85P EXPOSURE OF NEGATIVE CORRELATION BETWEEN THE OPERATIONAL AFFINITY AND EFFICACY OF NORADRENALINE AT α_1- ADRENOCEPTORS IN THE RAT SMALL MESENTERIC ARTERY

P.H. Van der Graaf.
Leiden/Amsterdam Center for Drug Research, Division of Pharmacology, PO Box 9503, 2300 RA Leiden, The Netherlands.

The idea that agonist affinity and efficacy are independent properties and can be estimated as such has been a central concept of pharmacological receptor theory (see Black & Leff, 1983; Leff 1995). In the past few years, however, the validity of this hypothesis has been called into question (see Leff, 1995). We have applied the operational model of agonism (Black & Leff, 1983) to analyse the interaction between noradrenaline (NA) and phenoxybenzamine (PBZ) in the rat small mesenteric artery (SMA) assay and now report that the model did not yield independent estimates of affinity and efficacy.

SMA's (internal diameter 239.8 ± 8.3 μm, n = 22) from male Wistar rats (225-350 g) were mounted as endothelium-denuded ring segments (~2 mm length) in a myograph as described before (Van der Graaf *et al.*, 1995). Tissues were exposed to five near-maximally effective concentrations of NA (10 μM), separated by 5 min washout periods. Effects were expressed as percentage of the response (6.68 ± 0.62 mN) produced by the fifth exposure to NA. After a 15 min washout period, tissues were incubated for 90 min with 30 μM cocaine and 6 μM timolol and a first NA concentration-effect (E/[A]) curve was obtained by cumulative dosing. Following a 15 min washout period, tissues were exposed to 0.1 nM (30 or 45 min) or 1 nM (10 or 15 min) PBZ and were subsequently washed for 30 min. Cocaine and timolol were then incubated as described above and a second NA E/[A] curve was obtained in each tissue.

NA (10 nM - 30 μM) produced concentration-dependent contraction of the SMA and the control E/[A] data (n = 19) were fitted to the Hill equation to provide estimates (mean ± s.e.mean) of midpoint location (pEC_{50} = 6.69 ± 0.07) and upper asymptote (α = 101.6 ± 0.9%). PBZ pretreatment produced significant rightward shift and depression of the upper asymptote of the NA E/[A] curve (pEC_{50} = 6.21 ± 0.06, 6.32 ± 0.13, 6.19 ± 0.14 and 6.12 ± 0.09; α = 90.3 ± 0.5%, 87.8 ± 4.2%, 56.3 ± 5.3% and 12.0 ± 1.4% following pretreatment with 0.1 nM for 30 min, 0.1 nM for 45 min, 1 nM for 10 min and 1 nM for 15 min, respectively). Co-incubation with 20 nM of the α_1-adrenoceptor antagonist, prazosin, produced complete protection against the effects of 20 min pretreatment with 1 nM PBZ (pEC_{50} = 6.16 ± 0.08 and 6.22 ± 0.05; α = 105.8 ± 4.5% and 104.0 ± 0.4% in the absence or presence of PBZ pretreatment, respectively, n = 3). Each pair of NA control and PBZ-treated E/[A] curves was fitted to the operational model of agonism to provide estimates of the maximum achievable effect (E_m = 120.2 ± 5.0%), the slope of the occupancy-effect relation (n = 1.88 ± 0.17), the agonist dissociation equilibrium constant (pK_A = 6.18 ± 0.10) and the efficacy parameter (log τ = 0.60 ± 0.08). Although the model appeared to provide an adequate fit of the E/[A] curve data (Van der Graaf, 1996), a highly significant, negative correlation was found between the estimates of pK_A and log τ (r = −0.80, P<0.0001), inconsistent with the assumption that affinity and efficacy are independent model variables (best line fit: $pK_A = -0.95 \times \log\tau + 6.75$). The pK_A and log τ estimates were not correlated with the pEC_{50} of the NA control curve (r = 0.31 and 0.30, respectively, P>0.1) or with the upper asymptote of the PBZ-treated curve (r = −0.33 and 0.14, respectively, P>0.1).

In conclusion, the operational model of agonism did not provide independent estimates of affinity and efficacy of NA at α_1-adrenoceptors in the rat SMA assay. Other models are currently under investigation to test whether these data can be accounted for by assuming the presence of a heterogeneous receptor population, the operation of a two-state mechanism of receptor activation or promiscuous coupling to multiple effector pathways.

