd (39%), in relation to the normal sodium group (Table 1). By contrast, membranes obtained from sodium loaded animals showed statistically significant ($P < 0.01$) decreases in Bmax (37%) and Kd (73%). Compared to rats with a normal sodium intake, adenosine A_1 receptor mRNA levels were significantly ($P < 0.01$) reduced (65%) in the high sodium group, whilst no significant ($P > 0.05$) change was noted in mRNA levels for the low sodium group (Table 1).

Table 1 Binding characteristics and mRNA levels for renal adenosine A_1 receptors in rats maintained on different sodium diets.

Dietary NaCl	Kd (pM)	Bmax (fmol mg^{-1})	mRNA (% levels in normal rats)
Normal	311 ± 9	5.9 ± 0.2	100 ± 18
Low	433 ± 20 *	8.6 ± 0.2 **	53 ± 26
High	83 ± 6 **	3.7 ± 0.4 **	35 ± 5 *

Bmax and Kd values are estimate ± s.e. estimate (8 d.f.) from nonlinear least squares regression analysis of binding isotherms and mRNA values are mean ± s.e. mean (n = 5); *$P < 0.05$, **$P < 0.01$ (Student's t-test), relative to normal sodium group.

The present study demonstrates that sodium depletion and sodium loading are associated with a significant increase and decrease, respectively, in renal adenosine A_1 receptor density. The latter changes in receptor density appear to result from decreased gene transcription. Receptor affinity was also influenced by sodium intake, with a decrease in sodium restricted rats and an increase in sodium loaded animals. Since A_1 receptors influence renal tubular function, such differential regulation of renal adenosine A_1 receptors by dietary sodium suggests that these receptors play a role in electrolyte homeostasis.

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286P TEMPERATURE SENSITIVITY OF CONTRACTIONS TO α-ADRENOCEPTOR AGONISTS IN CANINE SAPHENOUS VEINS *IN VITRO*

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Cutaneous blood vessels are very sensitive to changes in local temperature; thus in isolated canine saphenous veins, local cooling augments and local warming decreases the contractions induced by the endogenous neurotransmitter noradrenaline and other α-adrenergic agonists (Vanhoutte and Shepherd, 1970; Flavahan *et al.*,1985). These effects have been attributed to the temperature-sensitivity of the postjunctional α_2-adrenoceptors. An interesting observation was that temperature variations have the opposite effect on contractile responses caused by partial α_1-adrenoceptor agonists (Flavahan *et al.*,1985): these contractions are increased by warming and decreased by cooling; we discovered a similar profile of temperature dependence of contractile responses caused by the new partial non-selective α-adrenoceptor agonist S 18149 ((S)-spiro[(1,3-diazacyclopent-1ene)-5,2'-(7'-methyl-1',2',3',4'-tetrahydronaphthalene)] fumarate) (Cordi et al., 1995, Verbeuren et al.,1996). The aim of the present study was to further analyze the effects of the temperature changes on different α_1 and α_2-adrenoceptor agonists.

The experiments were performed on rings of isolated canine saphenous veins without endothelium mounted for isometric tension recording in organ chambers filled with oxygenated (95 % O_2 / 5 %CO_2) Krebs-Ringer solution at 37°C. The temperature of the Krebs-Ringer solution could be rapidly decreased from 37°C to 24°C (cooling) or increased from 37°Cto 42°C (warming).

In confirmation of earlier data, we found that the contractions induced by noradrenaline and by the α_2-adrenoceptor agonist, UK 14304 were augmented by cooling and decreased by warming. In contrast, the contractions caused by the partial α_1. adrenoceptor agonist, ST 587 were increased by warming and decreased by cooling (Table 1).

Table 1. Contractions (% KCl) to submaximally effective concentrations for the partial α--adrenoceptor agonists at different temperatures (mean ± s.e. mean; n ≥ 3).

Agonists	ST 587 (1 μM)	S 18149 (0.1 μM)	S 19014 (0.1 μM)
37 °C	31 ± 6	32 ± 7	27 ± 7
24 °C	2 ± 1	3 ± 1	1 ± 1
42 °C	49 ± 7	47 ± 4	50 ± 13

The contractions caused by the partial agonists, S 18149 and S 19014 (spiro [(1,3- diazacyclopent-1-ene) -5,2'- (5',6'- dimethylindane)] fumarate) at 1 μM are inhibited by both prazosin and rauwolscine indicating their α_1- and α_2-adrenergic nature; despite these non-selective α-adrenergic properties, the contractions to both agents showed a temperature sensitivity similar to that of ST 587 (Table 1). The contractions caused by the selective α_2-adrenoceptor agonists M-7 (0.01-1 μM) and dexmedetomidine (0.1-10 nM) were also influenced by cooling in a manner comparable to ST 587, but warming had no effect.

We conclude that the temperature sensitivity of the contractions of cutaneous veins caused by substances that interact with α_2-adrenoceptors is not uniform. Whether this depends on the different degree of interaction with postsynaptic α_1-adrenoceptors remains to be determined.

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287P DIFFERENT RECEPTOR MEDIATION OF DIRECT VASOCONSTRICTION AND POTENTIATION OF ADRENOCEPTOR-MEDIATED PRESSOR RESPONSES BY VASOPRESSIN IN THE RAT ISOLATED MESENTERY

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Vasopressin is known to enhance the sensitivity of the vasculature to other vasoconstrictor agents. In this study we investigated which vasopressin receptor subtype is mediating the potentiation by vasopressin of methoxamine (METH)-induced adrenoceptor-mediated vasoconstriction.

Mesenteric arterial beds isolated from male Sprague-Dawley rats were perfused at 37°C with oxygenated (95% O_2, 5% CO_2) Krebs solution at a constant rate of 4 ml min^{-1} (McGregor, 1965). Pressor responses were expressed as relative increases in perfusion pressure above the values measured immediately before drug administration. Responsiveness to METH (30 nmol, as bolus) was determined before and during infusion of vasopressin analogues. The data presented as mean±s.e.mean were statistically evaluated with the Wilcoxon test or Kruskal-Wallis H test followed by Dunn's test for multiple comparisons.

(Arg^8)vasopressin (AVP, 10 nM) increased perfusion by 55±10 mmHg and increased METH responses from 17±3 mmHg to 78±12 mmHg (n=8). The selective vasopressin V_{1A} receptor antagonist SR 49,059 (3 nM, Serradeil-Le Gal *et al.*, 1993) and the non-selective V_1 and oxytocin receptor antagonist (deamino-Pen^1,$Tyr(Me)^2$,Arg^8) vasopressin (45 nM, Bankowski *et al.*, 1978) markedly inhibited the direct vasoconstrictor action of AVP (8±2 mmHg and 3±1 mmHg, respectively; n=6-8, P<0.001 versus vehicle) but had no effect on the potentiation of the pressor response to METH (70±15 mmHg and 63±10 mmHg, respectively; n=6-8). The V_{1B}-selective agonist (deamino-Csy^1,ß-(3-pyridyl)-D-Ala^2,Arg^8)vasopressin (1 µM, Schwartz *et al.*, 1991) and the V_2-selective agonist (deamino-Cys^1,D-Arg^8)vasopressin (10 nM, Manning *et al.*, 1976) were devoid of any pressor activity and did not potentiate METH-evoked vasoconstriction. Thus the perfusion pressure rose only by 3±1 and 2±1 mmHg and the responses to METH amounted to 19±4 and 22±7 mmHg, respectively (n=6-8). In contrast, (1-triglycyl,Lys^8) vasopressin (1 µM) potentiated the METH responses (59±12 mmHg) without per se inducing vasoconstriction (5±1 mmHg, n=6).

We conclude that the direct vasoconstrictor effect of AVP and the peptide's action to enhance the sensitivity of vascular tissue to adrenoceptor stimulation are mediated by different vasopressin receptor subtypes. While AVP elicits direct vasoconstriction via V_{1A} receptors, the potentiating effect on pressor responses to METH does not involve any currently known vasopressin receptor subtype, such as V_{1A}, V_{1B}, V_2 or oxytocin receptors. The existence of a novel vasopressin receptor subtype has, therefore, to be considered. (1-triglycyl,Lys^8) vasopressin might be a selective agonist of this putative receptor.

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288P A COMPARISON OF THE RESPONSE TO BALLOON INJURY OF THE PORCINE CORONARY ARTERY PERFORMED *IN VIVO* AND *IN VITRO*

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The formation of a restenotic lesion following coronary balloon angioplasty results from medial smooth muscle cell (SMC) migration and proliferation and the accumulation of extracellular matrix. The porcine restenosis model most closely reflects the human lesion and, hence, clinical outcome (Jackson, 1994). Here we compare the changes induced in response to coronary balloon angioplasty in both an *in vitro* and *in vivo* porcine model. Pig hearts were transported from a local abattoir, the left anterior descending (LAD) coronary artery was dissected free and subjected to balloon angioplasty using a 3mm balloon catheter at 2 levels of injury - 6 or 9 atmospheres (atm) pressure. The artery rings were then maintained in organ culture for 14 days prior to preparation for morphological analysis. Male Large White Welsh Landrace cross breed pigs (13-17kg) were anaesthetised using a halothane/nitrous oxide mixture and the LAD coronary artery ballooned using a 3mm catheter inflated to 10 atm pressure. The animals were allowed to recover and 4 weeks later were euthanised and the vessels harvested. Vessel areas (µm²) and the degree of neointima (% of area lying within the internal elastic lamina, IEL) were quantified by planimetry. Groups were compared using one-way ANOVA followed by the Tukey test.

Organ culture of the artery rings resulted in a significant reduction in luminal area compared to baseline morphology. The area of the adventitia was significantly enhanced in those vessels which were injured prior to culture. Neointima formed in cultured vessels, the extent of which was significantly enhanced in injured vs non-cultured vessels but not compared to control cultured vessels (Table 1). Qualitative examination of the sections revealed a clear response to injury with IEL disruption. Endothelial regeneration was also commencing. Quantitative morphological analysis of the vessels from the *in vivo* balloon angioplasty group demonstrated a significant degree of restenosis in the injured vs control (left circumflex) artery. There were no changes in the areas of the adventitia, media or lumen as a result of balloon injury (Table 1). Qualitative analysis demonstrated a breach of the IEL in all vessel showing neointimal formation. The lesions were eccentric with endothelial cell regeneration. Two-way ANOVA showed a significantly enhanced contraction in injured vessels to potassium chloride, 5-hydroxytryptamine and phenylephrine. Vasorelaxation to SIN-1 was significantly attenuated and to calcimycin unchanged. To conclude, both the *in vitro* and *in vivo* porcine models of restenosis showed a significant degree of neointimal formation in response to injury. The *in vitro* model demonstrated enhanced growth of the adventitia following balloon angioplasty perhaps due to absence of the shear influences present *in vivo*. Qualitative examination demonstrated similarities between the lesions formed *in vitro* and *in vivo*.

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Table 1 Vessel layer area and extent of neointima in an *in vitro* and *in vivo* model of restenosis (mean ± s.e.mean, *p<0.05 vs non-cultured, Ψp<0.05 vs control)

		Lumen (µm²)	Media (µm²)	Adventitia (µm²)	Neointima (%)
In vitro	Non-cultured (n=10)	$1.433 \pm 0.113 \times 10^6$	$1.157 \pm 0.073 \times 10^6$	$1.322 \pm 0.177 \times 10^6$	1.022 ± 0.69
	Control cultured (n=10)	$0.268 \pm 0.111 \times 10^6$*	$1.514 \pm 0.090 \times 10^6$	$1.763 \pm 0.150 \times 10^6$	23.535 ± 9.192
	6 atm injury cultured (n=9)	$0.353 \pm 0.075 \times 10^6$*	$1.685 \pm 0.165 \times 10^6$	$2.193 \pm 0.242 \times 10^6$*	41.086 ± 9.583*
	9 atm injury cultured (n=9)	$0.377 \pm 0.042 \times 10^6$*	$1.557 \pm 0.225 \times 10^6$	$2.297 \pm 0.185 \times 10^6$*	39.614 ± 10.2*
In vivo	Control (n=7)	$0.779 \pm 0.120 \times 10^6$	$0.835 \pm 0.147 \times 10^6$	$1.207 \pm 0.154 \times 10^6$	6.885 ± 4.476
	10 atm injury (n=7)	$0.919 \pm 0.094 \times 10^6$	$1.093 \pm 0.097 \times 10^6$	$1.545 \pm 0.124 \times 10^6$	27.378 ± 5.506Ψ

289P EXOGENOUS ENDOTHELIN PRECONDITIONS THE HEART AGAINST ARRHYTHMOGENESIS INDUCED BY MYOCARDIAL ISCHAEMIA

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Exogenous administration of endothelin (ET-1) has been reported to be cardioprotective in the setting of myocardial ischaemia by reducing infarct size (Hide *et al.*, 1995). The aim of this study was to investigate the ability of ET-1 to precondition against ischaemic arrhythmias and to determine the receptor types involved. Male Sprague Dawley rats were anaesthetised with sodium pentobarbitone intraperitoneally (60 mg kg^{-1}) and subjected to 30 min of ischaemia induced by occlusion of the left anterior descending artery. The number and incidence of cardiac arrhythmias were recorded, in addition to mean arterial blood pressure (MABP) and heart rate. Animals were administered a bolus dose of ET-1 alone (1.6 nmol/kg, 5 min before occlusion, n=12) or in the presence of the ET_A receptor antagonist, BQ-123 (infused intravenously 10 min before and during 30 min of ischaemia in a dose of 50 μg/kg/min, n=10) or the ET_B antagonist, PD-161721 (1 mg/kg given as a bolus i.v. 10 min before occlusion, n=10). The effects of the antagonists alone, administered in the same doses and protocols were also investigated. Control animals received saline in lieu of drug administration (n=20). ET-1 significantly reduced the number of ventricular ectopic beats (VEBs) that occurred during the 30 min of ischaemia from 2775(1870-4041) [median(Q_1-Q_3)] to 1530(1204-2017) ($p < 0.05$) and reduced the incidence of ventricular fibrillation (VF) (65% to 17%, $p < 0.05$) and mortality (30% to 0%). ET-1 caused a significant transient fall in MABP from 107 ± 3 to 63 ± 3 mmHg which returned to control level before occlusion. PD161721, the ET_B antagonist, but not BQ123, the ET_A antagonist, partially blocked the transient fall in MABP produced by ET-1 (10 ± 2 vs 44 ± 5 mmHg, $p < 0.05$). In controls, MABP fell transiently from 106 ± 4 to 67 ± 4 mmHg at 1 min post-occlusion. ET-1 significantly attenuated this fall in MABP from 105 ± 6 to 89 ± 7 mmHg. Neither PD161721 nor BQ-123 modified the antiarrhythmic effect of ET-1 although both antagonists were in themselves anti-arrhythmic. The number of VEBs was significantly reduced from 2775(1870-4041) to 654(348-1489) and 782(432-1153) by BQ-123 and PD161721 respectively. The incidence of ventricular fibrillation was also significantly reduced from 65 to 10% ($p < 0.05$) by PD161721 but was unaffected by BQ-123 (50%). In conclusion, exogenously applied ET-1 can precondition against ischaemic arrhythmias and this may be related to its haemodynamic effect. The data also suggests that ET-1 released endogenously during ischaemia is arrhythmogenic and also plays a role in the fall in mean arterial blood pressure during ischaemia. The receptor subtype involved in the cardioprotective effect of exogenously applied ET-1 has not been elucidated.

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290P SYSTEMIC INFUSION OF AN ENDOTHELIN RECEPTOR ANTAGONIST INCREASES PLASMA ET-3 IN HUMANS

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We have previously shown that the increase in human plasma IR endothelin (ET) induced by the systemic administration of the ET antagonist, TAK-044 was not caused by increased synthesis because there was no change in plasma levels of big ET-1 or C-terminal fragment (Plumpton *et al.*, 1996). We hypothesised that this increase was caused by displacement of receptor bound peptide from ET_A receptors that predominate in the human vasculature although ET_B may also be involved. ET-3 has the same affinity as ET-1 for the ET_B receptor but in functional assays using human isolated vessels, has a lower affinity for the ET_A sub-type than ET-1 (Maguire and Davenport, 1995).

Therefore, to determine whether blockade of ET_B receptors also contributed to the rise in plasma ET, we used a selective assay to measure ET-3 following infusion into six healthy male volunteers (with local ethical committee approval), of two doses of TAK-044 (250 and 750 mg giving plasma concentrations of 27 and 80 nM) or placebo via a left forearm vein. Blood samples were withdrawn from the right antecubital vein prior to infusion and after 15 min of infusion of either TAK-044 or 50 ml placebo.

We developed an enzyme-linked immunosorbent assay to measure ET-3 which had a sensitivity of detection of 0.09 fmol/well with an inter- and intra-coefficients of variation of 2.2% and 3.0% ($n \geq 4$). The assay was highly selective for ET-3; cross-reactivity with TAK-044 was <0.00007; for big ET-3 <0.03% and <0.01% for ET-1, ET-2 or their corresponding precursors.

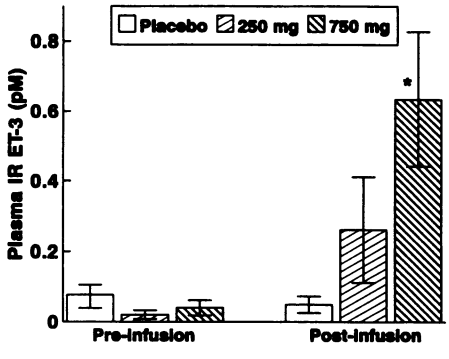

Figure 1. Levels of IR ET-3 in human plasma. (n=mean of 6 individual±s.e.m) *Significant increase compared with placebo control (Mann-Whitney U-test, $p<0.02$).

Basal levels of IR ET-3 were 0.07±0.03 pM in human plasma, about an order of magnitude lower than levels of IR ET measured using antisera that cross-react with all isoforms. Infusion of 750 mg TAK-044 resulted in a significant increase in plasma IR ET-3 after 15 min compared with pre-infusion levels. There was no significant change following infusion of the placebo. One explanation for these results is that infusion of TAK-044 causes blockade of ET_B 'clearing receptors' leading to accumulation of the peptide in the plasma. A second possibility is displacement of ET-3 from ET_B receptors, since the peptide is likely to be selective for this sub-type at the concentrations present in human plasma. Interaction of ET-3 with ET_B receptors may have a physiological role in humans.

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291P COMPARATIVE STUDY OF EFFECTS OF ET-1, BIG ET-1 AND SX6C IN RAT ISOLATED PULMONARY ARTERIES AND VEINS

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Previously, using the isolated perfused rat lung model, we have shown that ET-1 and sarafotoxin 6c (selective ET_B receptor agonist) act differentially to produce pulmonary vasoconstriction i.e. ET-1 causes venoconstriction whereas SX6C arterial constriction (Lal *et al.*, 1995). In the present study we have compared the effects of ET-1, big ET-1 and SX6C in rat isolated large pulmonary arteries (PA) and pulmonary veins (PV).

Male Wistar rats (300-350g) were anaesthetised with sagatal (6o mg/kg, i.p). The chest was opened and the heart and lung were removed, the main pulmonary veins and arteries were cleared of all visible connective tissue, and cut into rings of 2-2.5 mm in length. Rings were mounted on fine steel wires in Krebs' solution at 37 °C gassed with 20 % O_2 / 5% CO_2 / 75% N_2 and isometric contractions were measured. Each ring was equilibrated for 1 hour at the mean optimal resting tension (6.86 mN for PV and 9.89 mN for PA). Cumulative concentration-response curves for ET-1, big ET-1 or SX6C were constructed. Only one agonist was studied in each preparation. In separate experiments receptor antagonists were added into tissue baths 15 min prior to the addition of agonist.

ET-1 produced concentration dependent contractions of PA (EC_{50} ± s.e.m. 5.3±0.65 nM, n=8) and PV (EC_{50} 0.66 ± 0.24 nM, n=5) rings. The maximal contraction caused by ET-1 (300 nM) in PA, 5.5 ± 0.62 mN was significantly greater than that in PV (ET-1 10 nM) 1.7 ± 0.17 mN, ($p<0.001$). The ET_A receptor antagonist BQ123 (Ihara *et al.*, 1992) (3μM) inhibited the responses to ET-1 in PA and PV rings ($p<0.01$, n=4).

Big ET-1 produced slowly developing contractions in PA and PV rings. When compared with ET-1, big ET-1 was 10 fold less potent in producing contractions of similar magnitude in both vessels. 100 nM big ET-1 produced a response of 2.7 ± 1.1 mN, n=4 in PA and 1.5 ± 0.34 mN, n=4 in PV. BQ123 (3μM) abolished big ET-1 (100 nM)- induced responses in both PA and PV.

SX6C (0.01-30 nM) also produced concentration-dependent contractions in PA (EC_{50} 2.45 ± 0.68 nM, n=5) and PV (EC_{50} 0.61 ± 0.11 nM, n=5). The maximal response produced by SX6C in PV (1.04 ± 0.18 mN) was significantly greater than in PA (0.29 ± 0.08 mN, $p< 0.05$). The selective ET_B receptor antagonist BQ788 (Ishikawa *et al.*, 1994) (3μM) attenuated the responses to SX6C ($p< 0.01$, n=3).

In summary, ET-1 was a more potent vasoconstrictor than SX6C on PA and its effects were antagonised by BQ123. This suggests that, in the large PA, ET-induced vasoconstrictor responses are mediated predominantly by ET_A receptors. These findings are in agreement with Maclean *et al.*, 1994. Big ET-1 also induced vasoconstriction in PA and PV, which is inhibited by BQ123, suggesting a role for the ET_A receptor. In contrast ET-1 and SX6C were equipotent in PV. The responses to ET-1 in PV were blocked by BQ123 and the responses to SX6C were blocked by BQ788 suggesting the presence of both ET_A and ET_B receptors in large PV. Interestingly, and in contrast to our study in the perfused lung, SX6C produced a greater absolute response in PV than in PA.

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292P ENDOTHELIN-1 mRNA AND RENAL FIBROSIS FOLLOWING SUBTOTAL (5/6) NEPHRECTOMY IN THE RAT

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Endothelin receptor antagonists are renoprotective in models of renal disease (Benigni *et al*, 1996 & Nabokov et al, 1996). ET-1 gene expression has been shown to be induced in an animal model of renal fibrosis (Orisio *et al*, 1993), while effects on urinary ET-1 protein, thought to reflect renal production, remain controversial (Torralbo *et al*, 1995).