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86P DEVELOPMENT AND APPLICATION OF A GRAPHICAL TEST TO DETECT RECEPTOR DISTRIBUTION FROM NON-RECTANGULAR AGONIST CONCENTRATION-EFFECT CURVES

P.H. Van der Graaf.
Leiden/Amsterdam Center for Drug Research, Division of Pharmacology, PO Box 9503, 2300 RA Leiden, The Netherlands.

The operational model of agonism (Black & Leff, 1983) was built upon the assumption that the binding of an agonist to a receptor is independent of subsequent events, that is, affinity and efficacy are measurable independently. It has been argued, however, that receptor isomerisation or ternary complex formation may significantly reduce the number of available receptors. These processes may lead to a situation where receptors become distributed among unbound, bound and isomerised or complexed states and application of the operational model of agonism under these conditions may result in erroneous estimates of agonist affinity (see Black & Shankley, 1990). Black & Shankley (1990) have developed a graphical test to detect receptor distribution from rectangular hyperbolic agonist concentration-effect (E/[A]) curve data (see also Kenakin, 1993). An extension of this test is now presented which allows for analysis of non-rectangular hyperbolic curves.

The Hill-equation parameters, upper asymptote (α) and midpoint location (EC_{50}), are related to the operational model parameters E_m (maximum achievable effect), τ (efficacy parameter), n (slope of the occupancy-effect relation) and K_A (agonist dissociation equilibrium constant) as follows (Black & Leff, 1983; Kenakin, 1993):

$$\alpha = \frac{E_m \tau^n}{1+\tau^n} \qquad EC_{50} = \frac{K_A}{(2+\tau^n)^{1/n}-1}$$

From these equations, for any experimental curve:

$$\tau^n = \frac{\alpha}{E_m-\alpha} = \left(\frac{K_A+EC_{50}}{EC_{50}}\right)^n - 2$$

which can be rearranged as follows:

$$\log\left(\left(\frac{\alpha}{E_m-\alpha}+2\right)^{1/n}-1\right) = -\log EC_{50} + \log K_A$$

Therefore, a plot of individual values of $\log\{(\alpha/(E_m-\alpha)+2)^{1/n}-1\}$ against $-\log(EC_{50}/K_A)$ should yield a straight line with a slope of unity and an abscissa intercept of zero. This analysis was applied to a dataset of 19 pairs of control and phenoxybenzamine-pretreated noradrenaline E/[A] curves obtained in the rat isolated small mesenteric artery assay (Van der Graaf, 1996). The correlation between $\log\{(\alpha/(E_m-\alpha)+2)^{1/n}-1\}$ and $-\log(EC_{50}/K_A)$ was highly significant (r^2 = 0.99, P<0.0001). Regression yielded a slope which was not significantly different from unity (0.99 ± 0.02) and an abscissa intercept which was not significantly different from zero (0.01 ± 0.01; Figure 1). Overall, therefore, this analysis has not provided evidence for the existence of significant distribution of α_1-adrenoceptors in rat small mesenteric artery. Hence, the mechanism underlying the negative correlation between noradrenaline's affinity and efficacy estimates (Van der Graaf, 1996) remains to be elucidated.

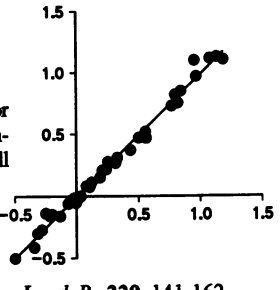

Figure 1
Plot of $\log\{(\alpha/(E_m-\alpha)+2)^{1/n}-1\}$ against $-\log(EC_{50}/K_A)$ for noradrenaline concentration-effect curves in rat small mesenteric arteries

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87P ACTIVATION OF SPONTANEOUS TRANSIENT OUTWARD CURRENTS (STOCs) BY PHENYLEPHRINE IN VASCULAR SMOOTH MUSCLE FROM THE RAT TAIL ARTERY

M.L. Earle, X.F. Li & C.R. Triggle. Smooth Muscle Research Group, Department of Pharmacology & Therapeutics, University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta, CANADA. T2N 4N1.

The α-adrenoceptor (α-AR) agonists cirazoline and phenylephrine (Phe) contracts vascular smooth muscle, in part, via the release of intracellular calcium. We have previously shown in freshly dispersed single rat tail artery vascular smooth muscle cells α_1-AR activation with 0.3μM cirazoline results in an increase in intracellular Ca^{2+} ($[Ca^{2+}_i]$) which is resistant to inhibition by nifedipine (Li et al., 1993). However the sustained tonic increase in $[Ca^{2+}_i]$ is significantly reduced ($p<0.05$) in the presence of a Ca^{2+}-free physiological saline solution containing 0.5mM EGTA (1240.25nM ± 235.81 to 767.84nM ± 114.5 phasic; 301.00nM ± 23.8 to 209.25nM ± 15.75 tonic, n=4-9). The nature of the ion channel(s) involved in facilitating extracellular Ca^{2+} entry is unknown and, therefore the aim of this investigation was to determine if Phe activates an influx pathway independent of the voltage gated L-type Ca^{2+} ion channel.

Using enzymatically dispersed vascular smooth muscle cells, as described by Li et al., (1993), from the tail artery of male Sprague-Dawley rats (350-400g) we investigated the effects of Phe (1-10μM) on the inward current carried by 10mM barium. We utilized the nystatin (200ng/ml) perforated patch clamp technique and as such were not clamping $[Ca^{2+}_i]$. We determined that under these conditions the inward current had the characteristics of an L-type voltage-dependent Ca^{2+} channel in that it was reduced by 0.1μM nifedipine (62.1 ± 10.3%, n=5) and enhanced by 2μM BayK8644 (113.8 ± 21.4%, n=6, $p<0.05$). Phe at these concentrations did not produce a significant change in the amplitude of the barium current (mean change in current -0.25pA ± 9.4, n=4), suggesting that this agonist did not affect the influx of calcium via an L-type calcium channel. In addition, steady state current and spontaneous transient outward currents (STOCs) were recorded under a constant voltage (-40mV) again using the nystatin perforated patch clamp technique with 5.4mM K^+ in the bath solution and 140mM K^+ in the pipette. STOCs are thought to be activated by spontaneous release of stored calcium, and are therefore a measure of the extent of calcium availability at the plasma membrane. In the presence of external tetraethylammonium (1mM) these currents were not observed (n=4), suggesting that they are carried by a calcium activated potassium channel. We found that in 10 out of 19 cells studied 0.3μM Phe activates either a transient or sustained inward current (n=5) which is normally preceded by an increased activation of STOCs. This inward current was not blocked by nimodipine (0.1μM) and was therefore not an L-type calcium current.

We conclude that activation of α-ARs results in release of stored calcium, activating the STOCs and subsequently enhancing an influx pathway. Thus, in freshly dispersed vascular smooth muscle cells, Phe may elicit an influx of calcium which is independent of an L-type calcium ion channel.

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88P ALPHA-PRESSOR RESPONSES IN PITHED RATS FED ON A LOW-CALCIUM DIET

V. López-Miranda, M. Puerro, A. Ortega & M.A. Aleixandre (Introduced by J. Tamargo) Dpto. Farmacología. Fac. Medicina, U. Complutense, 28040 Madrid, España.

Previous studies carried out in our laboratory showed that a low-calcium diet (Ca 0.1%) caused an increase in the arterial blood pressure (tail cuff method) of normotensive Sprague-Dawley rats (SDR) and spontaneously hypertensive rats (SHR), when compared with control animals fed on a similar semi-synthetic casein diet with a normal calcium content (Ca 1%). In the SHR strain the maximum differences in the systolic (SBP) and the diastolic (DBP) arterial blood pressure between animals fed on the Ca 0.1% diet and those fed on the Ca 1% diet were obtained in 8-week-old rats (López-Miranda et al., 1996) (see table 1). In this study, after being weaned at 3 weeks, male animals of both strains were randomized with "ad libitum" intake of the Ca 0.1% or the Ca 1% diet, and then we evaluated the pressor responses of methoxamine (MTX) (10-3000 μg/kg) and B-HT 920 (3-1000 μg/kg) with the pithed rat preparation (Shipley and Tilden, 1947), using 9-week-old (adult age) SDR and 8-week-old SHR. The increases in the SBP and the DBP were measured using a Panlab 4C Datasystem. Results are expressed as mean values ± s.e. mean for 6-8 rats. α-Adrenoceptor agonist non-cumulative dose-response curves were constructed, and the effect of the dietary Ca content on α-pressor responses was expressed as the area under each dose-response curve (AUC), taking the AUC for the control mean values as 100. For comparison of the areas Student's t-test was used.

The low-calcium diet did not significantly change the α-adrenoceptor agonist pressor responses either in SDR or in SHR (see table 1). The present results suggest that hypertension induced by dietary calcium deficiency in rats is not associated with an important increase in the pressor responses mediated by the stimulation of vascular α-adrenoceptors.