We describe in the rat subtotal (5/6) nephrectomy (SNx) model of fibrosis, induction of the preproET-1 gene, its sustained elevation throughout a 120 day time course and correlation of this with functional and morphological features characteristic of chronic progressive renal failure.

Male Wistar rats (300-400 g) underwent either surgical ablation of 5/6 of the kidney mass or sham operation. Groups of 4 to 6 animals were sacrificed at 7, 15, 30, 60, 90 and 120 days post SNx or sham operation. Northern blot analysis showed a progressive increase in ET-1 mRNA levels from day 7 in SNx compared to sham (Figure 1). This increase became statistically significant at day 15 (1.37 ± 0.13 vs 3.7 ± 0.97 volume density units, $p<0.05$) and reached a peak at day 90 (3.11 ± 0.33 vs 8.18 ± 1.9 volume density units, $p<0.05$).

In rats following SNx, ET-1 mRNA correlated with proteinuria and with glomerular and tubular/interstitial scores of fibrosis (Table 1).

	R^2	
Urine protein (mg/24hr)	0.87	$p<0.005$
Fibrosis score: Glomerulus	0.87	$p<0.005$
Tubule / Interstitium	0.91	$p<0.001$

Table 1 Correlation of renal ET-1 mRNA with proteinuria and renal fibrosis in rats following SNx.

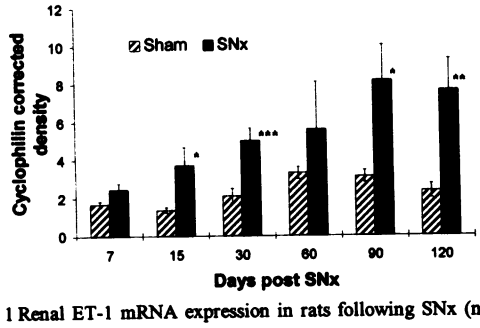

Figure 1 Renal ET-1 mRNA expression in rats following SNx (n=5 or 6)or sham operation (n=4 to 6).
Data are mean ± SEM, * $p<0.05$; ** $p< 0.01$; *** $p< 0.005$ vs sham.

These findings provide evidence that ET-1 may be an important mediator in the progression of renal fibrosis. Renal expression of the ET-1 gene is raised markedly in an experimental model of renal fibrosis and this correlates strongly with features of the disease suggesting that increased production of ET-1 may contribute to the development of chronic renal failure.

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293P EFFECTS OF ANGIOTENSIN II AND LOSARTAN ON FOREARM BLOOD FLOW IN PATIENTS WITH ESSENTIAL HYPERTENSION

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The effects of angiotensin II (Ang II) and the AT_1-receptor antagonist losartan may be modulated by hypertension-induced vascular changes. We investigated the forearm vascular responses to Ang II and the α_1-agonist methoxamine, and the inhibitory effects of losartan in essential hypertensive (HT) and normotensive (NT) subjects. The forearm ischemic response was measured to determine structural vascular changes.

Seven HT patients were selected (daytime 24 h ambulatory diastolic blood pressure > 90 mmHg), and 7 age, sex, and body mass index matched NT subjects. Patients were not on antihypertensive medication for at least 2 weeks prior to the study. Drugs were infused into the brachial artery. Forearm blood flow (FBF) was measured by venous occlusion plethysmography. Ang II (0.01-10 ng/kg/min) infusions were performed during infusion of sodium nitroprusside (SNP) to predilate the forearm vascular system. The effects of losartan (0.3, 1 and 3 µg/kg/min) on baseline FBF and on Ang II-induced vasoconstriction were determined. Methoxamine (0.2-2 µg/kg/min), as a RAS-unrelated vasoconstrictor, was co-infused with the nitric oxide synthase inhibitor L-NMMA. Drug effects were expressed as a change in FBF-ratio: $FAR=FBF_{infused\ arm}/FBF_{non\text{-}infused\ arm}$. Ang II at 10 ng/kg/min caused a significant but similar increase in mean arterial pressure in HT and NT subjects by 13±3% and 9±3% ($p>0.05$), respectively, which was maintained in the presence of losartan. Baseline FBF_i amounted to 2.56±0.80 and 2.66±0.25 ml/100ml/min in the HT and NT group respectively, and was increased by SNP to 5.46±0.92 and 5.42±0.40 ml/100ml/min, respectively. Ang II significantly decreased FAR with similar responses in the HT and the NT group ($p>0.05$). The maximal effects (E_{max}) amounted to -73±5% and -73±4%, respectively and the EC_{50} values -9.19±0.14 and -9.21±0.28 log[mol/L], respectively. Tachyphylaxis did not occur. Losartan did not change baseline flow levels in either group, but antagonised the Ang II-induced vasoconstriction in a dose-dependent manner ($p<0.05$). Losartan at 0.3, 1 and 3 µg/kg/min reduced the E_{max} to -48±7%, -47±5% and -14±6%, resp. in the HT group, and to -63±4%, -44±10% and -29±5%, resp. in the NT group, with no difference between groups ($p>0.05$). Methoxamine caused a significant but similar reduction in FAR in the HT and NT group, namely by 51±9% and 62±4%, respectively. Minimal FVR, after 10 min of forearm ischemia, was the same in Ht and NT, namely 3.2±0.7 and 3.2±0.4, respectively ($p>0.05$).

In conclusion, the vasoconstrictor effects of Ang II and methoxamine were comparable in HT and NT subjects, with a similar antagonistic efficacy of losartan. The forearm ischemic response was the same in both groups, indicating that in our study the forearm vascular bed does not show important structural and functional changes as a result of hypertension.

294P ANGIOTENSIN-CONVERTING ENZYME INHIBITION WITH RAMPIPRILAT PREVENTS ENDOTHELIAL DYSFUNCTION INDUCED BY ISCHAEMIA/REPERFUSION OF A CORONARY ARTERY IN THE DOG

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Previous investigations have shown that repeated administrations of angiotensin-converting enzyme (ACE) inhibitors may prevent the progression of endothelial dysfunction in chronic animal models of atherosclerosis and hypertension (Becker *et al.*, 1991; Clozel *et al.*, 1991). The present study was designed to determine whether an acute administration of the ACE inhibitor ramiprilat (R) prevents endothelial dysfunction induced by occlusion and reperfusion of a coronary artery in the dog. Additionally, the morphology of the endothelium of coronary arterioles of R-treated and -untreated dogs was assessed by means of scanning electron microscopy (SEM).

Dogs were anaesthetised with pentobarbital (40mg·kg^{-1} i.v.), thoracotomised, and an electromagnetic flow probe was placed around the left circumflex coronary artery (LCX). Proximal to the probe an indwelling cannula was inserted in the LCX for drug administration. Distal to the probe a snare was passed around the artery for the induction of the occlusion and reperfusion. The endothelium-dependent vasodilators ACh (5 and 10 µg.min^{-1} for 1 min) and 5-HT (50 and 100 µg·min^{-1} for 1 min) and the endothelium-independent vasodilator NTG (50 and 100µg·min^{-1} for 1 min) were given intracoronarily both prior to and after a 60 min occlusion and a 180 min reperfusion of the LCX. During occlusion and reperfusion the dogs received intracoronarily either saline (n=22) or R (40 ng/kg·min^{-1}, n=14). At the end of the experiment a 1 cm^3 myocardial biopsy of the first descending branch of the LCX was fixed and processed for SEM. The latter procedure was also carried out in 2 sham-operated dogs for assessment of normal endothelium morphology. Statistics: one-sided Wilcoxon signed rank test, at the 0.05 level.

Prior to occlusion and reperfusion, all three vasodilators induced a similar dose-related increase in coronary flow in both saline- and R-treated groups. In the saline-treated dogs, following ischemia and reperfusion, the responses to ACh and 5-HT were significantly blunted (ACh: -39 % at the lower dose ,and -34 % at the higher dose; 5-HT: -48 % and -49 %; $P<0.05$). Those to NTG were practically unchanged. R-administration prevented the blunting of the responses to the endothelium-dependent vasodilators (ACh: -5 %, and -10 %; 5-HT: -11 %, and -19 %;). At SEM, the myocardial arterioles (lumen ca. 300 µm) of the saline-treated dogs showed adhesion of leukocytes to the endothelium. This was less evident in the R-dogs.

Thus, an acute administration of the ACE inhibitor R significantly prevented the development of endothelial dysfunction induced by ischemia and reperfusion of a coronary artery in the dog. This could be shown by using functional as well as and morphological methods. The mechanism of action of this protective effect may rest on: *1)* inhibition of production of angiotensin II; *2)* the prevention of bradykinin degradation; *3)* an increased production of antioxidant defence mechanisms.

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295P LIGHT-EVOKED RELEASE OF NITRIC OXIDE (NO) FROM THE RABBIT RETINA

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In the vertebrate retina NO is believed to be involved in light adaptation and to act as a neuromodulator (Goldstein et al, 1996). In the rabbit retina nitric oxide synthase (NOS) has been demonstrated in some amacrine cells and bipolar cells (Perez et al, 1995) and there is circumstantial evidence that these cells release NO (Neal et al, 1997). The present study provides the first demonstration that NO is released from the retina when it is stimulated physiologically with light.

Adult New Zealand white rabbits were anaesthetised with urethane (1.5g/kg I.P.) and an eyecup prepared (Cunningham & Neal, 1985). Eye-cups were filled with Krebs bicarbonate medium (0.5ml) which was replaced every 10 min. The NO in the resulting samples was measured using nitrate reductase and an NO meter (WPI).

Stimulation of the dark-adapted retina with flickering (3Hz) or continuous light significantly increased the release of NO by 1.79±0.11 fold ($P<0.02$, n=11) and 1.63±0.10 fold ($P<0.01$, n=9) respectively. 2-Amino-4-phosphonobutyric acid (APB, 20μM), which specifically blocks transmission between photoreceptors and depolarising bipolar cells (DPBCs) abolished NO release evoked by both flickering and continuous light. This indicates that the light evoked release of NO does not originate from photoreceptors. Exposure of the retina to cis-2,3 piperidine dicarboxylic acid (PDA, 2mM), which blocks transmission between DPBCs, and amacrine and ganglion cells, decreased the NO release evoked by continuous light to 11±11% of control values ($P<0.05$, n=4), but had no effect on the flicker-evoked release of NO (108±19% of control values, n=4). This suggested that continuous light and flickering light evoked NO from different cells. In support of this suggestion, we found that glycine (2mM) reduced NO release evoked by continuous light (45±9.9% of control values, $P<0.04$, n=4) but had no effect on NO released by flickering light (120±19% of control values, n=3).

We conclude that stimulation of the retina with continuous light and flickering light causes the release of NO from different cells, tentatively identified as amacrine cells and DPBCs respectively.

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296P ENDOTHELIUM-DEPENDENT RELAXATION OF RABBIT AORTA EVOKED BY ACETYLCHOLINE

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Muscarinic agonists cause an endothelium-dependent vasorelaxation (Furchgott and Zawadzki, 1980). The aim of the present study was to examine various methodological aspects of the acetylcholine-evoked relaxation of rabbit isolated aorta. Furthermore, the effect of acetylcholine was compared with those of other muscarinic agonists.

Rings of rabbit thoracic aorta, either intact or endothelium-denuded, were preconstricted with phenylephrine and then acetylcholine or other muscarinic agonists were added. Results are expressed as mean ± s.e. m. Cumulative addition of acetylcholine (10^{-8} - 3 x 10^{-5} M) caused an endothelium-dependent relaxation. The Emax (%) was inversely related to the phenylephrine concentration (-log M): 79 ± 4 (7); 56 ± 7 (6); 45 ± 3 (5); 40 ± 2 (4); n = 4-5. In subsequent experiments, 4 x 10^{-7} M phenylephrine (~ EC_{80}) was used. Acetylcholine (10^{-8} - 3 x 10^{-5} M) added cumulatively, relaxed the intact aorta (Table 1). Identical responses were obtained when rings were derived from either the upper, middle or lower part of the thoracic aorta. Storage of the aorta in cold (4°C) salt solution for 24 h did not alter the acetylcholine-evoked relaxation. Two consecutive concentration-response curves obtained with acetylcholine did not differ with regard to pD_2 or Emax, *i.e.* no development of tachyphylaxis. Equieffective concentrations of acetylcholine (10^{-6} M) and carbachol (10^{-5} M) caused a rapid relaxation of intact aorta which markedly waned with time (6 h), *i.e.* desensitization. Vasorelaxation was also evoked by carbachol, pilocarpine and oxotremorine, while McN-A-343 essentially had no effect (Table 1).

Table 1. Relaxation of rabbit aorta evoked by muscarinic agonists

Agonist	pD_2 (-log M)	Emax (%)	*n*
Acetylcholine	6.37 ± 0.07	78 ± 2	37
Carbachol	6.42 ± 0.06	78 ± 6	6
Oxotremorine	6.87 ± 0.08	62 ± 8	6
Pilocarpine	5.24 ± 0.14	12 ± 4	6
McN-A-343	-	1 ± 6	5

We conclude that acetylcholine causes an endothelium-dependent relaxation of rabbit aorta which is uniform along the thoracic portion and not influenced by cold storage of the tissue. The acetylcholine-induced desensitization is not due to metabolic inactivation of acetylcholine. The individual responses which enter into the cumulative concentration-response curve for acetylcholine-evoked relaxation do not represent equilibrium responses. Acetylcholine and carbachol are full agonists. Pilocarpine and oxotremorine are partial agonists.

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297P ENDOTHELIUM-INDEPENDENT ATP-INDUCED RELAXATION IS MEDIATED BY THE A_{2A}-ADENOSINE RECEPTOR IN RAT PORTAL VEIN

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In vessels, ATP produces multiple effects in both arteries and veins. It induces vasoconstriction, mainly by activation of P2X-receptor located on smooth muscle and both endothelium-dependent and endothelium-independent relaxation ascribed to the activation of P2Y receptors of endothelial cells and smooth muscle cells respectively. The relationship between ectonucleotidase activity, which breaks down ATP to adenosine, and the vasoactive effects of ATP is unclear. The aim of this work was to determine how the P2-receptor agonist-induced endothelium-independent relaxation is influenced by the inhibition of ectonucleotidase activity and to define the receptor subtype(s) involved in this relaxation.

At 25°C, 1-100 µM ATP, 2-methylthio-ATP (2-MeSATP) and 0.1-10 µM 2-chloroATP (2-ClATP), dose-dependently inhibited spontaneous contractile activity of endothelium-denuded muscular strips from rat portal vein with potencies (pEC50 ± s.e.mean) of 4.8 ± 0.1 (n = 9); 4.7 ± 0.1 (n = 4) and 5.7 ± 0.1 (n = 7) respectively. Simultaneous measurements of contraction and ecto-ATPase activity estimated by the degradation of [γ-^{32}P]-ATP show that muscular strips rapidly (10-60 s) induced hydrolysis of ATP, which was inhibited by 56 ± 11 % (n = 3) by 200 µM $\alpha\beta$-methylene ATP ($\alpha\beta$-MeATP) previously identified as an ecto-ATPase inhibitor (Chen & Lin, 1997). In the presence of $\alpha\beta$-MeATP, the relaxing action of ATP, 2-MeSATP and 2-ClATP was strongly inhibited by 98 ± 2 % (n = 9); 99 ± 0.3 % (n = 7) and 73.7 ± 2.4 % respectively. Pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) (100 µM, 30 min), an antagonist of P2Y receptors, did not affect the relaxation induced by ATP, 2-MeSATP, and 2-ClATP (n = 4). In contrast, the highly selective A_{2A}-adenosine receptor antagonist ZM 241385 inhibited the ATP-induced relaxation in a concentration-dependent manner (1-100 nM) (n = 5). In the presence of 100 nM ZM 241385, the relaxing effect of 2-MeSATP (n = 4) and 2-ClATP (n = 4) was also virtually abolished. ADP, AMP, and adenosine also produced concentration-dependent inhibition of spontaneous contractions with potencies of 4.7 ± 0.1 (n = 4), 4.8 ± 0.1 (n = 7) and 4.8 ± 0.1 (n = 7), respectively. The relaxing effect of ADP, AMP and adenosine was insensitive to $\alpha\beta$-MeATP (200 µM) (n = 4-7) but was inhibited by ZM 241385 (100 nM) by 98.8 ± 0.1 % (n = 4); 90 ± 10 % (n = 4) and 81 ± 3.7 % (n = 7) respectively.

Taken together, these results suggest that in rat portal vein the endothelium-independent relaxing effect of ATP and other P2 receptor agonists was mediated through the activation of A_{2A}-receptors following breakdown of ATP by ectonucleotidase.

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Chen B.C. & Lin W-W. (1997) *Biochem. Biophys. Res. Commun.* 233, 442-446.

298P EVIDENCE FOR THE INVOLVEMENT OF POTASSIUM CHANNELS IN ANANDAMIDE-INDUCED AND EDHF-MEDIATED VASORELAXATIONS IN RAT ISOLATED MESENTERY

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We have recently proposed that an endocannabinoid may be an endothelium-derived hyperpolarizing factor (EDHF) (Randall *et al.*, 1996). We have now examined the effects of the selective K-channel inhibitors, charybdotoxin (which blocks large conductance calcium-activated K-channels, and also voltage-sensitive K-channels), and apamin (which blocks small conductance calcium-activated K-channels) and their use in combination (Zygmunt *et al.*, 1997) in order to define and compare the actions of anandamide and EDHF.

Male Wistar rats (250-350g) were anaesthetized with pentobarbitone (60 mg kg^{-1}, i.p.) and the mesenteric arterial bed was isolated as previously described (Randall *et al.*, 1997) and perfused with oxygenated Krebs-Henseleit solution containing indomethacin (10µM) and N$^{\omega}$-nitro-L-arginine methyl ester (100 µM) to inhibit prostanoid and nitric oxide synthesis respectively. Following a 30min equilibration period, methoxamine (1-2µM) was added to the buffer to increase perfusion pressure by 80-100mmHg. The vasorelaxant effects of carbachol (acting via EDHF) and anandamide were then assessed. In preparations receiving the toxins the agents were added to the buffer 15min before the construction of the dose-response curves. The data have been compared by ANOVA with Bonferroni's *post-hoc* test.

Carbachol caused dose-related relaxations (ED_{50}=1.81±0.71 nmol, mean±s.e.mean, and R_{max}=74.3±5.6%, n=7). In the presence of either 100nM charybdotoxin (n=6) or 500nM apamin (n=5) these responses were unaffected, with respective ED_{50} values of 999±187pmol and 792±113pmol and the respective R_{max} values were 81.9±2.6% and 77.5±1.3%. By contrast, in the presence of the combination of charybdotoxin (100nM) and apamin (500nM), the vasorelaxant responses to carbachol (n=5) were abolished. Anandamide (1nmol-3µmol) caused dose-related relaxations of tone, with an ED_{50}=28.6±11.5nmol and R_{max}=88.6±7.2% (n=9). Neither of the toxins alone affected anandamide-induced vasorelaxation (charybdotoxin, ED_{50}=21.1±12.2nmol and R_{max}=86.1±7.4%, n=5; apamin, ED_{50}=27.7±12.6nmol and R_{max}=73.3±8.0%, n=5). However, when charybdotoxin and apamin were used in combination, vasorelaxation to anandamide was substantially inhibited, with the only significant relaxation occurring at 3µmol (21.3±8.1% relaxation of tone) which was significantly ($P<0.001$) less than the maximum control response.

In the present investigation we have shown that EDHF and anandamide share a common site of action at a K-channel. Further, the profile of sensitivity to the different toxins points to important parallels in the pharmacology of EDHF and anandamide, which adds support to the proposal that an endocannabinoid may be an EDHF.

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299P POTENTIATION OF NO AND ENDOTHELIUM-INDEPENDENT RELAXATION THROUGH A LONG-LASTING SOLUBLE GUANYLYL CYCLASE STIMULATION BY YC-1 (3-(5'-HYDROXYMETHYL-2'FURYL)-1-BENZYL-INDAZOL)

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The vasomotor activity of 3-(5'-hydroxymethyl-2'furyl)-1-benzyl-indazol (YC-1; Ko *et al.*, 1994), a novel NO-independent activator of soluble guanylyl cyclase (sGC; Friebe *et al.*, 1996; Mülsch *et al.*, 1997), was studied on New Zealand White rabbit (4-5 months, 2.5-3.5 kg) isolated aortic rings with (E+) and without (E-) an intact endothelium precontracted with 1 µM phenylephrine or 0.1 µM $PGF_{2\alpha}$ and compared to that of NO donor-type sGC activators and vasodilators, NOC-18 (1-hydroxy-2-oxo-3,3-bis(3-aminoethyl)-1-triazine) or sodium nitroprusside (SNP). Similar to NO-donors, YC-1 alone caused a concentration-dependent relaxation of rabbit aortic rings, with EC50 values slightly lower in E+ vessels (20 µM versus 30 µM in E- vessels), and shifted the phenylephrine (PE)-induced contractile response towards higher concentrations (EC25 -YC-1 = 0.8 µM; EC25 +YC-1 = 10 µM). Surprisingly, when the interaction between YC-1 and NO was studied, endogenous (EDRF released by ACh) and exogenous (NO donors) sources of NO donors reacted differently. While the EDRF-induced submaximal relaxations were increased by YC-1 in efficacy (at 3 µM YC-1 and 1 µM PE from 80 ± 3 to 86 ± 2 % of preconstriction; at 30 µM YC-1 and 10 µM PE from 52 ± 4 to 68 ± 5* % of preconstriction; * indicates significant difference from control condition without YC-1, $p < 0.05$) but not in potency (EC50 ACh -YC-1 = 7.5 µM; EC50 Ach +YC- = 7 µM), exogenous NO donors always induced a maximal relaxation and, in the presence of YC-1, were potentiated by almost two orders of magnitude (at 300 µM YC-1 and 30 µM PE, from 1.5 µM to 40 nM SNP and from 30 µM to 4 µM NOC-18). Both the YC-1- and the NO donor-induced relaxations were greatly reduced by the sGC inhibitor ODQ (EC50 YC-1 from 8 µM to 30 µM) and thus due to activation of sGC as mechanism of action. However, their kinetics were different. The NO donor SNP caused an immediate relaxation, inhibition of PE-induced contraction and increase in intravascular cGMP levels that was readily reversed after washout of the compound (EC25 of PE: before YC-1, 0.15 µM; immediately after 30 µM SNP, 0.1 µM; after 150 min washout of SNP, 0.2 µM; cGMP response: t = 0 min, 677 ± 52 fmol cGMP/ mg protein; after 60 min washout of SNP, 0 ± 10 fmol cGMP/mg protein). In contrast, relaxation induced by YC-1 developed more slowly, inhibition of PE-induced contraction was not fully reversed even after extensive washout for over 60 min (EC25 of PE: before YC-1, 0.8 µM; immediately after 300 µM YC-1, 10 µM; after 150 min washout of YC-1, 3 µM); the latter condition was accompanied by a constant elevation of intracellular cGMP (t = 0 min, 257 ± 50* fmol cGMP/ mg protein; after 60 min washout of YC-1, 162 ± 47 fmol cGMP/mg protein; * indicates significant difference from 60 min value for SNP). Thus YC-1 is a highly effective vasodilator which not only substitutes for but also increases the effectiveness of endogenous vascular NO. The prolonged duration of action of YC-1 is unprecedented for any published sGC activator and therefore makes it a promising therapeutic lead to replace currently used, short-lived and tolerance-prone nitrovasodilators.