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

	% Dietary Ca	
	1	0.1
9-week-old SDR		
SBP (mm Hg)	144.0 ± 2.0	168.0 ± 3.0***
DBP (mm Hg)	111.0 ± 2.0	125.0 ± 2.0***
MTX AUC-SBP	100.0 ± 2.3	93.5 ± 2.8
MTX AUC-DBP	100.0 ± 1.9	102.7 ± 2.6
B-HT 920 AUC-SBP	100.0 ± 4.5	94.2 ± 2.1
B-HT 920 AUC-DBP	100.0 ± 3.4	92.5 ± 4.8
8-week-old SHR		
SBP (mm Hg)	191.0 ± 5.0	241.0 ± 4.0***
DBP (mm Hg)	140.0 ± 5.0	189.0 ± 7.0***
MTX AUC-SBP	100.0 ± 3.0	96.5 ± 6.0
MTX AUC-DBP	100.0 ± 6.0	101.6 ± 3.4
B-HT 920 AUC-SBP	100.0 ± 9.0	126.3 ± 10.5
B-HT 920 AUC-DBP	100.0 ± 6.2	124.0 ± 10.8

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

89P INVESTIGATIONS OF POSTJUNCTIONAL α_2-ADRENOCEPTOR SUBTYPES MEDIATING VASCULAR RESPONSES

Katherine T. Gavin & J. R. Docherty, Department of Physiology, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2.

α_2-Adrenoceptors belong to the G-protein coupled family of cell surface receptors, and three subtypes have been demonstrated: $\alpha_{2A/D}$-adrenoceptors, α_{2B}-adrenoceptors, α_{2C}-adrenoceptors. The predominant subtype of α_2-adrenoceptor present on peripheral nerves is the $\alpha_{2A/D}$-adrenoceptor subtype, e.g. in rat vas deferens (Smith et al., 1995). Postjunctional α_2-adrenoceptors of vascular smooth muscle have been less studied, due to the limited number of tissues in which they can be demonstrated.

Rats were pithed under ether anaesthesia, and respired with 100% O_2. Diastolic pressor responses (DBP) were obtained to the α_2-adrenoceptor agonists xylazine and oxymetazoline (1-10,000 $\mu g\ kg^{-1}$). Human saphenous veins were set up (Connaughton & Docherty, 1990) and contracted with noradrenaline (NA). Potency of antagonists was assessed as the ability to shift potency of xylazine or NA. The following antagonist drugs were used: ARC 239, BDF 8933, benoxathian, chlorpromazine, HV 723, prazosin, WB 4101, yohimbine (see Connaughton & Docherty, 1990; Smith et al., 1995).

The correlation with the postjunctional α_2-adrenoceptor mediating pressor responses in the pithed rat was best for the α_{2D}-adrenoceptor ligand binding site of rat submandibular gland (r=0.95, n=9, P<0.0001), as compared to correlations with the α_{2B}-adrenoceptor ligand binding site of rat kidney (r=0.90, n=9, P<0.001) and with the human recombinant α_{2C}-adrenoceptor ligand binding site (r=0.74, n=9, P<0.05). When the pressor potency of the α_{2D}-adrenoceptor selective agonist oxymetazoline was included in correlations, the correlation with α_{2D}-ligand binding site (r=0.91, n=10, P<0.0001) was much better than with the α_{2B}-ligand binding site (r=0.52, n=10, n.s.)

The correlation with the postjunctional α_2-adrenoceptor mediating contraction of the human saphenous vein was best for the human recombinant α_{2C}-adrenoceptor ligand binding site (r=0.92, n=8, P<0.001), as compared to correlations with the α_{2B}-ligand binding site of rat kidney (r=0.59, n=8, n.s.) and with the α_{2A}-ligand binding site of human platelet (r=0.38, n=8, n.s.).

It is concluded that the pressor responses to xylazine in the pithed rat preparation and contractile responses to NA in the human saphenous vein are mediated by α_{2D}- and α_{2C}-adrenoceptors, respectively.

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90P CHARACTERISATION OF THE RECEPTORS MEDIATING THE CONTRACTION OF RAT ISOLATED SMALL MESENTERIC ARTERY TO ARGININE VASOPRESSIN AND OXYTOCIN

W.B. Stam, P.H. Van der Graaf[1] & P.R. Saxena, Department of Pharmacology, Erasmus University Rotterdam. [1]LACDR, Leiden, The Netherlands.

The vasoconstrictor action of arginine vasopressin (AVP) is believed to be mediated via an action at smooth muscle V_1 receptors (see Yazawa, 1996). However, the V_1 receptors on resistance vessels have not yet been adequately characterised. Therefore, in this study we have examined the interaction between AVP and two nonpeptide, V_1 receptor antagonists, OPC-21268 (OPC) and SR 49059 (SR; see Serradeil-Le Gal *et al.*, 1994) on the rat isolated small mesenteric artery (SMA). Furthermore, since the presence of oxytocin (OT) receptors has recently been demonstrated in a vascular smooth muscle cell line (Yazawa *et al.*, 1996), we also studied the effects of OT and the selective OT receptor antagonist, atosiban (ATO, also known as ORF22164; Pettibone *et al.*, 1992).

SMA's (internal diameter 100-300μm) from male Wistar rats (225-300g) were mounted as ring segments in a myograph as described before (Van der Graaf *et al.*, 1995). Tissues were calibrated with a single contraction to 100μM phenylephrine (PE). Subsequently, the presence of the endothelium was confirmed by the relaxant response to 10μM methacholine. After a 30 min washout period, tissues were incubated for 60 min with antagonist or vehicle (distilled water for ATO, 10 % DMSO for OPC and SR). Single agonist concentration-effect (E/[A]) curves (n=4-11) were then obtained by cumulative dosing.

AVP and OT produced concentration-dependent contractions of SMA's and the individual E/[A] curves were fitted to the Hill equation to provide estimates of the midpoint location (pEC_{50}=9.48±0.04 and 6.76±0.04), midpoint slope (n_H=2.32±0.15 and 3.28±0.23) and upper asymptote (α=118.1±3.4% and 126.3±2.9% compared to the PE calibration contraction) for AVP (n=16) and OT (n=21), respectively. OPC (30-300nM), SR (3-10nM) and ATO (0.1-3μM) produced concentration-dependent parallel rightward shifts of the OT E/[A] curves and Schild analysis yielded slope parameters (b) not significantly different from unity (0.84±0.09, 0.86±0.10 and 1.10±0.21 for OPC, SR and ATO, respectively), thus allowing for the estimation of pK_B values (7.49±0.06, 9.38±0.05 and 6.34±0.11 for OPC, SR and ATO, respectively. OPC (0.1-1μM) also behaved as a competitive antagonist of AVP (b=1.27±0.15, pK_B=7.56±0.11). ATO (1-10μM) and SR (30-300nM), however, produced significant steepening (n_H=1.97±0.01, 2.14±0.10, 2.40±0.16 and 2.70±0.11 for 0, 1, 3 and 10μM ATO, respectively) and reduction of the upper asymptote (α=118.2±5.8%, 88.3±6.2%, 105.18±5.5% and 88.9±6.7% for 0, 30, 100 and 300nM SR, respectively) of the AVP E/[A] curves, respectively. Notwithstanding this complexity, the data were fitted to the Schild equation (b=1.06±0.10 and 0.97±0.12; apparent pK_B=6.48±0.07 and 9.20±0.09, for ATO and SR, respectively).

In conclusion, both AVP and OT constrict rat isolated SMA. AVP was ~500-fold more potent than OT but the intrinsic activity of the agonists was similar. The potency of OPC, SR and ATO was independent of the agonist, suggesting that AVP and OT act at the same receptor in the SMA. The similarity of our (apparent) pK_B values with previously published pK_i values for rat liver V_{1A} receptors (7.21-7.59, 9.10 and 6.65 for OPC, SR and ATO, respectively; Pettibone *et al.*, 1992; Serradeil-Le Gal *et al.*, 1994) suggests that this receptor has the characteristics of the V_{1A} receptor. The reason for the noncompetitive antagonism of AVP by SR and ATO remains to be elucidated.

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91P THE EFFECT OF EICOSAPENTAENOIC ACID UPON CONTRACTURES OF RAT ISOLATED AORTIC RINGS PRE-TREATED WITH α-ADRENOCEPTOR ANTAGONISTS

N. Bretherton, J.W Smith & K.A. Wilson. Pharmaceutical Sciences Institute, Aston University, Birmingham. B4 7ET.

Eicosapentaenoic acid (EPA) causes vasorelaxation, a mechanism thought to depend upon reduced intracellular calcium $[Ca]_i$ (Engler, 1992). This is not simply associated with a decrease in calcium entry through voltage-operated calcium channels (Bretherton *et al*, 1995), nor is it the result of a decrease in calcium released from stores linked to the inositol phosphate pathway (Bretherton *et al*, 1996). We have previously suggested that the action of EPA on the inositol phosphate pathway may be coupled to an α-adrenergic mechanism. In this study therefore, the effect of EPA on noradrenaline (NA)-induced contractures of rat aorta, was examined in the presence of prazosin (PZ) phentolamine (PH) or idazoxan (ID).