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300P EFFECTS OF OXIDISED CHYLOMICRON REMNANTS ON ENDOTHELIAL CELL FUNCTION IN THE PERFUSED AND FRESHLY ISOLATED RAT AORTA

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Atherosclerosis is known to impair endothelium-dependent relaxation due to incorporation of cholesterol from lipoproteins into the artery wall. Low density lipoprotein (LDL), considered to be strongly atherogenic due to its small size and high cholesterol content, has been shown to be taken up by the rat aorta *in vivo* (Cardona-Sanclemente *et al.*, 1994) and to impair its endothelial function (McPherson *et al.*, 1995). Oxidative modification of LDL is known to occur within the artery wall and this is thought to be responsible for its inhibitory effects on endothelium-dependent relaxation. There is now increasing evidence that chylomicron remnants (CMRs), which carry cholesterol of dietary origin to the liver, may also be taken up by the artery wall (Proctor & Mamo, 1996) and modulate endothelial function (Grieve *et al.*, 1997). The aim of this study was firstly, to measure uptake of oxidised CMRs (oxCMRs) by the isolated perfused rat aorta, and secondly, to investigate the effects of these lipoproteins on endothelial function both before and after perfusion.

CMRs were prepared as previously described (Lambert *et al.*, 1996) and oxidised by incubation with copper for 18 h. An *in vitro* system for perfusion of the rat aorta was developed using a perfusate consisting of 33% bovine serum replacement and 67% Krebs-Henseleit solution (KHS). Vessels from male Wistar rats (300-350 g) were perfused for 2 h with ^{125}I-labelled oxCMRs (16 µM cholesterol, specific activity 0.415 ± 0.061 kBq/mg protein) to measure uptake by the artery wall, or with or without similar concentrations of unlabelled oxCMRs for tests on endothelial function. In the latter case 3 mm rings of aorta were prepared (endothelium intact) for isometric tension recording. Rings were contracted with depolarising KHS to ensure their viability, then washed with KHS before cumulative concentration response curves were constructed to phenylephrine (PE; 1 nM to 10 µM) and to carbachol (CCh; 10 nM to 0.1 mM)) and S-nitroso-N-acetylpenicillamine (SNAP; 0.1nM to 10 µM) after vessel tone had been raised with PE (0.3 or 3 µM). Similar analyses were carried out with freshly isolated aortic rings in the presence and absence of oxCMRs (16 µM cholesterol). Data are expressed as mean ± s.e.m. (n = 4 to 6). Statistical significance ($P<0.05$) was determined by a paired or unpaired Student's t test, where appropriate.

Perfusions with ^{125}I-labelled oxCMRs showed that only small amounts (0.216 ± 0.082 ng protein/mg tissue) were taken up by the artery wall. In freshly isolated vessels, oxCMRs significantly increased vessel sensitivity to PE (EC_{50} decreased from 74.7 ± 11.5 to 37.6 ± 9.9 nM), and significantly decreased both maximum % relaxation (76.9 ± 7.8 to 42.9 ± 8.0) and vessel sensitivity (EC_{50} increased form 0.60 ± 0.17 to 1.92 ± 0.42 µM) to CCh but had no significant effect on SNAP-induced relaxations. In vessels which had been perfused with oxCMRs the maximum response to PE was significantly increased (0.34 ± 0.06 to 0.51 ± 0.04 g/mg tissue), the maximum % relaxation to CCh was significantly decreased (91.6 ± 2.4 to 71.5 ± 7.2) and the responses to SNAP were unaffected.

OxCMRs potentiated contractions to PE and inhibited relaxations to CCh, without affecting responses to SNAP in the rat aorta. These effects were observed both in the presence of oxCMRs and in vessel rings which had been perfused with lipoprotein, but were more pronounced when freshly isolated vessels were used. These results suggest that oxCMRs may influence vascular endothelial function by interfering with the L-arginine-nitric oxide pathway. The potentiation of contraction to PE may be due to inhibition of the basal release of nitric oxide or to the release of contractile factors. The observed effects persist after the lipoprotein is removed from the solution bathing the artery wall although only small amounts of oxCMRs are taken up during the perfusions. These findings may have important implications for the vasospasm seen to accompany atherosclerosis.

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301P INHIBITION OF NITRIC OXIDE SYNTHASE INCREASES THE POSITIVE INOTROPIC RESPONSE TO SYMPATHETIC NERVE STIMULATION IN GUINEA-PIG ISOLATED ATRIA

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Neuronal nitric oxide synthase (nNOS) has been implicated in cardiac sympathetic neurotransmission. Schwarz *et al.* (1995) showed that the non-selective NOS inhibitor Nω-nitro-L-arginine (L-NA) increased the release of noradrenaline (NA) from the rat heart during sympathetic nerve stimulation (SNS). To investigate whether NO is involved in the sympathetic control of cardiac contraction, we tested the effects of two relatively selective nNOS inhibitors (1-(2-trifluoromethylphenyl) imidazole (TRIM; Handy *et al.*, 1996)) and 7-nitro indazole (the sodium salt, 7-NiNa; Silva *et al.*, 1995) on the positive inotropic response to SNS.

Double atrial preparations with the right stellate ganglion attached were dissected from guinea-pigs (150-200g), placed in Tyrode solution and paced at 6Hz (5V, 1ms duration). The ganglion was stimulated every two minutes at 1, 2, 3 or 5Hz (5-15V, 1ms duration, 30sec; 15min duration) and changes in the force of contraction (mN) with SNS were measured. This protocol was repeated after the addition of either: (1) TRIM (100μM; 20min duration; n=6) or (2) 7-NiNa (100μM; 15min duration; n=6). L-arginine (1mM; 15min duration; n=6) was subsequently added on top of the NOS inhibitors.

TRIM or 7-NiNa caused no change in the basal force of contraction. However, both NOS inhibitors increased the positive inotropic response to SNS at all frequencies, except 5Hz. TRIM increased the inotropic response to SNS from 0.32±0.13 to 0.42±0.15mN (mean±sem; 1Hz; $p<0.03$, paired t-test), from 0.56±0.13 to 0.86±0.23mN (2Hz; $p<0.006$), and from 0.93±0.20 to 1.20±0.23mN (3Hz; $p<0.003$; Fig.1). 7-NiNa increased the inotropic response to SNS from 0.35±0.08 to 0.49±0.10mN (1Hz; $p<0.04$), from 0.75±0.19 to 0.97±0.18mN (2Hz; $p<0.004$), and from 1.0±0.21 to 1.31±0.21mN (3Hz; $p<0.003$). These effects were reversed with L-arginine (Fig. 1).

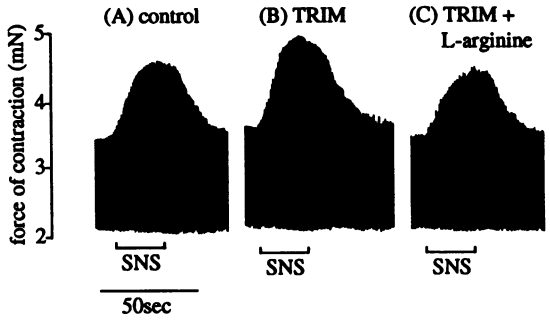

Figure 1. Raw data traces showing changes in force of contraction with SNS (3Hz, 10V, 1ms duration, 30sec): (A) control; (B) addition of TRIM (100μM); (C) addition of TRIM (100μM) + L-arginine (1mM).

In conclusion, nNOS inhibitors increase the positive inotropic response to SNS. This is consistent with the idea that neuronal NO could inhibit the release of NA during cardiac SNS.

We are grateful to the BHF for their support.

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302P DIFFERENT MECHANISMS MEDIATE RELAXATION TO ENDOTHELIUM-DERIVED NO AND THE NO DONOR SIN-1 IN THE RABBIT ISOLATED FEMORAL ARTERY

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Acetylcholine (ACh)-evoked relaxation and repolarisation of the rabbit isolated femoral artery can be fully accounted for by the release of endothelium-derived nitric oxide (NO; Plane *et al.*, 1995). However, although both endothelium-derived and authentic NO can cause glibenclamide-sensitive smooth muscle repolarisation in this vessel, relaxation to NO appears to occur by mechanisms independent of changes in membrane potential. In this study, the mechanisms underlying relaxation of the rabbit isolated femoral artery to both endothelium-derived NO (released by ACh) and the NO donor 3-morpholino-sydnonimine (SIN-1) were investigated.

New Zealand White rabbits of either sex were anaesthetised (sodium pentobarbitone, 60 mg kg^{-1}) and killed by rapid exsanguination. Segments of femoral artery (2 mm in length) were mounted in a myograph under an optimal preload of 1 g and maintained at 37°C in oxygenated Krebs buffer containing indomethacin (1 μM). All data are expressed as mean ± s.e. mean and differences between mean values were calculated using the Students t-test.

ACh (0.01-10 μM) evoked endothelium-dependent relaxation of arterial segments pre-contracted with phenylephrine (PE; 1-3 μM; maximal relaxation 95.0 ± 1.1 %; n=12). Exposure to 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; 10μM; 10 mins), an inhibitor of soluble guanylyl cyclase, significantly inhibited relaxation to ACh, reducing the maximum relaxation to 20.9 ± 4.6 % (n=5; $P<0.01$). In the presence of 30 mM KCl, or following pre-incubation with either charybdotoxin (ChTX; 50 nM; 10 mins) or glibenclamide (10 μM; 10 mins), relaxations to ACh were unaltered (n=4 in each case; $P>0.01$). Furthermore, exposure to the combination of ODQ and ChTX did not further depress the response to ACh compared to ODQ alone (maximal relaxation 14.9 ± 0.5 %; n=4; $P>0.01$). However, in the presence of a combination of ODQ and glibenclamide (10 μM), or ODQ and 30 mM KCl, relaxations to ACh were abolished (n=4 in each case; $P<0.01$).

SIN-1 (0.01-10 μM) evoked concentration-dependent relaxation in PE-stimulated arterial segments, which was not altered by the presence of the endothelium (n=4; $P>0.05$). As with ACh, relaxation to SIN-1 was not altered by glibenclamide (n=4; $P>0.05$) but pre-incubation with ODQ reduced the maximum relaxation to SIN-1 by around 80 % (maximum relaxation 21.6 ± 6.8 %; n=4; $P<0.01$). However, in contrast to ACh, responses to SIN-1 were inhibited by around 50 % in the presence of either 30 mM KCl, or the potassium channel inhibitor The maximum relaxation to SIN-1 was reduced to 48.9 ± 1.2 % and 57.1 ± 6.8 % (n=4 in each case ; $P<0.01$), respectively. Exposure to a combination of ODQ with either 30 mM KCl or ChTX abolished relaxation to SIN-1 (n=4; $P<0.01$), but pre-incubation with glibenclamide and ODQ did not further depress relaxations to SIN-1, compared with ODQ alone (n=4; $P>0.05$).

These data demonstrate that in the rabbit isolated femoral artery relaxation to endothelium-derived NO can be largely accounted for (around 80 %) by voltage-independent mechanisms mediated by cyclic GMP. However, a KCl- and glibenclamide-sensitive mechanism does become apparent when activation of this enzyme is inhibited, although it appears that it only mediates around 20 % relaxation of induced tone. In contrast, although relaxation to the NO donor SIN-1 is also largely dependent upon the activation of soluble guanylyl cyclase (around 80 %), a significant component (40 %) of the response is also sensitive to KCl and the potassium channel inhibitor ChTX. Thus, it appears that three mechanisms can contribute to relaxation to SIN-1 in this vessel : (i) a cyclic GMP-dependent, ChTX-sensitive pathway (ii) a cyclic GMP-mediated, voltage-independent pathway and (iii) a cyclic GMP-independent, ChTX-sensitive pathway.

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303P GENERATION OF NITRIC OXIDE BY XANTHINE OXIDASE

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Human vascular smooth muscle and endothelial cells have been shown to bioactivate organic nitrates to nitric oxide (NO) by enzymatic pathways in sufficient quantities to influence platelet function (Benjamin et al. 1991, Feelisch et al. 1995). There is increasing interest in the potential of xanthine oxidase (XO) to convert nitrate to nitrite in hypoxic conditions, using an electron donor. The effect of XO on conversion of glyceryl trinitrate (GTN) to NO, using platelet aggregation in a suspension of platelet rich plasma as a bioassay, was evaluated. Statistical comparisons were carried out using a paired t-test. Xanthine oxidase incubated with GTN (220 μM) at 37°C for 1 minute, inhibited U46619 (2 μM, a thromboxane mimetic) induced platelet aggregation in a dose dependent fashion (Table 1). The dose response curve to XO was shifted to the left on addition of superoxide dismutase (SOD, 100 IU/ml) (Figure 1, n=6 mean ± SEM). The anti-aggregant effect of GTN and XO was attenuated by allopurinol (100 μM allowed 56 ± 12 % platelet aggregation at top dose XO, n=4, mean ± SEM) and oxyhaemoglobin (HbO_2, dose range 1-10 μM) (Table 2). We conclude that organic nitrate was converted to NO by XO. This has important therapeutic implications, particularly in conditions where nitrovasodilators are used in the treatment of cardiovascular disease.

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Table 1. Dose response curve to XO (n=10, mean ± SEM)

[XO] U/L	% Inhibition
340	94.5 ± 1.7
170	49.6 ± 9.1*
85	14.9 ± 3.9*
42	10.6 ± 3.2*

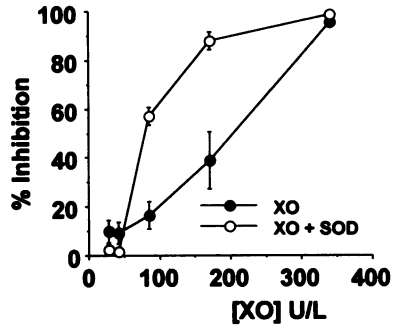
*p < 0.001 difference from baseline

Table 2. The effect of HbO_2 on the anti-aggregant effects of XO, 340 U/L, and GTN, 220 μM (n=4, mean ± SEM)

[HbO_2] μM	% Inhibition
0	98.5 ± 0.8
1 μM	76.4 ± 3.2*
5 μM	40.7 ± 2.6*
10 μM	35.3 ± 4.8*

*p < 0.001 difference from baseline

Figure 1 Effect of XO and XO + SOD on platelets



304P β-AMYLOID INDUCES CYCLOOXYGENASE-2 AND INDUCIBLE NITRIC OXIDE SYNTHASE IN MICROGLIAL CELLS: INHIBITION OF PGE_2 AND NO PRODUCTION BY SELECTIVE COX-2 AND iNOS INHIBITORS

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Alzheimer's disease (AD) has an inflammatory component that contributes to the neurodegenerative process. Non steroidal anti-inflammatory drugs (NSAIDs) have been found in retrospective epidemiological studies and in a small clinical trial to delay onset and progression of AD (Breitner *et al.*, 1995; Rogers *et al.*, 1993). Moreover, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) are up-regulated in AD brains. The inflammatory process is thought to involve microglial cells which appear chronically activated within amyloid plaques (see McGeer & McGeer, 1995). We have investigated the up-regulation of COX-2 and iNOS in microglial cells activated by aggregated β-amyloid (1-42; βA). In addition, the effect of the selective COX-2 inhibitor, SC58125 (Reitz *et al.*, 1994) and iNOS inhibitor, 1400W (Garvey *et al.*, 1997) on the production of prostaglandin E_2 (PGE_2) and nitric oxide (NO) from activated microglia was examined.

NTW8 microglial cells (Anderson *et al.*, 1997) were cultured in HEPES-buffered Dulbecco's modified Eagles medium with 10% (v/v) foetal calf serum and incubated at 37°C in a humidified 5% CO_2, 95% air atmosphere. NO was detected in medium using the Griess assay and ELISA's were used to measure PGE_2 and tumour necrosis factor-α (TNFα). COX-2 and iNOS protein were detected by western blotting. Treatment for 24h of NTW8 cells with βA (30μM) or lipopolysaccharide (LPS; 10ng ml^{-1}, positive control) in combination with interferon-γ (IFNγ; 100U ml^{-1}) caused the up-regulation of COX-2 and iNOS protein with concomitant increase of PGE_2 and NO (Figure 1). Control or IFNγ alone had no effect. SC58125 (0.1-300nM) or 1400W (0.3-1000μM) caused a concentration-dependent inhibition of LPS+IFNγ (10ng ml^{-1}+10U ml^{-1}) induced release of PGE_2 or NO, respectively (IC_{50} values; SC58125 1 [0.9-1.1]nM, n=4; 1400W 1.4 [1.3-1.5]μM, n=4; geometric mean with [95% confidence limits]). SC58125 did not block increases in NO and TNFα nor did 1400W prevent increases in PGE_2 or TNFα. SC58125 or 1400W did not reduce levels of COX-2 or iNOS.

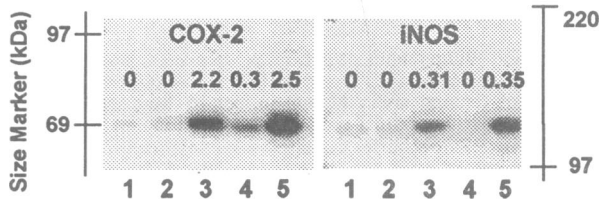

Figure 1: Induction of COX-2 and iNOS following treatment (24h) with 1. control, 2. IFNγ, 3. LPS+INFγ, 4. βA, or 5. βA+INFγ. PGE_2 (ng mg $protein^{-1}$) or NO (μmol mg $protein^{-1}$) levels given above lanes.

These results demonstrate that βA activated microglial cells up-regulate COX-2 and iNOS. Inhibition of COX-2 in βA activated microglial cells provides a possible mechanism to explain the therapeutic benefit of NSAIDs in AD.

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305P DIFFERING EFFECTS OF NITRIC OXIDE COMPARED WITH PEROXYNITRITE ON MAST CELL DEGRANULATION *IN VITRO*

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Mast cells (MCs) are involved in allergic and inflammatory responses and may have an important role in cardiac disorders (Jeziorka *et al*, 1997) and a variety of forms of angiogenesis (Starkey *et al*, 1988). Nitric oxide (NO) attenuates rat serosal MC responses in vitro (Salvemini *et al*, 1991), suggesting that NO may modulate MC mediator release in pathophysiological conditions. However, NO formed under pathophysiological conditions is largely derived from inducible nitric oxide synthase (iNOS) and is formed in high, sustained amounts; conditions which may facilitate formation of peroxynitrite ($ONOO^-$; adduct of NO and O_2^-). The effects of $ONOO^-$ on MC responses have yet to be investigated. The RBL-2H3 cell line has MC characteristics including degranulation responses to a variety of stimuli and provides a convenient system to study MC responses in vitro. The aims of the present study were i) to compare the effects of cGMP and NO on RBL-2H3 degranulation, with those previously described in rat serosal MCs, and ii) to examine the effects of $ONOO^-$ on RBL-2H3 responses.

Cells at confluency in 24 well plates were incubated with [3H]-serotonin (5-HT; 1μCi, 20-24 hrs). To elicit [3H]-5-HT release, cells were exposed to A23187 (3μM or 1μM, 30 min, 37°C). To determine the effects of cGMP or NO on 5-HT release, either 8-bromo cGMP (10-300μM, 30 min, 37°C), SNAP (10-100μM) or detaNONOate (0.3-3 mM, 1 hr, 37°C) were incubated with the cells prior to A23187 addition. For $ONOO^-$ exposure, cells were treated with $ONOO^-$ (1μM-1mM, 5 min, room temp) immediately prior to A23187. Under these conditions, addition of $ONOO^-$ did not cause significant cytotoxicity. Results are expressed as a percentage (mean±sem) of the release of [3H]-5-HT due to A23187. Data were analysed by ANOVA followed by Dunnett's test, $P<0.05$ was considered to be significant.

Pre-treatment with either 8-bromo cGMP, SNAP or detaNONOate, at all concentrations used, had no effect on [3H]-5-HT release in the presence of 1μM A23187 (data not shown). In contrast, $ONOO^-$ (1-10μM) significantly enhanced [3H]-5-HT release (213±26% at 3μM $ONOO^-$). At 3μM A23187, 8-bromo cGMP, detaNONOate and SNAP attenuated the [3H]-5-HT release to: 62±15% (8-bromo cGMP, 300μM), 78±3% (detaNONOate, 3mM) and 53±8% (SNAP, 100μM), whereas the low concentrations of $ONOO^-$ (1-10 μM) did not affect [3H]-5-HT release. However, higher concentrations (0.1-1 mM) of $ONOO^-$ did attenuate [3H]-5-HT release to 66±14% (1 mM).

Cyclic GMP or NO attenuates RBL-2H3 degranulation responses suggesting that the RBL-2H3 cell line is appropriate to study the effects of NO and/or its metabolites on MC responses. $ONOO^-$ has a biphasic effect on RBL-2H3 degranulation; enhancement at low concentrations and inhibition at higher concentrations. In conclusion, the nature of the iNOS product (NO or $ONOO^-$), may be crucial in determining the influence of NOS activity on MCs.

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306P ENDOTHELIUM-DEPENDENT RELAXATION IN CAROTID ARTERIES FROM DIABETIC AND NON-DIABETIC BIOBRED RATS

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Carotid artery atherosclerosis is increased significantly in patients with diabetes (Podobnik-Sarkanji *et al.*, 1995) and may contribute to the increased incidence of stroke in this condition (Malkoff *et al.*, 1997). Increased atherogenesis may be due to endothelial cell (EC) dysfunction, which has been demonstrated in aortae from BioBred (BB) spontaneously diabetic rats (Durante *et al.*, 1988). The effect of diabetes on endothelium-dependent relaxation has not been investigated in carotid arteries from these animals. The aim of the present study was to determine whether EC function was impaired in carotid arteries from the diabetic BB rat.