Male Wistar rats (200-250 g) were stunned and killed by cervical dislocation. Isolated aortic rings (5-7 mm), devoid of endothelium were suspended in Krebs buffer containing propranolol (10^{-6} M), EDTA (10^{-5} M) and ascorbic acid ($5x10^{-5}$ M). The tissues were maintained at 37°C, gassed with carbogen, and placed under a resting tension of 2 g. Isometric contractures were recorded. Following a 1 hr equilibration period, contractures to NA ($3x10^{-6}$ M) were elicited; the sensitising response. Non-cumulative concentration response curves to NA were constructed 30 min after treatment with ethanol (ETH 1%; the EPA vehicle) or EPA ($5x10^{-5}$ M). In some experiments, prazosin (PZ, $5x10^{-9}$ M), phentolamine (PH, 10^{-6} M) or idazoxan (ID, $5x10^{-7}$ M) was added to the tissues 15 min prior to EPA or ETH.

NA contractures were reduced by the treatment of the tissues with PZ, PH or ID. A rightward shift of the concentration-response curves was seen. After treatment with EPA, NA concentration-response curves were moved further to the right, as indicated by the PD_2 values in table 1, together with a decrease in E_{max} in the presence of prazosin.

Table 1	PD_2 values	E_{max} (% sensitising response to NA)
ETH	8.14 ± 0.21 (n=5)	120.5 ± 9.76
ETH + PZ [1]	6.17 ± 0.08 (n=8)	104.4 ± 8.79
ETH + PH [2]	7.08 ± 0.03 (n=7)	85.3 ± 9.45
ETH + ID [3]	7.38 ± 0.05 (n=5)	148.4 ± 13.33
EPA + ETH + PZ	5.84 ± 0.11 (n=7)*	65.1 ± 7.72*
EPA + ETH + PH	6.62 ± 0.10 (n=7)†	78.9 ± 9.94
EPA + ETH + ID	6.87 ± 0.14 (n=5)#	152.4 ± 37.09

Values are mean ± s.e.mean. Statistical significance was determined using Students unpaired *t*-test; * compared with [1]; † compared with [2]; # compared with [3] where $P<0.05$.

The results would suggest that vasorelaxation induced by EPA is not the result of an α_1-adrenoceptor-mediated mechanism as pre-treatment of the tissues with PZ did not antagonise the effect of EPA. However, the antagonism of the effect of EPA induced by PH and ID may indicate an α_2-adrenergic-related mechanism.

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Bretherton, N., Richardson, A.G., Smith, J.W. *et al.* (1996) *Br. J. Pharmacol.* (Leicester meeting; in press)

92P INVESTIGATION OF α_1-ADRENOCEPTOR SUBTYPES IN RABBIT CUTANEOUS ARTERIES

K.M. Smith, J.B. Macmillan & J.C. McGrath, Clinical Research Initiative in Heart Failure, IBLS, West Medical Building, University of Glasgow, Glasgow G12 8QQ

Although the subclassification scheme for α_1-adrenoceptors is open to interpretation, some compounds are attributed subtype-selectivity across a range of tissues, and so were used in this study. Cutaneous resistance arteries (internal diameter 290.3±8.3μm) from New Zealand White rabbits were mounted as ring preparations (2mm length) on a wire myograph in Krebs (at 37°C), bubbled with $95\%O_2/5\%CO_2$ and normalised using the method outlined by Mulvany & Halpern (1977). Cumulative concentration-response curves (CCRCs) were constructed for the agonists noradrenaline, (R) A-61603 and phenylephrine (PE) (in the presence of cocaine 3μM, propranolol 1μM and corticosterone 10μM). The effects of the competitive α_1 antagonists, prazosin, WB4101, 5-Me-Urapidil and HV723 (Muramatsu et al.,1990), and the irreversible α_{1B} selective compound chloroethylclonidine (CEC) were examined versus the potency and maximum response to noradrenaline.

Table 1: α-adrenoceptor agonists

agonist	pD_2	maximum (mN/mm^2)
noradrenaline	7.02±0.16 (n=11/6)	51.00±5.56 (n=11/6)
phenylephrine	6.08±0.13 (n=8/4)	37.08±4.80 (n=8/4)
(R) A-61603	8.78±0.12 ***(n=8/4)	37.02±5.42 (n=8/4)

***$p<0.001$, paired Student's t test: (R)-A-61603 compared to phenylephrine, (n=tissues/animals).
The potency of A-61603 relative to PE has been shown to differentiate functional α_{1A} and α_{1B} from α_{1D}-adrenoceptors (Knepper et al., 1995): the results suggest the presence of functional α_{1A} or α_{1B} as opposed to an α_{1D}-subtype. The maximum response was reduced in the presence of PE and (R) A-61603, relative to noradrenaline. The CCRC to noradrenaline was shifted to the right and the maximum response to noradrenaline was decreased by preincubation with CEC (1E-4M for 60 mins, followed by 10xwash over 30mins). Together with the low K_B value for 5-Methyl-Urapidil, this supports the presence of functional α_{1B}-adrenoceptors rather than α_{1A}.

Table 2: α-adrenoceptor antagonists

α_1-antagonist	pK_B	NAdr max (mN/mm^2)
prazosin	8.54±0.15 (n=6/6)	43.78±6.00 (n=6/6)
WB4101	8.74±0.13 (n=5/5)	52.67±9.35 (n=5/5)
5-Me-Urapidil	5.62±0.17 (n=4/4)	46.07±1.14 (n=4/4)
HV723	8.24±0.17 (n=4/4)	57.46±6.08 (n=4/4)

The low potency of prazosin indicates the additional involvement of prazosin-insensitive functional α_1-adrenoceptors, which is further supported by an extremely low K_B value for 5-Methyl-Urapidil. Taking this into account, the relatively low potency of HV723 suggests the presence of a functional α_{1L}-adrenoceptor (Muramatsu et al.,1990).
We suggest that the rabbit cutaneous resistance arteries express a functional α_{1L}- and an α_{1B}-adrenoceptor, on the basis of the present classification.

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93P PRELIMINARY EVIDENCE FOR THE α_{1B}- AND α_{2A}-ADRENOCEPTOR BINDING SITES ON THE PORCINE ISOLATED THORACIC AORTA

N.A.Blaylock, J.M.Allfree, D.A.Kendall, I.K.Wright & V.G.Wilson. Dept. of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

Recent advances in pharmacological techniques and in molecular biology have indicated the existence of multiple subtypes of α_1- and α_2-adrenoceptor (see Bylund *et al.*, 1994). We have previously reported the presence of α_1-adrenoceptor and α_2-adrenoceptor binding sites on the porcine thoracic aorta which appear to mediate contraction (Wright *et al.*, 1995). Here we have characterised the subtypes of the α_1-adrenoceptor and α_2-adrenoceptor binding site on the porcine thoracic aorta.

Porcine aortae were homogenised, and the P_2 pellet prepared as previously described (Wright *et al., 1995*). The membranes were resuspended in 50mM Tris, pH 7.4 at 25°C, and stored at -20°C until use. Membranes were incubated with either [^{3}H]-prazosin (0.18-0.22nM) or [^{3}H]-RX821002 (0.67-0.89nM) in the presence or absence of increasing concentrations of competing ligands in Tris buffer (50mM, pH7.4) was added to a final volume of 0.5ml and the incubation proceeded at room temperature for 1 hour. Non-specific binding was defined using 10μM noradrenaline in all experiments. Data is expressed as the mean pK_i±sem and mean Hill slope (nH) ±sem of at least three separate experiments.

Table 1. Mean pK_i and nH values against [^{3}H]-prazosin (n=4-6)

Ligand	pK_i	Hill slope
prazosin	9.6±0.20	0.92±0.07
spiperone	8.5±0.04	1.19±0.07
oxymetazoline	6.7±0.04	0.92±0.10
Phentolamine	7.6±0.07	1.00±0.05
WB4101	8.1±0.16	1.19±0.06
Rauwolscine	5.22±0.13	1.19±0.14

Table 2. Mean pK_i and nH values against [^{3}H]-Rx821002 (n=3)

Ligand	pK_i	Hill slope
prazosin	5.6±0.04	0.85±0.06
spiperone	6.0±0.10	0.95±0.09
oxymetazoline	7.8±0.07	0.97±0.10
Phentolamine	7.9±0.15	0.86±0.02
WB4101	7.5±0.16	0.96±0.01
RX821002	8.7±0.21	1.03±0.06
Rauwolscine	8.5±0.07	1.10±0.09

All ligands produced concentration-dependent competition displacing both radio-ligands to the level of non-specific binding. Hill slopes were close to unity indicating that the radio-ligands labelled single sites. Prazosin and rauwolscine exhibited 1000-fold selectivity for [^{3}H]-prazosin and [^{3}H]-RX821002 labelled sites respectively, indicating the presence of both α_1- and α_2-adrenoceptors. On the other hand, phentolamine failed to discriminate between the two sites (< 2-fold). Spiperone and WB4101 were equipotent at α_1-adrenoceptors providing preliminary evidence for the α_{1B} subtype. Prazosin exhibited lower affinity for the [^{3}H]-RX821002 labelled binding site and was less (100-fold) potent than oxymetazoline (characteristic of an $\alpha_{2A/D}$ site), while rauwolscine was 7-fold potent than phentolamine (indicating an α_{2A} site).