Functional responses of carotid arteries from male, diabetic (age 179±8 days, wt.405±16g; daily insulin dose 2.5±0.1U; n=8) and matched non-diabetic BB rats (age 182±5 days, wt. 426±7g; n=8) were assessed using the protocol described. Left carotid arteries were mounted in a myograph containing physiological salt solution (PSS) at 37°C, perfused with 95%O_2; 5%CO_2 (pH 7.4), for measurement of isometric responses. Arteries were stretched to their optimum resting force (1.5g; Antonaccio *et al.*, 1994) and contracted with high (125mM) potassium PSS. Cumulative concentration-response curves were constructed for the vasoconstrictors noradrenaline (NA, 10^{-9}-$3x10^{-5}$M) and endothelin-1 (ET-1; 10^{-11}-$3x10^{-6}$M), the endothelium-dependent vasodilator acetylcholine (ACh; 10^{-9}-10^{-5}M), and the endothelium-independent nitric oxide donor 3-morpholinosydnonimine (SIN-1; 10^{-9}-$3x10^{-5}$M). Vasodilator responses were investigated following sub-maximal contraction with NA ($3x10^{-7}$M). Results are mean ± s.e.mean. Comparisons were made using Student's t-test.

Diabetic BB rats had significantly higher plasma glucose (22.3±3.8 vs 6.6±0.1mmol/l; $P<0.01$) and glycated haemoglobin (9.6±1.1 vs 3.8±0.1%; $P<0.001$), but not cholesterol (2.5±0.09 vs 2.36±0.11 mmol/l; $P>0.1$) concentrations than controls. Carotid arteries from both diabetic and non-diabetic BB rats responded well to the endothelium-independent vasodilator, SIN-1, but not to the endothelium-dependent agonist ACh (Table 1). The ET-1-induced contraction failed to reach a steady maximum in the concentration range used, preventing calculation of the sensitivity to this agonist. Maximum responses to the vasoconstrictors, and sensitivity to NA, were similar in vessels from diabetic and non-diabetic BB rats (Table 1).

Rat carotid arteries have been shown previously to relax in response to ACh (Antonaccio *et al.*, 1994). The poor ACh-induced relaxation, but maintained response to exogenous nitric oxide (SIN-1), in the present investigation suggests an impairment of EC function. The demonstration of this impairment in non-diabetic, as well as diabetic, BB rats suggests that it is independent of the development of diabetes. Similar results obtained using resistance arteries from these animals (Hadoke et al., 1997) indicate that this dysfunction is not restricted to conduit vessels.

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Table 1. Maximum relaxation (M.R.; %) maximum contraction (M.C.; mN/mm), and sensitivity data for diabetic and non-diabetic rats.

	ACh		SIN-1		NA		ET-1	
	M.R.	$-LogIC_{50}$	M.R.	$-logIC_{50}$	M.C.	pD_2	M.C.	pD_2
Non-diabetic	65.7±16.9	6.17±0.26	106.1±6.8	6.24±0.16	1.97±0.32	7.86±0.08	2.68±0.45	*
Diabetic	59.3±11.6	6.40±0.26	101.6±9.2	6.18±0.17	1.97±0.23	7.65±0.06	2.23±0.54	*
P	0.76	0.55	0.70	0.80	1.00	0.08	0.53	*

Results are mean ± s.e.mean. * could not be calculated.

307P EVIDENCE FOR A ROLE OF PROSTANOIDS AND SPHINGOSINE IN TUMOUR NECROSIS FACTOR-α MEDIATED CORONARY VASOCONSTRICTION IN THE ISOLATED PERFUSED RAT HEART

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Tumour necrosis factor-α (TNFα), an important mediator endotoxic shock and bacterial sepsis, can mimic some of the profound circulatory changes which are observed in these conditions (Tracey *et al.*, 1986). Endotoxic shock is characterised by a massive drop in blood pressure, a decline in cardiac output and intense regional vasoconstriction, eventually leading to underperfusion of vital organs. Clearly any changes in the hearts ability of autoregulate the coronary circulation during shock could have severe consequences on cardiac function and hence organ perfusion. Therefore, we have examined the actions of TNFα on the coronary circulation in the isolated rat heart. We present evidence to show that TNFα can constrict the coronary vessels, and suggest a role for prostanoids and sphingosine in this action.

Hearts from male Wistar rats (280-310g) were isolated and perfused, via the aorta, according to the Langendorff technique, with recirculating (50ml) Krebs-Henseleit solution of the following composition: NaCl 118mM, $NaHCO_3$ 25mM, KCl 4.7mM, KH_2PO_4 1.2mM, $MgSO_4$ 1.2mM, CaCl 1.23mM and D-glucose 11.6mM (oxygenated with 95% O_2, 5% CO_2, pH 7.4, 37°C and a constant flow of 10ml/min). Recombinant human TNFα was added after 25min. The ceramidase inhibitor, N-oleoyethanolamine (NOE, 1μM), or indomethacin (10μM) were added 20min before TNFα.

The increase in coronary perfusion pressure seen upon TNFα treatment is indicative of coronary vasoconstriction (figure 1). This increase was observed within 5min, and was evident throughout the remainder of the experiment. The vasoconstriction was completely blocked by inclusion of either NOE or indomethacin in the perfusate (figure 1), neither of which affected basal coronary tone.

These data, together with the observations that sphingosine can contract coronary vessels via the release of prostanoids (Murohara *et al.*, 1996), and that TNFα can activate the sphingomyelinase pathway (Dressler *et al.*, 1992), indicate that TNFα may depress cardiac function indirectly via a sphingosine/prostanoid mediated coronary vasoconstriction.

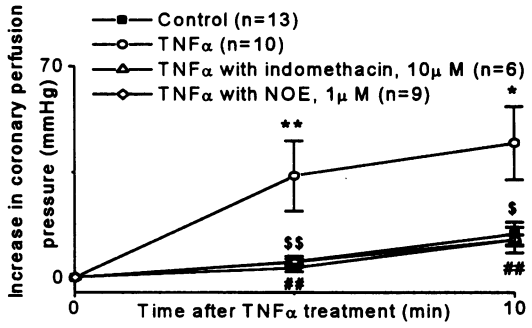

Figure 1. TNFα caused an increase in coronary perfusion pressure. This was blocked by NOE and indomethacin. * p<0.05,** p<0.01 TNFα vs. control. ## p<0.01 TNFα vs. NOE, $ p<0.05,$$ p<0.01 TNFα vs. indomethacin. Oneway ANOVA coupled to Dunnetts.

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308P EFFECTS OF SHEEP POLYCLONAL ANTIBODIES TO HUMAN TNF-α AND IL1β ON CARDIOVASCULAR RESPONSES TO THE CYTOKINES IN CONSCIOUS RATS

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In conscious rats, co-administration of human TNF-α and IL-1β evokes cardiovascular responses which, in several respects, resemble the effects of lipopolysaccharide (Gardiner *et al.*, 1997). Now we have assessed the ability of a mixture (50:50) of polyclonal antibodies (Ab) against TNF-α and IL-1β (Ruetten *et al.*, 1996) to influence the cardiovascular responses to the cytokines. Male, Long Evans rats (350-450g) were chronically instrumented with pulsed Doppler flow probes and intravascular catheters to measure regional haemodynamics. All surgery was performed under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p. supplemented as required). In different experiments, animals were infused i.v. with saline premixed with the cytokines (TNFα (250 μg kg^{-1}), IL-1β (10 μg kg^{-1}) in 2.5 ml delivered over 45 min; n = 8), or Ab (300 mg kg^{-1}) premixed with the cytokines (n = 8), or Ab given as a pre-treatment starting 1h before cytokine infusion (n = 8). Table 1 summarises some of the results. Administration of the cytokines caused a biphasic tachycardia, hypotension and vasodilatation; the temporal pattern of vasodilatation differed in the 3 vascular beds. In the animals given the Ab premixed with the cytokines, all the early effects of the cytokines were prevented, but there was tachycardia at 24h. In the animals pretreated with Ab, the hypotension and mesenteric and hindquarters vasodilatations were suppressed. However, the biphasic tachycardia was still present and there was renal vasodilatation, but this was slower in onset, and more sustained than in the absence of Ab. Both groups given Ab showed mesenteric vasoconstriction. Thus, Ab reduced most, but not all, of the effects of the cytokines. At present, we do not have a ready explanation for the tachycardia seen 24 h after administration of Ab premixed with the cytokines. The responses observed in the animals pretreated with Ab could be explained by differential access of the cytokines and Ab to, for example, the central nervous system.

Table 1. Resting cardiovascular variables (HR = heart rate, beats min^{-1}; MBP = mean blood pressure, mm Hg; RVC, MVC, HVC = renal, mesenteric and hindquarters vascular conductance, respectively, [kHz mm Hg^{-1}]10^3)and changes in response to infusion of saline and cytokines (A), Ab premixed with cytokines (B) or Ab given as a pretreatment 1h before cytokines (C) in conscious rats. Values are mean ± s.e. mean. *P < 0.05 vs baseline (Friedman's test).

		Rest	Time after start of cytokine infusion 0.25 h	1 h	24 h
HR	A	338±8	+110±12*	+12±8	+66±10*
	B	342±5	-2±5	+17±9	+61±10*
	C	332±5	+80±20*	+8±5	+51±6*
MBP	A	104±2	+2±2	-18±2*	-7±2*
	B	101±1	+3±2	+2±1	+1±1
	C	105±1	+3±1	+2±1	-3±1
RVC	A	55±4	+15±3*	+28±4*	+1±3
	B	64±6	-1±2	0±2	+8±4
	C	60±3	+8±4	+11±4*	+37±5*
MVC	A	53±5	+10±4*	+7±5	+5±5
	B	61±5	-1±2	-1±2	-18±3*
	C	58±5	-7±3	-21±3*	-16±5*
HVC	A	42±3	+2±6	+25±6*	+16±5*
	B	52±3	-4±2	-6±2*	+8±4
	C	47±3	+1±3	-2±2	-1±2

Gardiner, S.M. *et al.* (1997). J.Vasc.Res., **34** (Suppl. 1) **17**.
Ruetten, H. *et al.* (1996) Br.J.Pharmacol., **119**, 19P

309P EFFECT OF SHEEP ANTIBODIES TO HUMAN TNF-α AND IL-1β ON CARDIOVASCULAR RESPONSES TO LIPOPOLYSACCHARIDE (LPS) IN CONSCIOUS RATS

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Since some of the cardiovascular effects of co-administration of human TNF-α and IL-1β resemble those of LPS administration (Gardiner *et al.*, 1997), we have examined the effects of pretreatment with a mixture (50:50) of sheep polyclonal antibodies (Ab) to TNF-α and IL-1β (Ruetten *et al.*, 1996) on the haemodynamic responses to LPS infusion in conscious, male Long Evans rats (350-450 g). Animals were chronically instrumented with pulsed Doppler flow probes and intravascular catheters for recording renal, mesenteric and hindquarters haemodynamics; all surgery was carried out under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p. supplemented as required). The Ab was administered i.v. 1 h before LPS infusion (*E.Coli* serotype 0127 B8, Sigma, 150 μg kg^{-1} h^{-1} i.v.) at a dose of 300 mg kg^{-1} in 2.5 ml saline infused over 45 min (n = 8). Control animals received saline starting 1 h before LPS infusion (n = 9). There were no differences between the cardiovascular variables in the 2 groups under baseline conditions or prior to administration of LPS (Table 1).

During LPS infusion (Table 2) in saline pretreated rats, the regional haemodynamic profile was generally as described previously (Gardiner *et al.*, 1995). Pretreatment with Ab had no significant effects on the responses to LPS, with the exception of an attenuation of the early hindquarters vasodilatation. These results contrast with those of Ruetten *et al.* (1996), who used the same Ab and found attenuation of the hypotension and rise in plasma TNFα and iNOS activity in anaesthetised rats given bolus doses of LPS. Hence, the involvement of cytokines in the cardiovascular sequelae of endotoxaemia may vary with the model studied.

Table 1. Heart rate (HR; $bmin^{-1}$), mean arterial pressure (MAP; mmHg) and renal (R), mesenteric (M) and hindquarters (H) vascular conductance (VC (kHz mm Hg^{-1})10^3) under resting conditions and just prior to LPS infusion in animals treated with saline (Sal; n = 9) or Ab (n = 8). Values are mean ± s.e. mean.

	Rest		Pre-LPS	
	Sal	Ab	Sal	Ab
HR	321 ± 11	341 ± 7	314 ± 12	324 ± 7
MAP	103 ± 1	103 ± 1	102 ± 1	104 ± 1
RVC	57 ± 3	63 ± 6	53 ± 2	62 ± 6
MVC	72 ± 9	63 ± 5	70 ± 8	65 ± 5
HVC	40 ± 4	44 ± 4	38 ± 4	38 ± 2

Table 2. Cardiovascular changes (mean ± s.e. mean) during LPS infusion after pretreatment with saline (Sal) or Ab. For abbreviations and units, see Table 1.

		Time after onset of LPS infusion		
		1 h	8 h	24 h
HR	Sal	+42 ± 8*	+61 ± 8*	+71 ± 11*
	Ab	+21 ± 11*	+61 ± 13*	+94 ± 11*
MAP	Sal	-14 ± 3*	-1 ± 2	-5 ± 2*
	Ab	-14 ± 4*	-3 ± 1	-7 ± 2*
RVC	Sal	+22 ± 3*	+19 ± 5*	+44 ± 8*
	Ab	+33 ± 7*	+28 ± 5*	+58 ± 9*
MVC	Sal	+12 ± 4*	-13 ± 4*	-12 ± 5*
	Ab	-3 ± 8	-15 ± 4*	-3 ± 3
HVC	Sal	+16 ± 3*	+11 ± 2*	+20 ± 2*
	Ab	+7 ± 3*†	+6 ± 2*	+22 ± 5*

*$P < 0.05$ versus baseline (Friedman's test), †$P < 0.05$ versus Sal (Mann Whitney U test)

Gardiner, S.M. *et al.* (1995) Br.J.Pharmacol., **116**, 2005-2016.
Gardiner, S.M. *et al.* (1997) J.Vasc.Res., **34** (Suppl. 1), 17.
Ruetten, H. *et al.* (1996) Br.J.Pharmacol., **119**, 19P

310P A COMPARISON OF THE VASOCONSTRICTOR EFFECT OF 8-EPI PROSTAGLANDIN $F_2\alpha$ IN RAT ARTERY AND VEIN

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We have previously shown that the isoprostane, 8-epi prostaglandin (PG) $F_{2\alpha}$ is a constrictor in isolated porcine, bovine and human coronary arteries (Kromer *et al.*, 1997; Kromer & Tippins, 1996a). 8-epi $PGF_{2\alpha}$ is also a vasoconstrictor in the Langendorff perfused rat heart, but only after oxidant stress (Kromer & Tippins, 1996b, 1997). In porcine and ovine coronary arteries 8-epi $PGF_{2\alpha}$ acts as a partial agonist at the TP receptor (Kromer & Tippins, 1996a). The present study was undertaken to determine whether the isoprostane constricts venous tissue and, if so, compare the responses with those of arterial tissue.

2-3mm rings of rat aorta and jugular vein were suspended in Krebs at 37°C. Tension was adjusted by the length / tension relationship using $0.9L_{100}$ aorta and $0.9L_{20}$ for jugular vein. The endothelium was removed in aortas by rubbing the luminal surface with forceps. Responses to 80mM KCl were recorded and the presence of an endothelium determined by relaxation of pre-contracted tissues (1μM noradrenaline) with 1μM acetylcholine. Cumulative concentration-response curves for 8-epi $PGF_{2\alpha}$ and the thromboxane mimetic U46619 were recorded and concentration-response curves to U46619 were repeated in the presence of 8-epi $PGF_{2\alpha}$, 0.3, 1, 3 and 10μM in aortas and 0.1, 0.3 and 1μM in veins. Responses are expressed as a percentage of the response to KCl.

Both U46619 and 8-epi $PGF_{2\alpha}$ constricted rat aorta and jugular vein with the following EC_{50}s: 6.8±1.6, 4.5±1.0 for U46619 on aorta, +/- endothelium respectively; 455±52, 268±34 for 8-epi $PGF_{2\alpha}$ on aorta +/- endothelium respectively; and 54±10 and 303±48 for U46619 and 8-epi $PGF_{2\alpha}$ respectively on jugular vein (nM, mean±s.e.mean). Responses of the jugular vein in the presence of endothelium were not recorded. Maximal responses to 8-epi $PGF_{2\alpha}$ in rat aorta were slightly smaller than those to U46619 in the presence of endothelium (116±3 for 8-epi $PGF_{2\alpha}$ and 139±2 for U46619, $P<0.0001$, unpaired t test). However, the absence of endothelium shifted the EC_{50} slightly to the left for both agonists and increased the maximal response to 8-epi $PGF_{2\alpha}$ (116±3% and 129±4%, $P < 0.05$, unpaired t test). In the vein maximal responses to both agonists were comparatively smaller than in aorta (90±7%, $p<0.0001$ and 98±9%, $p<0.05$, unpaired t test; for 8-epi $PGF_{2\alpha}$ and U46619 respectively). When tested in the presence of 8-epi $PGF_{2\alpha}$, responses to low concentrations of U46619 in both aorta and vein were augmented ($P<0.001$, ANOVA). However, responses to higher concentrations of U46619 were not inhibited by 8-epi $PGF_{2\alpha}$.

Our results show that 8-epi $PGF_{2\alpha}$ is a vasoconstrictor in both artery and vein with a similar potency in both tissues. Maximal responses to 8-epi $PGF_{2\alpha}$ are smaller than those to the thromboxane mimetic U46619 in artery in the presence of endothelium but of a similar size in vein in the absence of endothelium . The inability of 8-epi $PGF_{2\alpha}$ to inhibit responses to U46619 in both tissues suggests that in these tissues, in contrast to the coronary arteries, 8-epi $PGF_{2\alpha}$ is not a partial agonist.

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311P EFFECTS OF SC-58635, A SELECTIVE INHIBITOR OF CYCLOOXYGENASE 2 ON HAEMODYNAMICS AND ORGAN FUNCTION OF RATS WITH ENDOTOXAEMIA

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Here we compare the effects of SC-58635 (SC), a selective inhibitor of the activity of cyclooxygenase-2 (COX-2) (Penning *et al.*, 1997), with those of dexamethasone on haemodynamics and organ dysfunction in a rodent model of endotoxic shock.

Male Wistar rats (240 - 290 g) were anaesthetised with sodium thiopentone (120 mg·kg^{-1}, i. p.). The trachea was cannulated to facilitate respiration, and rectal temperature maintained at 37 °C. The right carotid artery was cannulated and connected to a pressure transducer for the measurement of mean arterial blood pressure (MAP). The femoral vein and bladder were cannulated for the administration of drugs and the collection of urine respectively. At completion of the surgical procedure, animals were allowed to equilibrate for 15 min after which they received injections of SC-58635 (3 mg·kg^{-1} i. p.) (n = 9), dexamethasone (Dex, 3 mg·kg^{-1} i. p.) (n = 6) or the vehicle for SC-58635 (0.3 ml 10 % Dimethylsulfoxide, DMSO) (n = 5). This dose of SC-58635 abolishes COX-2 activity in rats with endotoxaemia (Hamilton L.C. *et al*). Sixty minutes later (30 min with dexamethasone) animals received an infusion of *E. coli* lipopolysaccharide (LPS, 6 mg·kg^{-1} i.v. for 15 min) followed by saline (1.2 ml·h^{-1}). It has been previously shown in our laboratory that endotoxaemia within 6 h leads to a substantial increase in COX-2 protein and activity (Ruetten and Thiemermann, 1997). All values are expressed as mean ± s. e. mean.

Endotoxaemia for 6 h resulted in a significant rise in the serum levels of urea, creatinine (Creat, an indicator of renal dysfunction/failure) and alanine aminotransferase (ALT, an indicator of liver injury) (Table 1). Pretreatment of rats with SC-58635 did not affect the degree of renal dysfunction or liver injury caused by LPS whereas these were reduced by dexamethasone.
In rats not receiving LPS (control), neither SC-58635 nor dexamethasone affected the parameters measured; similarly neither vehicle (saline/DMSO) had any effect on organ injury in the LPS rats (data not shown).

Table 1: Effect of SC-58635 or dexamethasone on MAP and organ injury/dysfunction caused by LPS (*p < 0.05 vs LPS + saline. ANOVA followed by Bonferoni's test.)

	Urea (mmol/l)	Creat (μmol/l)	AST (iu/l)	ALT (iu/l)	MAP (mmHg)
Control (n = 8)	8 ± 1*	35 ± 2*	241 ± 19*	83 ± 5*	100 ± 5
LPS (n = 9)	20 ± 1	61 ± 6	502 ± 43	201 ± 28	90 ± 6
LPS + SC (n = 9)	23 ± 2	70 ± 7	501 ± 88	179 ± 18	75 ± 3
LPS +Dex (n = 6)	9 ± 1*	28 ± 1*	318 ± 25	97 ± 10*	106 ± 2

Thus, selective inhibition of COX-2 activity with SC-58635 does not affect the renal dysfunction or liver injury caused by LPS in the rat. In addition, these results suggest that the reduction of the organ dysfunction/injury provided by dexamethasone is not due to it inhibiting the expression of COX-2.

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312P THE EFFECTS OF PROSTAGLANDIN E_2 AND EP_2 RECEPTOR MIMETICS ON THE HUMAN ISOLATED UMBILICAL ARTERY

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Prostaglandins cause constriction of human umbilical artery (Hillier *et al.*, 1968) but as yet the receptor population has not been defined. In the present study an attempt has been made to characterise the EP-receptor population, on human umbilical artery, using the prostaglandin E_2 and EP_2-receptor agonists, butaprost (TR 4979, 15-deoxy-16-hydroxy, 17 cyclo butyl PGE_1) and AH 13205 (*trans*-2-[4-(1-hydroxyhexyl) phenyl]-5-oxocyclo penta-heptanoic acid) alone and in conjunction with, the TP-receptor antagonist, Bay u3405 (McKenniff *et al.*,1989).

Samples of human umbilical cord were obtained from full term pregnancies (all women gave written consent) and placed immediately into Krebs' solution at room temperature. The cords were transported to the laboratory within 60 minutes. Rings of umbilical artery (with intact endothelium) were suspended in Krebs' solution containing indomethacin (2.79 μM) at 37°C in a 10ml organ bath and oxygenated with 2.5 % O_2/ 8 % CO_2/balance N_2 as described previously (Amin *et al.*, 1995). Cumulative concentration-effect curves were constructed to PGE_2, butaprost and AH 13205. Where the antagonist was used it was allowed to equilibrate for at least 30 minutes before the agonist concentration-effect curve was repeated. In all cases n=5.

PGE_2 and the EP_2 receptor mimetics butaprost and AH 13205 all evoked constrictor responses (EC_{50}s (M) were 5.0 x 10^{-7}, 2.0 x 10^{-6}, 4.0 x 10^{-7} respectively on unstimulated arterial rings.

In the presence of the selective TP-receptor antagonist, Bay u3405 (10^{-6}M), the concentration response curves to PGE_2, butaprost and AH 13205 were depressed between the doses of 3 x 10^{-6}M and 3 x 10^{-5}M (P<0.05-P<0.001). The maximum attainable responses to PGE_2 and butaprost in the presence of Bay u3405 were reduced by approximately 41 and 46% respectively. In the absence of Bay u3405, AH 13205 achieved a maximum response but in the presence of Bay u3405 the maximum response was reached but was reduced by 46%,

Previous studies in this laboratory have suggested the presence of constrictor TP- and FP-receptors on human umbilical artery (Amin *et al.*, 1995, 1996). These results suggest that butaprost and AH 13205 exert a constrictor effect on human umbilical artery which maybe mediated through the TP-receptor. This is an unexpected finding since butaprost and AH 13205 have been shown to be EP_2-receptor selective in most tissues (Coleman *et al.*, 1987).

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313P CHARACTERISATION OF A PROSTAGLANDIN DP-RECEPTOR POPULATION ON THE HUMAN ISOLATED UMBILICAL ARTERY

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Umbilical arteries are unique vessels in the sense that they must remain patent *in utero* but must close completely and quickly after delivery. In the present study an attempt has been made to characterise the DP-receptor population on human umbilical artery using prostaglandin D_2 (PGD_2) and the DP-receptor selective agonist, BW 245c (Giles *et al.*, 1989).

Samples of human umbilical cord were obtained from full term pregnancies (all women gave written consent) and placed immediately into Krebs solution at room temperature. The cords were then transported to the laboratory within 60 minutes. Rings of umbilical artery (with intact endothelium) were suspended in Krebs solution containing indomethacin (2.79 μM) at 37°C in a 10ml organ bath and oxygenated with 2.5 % O_2/ 8 % CO_2/balance N_2 as described previously (Amin *et al.*, 1995). Cumulative concentration-effect curves were constructed to PGD_2 and BW 245c. Bay u3405 (10^{-6}M), a TP-receptor anatgonist (McKenniff *et al.*,1989) and AH 6809 (10^{-5}M), a DP receptor antagonist (Keery & Lumley, 1985), were used to investigate whether the responses were TP- or DP-receptor mediated. The antagonists were allowed to equilibrate for at least 30 minutes before the agonist concentration-effect curves were repeated. As this tissue does not possess any tone then histamine was used (10^{-6} M) to investigate vasodilator responses. In all cases n=5.

PGD_2 and BW245c evoked both vasoconstrictor (EC_{50}s for PGD_2 and BW245c were 1.0 x 10^{-7}M and 2.0 x 10^{-7}M respectively) and vasodilator (IC_{50}s for PGD_2 and BW 245c were 7.0 x 10^{-9}M and 3.0 x 10^{-9}M respectively) responses.

In the presence of Bay u3405 and AH 6809 the vasoconstrictor responses to PGD_2 were attenuated. Between the doses of 10^{-6}-10^{-5}M AH 6809 significantly attenuated ($P<0.05$) the constrictor responses to PGD_2 the maximum response in the presence of the antagonist being reduced by approximately 50%. The maximum attainable response to BW 245c was reduced in the presence of AH 6809 by approximately 70% ($P<0.001$). Bay u3405 caused a marked reduction ($P<0.01$-$P<0.001$) of the PGD_2 constrictor response, the maximum response being reduced by approximately 76%. Previous studies in this laboratory have suggested the presence of a TP-receptor and an FP-receptor on human umbilical artery (Amin *et al.*, 1995, 1996). These results suggest the possibility of a DP-receptor mediating vasodilation whilst the vasoconstriction seen maybe, in part, through the TP-receptor.

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314P RUBIDIUM AFFECTS RESPONSES TO POTASSIUM CHANNEL MODULATORS IN RAT PORTAL VEIN

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Previous studies in our laboratories showed that rubidium (Rb) reduced the relaxant responses to potassium channel openers (KCOs) on the mouse ileum bathed in Krebs' solution (KS) in which potassium (K) was replaced by Rb (Yeung *et al.*, 1996). However, the extent of reduction in the effects of KCOs may be tissue-dependent. In the present study, the effects of Rb on responses to KCOs are investigated in the isolated rat portal vein bathed in KS supplemented with either RbCl or KCl and contracted by carbachol (CARB).

Portal veins from male Hooded Lister rats (210-440g, Bradford strain) were placed under 0.5g tension in KS supplemented with either 10mM KCl (KS+KCl) or 10mM RbCl (KS+RbCl) Following 40min equilibration, an isometric contraction was elicited by a single concentration of CARB (3μM, giving approximately 95% maximal contraction). Cumulative concentration-response curves to pinacidil (PIN, 3μM-1mM), cromakalim (CROM, 0.3-300μM), SDZ PCO400 (SDZ, 0.1-200μM) or vehicle (alcohol) were then constructed. The same procedure was performed with the L-type calcium channel blocker, verapamil (VP, 0.3-100μM), in order to assess the influence of Rb on an agent with a relaxation mechanism of action other than K channel opening. The EC_{50}s (the concentration of relaxant required to produce 50% maximium relaxation of CARB-induced contraction) were calculated and expressed as geometric means with 95% confidence limits. Differences between observed EC_{50}s in tissues bathed in KS+KCl or KS+RbCl were analysed using Student's unpaired t-test.

The magnitude of the contractile response to 3μM CARB was not significantly different in preparations bathed in either KS: KS+KCl, 0.93±0.17g; KS+RbCl, 0.96±0.18g. PIN, CROM, SDZ and VP caused concentration-dependent reduction of CARB-induced contraction, VP being the most potent (Table 1). The EC_{50} values indicated that the potencies of PIN, CROM and SDZ were attenuated in preparations bathed in KS containing 10mM Rb by approximately 3, 6 and 13 fold respectively, whereas the EC_{50} for VP was unaffected (Table 1). No vehicle effect was observed (n≥4). Thus Rb attenuated KCO-induced relaxations but not those due to calcium channel blockade in portal vein, as in mouse ileum (Yeung *et al.* 1995).

Table 1. Relaxant potencies of pinacidil, cromakalim, SDZ PCO400 and verapamil in the rat portal vein bathed in Krebs' solution (KS) supplemented with either 10mM KCl or 10mM RbCl and contracted by carbachol 3μM.

Relaxant	KS+10mM KCl EC_{50} μM (95% CL)	KS+10mM RbCl EC_{50} μM (95% CL)	n
Pinacidil	12.9 (4.2-24.7)	35.0 (12.5-65.0)*	4
Cromakalim	1.9 (1.3-2.5)	10.7 (16.0-52.5)**	4
SDZ PCO400	4.7 (3.9-5.5)	59.6 (9.7-129.3)***	4
Verapamil	0.6 (0.4-0.8)	0.5 (0.2-0.8)	4

*$P<0.05$, **$P<0.01$, ***$P<0.001$; EC_{50} of relaxants in preparations bathed in KS+10mM KCl *vs* EC_{50} of relaxants in preparations bathed in KS+10mM RbCl. Student's unpaired t-test.

The results obtained in the present study were in good agreement, despite the use of tissues from different animals for the evaluation of the inhibitory effects of Rb on the relaxant responses to KCOs, with those of Greenwood & Weston (1993) in the rat aorta and Yeung *et al.* (1996) in the mouse ileum.

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315P ENHANCED ROLE OF THE HYPERPOLARIZING PATHWAY IN ENDOTHELIUM-DEPENDENT RELAXATIONS IN RABBIT CAROTID ARTERIES WITH A NEOINTIMA

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Intimal thickening, the soil for atherosclerosis, can be induced in the carotid artery of rabbits by the placement of a perivascular collar for 2 weeks. In the collared segments, the endothelium-dependent relaxation to acetylcholine after contraction with phenylephrine is preserved (Van Put *et al.*, 1995). Recently, it has been proposed that endothelium-dependent relaxations not only depend on nitric oxide (NO), acting via cyclic GMP, but rely in part on an endothelium-dependent hyperpolarizing pathway, acting via the opening of K^+ channels. By measuring relaxations in a high K^+ solution, and hence precluding the hyperpolarizing pathway, we investigated its contribution to the relaxing response in collared artery segments.

Male New Zealand white rabbits (3 kg) were anaesthetized with sodium pentobarbitone (30 mg/kg) and silastic collars were placed around both carotid arteries. Segments proximal to the collars served as controls. Two weeks later the rabbits were killed by an overdose of pentobarbitone, and both arteries were removed. Histological examination showed the absence of an intimal layer in the control rings and a discrete neointima with a thickness of 13±3 μm in the collared segments. Results are expressed as mean ± s.e.mean; n is the number of carotid arteries.

In the organ bath, control and collared carotid artery segments, contracted isometrically with the EC_{50} of phenylephrine, relaxed to cumulative concentrations of acetylcholine with comparable sensitivity ($-\log EC_{50}$ 7.57±0.06 and 7.50±0.20, respectively; n=8) and complete relaxation was attained in both groups. Relaxation to acetylcholine upon contraction in a depolarizing solution of 30 mM K^+ was significantly ($p<0.05$) reduced in control rings ($-\log EC_{50}$ 6.93±0.04; n=7), which illustrates the contribution of a hyperpolarizing pathway in the response to acetylcholine. In collared rings exposed to 30 mM K^+, the response to acetylcholine was abolished (n=2) or significantly reduced ($-\log EC_{50}$ 6.49±0.20; $p<0.05$; n=5) as compared to control rings. The maximum relaxation was 60±3 % in control segments and 39±10 % in collared segments (n=7). In both the control and the collared rings, the already reduced responses to acetylcholine were completely suppressed by $3x10^{-4}$ M N^G-nitro-L-arginine, which points to the NO/cyclic GMP-dependent pathway as the sole mediator of the relaxations observed in a high K^+ solution.

Our results suggest that the NO/cyclic GMP pathway is less active in artery rings with a thickened intima, which appears to be compensated by an increased contribution of the hyperpolarizing pathway, leaving the overall relaxation to acetylcholine intact. Similar observations have been made in the carotid arteries of rabbits on a cholesterol diet for 10 weeks (Najibi *et al.*, 1994). Therefore, the collar model appears to be a valuable tool not only for the study of intimal thickening but also of early dysfunctions in the underlying mechanisms of relaxation.

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Van Put, D.J.M., Van Hove, C.E., De Meyer, G.R.Y. *et al.* (1995) *Eur. J. Pharmacol.* 294, 753-761.

316P ADMINISTRATION OF THE NOVEL Na^+/H^+ EXCHANGE INHIBITOR HOE 642 PRIOR TO REPERFUSION REDUCES INFARCT SIZE IN THE PIG

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Inhibitors of the Na^+/H^+ exchanger have been reported to significantly reduce infarct-size when given prior to occlusion and reperfusion. However, there is a controversy in the literature with regard to the effect of these agents when administered prior to reperfusion. They have been found to have either no effect (Klein *et al.*, 1995), or to reduce the size of infarction (Rohmann *et al.*, 1995). Thus, in the present study we investigated the effect of a novel Na^+/H^+ exchange inhibitor, HOE 642, on infarct-size, when given prior to reperfusion in the pig.

Eighteen pigs (25-30 kg) were anaesthetised with pentobarbital (25 mg kg^{-1} plus 20 mg $kg^{-1} h^{-1}$ i.v.), thoracotomised, and a snare was passed around the left anterior descending coronary artery (LAD) distal of the first branch for the induction of the occlusion and reperfusion. Left ventricular pressure (LVP) and its first derivate (dP/dt) were measured with A 5F Millar catheter-tipped manometer. Mean arterial pressure (MAP) was measured via a catheter placed in the left femoral artery. The LAD was occluded for 60 min and then reperfused for 180 min. The Na^+/H^+ exchange inhibitor, HOE 642 (10 mg kg^{-1}, n=7) or its vehicle (saline, 10 ml, n=11) were given into the left ventricle 1 min prior to reperfusion. Global haemodynamic parameters, including MAP, heart rate (HR), LV end-diastolic pressure (LVEDP), dP/dt_{max} were recorded prior to occlusion, before reperfusion and at the end of the experiment. Infarct-size (IS) was determined as percentage of area at risk (tetrazolium method). Statistics: unpaired Student's t test, $P<0.05$ was considered statistically significant. Data are expressed as mean±s.e.mean.

In the control group, 5 out of 11 pigs died during reperfusion, while in the HOE 642 treated group, only 1/7 animal died. Global haemodynamic parameters were not significantly different between both groups (n=6) prior to occlusion (C: MAP: 93±7 mmHg; HR: 98±5 beats min^{-1}, LVEDP: 4.3±0.9 mmHg, dP/dt_{max}: 1750±136 mmHg sec^{-1}; HOE 642: MAP: 95±5 mmHg; HR: 108±2 beats min^{-1}, LVEDP: 6.0±0.8 mmHg, dP/dt_{max}: 1375±116 mmHg sec^{-1}). In both groups, ischaemia for 60 min resulted in a moderate increase in LVEDP in both groups (C: 1.7±1.3 mmHg; HOE 642: 4.2±2.2 mmHg). In the C group, 180 min of reperfusion caused a significant decrease in MAP and dP/dt_{max} (MAP: -25±7 mmHg; dP/dt_{max}: -650±65 mmHg sec^{-1}; $P<0.05$). Treatment of pigs with HOE 642 prior to reperfusion significantly improved dP/dt_{max} (-150±133 mmHg sec^{-1}), while it had no effect on MAP (-21±7 mmHg). In both groups, area at risk was identical (HOE 642: 42±3.4%; C: 42±2.4%). Treatment with HOE 642 significantly reduced IS when compared to C group (HOE 642: 45.7±2.2%; C: 66.3±2.8%; $P<0.05$).

This study shows that a high dose of HOE 642 given prior to reperfusion results in a reduction of infarct-size. Probably, a high concentration of the inhibitor is necessary at the exchanger site in the first few seconds after reperfusion, in order to achieve a protective effect.

Klein, H.H. *et al.* (1995) *Circulation* 92: 912-917.
Rohmann, S. *et al.* (1995) *Cardiovasc. Res.* 30: 945-951.

317P IMPACT OF INACTIVATION OF G-PROTEINS ON INTRACELLULAR CALCIUM SENSITIVITY OF TENSION IN THE RAT TAIL ARTERY

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In vascular smooth muscle cells, KCl elicits a small vasoconstrictor response with pronounced calcium ($[Ca^{2+}]_i$) mobilisation whereas noradrenaline elicits a marked vasoconstrictor response with weak $[Ca^{2+}]_i$ mobilisation (Karaki, 1989). This difference in $[Ca^{2+}]_i$ sensitivity suggests an amplification of the pharmacomechanical transduction. The object of this study was to investigate whether pertussis toxin (PTX)-sensitive G-proteins are involved in $[Ca^{2+}]_i$ sensitivity of tension.

The tail artery was dissected out from adult, male, Wistar rats (600±10 g, n=5-9 per group) under sodium pentobarbitone anaesthesia (60 $mg.kg^{-1}$, ip). A 1-cm segment was cannulated, mounted in a perfusion/cuvette system placed in a dual wavelength spectrofluorometer and perfused at a constant rate with PSS. Endothelium was disrupted by coperfusion with air (0.4 ml/min, 10 min) and arteries were loaded with Fura 2/AM (5µM, 90 min) and PTX (0, 30, 100, 300, 1000 ng/ml, Osol *et al.*, 1993). They were then stimulated either with high KCl depolarising solution (15, 30, 60, 80, 120 mM, 2 min) or noradrenaline (0.1, 0.3, 1, 3, 10, 100 µM, 2 min; NA). KCl- or NA-evoked increases in $[Ca^{2+}]_i$ ($\Delta[Ca^{2+}]_i$, arbitrary units, a.u.) and perfusion pressure (ΔP mmHg) were measured. Values are mean±SEM. Significant differences (*P < 0.05; *versus* Control) were determined by the Bonferroni test.

PTX produced no change in vasoconstriction elicited by KCl but decrease vasoconstriction elicited by NA (Fig 1).

PTX produced no change $[Ca^{2+}]_i$ mobilisation elicited by KCl or NA (Fig 2).

Fig 1 : Impact of PTX on vasoconstriction elicited by KCl and NA

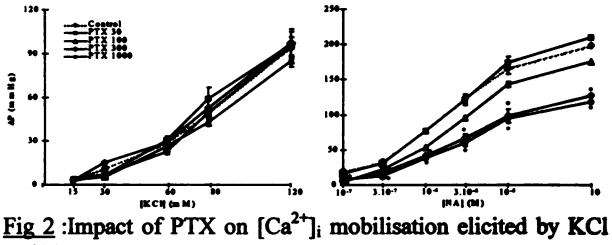
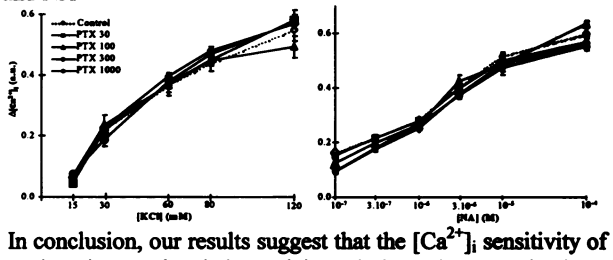


Fig 2 :Impact of PTX on $[Ca^{2+}]_i$ mobilisation elicited by KCl and NA



In conclusion, our results suggest that the $[Ca^{2+}]_i$ sensitivity of tension is regulated by calcium independent mechanisms mediated by PTX-sensitive G-proteins. These are of the G_i type (Petitcolin *et al.*, 1997)

Karaki., H., (1989). *Trends Pharmacol.Sci.*, 10 : 320-325

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Petitcolin, M.-A., Bueb J.-L., Spitzbarth, E. *et al* (1997). This meeting, P222.

318P EVIDENCE OF AN INCREASE IN VASODILATOR EFFICACY IN ISOLATED THORACIC AORTA FROM GUINEA-PIGS AFTER CHRONIC ASCENDING AORTIC BANDING

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Syncope due to abrupt and often unexplained hypotension is a recognised complication of severe aortic stenosis. To explain whether alteration in vascular reactivity may occur in left ventricular (LV) outflow obstruction following aortic banding in guinea pigs, we studied changes in vascular reactivity using an aortic ring preparation. Responses to phenylephrine (PE), acetylcholine (Ach), isoprenaline (Iso) and angiotensin II (AII) were examined 58 ± 2d and 158 ± 8d after ascending aortic banding and in age and weight matched sham controls. Animals were sacrificed, the thoracic aorta removed, cut into 2.5-3 mm rings and mounted in tissue baths. After equilibration at a 3g resting tension, responses to 60mM K^+ solution and cumulative doses of: PE (10^{-6} -3x10^{-5} M), AII (10^{-7} -3x10^{-6} M), Iso (10^{-7} -3x10^{-6} M) and Ach (10^{-7} -3x10^{-6} M) were determined. Constriction was expressed as a percentage of maximum response to a 60mM K^+ solution and dilatation as percentage relaxation from submaximal preconstriction with PE (3x10^{-5} M). Dose response curves were analysed by fitting sigmoidal curves using non-linear regression analysis; EC_{50} and maximum values were obtained for each experiment and analysed using unpaired t-tests with Welch's correction for unequal variances .

After 58 ± 2d aortic banding, there was significant ($p<0.01$) LV hypertrophy (LVH) with a 44% increase in LV weight / body weight ratio compared with controls. This represented an increase in LV mass as there was no significant difference in body weight. However there was no significant difference in response of aorta from banded animals or corresponding sham controls.

After 158 ± 8d aortic banding, the established LVH was accompanied by significant ($p<0.001$) increases in both right ventricle (79%) and lung (60%) weight to body weight ratio compared with sham controls. In isolated aortic ring preparations, there was no significant difference in maximum constriction to 60mM K^+ or in constriction response to PE and AII between banded and control groups. However, there was a significant ($p<0.05$) increase in maximum dilator response to both Iso and Ach, (with no significant change in EC_{50} values) in aorta from banded animals (table 1).

		PE	AII	Iso	Ach
SHAM	Max (%)	112.7 ± 16.7	24.2 ± 1.2	7.2 ± 0.1	9.7 ± 1.8
	EC_{50} (10^{-7}M)	10.0 ± 4.7	3.8 ± 1.4	5.3 ± 0.2	4.9 ± 3.1
BANDED	Max (%)	108 ± 16.0	23.4 ± 0.3	21.7 * ± 0.7	24.9 * ± 2.5
	EC_{50} (10^{-7}M)	5.7 ± 3.2	3.5 ± 0.4	4.1 ± 0.5	4.8 ± 1.8

Table 1: data from dose response curves in isolated aorta from 158 ± 8d banded and sham control animals. * p<0.05

In conclusion we found an increased efficacy of Iso and Ach mediated dilatation in aortic rings taken from guinea pigs banded for 158 ± 8d. These changes were not apparent in aorta taken from animals banded for 58 ± 2d despite established LVH. We postulate that second messenger dependent protein kinase activity may be up regulated in the transition from 58 ± 2d to 158 ± 8d after ascending aortic banding as the animals progress from LVH towards decompensation and heart failure.

319P DIFFERENTIAL SENSITIVITY TO CANNABIDIOL OF THE TWO COMPONENTS OF DELAYED RECTIFIER POTASSIUM CURRENT IN GUINEA-PIG ISOLATED VENTRICULAR MYOCYTES

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The delayed rectifier potassium current (I_K) is believed to play an important role in the repolarisation of cardiac action potentials. Two components of I_K have been separated, i) a rapidly activating component (I_{Kr}) sensitive to the class III antiarrhythmic agent E4031 and maximally activated at 0mV (Sanguinetti & Jurkiewicz 1990); and ii) a slowly activating component (I_{Ks}) which exhibits activation at more positive potentials than I_{Kr}, and is sensitive to general anaesthetics (Takahashi & Terrar, 1995). We have investigated the effects of the cannabinoid cannabidiol on I_{Kr} & I_{Ks} in guinea pig isolated ventricular myocytes.

Myocytes were enzymatically isolated from guinea-pig ventricle and superfused with a balanced salt solution containing 2.5 mM calcium. I_K was activated by step depolarisations from -40 mV to either +40 mV or 0 mV for 10 to 800 ms and measured as outward tail currents on repolarisation to -40 mV. (switched voltage clamp; 36°C). Components of I_K were separated with: i) 5 μM E4031 to selectively block I_{Kr} and hence record I_{Ks}; ii) step depolarisations to 0 mV to selectively activate I_{Kr}.

Exposure of myocytes to cannabidiol was associated with concentration-dependent inhibition of I_{Kr} and I_{Ks} over a range of concentrations from 100 nM to 3.3 μM, as illustrated in Figure 1. Furthermore, it appeared that I_{Ks} was more sensitive to the effects of cannabidiol than I_{Kr} under the conditions of our experiments at concentrations between 100 nM to 3.3 μM cannabidiol ($P<0.05$); for example following exposure to 3.3 μM cannabidiol, I_{Ks} at 400 ms was reduced by 83±2% ($P<0.05$, n=4) whereas I_{Kr} at 400 ms was reduced by 21±3% ($P<0.05$, n=5).

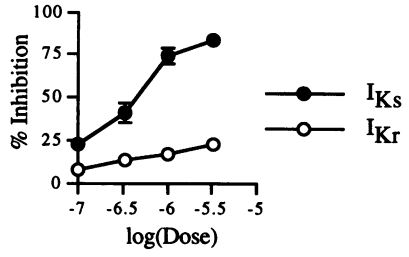

Figure 1. Log(dose)-response curve for inhibition of the 2 components of I_K : symbols represent measurements from 3-5 cells and bars show SEM where larger than symbol.

In conclusion, we have observed that exposure of guinea-pig isolated ventricular myocytes to cannabidiol was associated with an inhibition of I_{Kr} and I_{Ks}, although I_{Ks} appears to exhibit a greater sensitivity to the inhibition by cannabidiol than I_{Kr} under the conditions of our experiments.

This work was supported by the British Heart Foundation.

Sanguinetti M.C. & Jurkiewicz N.K. (1990) *J. Gen. Physiol.* 96. 195-215.
Takahashi, H. & Terrar D.A. (1995) *Br. J. Pharmac.* 115, 20P.

320P EFFECT OF NIFEDIPINE ON SALT-DEPENDENT CARDIAC HYPERTROPHY IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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We have previously shown that salt loading produces an increase of cardiac mass accompanied by an increased expression of preproendothelin-1 (ET-1) mRNA in spontaneously hypertensive stroke-prone rats (SHR-SP; Feron *et al.*, 1995) and we have also reported that a non-hypotensive dose of lacidipine, a long acting 1,4-dihydropyridine calcium antagonist, reduces both ET-1 gene expression as well as cardiac hypertrophy (Feron *et al.*, 1996). It is worth to know whether these cardiac effects are specific to lacidipine or if they are shared by other calcium antagonists. Therefore we have studied the actions of nifedipine, a short acting 1,4-dihydropyridine, on cardiac hypertrophy and ET-1 gene expression induced by salt load in SHR-SP.

Male SHR-SP (42 rats) at age of 8 weeks were divided at random in 3 groups: one control- salt free water and standard chow; two groups-1% NaCl drinking water and the ordinary chow or the chow containing nifedipine (Bayer, Germany) for a daily mean intake of 8 $mg.kg^{-1}$. The systolic blood pressure (SBP) was measured by the tail-cuff method in conscious animals (Physiograph Narco). At age of 14 weeks rats (weight 255 ±5 g) were killed by decapitation, plasma and heart samples were collected and processed for plasma renin activity (PRA) or mRNA expression. PRA was determined with a commercial radioimmuno-assay kit (Medix biochemica). PolyA$^+$ mRNA was isolated from weighed ventricles by using Fast Track 2.0 Kit (Invitrogen). Isolated mRNA were size fractionated, transferred to nylon membranes (Feron *et al.*, 1996) and hybridised with ^{32}P-labeled cDNA probes for rat ET-1, a 0.4 kb PvuII/PvuII fragment, human collagen I (α1), a 1.5 kb PvuII/PvuII fragment and rat glyceraldehyde-3-phosphate dehydrogenase, a 1.3 kb PstI/PstI fragment.

The measured chow intake showed no differences between the groups of SHR-SP, as indicated by the mean body weight at the end of treatment. Water intake was almost double in salt-loaded groups (SL-SHR-SP). The SBP at the 14th week of age were slightly different between SL- and non SL-SHR-SP. Salt load induced cardiac hypertrophy, as shown by the measurement of ventricle:body weight ratio (4.03±0.1 vs 3.44±0.06 $mg.g^{-1}$), and also a paradoxical 3-fold increase of PRA ($P<0.01$ for both parameters). Nifedipine treatment reduced SBP (by 14%; $P<0.05$), ventricle weight:body weight ratio (by 18%; $P<0.01$) and normalised PRA ($P<0.01$) in SL-SHR-SP.

Densitometric scanning of the autoradiograms showed that the expression of the ET-1 (2.3 kb) and collagen I (4.7 and 5.7 kb) gene transcripts were respectively 2.5-fold ($P<0.01$) and 1.6-fold greater ($P<0.01$) in SL-SHR-SP than in non-SL-SHR-SP. The six weeks nifedipine treatment blunted the augmentation of the levels of mRNA for ET-1 and for collagen I in SL-SHR-SP ($P<0.01$).

In conclusion, this study shows that a therapeutic dosage of nifedipine reduced overexpression of ET-1 and collagen I genes and normalised PRA in SL-SHR-SP. Whether the beneficial effect of nifedipine on salt-load induced cardiac hypertrophy is due only to reduction of blood pressure or mediated, at least partly, by a direct action on PRA and/or cardiac ET-1 gene expression, remains unsettled.

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Feron, O., Salomone, S., & Godfraind, T. (1996) *Br. J. Pharmacol.* 118, 659-664

321P STIMULATION OF THE AT_2 RECEPTOR CAUSES HYPERTROPHY IN ADULT RAT VENTRICULAR CARDIAC MYOCYTES

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Angiotensin II (AII) may play a crucial role in the pathophysiology of cardiac hypertrophy since selective antagonists at AII receptors cause regression of cardiac hypertrophy *in vivo*. Both AT_1 and AT_2 receptor subtypes are equally distributed throughout the rat heart (Sechi *et al.*, 1992). In adult cardiac myocytes the AT_1 receptor subtype has been implicated in the development of AII-induced hypertrophy, but a role for the AT_2 receptor subtype has not yet been elucidated; indeed, in neonatal rat ventricular myocytes an antigrowth effect of AII mediated via the AT_2 receptor has been reported by Booz & Baker (1996). However, as myocardial hypertrophy is a disease associated with the adult state it would be important to investigate the hypertrophic actions of AII in adult cardiac myocytes.

Cardiomyocytes isolated from 12 week old male Sprague-Dawley rats were maintained in short-term, serum-free, primary culture for 24 hours. Hypertrophic effects were determined by measuring the total mass of protein, and, incorporation of l-U-[^{14}C]-phenylalanine into cellular protein as a marker of *de novo* protein synthesis. Data, adjusted for cell number (DNA content), are given as mean ± s.e. mean of 4-10 experiments. Insulin, which stimulates hypertrophy via a tyrosine kinase-linked receptor, was used as a positive control.

The total mass of cellular protein in the presence of insulin (1unit.ml^{-1}) was 38.8±17.9% greater than the basal value (47.4±6.4 μg.μg^{-1} DNA). The incorporation of l-U-[^{14}C]-phenylalanine in the presence of insulin (1 unit.ml^{-1}) was significantly increased to 80.1±12.3% above the basal value (707±72 dpm.μg^{-1} DNA). AII and the selective agonist at the AT_2 receptor, p-NH_2-phe-AII (pAII) (Speth & Kim, 1990), markedly increased protein mass and the incorporation of l-U-[^{14}C]- phenylalanine at all concentrations used (Table 1). In the presence of the selective AT_2 receptor antagonist, Nicotinoyl-Tyr-Lys(Z-Arg)-His-Pro-Ile-OH (Whitebread *et al.*, 1989), while no inhibition of the insulin response was observed, incorporation of l-U-[^{14}C]-phenylalanine in response to AII was decreased at all concentrations used and the difference was significant at 10pM (Table 1).

Table 1: Effects of AII (in the absence or presence of Nicotinoyl-Tyr-Lys(Z-Arg)-His-Pro-Ile-OH) and p-NH_2-phe-AII (10pM - 100nM) on protein mass and incorporation of l-U-[^{14}C]-phenylalanine in rat ventricular cardiomyocytes (expressed as % increase over respective basal value); $p < 0.05$ with respect to *basal value (Dunnett's t test) or †value (paired t test) in the absence of antagonist.

	10pM	100pM	1nM	10nM	100nM
Protein Mass					
AII	14.1±5.5	17.0±11.8	18.1±10.4	24.0±10.8*	21.9±13.9*
pAII	12.5±11.8	12.5±13.5	14.1±13.5	16.9±6.5	33.2±22.0*
l-U-[^{14}C]-Phenylalanine Incorporation					
AII	19.8±2.1*	19.1±5.1*	22.5±4.0*	12.5±3.1*	23.1± 5.5*
AII + AT_2 receptor antagonist (100nM)					
	13.7±3.4†	16.5±3.0	18.0±4.1	7.2±2.6	18.9±4.9
pAII	20.4±3.4*	11.2±4.0	15.3±2.2*	17.7±5.5*	22.4±9.4*

The results show that stimulation of AT_2 receptors causes increased ventricular cell growth rather than an inhibition as has been observed in neonatal cells. The partial inhibition of the AII-mediated response by an AT_2 receptor antagonist provides a further indication that AT_1 receptors are involved in the hypertrophy of rat heart. That both receptor subtypes are linked to the hypertrophic response may have implications for treatment with receptor-selective blocking agents.

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Sechi, L.A., Griffin, C.A., Grady, E.F. *et al.* (1992) *Circ. Res.* 71, 1482-1489
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Whitebread, S., Mele, M., Kamber, B. et al., (1989) *Biochem. Biophys. Res. Comm.* 163, 284-291

322P ACTIONS OF CANNABIDIOL ON CONTRACTION OF GUINEA-PIG ISOLATED VENTRICULAR MYOCYTES

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Previous studies investigating the effect of cannabinoids in the mammalian heart have given evidence of decreased contractile force with varying effects on rate and coronary flow (Nahas & Trouve, 1985). Mechanisms of action suggested include inhibition of the calcium-ATPase (Collins & Haavik, 1979); this study examines the action of cannabidiol on excitation-contraction coupling and in particular on the availability of calcium.

Myocytes were isolated from guinea-pig ventricular muscle and superfused with a balanced salt solution containing 2.5 mM calcium (36°C). Contraction was measured with a non-invasive optical method using computer analysis of a video image of the cells viewed via a microscope. Cannabidiol was dissolved in Tween 80 (maximum final solvent concentration 0.01% v/v, a concentration which reduced contraction by 11±4%). Intracellular microelectrodes were used to record action potentials and voltage clamp methods used to record L-type calcium currents. Calcium transients were constructed from calcium-activated tail currents (Terrar & White, 1989).

Cannabidiol caused a dose-dependent decrease in contraction (Fig. 1), and the contraction was reduced by 70±7% with 3.3 μM. Action potential duration at 20% repolarisation was reduced from 136±6ms to 80±14ms after 10 min exposure to 1 μM cannabidiol ($P<0.05$, n=4). This dose of cannabidiol also decreased the calcium transient by 84±9% and reduced the peak L-type calcium current by 31±5% (step depolarisations to 0 mV, $P<0.05$, n=4).

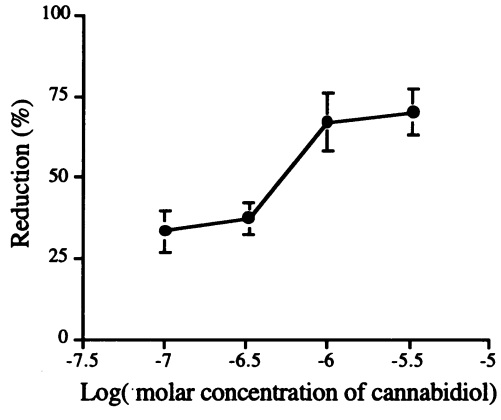

Figure 1. Log(dose)-response curve for the action of cannabidiol on contractions accompanying action potentials (stimulation rate 1 Hz); bars show s.e.mean, n=6 cells for each dose.

These observations are consistent with a reduction of cardiac myocyte contraction by cannabidiol at least in part as a consequence of reduced calcium entry via L-type calcium channels and a suppression of the calcium transient.

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Terrar, D.A., White, E. (1989) *Proc. R. Soc. Lond. B* 238, 171 - 188

323P MODULATORY EFFECT OF Δ^9-TETRAHYDROCANNABINOL ON $[Ca^{2+}]_i$ IN DDT$_1$MF-2 CELLS

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Δ^9-Tetrahydrocannabinol (THC) is the active component of marijuana, and mediate its physiological effects. These actions are the consequence of interaction with specific cannabinoid receptors, namely CB_1 (mainly present in nervous system) and CB_2 (present in peripheral tissues). Both receptors belong to the G-protein coupled receptor family, and are able to inhibit adenylate cyclase and voltage dependent Ca^{2+} channels. However, less is known about the modulation of intracellular Ca^{2+} homeostasis by cannabinoids. Therefore, in the present work, we investigated the effects of THC on $[Ca^{2+}]_i$ in DDT$_1$MF-2 cells, a typical smooth muscle cell line.

We measured $[Ca^{2+}]_i$ in DDT$_1$MF-2 cell suspension (~ 10^6 cells/ml), at 37° C after loading with 2 μM Fura2-AM during 30 min, at room temperature in the dark. When the downregulation of functional Ca^{2+} stores was necessary, the cells were incubated for 48 h with 1 μM thapsigargin (thapsi).

THC induced a biphasical increase in $[Ca^{2+}]_i$, with a threshold of 0.3 μM, the maximal response (first phase) being not reached even at 100 μM (table 1). The effects of THC are not mimicked by the specific CB_2 receptor agonist, palmitoylethanolamide (n=4), which failed to influence the basal $[Ca^{2+}]_i$ of 148 ± 19 nM (n=48). The specific CB_1 receptor antagonist, SR 141716A (1 μM), reduced the effects of 32 μM THC on $[Ca^{2+}]_i$ to 78 ± 3 % of control values (n=6). In external Ca^{2+} free medium, THC induced an unusual biphasic transient increase in $[Ca^{2+}]_i$. The first phase has a small amplitude (12 ± 2 nM) and reaches a maximum after 26 ± 3 s. The second is faster and larger (63 ± 10 nM and 31 ± 2 s, n=6). Only the second phase can be blocked by SR141716A (n=4). Downregulation of functional Ca^{2+} stores also abolished the second phase of THC-induced $[Ca^{2+}]_i$ increases in Ca^{2+} free medium, but did not affect the first one.

Table 1 $[Ca^{2+}]_i$ increase over basal (nM, n≥4)

THC	ECS	Ca^{2+} free	SR	thapsi
0.32 μM	16 ± 3	0	n.d.	0
3.2 μM	254 ± 65	21 ± 5	n.d.	239 ± 45
32 μM	327 ± 67	63 ± 10	51 ± 18	319 ± 51
100 μM	378 ± 56	84 ± 15	66 ± 14	n.d.

n.d.- not determined ECS-extracellular solution

To further investigate the modulatory effect of THC on $[Ca^{2+}]_i$ we compared its effects with other agonists. THC (100 μM) effects on $[Ca^{2+}]_i$ are not influenced by pretreatment with arachidonic acid (100 μM, 356 ± 45 nM vs. 378 ± 56 nM, p=0.87 with unpaired Student t-test). Histamine (100 μM) induced $[Ca^{2+}]_i$ increases are abolished by pretreatment with 100 μM THC (n=4). In contrast, ATP (100 μM) is able to induce a further increase of 121 ± 23 nM (n=4), when added after THC.

In conclusion, in DDT$_1$MF-2 cells, THC is able to induce $[Ca^{2+}]_i$ increases through Ca^{2+} release from thapsigargin sensitive and insensitive Ca^{2+} stores, as well as Ca^{2+} influx.

THC is also able to modulate the Ca^{2+} responses induced by stimulation of the histaminergic receptor system.

324P INHIBITION OF Ca^{2+}-ACTIVATED Cl^--CURRENTS IN SMOOTH MUSCLE CELLS BY COMPOUNDS STRUCTURALLY SIMILAR TO NIFLUMIC ACID

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Ca^{2+}-activated Cl^--currents ($I_{Cl(Ca)}$) have been observed in many smooth muscle cell types. We have studied the pharmacology of $I_{Cl(Ca)}$ and showed that in rabbit portal vein smooth muscle cells niflumic acid (NFA) blocks $I_{Cl(Ca)}$ with an IC_{50} of about 5 μM and only affects other conductances at concentrations ≥ 200 μM (Hogg *et al.*, 1994; Greenwood & Large, 1995). Niflumic acid is therefore a relatively selective agent for probing the functional role of $I_{Cl(Ca)}$. In an attempt to develop a more selective probe for $I_{Cl(Ca)}$ we have studied the effects of two compounds structurally similar to NFA, namely 3, 5-dichlorophenylamine 2-carboxylic acid (DCDPC) and tolfenamic acid (TFA) on $I_{Cl(Ca)}$ in smooth muscle cells to determine if these agents were more potent and/ or selective inhibitors than NFA.

Single smooth muscle cells were isolated from the portal veins of female New Zealand White rabbits (2-2.5 kg) by enzymatic digestion using collagenase and protease and ion currents were recorded at -50 mV using the perforated patch technique (at 22-25°C). Spontaneous transient inward chloride currents ($I_{Cl(Ca)}$, STICs) recorded in K^+-free conditions were well sustained for over 20 min in the absence of any pharmacological agent and had a mean decay time constant (τ) of 78 ± 5 ms (n=15 cells). DCDPC rapidly inhibited STICs in a concentration dependent manner (mean IC_{50} = 1.4 ± 0.2 μM; n=6) which was accompanied by a prolongation of τ (e.g τ in 1 μM DCDPC was 123 ± 16 ms). DCDPC also inhibited $I_{Cl(Ca)}$ evoked by Ca^{2+} influx through voltage-dependent Ca-currents (I_{tail}, mean IC_{50} = about 4 μM) with no significant effect on I_{Ca} and the decay of I_{tail} was prolonged by DCDPC. In comparison, TFA inhibited STICs with a mean IC_{50} of 5 ± 0.1 μM (n=5) but did not affect the decay significantly (69 ± 13 ms and 72 ± 5 ms in the absence and presence of 5 μM TFA, respectively). In K^+-conditions using a KCl-rich pipette solution and a K^+-containing external solution application of DCDPC and TFA (20-100 μM) activated a 'noisy' current at 0 mV which was tetraethylammonium-sensitive and which rectified in the outward direction.

These data show that DCDPC and TFA inhibit $I_{Cl(Ca)}$ with a similar potency to NFA but both agents activated a K^+-current at concentrations far lower than NFA. Consequently, DCDPC and TFA are less selective inhibitors of $I_{Cl(Ca)}$ than NFA which remains the preferential agent to probe the functional role of $I_{Cl(Ca)}$.

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325P INFLUENCE OF STIMULATION RATE ON THE EFFECT OF cADP-RIBOSE ANT ITS ANTAGONIST, 8-AMINO-CADP-RIBOSE, ON CONTRACTIONS OF GUINEA-PIG VENTRICULAR MYOCYTES

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Cyclic ADP-ribose (cADPR) has been suggested to enhance Ca^{2+} release through ryanodine receptors in mammalian heart cells (Rakovic et al., 1996), although negative observations have also been reported (Guo et al., 1996). Temperature appears to be an important influence on the actions of cADPR (Iino et al., 1997). In the present study, we have investigated the influence of stimulation rate on the effects of cADPR and its antagonist, 8-amino-cADPR, on contractions of guinea-pig ventricular myocytes .

Myocytes were isolated from guinea-pig ventricular muscle and superfused with a bath solution containing 118.5 mM NaCl, 14.5 mM $NaHCO_3$, 4.2 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$ and 11.1 mM glucose (pH 7.4, 36°C). Contractions in response to action potentials were measured from the video images of cells viewed microscopically. First a permeabilised whole-cell patch (200 mg/L amphotericin B in the electrode solution) was made for the measurement of control contractions. Each cell was stimulated at one rate (0.1, 1 or 3 Hz) for at least 5 min under the perforated whole-cell condition and then changes in contraction in response to intracellular application of cADPR or its antagonist via the electrode were measured 5 min after rupture of the patch membranes (conventional whole-cell condition). The electrode solution contained, in mM: KCl 140; NaCl 5; $MgCl_2$ 2; K_2ATP 1; HEPES 5 (pH 7.2). The drugs under study were added to the electrode solution. Data are mean ± standard error of the mean.

The effects of cADPR on the contraction of isolated guinea-pig ventricular muscle were larger at lower rate of stimulation. At 3 Hz stimulation, the applications of both 10 nM and 5 μM cADPR did not show significant effect on peak contractions ($P > 0.05$ in paired t test; 2±4 % decrease, n=7 cells and 6±5 % increase, n=6 cells, respectively). At 1 Hz, both concentrations showed significant change ($P < 0.05$; 11±3 % decrease, n=8 cells and 33±4 % increase, n=15 cells, respectively). At 0.1 Hz, changes of peak contraction by cADPR were significantly larger than at 1 Hz. Both 10 nM and 5 μM showed significant increase ($P < 0.05$; 47±10 %, n=7 cells and 91±20 %, n=6 cells, respectively). In contrast, the effects of 8-amino-cADPR on the contraction were larger at higher rate of stimulation. The application of 5 μM 8-amino-cADPR did not cause significant change in peak contraction at 0.1 Hz stimulation ($P > 0.05$; 3±8 % increase, n=6 cells). At 1 Hz, the peak contractions were decreased significantly by 8-amino-cADPR ($P < 0.05$; 15±2 %, n=7 cells). At 3 Hz, the change was significantly larger than at 1 Hz and showed 28±4 % decrease ($P < 0.05$; n=6 cells).

Our observations demonstrate that the effects of cADPR on ventricular contraction have a strong dependence on the rate of stimulation and that the effects of its antagonist, 8-amino-cADPR, have a opposite dependence. One possibility to account for our observations is that the synthesis of cADPR is enhanced as the stimulation rate is increased, leading to greater effects of the antagonist (which reduces the enhancing effect of endogenous cADPR on contraction) and to smaller effects of additional exogenous cADPR.

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326P POSSIBLE REQUIREMENT OF CALMODULIN FOR ACTIONS OF cADP-RIBOSE ON CONTRACTION OF GUINEA-PIG ISOLATED VENTRICULAR MYOCYTES

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cADP-ribose (cADPR) has been shown to regulate the calcium release from sarcoplasmic reticulum stores in heart cells (Rakovic et al, 1996; Iino et al, 1997). The mechanism is not yet fully elucidated, though it has been reported that calmodulin is a regulator of cADPR -induced intracellular calcium mobilisation in sea urchin egg preparations (Lee et al, 1994; Tanaka & Tashjian, 1995). The aim of the present study was to investigate whether calmodulin might play a role in the actions of cADPR on contraction of myocytes isolated from guinea-pig ventricles.

Myocytes were isolated from guinea-pig ventricular muscle and superfused with a balanced salt solution containing 2.5 mM calcium (36°C). Permeabilized patch-clamp techniques (amphotericin B in the pipette solution) were used to apply current stimuli to fire action potentials at 1 Hz. The accompanying contractions were measured using an edge-detection technique applied to the video image of cells viewed via a microscope. The drugs under study were added to the patch pipette solution. Student's paired 't' test was used as to quantify statistical significance, except where indicated.

Control records were obtained with the permeabilized patch and the membrane was ruptured to allow access of drugs to the cytosol . Under these conditions 5 μM cADPR increased contraction by 30±5% (compared with contraction before rupture of the patch, n=9, $P<0.05$). When the calmodulin antagonists, W7 (50 μM) or calmidazolium (5 μM), were applied to the cytosol, there was a decrease in contraction (reduction of 20±3% with W7; n=8; $P<0.05$ and 21±6% with calmidazolium; n=5; $P<0.05$). When 5 μM cADPR was added with W7 or calmidazolium the changes in contraction were a reduction of 16±6% with cADPR combined with W7 (n=8; $P<0.05$), and 18±6% with cADPR combined with calmodizolium (n=7; $P<0.05$); since these changes are not significantly different from those with W7 or calmidazolium alone ($P>0.05$, two sample 't'-test), it appears that 5 μM cADPR failed to increase contraction when combined with these drugs.

These observations are consistent with the hypothesis that calmodulin-dependent processes may be involved in the action of cADPR to increase contraction in guinea-pig ventricular cells.

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327P INFLUENCE OF RYANODINE ON THE POSITIVE CHRONOTROPIC RESPONSE TO β-ADRENERGIC STIMULATION WITH ISOPRENALINE IN GUINEA-PIG SINO-ATRIAL NODE

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Beta-adrenergic stimulation of the cardiac pacemaker results in a positive chronotropic response which is mediated through the modulation of a number of ionic currents at the plasma membrane (see Irisawa *et al.*, 1993). We have demonstrated recently that, in the absence of autonomic regulation, inhibition of calcium release from the sarcoplasmic reticulum (SR) leads to a reduction in heart rate in the guinea-pig (Rigg & Terrar, 1996). It is conceivable therefore that the SR might influence changes in rate in response to beta-adrenergic stimulation. We have therefore investigated the effects of the release channel inhibitor, ryanodine, on the ability of the beta-adrenergic agonist, isoprenaline (iso), to increase the rate of beating of guinea-pig intact sino-atrial node/atrial preparations.

Male guinea-pigs (220-300g) were killed by cervical dislocation following stunning. The heart was quickly removed and the atria were dissected and pinned out in a Sylgard based flow chamber. An extracellular electrode was positioned close to the sino-atrial node region to monitor heart rate. A period of one hour was allowed for the tissue to stabilise. The preparation was superperfused with oxygenated physiological saline solution at 35-36°C and 2.5 mM Ca^{2+}. Results are expressed as mean ± S.E.M. Significance was determined using Student's t-test.

Figure 1 shows the effects of ryanodine on the percentage change in heart rate following the application of isoprenaline at doses between 1 and 50 nM. Ryanodine markedly suppressed the dose response curve to iso, especially at higher concentrations. In the absence of any drug, 50 nM isoprenaline increased rate by 36 ± 2 %. Following 30 minutes exposure to 2μM ryanodine, this concentration of isoprenaline caused a significantly smaller increase of 21 ± 3 % ($P < 0.05$, n = 6).

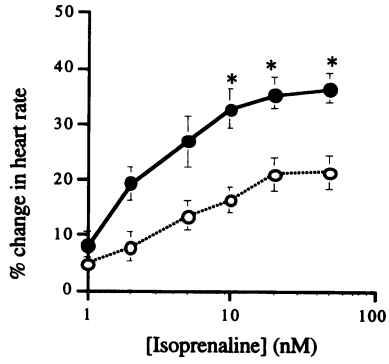

Figure 1: Filled circles: isoprenaline alone. Open Circles: isoprenaline following application of 2μM ryanodine. * represents significant difference, $P < 0.05$.

Our results are consistent with the hypothesis that calcium release from the sarcoplasmic reticulum plays an important part in the response of intact guinea-pig sino-atrial node to application of the beta-adrenergic agonist, isoprenaline. It is possible that calcium may regulate a variety of pacemaker currents including sodium-calcium exchange, the delayed rectifier potassium current and the hyperpolarisation-activated current.

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328P POSSIBLE INFLUENCE OF CYTOSOLIC CALCIUM ON RATE OF BEATING OF SINO-ATRIAL NODE CELLS ISOLATED FROM GUINEA-PIG HEART

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Cardiac pacemaking is accomplished through the sequential activation of several ionic currents across the plasma membrane (Irisawa *et al.*, 1993). Recent evidence shows that calcium release from the sarcoplasmic reticulum (SR) may play an important role in pacemaker mechanisms through regulation of these currents (Rigg & Terrar, 1996,1997; Hata *et al.*, 1996). Here we investigate the effects of SR inhibitors (ryanodine and cyclopiazonic acid ,CPA) and the calcium chelator, BAPTA, on the amplitude and frequency of calcium transients in single sino-atrial node cells isolated from the guinea-pig.

Spindle shaped, single sino-atrial node cells were isolated using a combination of previously described techniques (Anuwomono *et al.*, 1992, Denyer & Brown, 1990). Calcium transients were measured using indo-1 (cells loaded with 5 μM indo-1 AM for 20 minutes). Excitation light was delivered from a Xe arc lamp at 360 nm and was directed to the cell of interest via a 100 μm diameter quartz fibre optic. Two photomultipliers collected emitted light at 410 and 495 nm wavelengths. Calcium transients were displayed as the ratio of 410/495 signals. Solutions: Cells were superfused with oxygenated physiological saline solution at 35-36°C and 2.5 mM Ca^{2+}. Calcium transients were measured in the continuous presence of 100 nM isoprenaline since beating appeared to be suppressed by loading with the fluorescent dye, perhaps as a consequence of calcium buffering by indo-1. Statistical significance was assessed using a Wilcoxon signed-rank test. Means are shown ± S.E.M.

In cells exposed to 2 μM ryanodine rate of beating was consistently slowed (of 6 cells studied, 2 slowed and 4 became quiescent during a 10 minute exposure). The amplitude of the calcium transient, measured at 5 to 7 minutes (before cessation of beating) was also consistently reduced (peak value reduced by 25 ± 2 % and difference between diastolic and peak (p - d) values by 56 ± 5 %; $P < 0.05$ in both cases). With 30μM CPA rate of beating and amplitude of the calcium transient were again consistently reduced (rate by 77 ± 6 %; peak by 25 ± 5 % and p - d by 33 ± 7 %; $P < 0.05$ in all cases). In cells loaded with the calcium chelator BAPTA (5 μM BAPTA-AM for 5 to 7 minutes) rate was reduced by 36 ± 7 %, peak amplitude by 41 ± 3 % and p - d by 72 ± 5% ($P < 0.05$ in all cases). It appears therefore that reduction of the calcium transient by all three compounds was associated with a reduction in rate of beating.

Our results are consistent with a regulatory role for cytosolic calcium in the control of spontaneous pacemaker activity in sino-atrial node cells isolated from the guinea-pig.

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329P EFFECT OF THE PREDOMINANTLY T-TYPE CALCIUM CHANNEL BLOCKER MIBEFRADIL ON SPONTANEOUS AND STIMULATED MYOGENIC ACTIVITY IN THE RAT ISOLATED PORTAL VEIN

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The rat portal vein is an often used model to investigate drug effects on spontaneous and stimulated vascular myogenic activity. Although located in the venous part of the circulation, this vessel shows properties quite untypical for a vein, such as the sensitivity towards L-type calcium channel blockade by specific compounds like 1,4-dihydropyridines, phenylalkylamines and benzothiazepines. The spontaneous but also the stimulated contractile activity can be inhibited by these drugs. It has been suggested that besides L-type other types of calcium channels might be involved in the generation and maintenance of the myogenic contractions (Loirand et al, 1989). The role of T-type calcium channels in the rat portal vein remains to be elucidated.

The aim of the present study was to investigate the effects of a predominantly T-type calcium channel blocker, mibefradil (Mishra & Hermsmeyer, 1994), on the spontaneous and angiotensin II-stimulated contractile activity.

We compared the inhibitory effects of mibefradil, the 1,4-dihydropyridine nifedipine, the phenylalkylamine verapamil and the benzothiazepine diltiazem on the spontaneous myogenic activity and angiotensin II-induced contractions of isolated rat portal vein.

After male Wistar rats of 250-300 g were sacrificed, the portal vein was excised and mounted in a 20 ml organ bath. The isometric force of contraction was recorded under a constant pre-tension of 10 mN. For the experiments with angiotensin II-stimulation, the Tyrode's solution contained 0.9 mM calcium, for all other experiments 2.5 mM. The spontaneous myogenic activity was recorded for one hour before the calcium antagonists were added. After another hour of equilibration in the presence of the drugs, a cumulative angiotensin II concentration-response curve was constructed (0.1 - 30 nM). IC_{50} values were calculated using the maximal response to angiotensin II in the presence of the various concentrations of the particular calcium antagonist.

All results are given as mean values ± standard deviation of the mean of at least 6 experiments.

In the isolated rat portal vein the pIC_{50} for inhibiting the spontaneous myogenic activity was 7.84 ± 0.02 for nifedipine, 7.03 ± 0.10 for verapamil and 6.53 ± 0.07 for diltiazem whereas for mibefradil no effect could be observed up to a concentration of 10 μM. The angiotensin II-induced contractions were inhibited with a pIC_{50} of 8.32 ± 0.15 for nifedipine, 7.0 ± 0.03 for verapamil, 6.53 ± 0.02 for diltiazem and 6.15 ± 0.10 for mibefradil, respectively.

This pattern of inhibitory potency on agonist stimulated increase of contractile tone and the lack of influence on the spontaneous myogenic activity of mibefradil in the rat isolated portal vein suggest that T-type calcium channels do not play a major role, neither in the signal transduction of angiotensin II nor in the generation and/or propagation of spontaneous myogenic activity. In contrast L-type calcium channels were found to be crucially involved in both processes .

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330P UPREGULATION OF CARDIAC PRECONDITIONING IN ISOLATED HEARTS FROM ENDOTOXAEMIC RATS

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Short periods of ischaemia protect the heart against prolonged ischaemia and this protective effect has been termed cardiac preconditioning (Murry *et al.*, 1986). Recently Rowland *et al.* (1997) have reported that transient ischaemia enhances the cardioprotective effects of endotoxaemia. In the present investigation, the effects of preconditioning were assessed in hearts from endotoxaemic rats which had received a continuous infusion of lipopolysaccharide (LPS) (Waller *et al.*, 1994).

Male Long Evans rats (400-500g) were anaesthetized with sodium methohexitone (40-60mg kg^{-1}, i.p.) and were implanted with arterial (for blood pressure measurements) and venous (for LPS administration) catheters (Waller *et al.*, 1994). At least 24h later the rats were infused with LPS (*E. coli*, serotype 0127 B8; 150μg kg^{-1} h^{-1}) for a further 24h, while control rats received saline (0.4ml h^{-1} of 154mM NaCl). At the end of the treatment period the rats were heparinized (1,000U kg^{-1} i.p.) and anaesthetized with sodium pentobarbitone (40mg kg^{-1} i.v.). Following a thoracotomy, the hearts were rapidly excised and perfused in the Langendorff mode at constant flow (20ml min^{-1}) with oxygenated Krebs-Henseleit buffer (Randall *et al.*, 1997). A fluid-filled balloon catheter was inserted in to the left ventricle in order to measure left ventricular developed pressure (LVDP), from which heart rate was derived. After 30min equilibration, preconditioning was induced by 3 cycles of 4min ischaemia with 6min reperfusion prior to a 30min ischaemic insult, which was followed by 60min reperfusion (15ml min^{-1}). The non-preconditioned hearts were continuously perfused prior to the 30min ischaemia.

During the 1st min of *ex vivo* perfusion LVDP was substantially depressed in hearts from LPS-treated rats compared to control (LPS, 17.6±3.7mmHg, mean±s.e.mean, n=20; saline, 68.7±7.2mmHg, n=16, $P<0.001$, ANOVA with Bonferroni's post-hoc test). After 30min of perfusion LVDP had improved in the hearts from both groups but was still greater in the controls (103±8mmHg) compared to the hearts from LPS-treated rats (76.1±8.2mmHg, $P<0.05$). In both saline and LPS-treated groups the 30min ischaemia in the absence of preconditioning resulted in severe impairment of mechanical performance with LVDP values of 11.0±3.3mmHg (saline, n=8) and 13.1±5.6mmHg (LPS, n=8) after 60min of reperfusion. By contrast preconditioning resulted in protection of systolic function in both groups such that after 60min of reperfusion the LVDP values were 22.3±6.3mmHg (saline, n=8) and 44.4±4.3mmHg (LPS, n=12). Furthermore, LVDP following 60min of reperfusion was significantly ($P<0.01$) greater in the LPS-treated group compared to controls.

The results of the present study clearly indicate that endotoxaemia, induced by LPS, whilst not affording cardioprotection *per se*, may upregulate the protective effects of ischaemic preconditioning.

SBT is a Sir Francis Hill Scholar.

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331P VASCULAR ACTIVITIES OF METHOXAMINE AND NORADRENALINE IN ISOLATED PERFUSED MESENTERIC ARTERIAL BEDS FROM ENDOTOXAEMIC RATS

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Reduced constrictor responses to noradrenaline (NA) have been widely reported in conduit vessels from endotoxaemic animals (Stoclet *et al.*, 1993). However, recent evidence also indicates that reduced vasoconstrictor responses are not universally observed in resistance beds removed from endotoxaemic rats (Mitchell *et al.*, 1993). *In vivo*, we have observed attenuated regional constrictor responses to methoxamine (ME), but not to NA, after a 24h infusion of lipopolysaccharide (LPS, Tarpey *et al.*, This meeting). We have now investigated the effects of NA and ME in isolated mesenteric vascular beds removed from the same rats after 24h LPS infusion. The effects of the nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) have also been examined against these responses.

Male, Long Evans rats (350-450g) from Tarpey *et al.* (1997) at the end of the 24h i.v. infusion of either saline (0.4ml h^{-1}) or LPS (150μg kg^{-1} h^{-1}; *E coli* serotype 0127:B8), were anaesthetised with pentobarbitone (44mg kg^{-1} i.v.) and the mesenteric arterial bed was cannulated and perfused with oxygenated Krebs-Henseleit solution (Randall & Hiley, 1988). Following 30min equilibration, responses to NA and ME were determined in the absence and presence of 100μM L-NAME.

Basal mesenteric perfusion pressures were similar between the saline (n=12) and LPS (n=14) treated groups (9.1±1.8mmHg (mean ±s.e.mean) and 11.1±3.4mmHg, respectively). Vascular responses to NA were not different in the isolated mesenteric vascular beds from rats treated with saline (R_{max}=140±12mmHg; ED_{50}=38.0±10.0μmol) compared with those from LPS-treated rats (R_{max}=118±10mmHg; ED_{50}=28.4±5.6μmol). Responses to ME were also not different between the saline (R_{max}=128±14mmHg; ED_{50}=166±28μmol) and LPS (R_{max}=100±8mmHg; ED_{50}=224±56μmol) treated groups. Addition of 100μM L-NAME did not significantly alter the basal mesenteric perfusion pressures in either the saline or LPS groups (12.4±2.4mmHg and 13.5±1.8mmHg, respectively). NA responses in mesenteries from both the saline and LPS-treated groups in the presence of L-NAME were not significantly different from its absence. Similarly, responses to ME in the saline-treated group were not altered in the presence of L-NAME. However, in mesenteries from LPS-treated rats L-NAME caused a significant ($P<0.05$; ANOVA with Bonferroni's *post-hoc* test) increase in the R_{max} for ME (136±6mmHg) but the ED_{50} was unchanged (149±20μmol).

The major finding of this study is that responses to NA and ME are unaltered in mesenteric vascular beds removed from rats after a 24h infusion of LPS from Tarpey *et al.* (This meeting). This is in contrast to our findings *in vivo* where at 24h responses to ME are depressed in the mesenteric vascular bed, but responses to NA are not (Tarpey *et al.*, This meeting). A further finding, that responses to ME in the LPS group are enhanced in the presence of L-NAME, indicates that NO may play a role in modulating the responses to ME (but not NA) following LPS-treatment.

S.B. Tarpey holds a Sir Francis Hill studentship.

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332P DIFFERENTIAL CHANGES IN CARDIOVASCULAR RESPONSES TO METHOXAMINE AND NORADRENALINE DURING ENDOTOXAEMIA IN CONSCIOUS RATS

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We have recently shown that during continuous infusion of lipopolysaccharide (LPS), the pressor responses to angiotensin II (AII) and vasopressin (AVP) are reduced when the vasoconstrictor responses are normal or enhanced (Tarpey *et al.*, 1996). In the present study we measured the pressor and regional vasoconstrictor responses to the α_1-adrenoceptor agonist, methoxamine (ME), and the mixed adrenoceptor agonist noradrenaline (NA) during LPS infusion, in order to determine if our previous finding are peculiar to AII and AVP.

Male Long Evans rats (350-450g), were chronically instrumented with pulsed Doppler flow probes and intravascular catheters for recording renal, mesenteric, and hindquarters haemodynamics (Gardiner *et al.*, 1995). All surgery was performed under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p., supplemented as required) and experiments began 24 h after the last procedure (catheterisation). Increasing bolus doses of ME (100, 300, 500 nmol kg^{-1}) and NA (75, 375, 750 nmol kg^{-1}) were administered i.v. at 10 min intervals before and 2, 6, and 24 h after the onset of infusion of saline (0.4 ml h^{-1}, n=11) or LPS (150μg kg^{-1} h^{-1}; *E coli* serotype 0127:B8; n=12).

Resting cardiovascular variables in the saline and LPS -infused groups respectively were:- heart rate (HR) 356±10, 339±8 beats min^{-1} (mean±s.e.mean), mean arterial pressure (MAP) 98±2, 98±2 mmHg; renal, mesenteric and hindquarters vascular conductances (RVC; MVC; HVC), respectively:- 70±9, 63±8; 75±7, 67±4; 69±4, 58±5 (kHz $mmHg^{-1}$)10^3. During infusion of LPS, the changes in baseline haemodynamics were as described previously (Gardiner *et al.*, 1995). Some of the results obtained with NA and ME are summarised in Table 1.

Table 1. Changes in cardiovascular variables in response to ME (300nmol kg^{-1}) and NA (375nmol kg^{-1}) 2 h and 24 h after the onset of saline (S) or LPS (L) infusion. For abbreviations see text. † $P<0.05$ compared to S (Mann Whitney U test)

		2 h		24 h	
		ME	NA	ME	NA
HR	S	-162±14	-48±4	-161±15	-45±10
	L	-91±11†	-24±5†	-79±7†	-36±14
MAP	S	+55±2	+40±4	+54±2	+35±3
	L	+32±3†	+16±2†	+21±2†	+29±3
RVC	S	-59±9	-47±6	-50±7	-45±5
	L	-53±7	-30±4†	-44±4	-60±6
MVC	S	-62±6	-40±5	-69±5	-49±5
	L	-46±3†	-29±2†	-45±5†	-42±3
HVC	S	-39±4	-23±2	-34±3	-25±3
	L	-17±2†	-7±2†	-20±3†	-22±4

The pressor, bradycardic and mesenteric and hindquarters responses to ME were reduced at 2 h and at 24 h after the onset of LPS infusion. However, the cardiovascular responses to NA were reduced after 2 h but not after 24 h of LPS infusion (Table 1). Thus, in contrast to our previous results obtained with AII and AVP (Tarpey *et al.*, 1996), the present findings show that a reduced pressor response to NA and ME during LPS infusion is associated with reduced vasoconstrictor responses. The interesting difference between NA and ME may be due to changes in β-adrenoceptor function, catecholamine uptake mechanisms and/or cardiac mechanisms.

S.B. Tarpey holds a Sir Francis Hill studentship.

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333P ANTI-ISCHAEMIC ACTION OF TRANSFORMING GROWTH FACTOR β-1 IN THE RAT ISOLATED HEART IS ABOLISHED BY THE p38 MAPK INHIBITOR SB203580

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There is evidence that various endogenous growth factors may be cardioprotective in the setting of myocardial ischaemia-reperfusion injury. Transforming growth factor-β1 (TGF-β1) has been reported to limit myocardial ischaemia-reperfusion injury (Lefer *et al.*, 1990) but the mechanism of protection is unknown. In the present study we have examined the ability of TGF-β1 administration to limit myocardial ischaemia-reperfusion injury and the possible signalling pathways associated with protection. We have examined the involvement of protein kinase C (PKC) and the p38 mitogen activated protein kinase (MAPK) by using chelerythrine and SB203580 which are potent and specific inhibitors of PKC and p38 MAPK respectively (Herbert *et al.*, 1990; Cuenda *et al.*, 1995).

Male Sprague-Dawley rats (300-350 g) were deeply anaesthetised with pentobarbitone sodium (50 mg/kg i.p.). The hearts were excised and Langendorff perfused with Krebs-Henseleit buffer at constant pressure (80 mm Hg). All hearts were subjected to 35 min left coronary artery occlusion followed by 120 min reperfusion. The risk zone size was delineated using fluorescent microspheres and infarcted tissue was determined with triphenyltetrazolium staining. Computerised planimetry was used to quantitate these volumes and the percentage infarction within the risk zone (I/R %) was calculated.

Hearts were randomised into 6 experimental groups. Group 1 was control hearts which underwent coronary occlusion but received no drug intervention. Group 2 hearts were perfused with TFG-β1 0.2 ng/ml for 10 min immediately prior to coronary occlusion. In Group 3 and Group 4 hearts were perfused with chelerythrine (10 μM) or SB203580 (10 μM) respectively for 15 min, beginning 5 min prior to the commencement of TGF-β1 perfusion. Group 5 and Group 6 hearts were perfused with chelerythrine (10 μM) or SB203580 (10 μM) for 15 min prior to coronary occlusion.

Table 1. Infarct data

Group	n	I/R (%)
1: control	7	40.8 ± 3.1
2: TGF-β1	7	17.5 ± 2.4*
3: TGF-β1 + chelerythrine	7	23.2 ± 3.8*
4: TGF-β1 + SB203580	5	42.0 ± 2.2
5: chelerythrine	5	40.6 ± 1.9
6: SB203580	5	38.5 ± 2.2

Results are expressed as mean ± s.e.mean. * P < 0.01 versus Group 1 (control).

Administration of TGF-β1 prior to coronary occlusion significantly limited infarct size. This protective effect was abolished in the presence of the p38 MAPK inhibitor SB203580. However, the protection was only slightly and not significantly attenuated by chelerythrine, a PKC inhibitor. The kinase inhibitors alone did not influence infarct size. We conclude that TFG-β1 has anti-ischaemic properties and these may be mediated through p38 MAPK signalling. The action of TGF-β1 appears to be independent of PKC in this model.

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334P BRADYKININ-INDUCED VASORELAXATION IN THE RAT ISOLATED PERFUSED HEART: IMPORTANCE OF PREFORMED POOLS OF NO-CONTAINING FACTORS

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To study whether the vasorelaxant effect of bradykinin (BK) in the coronary vascular bed depends on the release of NO from preformed pools and/or *de novo* NO synthesis resulting from BK-induced NO synthase (NOS) stimulation, hearts obtained from Wistar rats (300-400 g) were perfused at 37 °C with Tyrode's buffer according to Langendorff. Coronary flow (CF) was measured continuously using an ultrasound flow probe. Concentration/response (C/R) curves were constructed with BK (0.1-100 nM, final concentration in aorta) and the NO-precursor L-arginine (L-Arg) (0.3-30 mM) under control conditions, after downregulation of NOS by exposing the heart to high NO concentrations (10 μM; n=6-7) and during chronic NOS inhibition, obtained by perfusing the heart for 30-45 min with 0.1 mM L-NAME (n=7). The effect of acute NOS inhibition was studied by infusing single doses of BK (10 nM) or L-Arg (15 mM) in the absence or presence of 0.1 mM L-NAME (n=5). CF (baseline 9±2 ml min^{-1}; mean±SD) increased to maximally 23±6 ml min^{-1} with BK and to 16±4 ml min^{-1} with L-Arg. Maximal CF, established as the maximal effect to NO, was 22±4 ml min^{-1}. EC_{50} values were 10±7 nM (BK) and 17±10 mM (L-Arg). Chronic NOS inhibition reduced CF to 4±2 ml min^{-1}, illustrating the importance of endogenous NO production. CF did not change after NOS downregulation by NO. Downregulation nor acute inhibition of NOS affected the response to BK, whereas chronic NOS inhibition blocked the BK-induced increase in CF by >90%. L-Arg did no longer increase CF under all tested conditions. We conclude that preformed pools of NO-containing complexes exist within the isolated perfused heart and that BK exerts its vasorelaxant effect at least in part by the mobilization of these preformed pools. The vasorelaxant effect of L-Arg depend on its conversion to NO by NOS. These data may reconcile previous discrepancies (e.g. Rees *et al.*, 1990; Bjornstad-Ostensen *et al.*, 1997) about the (lack of) effect of NOS inhibitors on BK-induced coronary vasodilatation.

Table Maximal CF (ml min^{-1}) values under experimental conditions.

	Baseline	*BK*	*L-Arg*
C/R curves			
Control	9±2	23±6*	16±4*
High NO	9±1	22±3*	11±1
L-NAME	4±2	6±2	4±1
Single dose			
Control	8±2	24±3*	12±2*
L-NAME	8±3	24±2*	9±2

*, *P<0.05 vs. baseline*

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335P THE CHARYBDOTOXIN-SENSITIVE Ca-DEPENDENT K-CHANNEL IS INVOLVED IN POST-ISCHAEMIC VASODILATION IN RAT MESENTERIC ARTERY

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Postocclusive hyperemia is used as a means to induce maximal vasodilation in the human forearm vascular bed. Although both endothelium dependent and independent factors have been mentioned to contribute to postocclusive hyperemia, the exact mechanism is still unclear. Postischemic vasodilation was studied in isolated rat mesenteric resistance arteries.
Mesenteric resistance arteries were collected from 250-300 gram male Wistar rats and mounted in the small vessel myograph according to standard methods. Precontraction was established using $5x10^{-6}$ M phenylephrine. After a 20 min. equilibration period 95% O_2/5% CO_2 was replaced for 20 min by 95% N_2/5% CO_2. Then aeration with 95% O_2/5% CO_2 was resumed and after again 20 min phenylephrine was washed away. Next, in the same vessel, following the same experimental procedure, the effect of changing the K concentration or adding blockers of K-channels were studied. In all experiments the vascular contractility in mN at the moment O_2 was reinstalled (p) and when vasodilation was maximal after reintroduction of O_2 (q) was measured and the percentage of vasodilation was calculated as [(p-q)/p] x 100%. Finally, the effect of charybdotoxine on verapamil induced vasodilation was assessed.
After reintroduction of O_2 a transient vasodilation was observed, which was maximal at 5 minutes and lasted about 15 minutes. When this experiment was repeated in the same vessel, the same result was obtained. Postischemic vasodilation was completely abolished after replacing Na with 125 mM K (n=10), whereas with 40 mM K this vasodilation was still present (n=6). These experiments implicate that opening of a K-channel is involved. Charybdotoxine and not apamine nor glibenclamide were able to ameliorate the postischemic vasodilation (Table 1). Charybdotoxine did not affect 10^{-8} M verapamil induced vasodilation (27 ± 7% vs 34 ± 15%; n=7)
We conclude that charybdotoxine sensitive Ca-dependent K-channels may be involved in postischemic vasodilation. Since charybdotoxine did not effect verapamil induced vasodilation, we conclude this effect of charybdotoxine is specific for this phenomenon.

Table 1. Percentage postischemic vasodilation before (A) and after (B) addition of several K-channels blockers (n=12, means ± SD).

	A	B	p*
control	48 ± 20	42 ± 20	NS
glibenclamide (10^{-6} M)	53 ± 21	51 ± 23	NS
apamine ($5x10^{-7}$ M)	36 ± 15	38 ± 18	NS
charybdotoxine (10^{-7} M)	53 ± 19	28 ± 13	<0.01

* Difference between A and B. Paired Wilcoxon test

336P ACTIVE SECRETION AND INTRACELLULAR DISPOSITION OF LUCIFER YELLOW IN RENAL PROXIMAL TUBULES

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Introduction: Secretory transport of anionic drugs by the kidney have been studied extensively by using p-aminohippurate (PAH) as a model substrate for the organic anion system. During secretion the drug may accumulate within proximal tubular cells. The membrane steps for PAH secretion are well defined, however, its intracellular disposition is still unknown. To gain more insight in tubular cell accumulation of organic anions, confocal microscopy can be applied but this technique requires a compound with good fluorescent properties.
Methods: In the present investigation, the fluorescent dye Lucifer Yellow was used to examine its utility as a marker for renal disposition of anionic drugs. For this purpose, the transport and accumulation characteristics of the dye were studied in the isolated perfused rat kidney (IPK), in freshly isolated rat kidney proximal tubular cells (PTC; weight of rats used was 200-230 g), and in killifish (*fundulus heteroclitus*) renal proximal tubules.
Results: After exposing perfused kidneys to 10 µM Lucifer Yellow, the ratio of renal clearance over glomerular filtration clearance (CL_R/GF) was 1.6 ± 0.2, indicating active secretion of the dye. Pretreatment of the kidneys with 0.5 mM probenecid or 1.15 mM α-ketoglutarate resulted in a significant inhibition of renal Lucifer Yellow clearance ($P<0.05$). Moreover, accumulation of the dye in the IPK was significantly reduced to a similar extent by these inhibitors ($P<0.05$). These results suggest that the basolateral organic anion system is involved in Lucifer Yellow secretion and accumulation in the IPK. In killifish proximal tubules uptake and secretion were followed by confocal microscopy and a significant inhibition of both uptake and secretion was found with ouabain, PAH and probenecid ($P<0.01$), which is in agreement with the results found for Lucifer Yellow in the IPK. To investigate the intracellular disposition of the dye, freshly isolated PTC were incubated in medium with 2 µM Lucifer Yellow and cellular uptake was studied by means of confocal microscopy. Uptake of the dye was significantly reduced after preincubating the cells with 1 mM PAH, 0.5 mM probenecid and 1 mM α-ketoglutarate ($P<0.05$), confirming the contribution of the organic anion system in cellular Lucifer Yellow uptake. Confocal images revealed that intracellularly the drug is compartmentalized in vesicular structures that are susceptible to nocodazole, an inhibitor of microtubule motors.
Conclusion: The present study indicate that Lucifer Yellow may be a good model substrate to study intracellular accumulation of anionic drugs during secretory transport by the kidney.
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337P CHARACTERISATION OF THE G-PROTEINS IN THE RAT TAIL ARTERY SMOOTH MUSCLE

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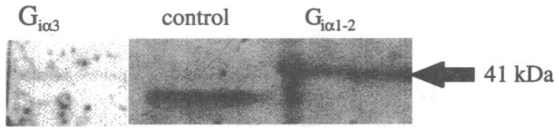
Following pharmacomechanical stimulation, contraction shows a high-intracellular calcium-sensitivity (Capdeville-Atkinson *et al.*, 1995). This amplification pathway is thought to be modulated by pertussis toxin (PTX)-sensitive G-proteins (Spitzbarth *et al.*, 1997). The aim of this study was to isolate G_i proteins and confirm their sensitivity to PTX in the rat tail artery.

The tail artery was dissected out from adult, male, Wistar rats (350-400 g). Endothelium was removed by rubbing the intimal surface with a stainless steel wire. Briefly, membranes were isolated by consecutive centrifugation (900 *g*, 10 min ; 10 000 *g*, 10 min ; 60 000 *g*, 45 min) (Kwan *et al.*, 1983). Membrane fractions were resuspended in 15 mM Tris and 1% cholic acid and the protein concentration determined (Lowry *et al.*, 1951). Membranes were next dissolved in SDS-PAGE buffer, loaded onto a 16.5% polyacrylamide gel (Laemmli, 1970), transferred to a nitrocellulose membrane and immunoblotted using rabbit antibodies against $G_{i\alpha}$ subunits 1-2 and 3 and $G_{o\alpha}$ subunit (Calbiochem, San Diego, CA). Transfer of ADP-ribose by PTX using [α^{32}P]nicotinamide adenine dinucleotide was used to assess G-protein function. PTX was activated with 5 mM dithiothreitol (DTT) and diluted to 1 µg/ml after DTT dialysis. Samples were submitted to PAGE and immunoblotted as described above. The dried blots were then apposed to an X-ray film. Densitometry analysis was performed using the NIH *Image®* programme (v.1.58, Wayne Rasband, NIH, USA).

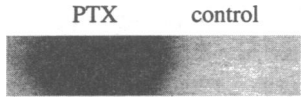
G_i proteins were detected in rat arterial smooth muscle cell membranes (Figure 1). $G_{i\alpha 1\text{-}2}$ proteins were the predominant form in comparison to $G_{i\alpha 3}$ (ratio 2/1). A low level of G_o was measurable in the same membrane preparations.

Figure 1 : $G_{i\alpha 1\text{-}2}$ and $G_{i\alpha 3}$ antibody labelling in rat arterial smooth muscle (n=3).



PTX induced ADP-ribosylation of $G_{\alpha i/o}$ subunits (Figure 2).

Figure 2 : ADP-ribosylation of G-protein with PTX.



In conclusion, in the rat tail artery, the inactivation of $G_{i/o}$ proteins is involved in the PTX-induced loss of reactivity. This may explain a lower agonist-induced vasoconstrictor response in PTX-treated arteries (Spitzbarth *et al.*, 1997).

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338P MEASUREMENT OF INTRACELLULAR pH USING BCECF IN CELLS CONTAINING ORGANIC ANION TRANSPORTERS

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We have previously reported that the fluorescent pH indicator, BCECF, accumulates to lower levels in resistant (R) lung tumour cells overexpressing multidrug resistance associated protein (MRP), than in their parental (P) cells that contain little MRP. If loaded for 1hr at 37°C, fluorescence observed 15min later is spread evenly throughout the P cells but is localised to vesicles in the R cells (Francis et al, 1997). In the present study, these differences have been investigated further.

Cells grown on coverslips were mounted in a standard cuvette for fluorescence measurement (excitation at 440 and 502 nm, emission 526nm). When cells were loaded by exposure to the non-fluorescent precursor, BCECF-AM, at concentrations ≤ 0.5 µM at 37°C, the fluorescence with P cells increased linearly for more than 15min while that with R cells first increased rapidly then at a much slower rate. At intervals, with the coverslip holding the cells transiently removed, fluorescence in the medium was measured. This remained low with P cells but increased with the R cells. At 15°C, both R and P cells loaded to a similar extent in a linear fashion. Whereas P cells retained their fluorescence for long periods ($t_{1/2}$ >> 30min) at 37°C, R cells retained their fluorescence at 15°C but lost it rapidly at 37°C (half-loss typically within 5min). These results are consistent with a similar rate of BCECF production in P and R cells, combined with the presence of a strongly temperature-sensitive efflux process for BCECF in R but not P cells. The temperature dependence is consistent with efflux by an active process, e.g. extrusion by MRP. BCECF is thought to be a substrate for MRP (Draper et al, 1997a).

The efflux process could be inhibited by 50 µM MK-571 and 20µM indomethacin which have been reported to inhibit MRP activity (Leier et al, 1994; Draper et al 1997b). Once loading of R cells at 37°C had approached a plateau, addition of either compound led to a further increase in loading. When added during washout, either compound reduced the rate of efflux.

Intracellular pH was estimated in P and R cells during loading at 15°C and immediately after loading with the temperature increased to 37°C. During loading, increases in fluorescence at 502nm and at 440nm were used to calculate ratios. After loading, ratios were calculated from fluorescence values with background subtracted. These ratios were converted to pH by linear interpolation between standard values measured at the end of each experiment using nigericin.

During loading at 15°C the observed values were (mean±s.e.m.) 7.6±0.06 (n=10) for P cells and 7.4±0.08 (n=11) for R cells. After loading at 37°C, the values were 7.5±0.03 (n=10) for P cells and 7.3±0.08 (n=12) for R cells. The differences between the P and R cells are both significant ($p<0.02$ and $p<0.03$ respectively). These results confirm the differences reported previously (Francis et al, 1997).

There are difficulties in obtaining accurate values of internal pH in cells containing anion transporters but these can be overcome if measurements are made sufficiently rapidly.

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339P PHARMACOKINETICS OF THE BICYCLIC PEPTIDE TACHYKININ NK_2 RECEPTOR ANTAGONIST MEN 11420 IN RATS AND GUINEA-PIGS

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MEN 10627 is a bicyclic hexapeptide endowed with potent and selective antagonism at NK_2 receptors (Maggi et al 1994). The need for a less lipophilic molecule led to the synthesis of MEN 11420. This glycosylated water soluble derivative shows nearly identical antagonist potency and selectivity *in vitro*, but it appears to have more potent and longer lasting activity *in vivo* (Catalioto et al, in press). To assess whether these findings can be related to differences in metabolism and pharmacokinetics, we studied the lability of MEN 10627 and MEN 11420 to *in vitro* degradation by rat liver preparations and evaluated their plasma kinetics after i.v. administration to rats and guinea pigs.

Analytical conditions (HPLC): Analytes were separated on a Nucleosil 100 C18 5 µm reverse-phase column, eluted with water/acetonitrile/methanol (each containing 0.1% TFA) in the ratio 50/40/10 and 60/30/10 v/v, for MEN 10627 and MEN 11420, respectively. Peaks were detected by spectrofluorometry (λex 280 nm, λem 350 nm). Sensitivity was 20 ng ml^{-1}.

In vitro metabolism: Three pooled livers from male Wistar rats (300-400 g) were homogenized in TRIS buffer and microsomes prepared by differential centrifugation (Lippi et al 1996). Incubation mixtures contained 2 nmol of test compound in 400 µl of crude homogenate or microsomal suspension plus cofactors. Before and after 6-h and 1-h incubation at 37°C, with liver homogenate or microsomes, respectively, 50-µl samples were deproteinized with 250 µl of acetonitrile and centrifuged; the supernatant was evaporated to dryness under N_2 gas; the residue was dissolved in 100 µl of water and analyzed by HPLC. MEN 11420 was not affected (100% unchanged) by the incubation with both preparations while MEN 10627 was 62% (mean value from two incubations) degraded after incubation with homogenate and 65% with microsomes.

Pharmacokinetics: Male rats (Sprague Dawley, 300-400 g) and guinea pigs (Dunkin Hartley, 300-400 g), implanted with a silastic cannula in a jugular vein 18 h before treatment, were injected with MEN 10627 or MEN 11420 (1 mg kg^{-1}, i.v.). 0.5-ml blood samples were collected from the cannula into heparinized tubes, at suitable intervals up to 360 min from the administration. Plasma, obtained by centrifugation, was deproteinized and analysed as reported above. Individual plasma concentration-time curves were fitted to a bi-exponential model by the EasyFit for Macintosh software and computed parameters are shown in Table 1.

No marked species-related differences in pharmacokinetics were observed. In both species MEN 11420 showed significantly (Student's t test) longer half-life and greater AUC, resulting in reduced systemic clearance, as compared to MEN 10627.

We conclude that chemical modification of MEN 10627 to obtain MEN 11420, besides affecting solubility, produced a considerable change in the pharmacokinetic behaviour, possibly through an increased resistance to hepatic hydrolases and oxidases. Higher and longer lasting plasma levels of MEN 11420 appear to agree with its more prolonged activity, as compared to MEN 10627.

Table 1. Pharmacokinetic parameters after i.v. treatment. Values are mean±SD, n=4 for rats, 3 for guinea pigs. *: P<0.05 vs. MEN 10627

	Rat		Guinea pig	
	MEN 10627	MEN 11420	MEN 10627	MEN 11420
t1/2α (min)	1.5±1.2	4.6±4.5	2.9±1.1	4±3
t1/2β (min)	16±2	44±7 *	24±6	57±8 *
AUC (µg min ml^{-1})	129±62	285±23 *	101±71	384±57 *
Vd (ml kg^{-1})	247±179	225±53	340±105	212±1 *
Cl (ml min^{-1} kg^{-1})	10±7	3.5±0.3	13±6	2.6±0.4 *

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340P ADAPTATION TO MOVEMENT AND GENDER DIFFERENCES IN THE DEVELOPMENT OF MOTION SICKNESS IN SUNCUS MURINUS

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Suncus murinus is reported to be a useful experimental model for motion sickness research (Ueno *et al.*, 1987). We have shown that the vomiting response is related to the frequency but not the amplitude of shaking (Javid & Naylor, 1997). The aim of the present study was to identify the possibility of adaptation to motion stimuli and also to investigate gender differences to motion sickness in *Suncus murinus*.

Adult Japanese House Musk shrews, Suncus murinus (38-88 g) of either sex were used. Each animal was placed individually in a transparent cage (100wx150Lx150H mm) of 6 linked units. After 3 min, a horizontal motion stimulus commenced with various amplitudes (peak-to peak displacement: 7, 13 or 40 mm) and frequencies (0.5, 1, 2 or 3 Hz). For the study of adaptation, two groups of males (n=6) were exposed to repeated motion sickness every 2 days for a period of 30 days. Using an amplitude of 40 mm, one group received a shaking frequency of 0.5 Hz and the other group received a frequency of 1 Hz. Four groups of both males and females were also exposed to different amplitudes (13 and 40 mm) and frequencies (0.5-3 Hz) once every week for a period of 28 days. The latency of onset (s) and the number of vomits were recorded over a 10 min period. Data was expressed as the mean+s.e.mean of n=6 and analysed using a paired or unpaired t-test

Animals acquired adaptation with repeated exposure to motion stimuli when they were used once every 2 days. The number of vomits for the two groups of males that were exposed to the motion stimuli once every 2 days, after 15 trials (group 1, 1 Hz and 40 mm) and 11 trials (group 2, 0.5 Hz and 40 mm) were 2.8±1.5 and 0.3±0.3, respectively, which were significantly ($p<0.001$) lower as compared to the number of vomiting responses in the first trial: 12.3±4 and 4.16±1.8, respectively. The onset of the vomits after 11 and 15 trials were significantly ($p<0.05$, $p<0.001$) delayed: 588±12 s (group 1) and 398±103 s (group 2), as compared to the onset of responses in the first trial: 335.8±120 s and 109±28 s, respectively. However adaptation was not observed in animals that were exposed to the motion stimuli once a week ($p>0.05$). In females the number of vomits summated from the 3 frequencies used at 1, 2 and 3 Hz were significantly ($p<0.05$) higher than those in males. Also, the latency for the start of the first vomit at 1 and 2 Hz in females was significantly ($p<0.05$) lower than in males, Figure 1.

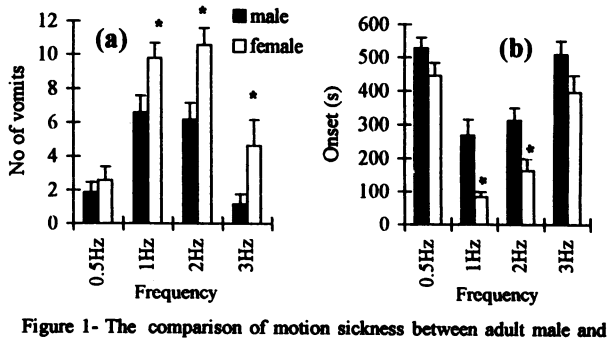

Figure 1- The comparison of motion sickness between adult male and female *Suncus murinus*: (a) the number of vomits and (b) the latency to first vomit during repeated shaking (0.5, 1, 2 and 3 Hz using the amplitude of 40 mm) for 10 min. Each histogram represents the mean+s.e.mean; n=6. * $p<0.05$ compared to male control values.

The data indicate that adaptation to motion sickness in *Suncus murinus* will occur if the animals are exposed to motion stimuli when challenged every two days but not weekly. Also, females are more sensitive than males to the emetic stimulus of motion.

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