In summary, our preliminary results with competitive ligands suggests the presence of the α_{1B}- and α_{2A}-adrenoceptor subtypes on the porcine thoracic aorta.

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94P SEDATIVE EFFECTS AND PHARMACOKINETICS OF MEDETOMIDINE AND ATIPAMEZOLE IN RAINBOW TROUT (*ONCHORHYNCHUS MYKISS*)

T.E. Horsberg, J.F. Burka[1] & R.A.R. Tasker[1], Norwegian College of Veterinary Medicine, Oslo, Norway and [1]Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada, C1A 4P3.

The effects of α_2-adrenergic agonists and antagonists have been studied in mammalian species (Short, 1992; Jalanka, 1993). Information about the effects of such agents upon aquatic species is virtually lacking. The aim of the current work was to study the effects and plasma concentrations of the α_2-adrenergic agonist, medetomidine, and the antagonist, atipamezole, on rainbow trout held in fresh water.

Rainbow trout averaging 250g and held in fresh water (10°C) were used. One group of 18 fish was exposed to 5 mg/l medetomidine for 10 min, then transferred to fresh water. A control group was treated identically to those receiving drugs. Another group was exposed to 5 mg/l of medetomidine for 10 min, then to 30 mg/l of atipamezole for 10 min before being transferred to fresh water. The effects on behaviour and respiration were observed for five days. Trout were sampled and blood withdrawn from the caudal vein at the end of exposure to medetomidine and atipamezole, and 10 min and 6 and 24h. Plasma was assayed for medetomidine and atipamezole by HPLC.

After exposure to 5 mg/l medetomidine, the fish remained active for 30-50s before settling down. They rested on the bottom; all swimming activities ceased; and no movement of any fins could be seen. Five of 18 fish (28%) lost their equilibrium during the exposure to 5 mg/l medetomidine and either rested on their sides on the bottom of the tank or turned belly-up. The rest remained in an upright position. Opercular movements dropped from approximately 80/min to approximately 40/min during the first 5 min and thereafter declined slowly. All fish turned pale within 5 min of exposure to medetomide, indicating aggregation of chromatosomes in the skin. After transfer from medetomidine to atipamezole, the fish stayed on the bottom for 3-4 min, then started moving vigorously for approximately 15s before settling down approximately. These movements were not well coordinated and the fish tired easily. Respiration rate rapidly increased and was back to the original rate 7 min post exposure. The fish also recovered their normal coloration. All fish recovered their equilibrium rapidly following transfer to fresh water containing no drugs.

Immediately after the end of exposure to 5 mg/l medetomidine the average plasma concentration was 1.58 ± 0.57 ($\bar{x}$ ± SD) μg/ml. The plasma $t_{½}$ of medetomidine was estimated to be 2.40 ± 0.23h. The average plasma atipamezole concentration following exposure to 30 mg/l atipamezole was 21.45 ± 0.40 μg/ml and the $t_{½}$ was estimated to be 1.84 ± 0.23h.

These experiments demonstrated that the α_2-agonist medetomidine was an effective sedative in the rainbow trout and that the effect could be reversed with the α_2-antagonist atipamezole. The efficacy and time-course of these agents suggest α_2-agonists may be useful agents to sedate fish for brief manipulation in laboratory or aquaculture settings.

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95P MEFLOQUINE, AN ANTI-MALARIAL DRUG: EFFECTS ON THE CONTRACTILE FUNCTION AND EFFECTIVE REFRACTORY PERIOD OF GUINEA-PIG ISOLATED CARDIAC MUSCLE PREPARATIONS

S.J. Coker & A.J. Batey, Department of Pharmacology & Therapeutics, University of Liverpool, Ashton Street, Liverpool L69 3BX.

A recent report suggests that mefloquine may potentiate QTc prolongation induced by another antimalarial drug, halofantrine (Nosten *et al.*, 1993). This has prompted us to examine the effects of mefloquine on the heart, to allow comparison with those of halofantrine (Lightbown *et al.*, 1996). Male Dunkin-Hartley guinea pigs (380 to 530g) were killed by a blow to the head followed by exsanguination. Hearts were removed rapidly and the left atrium, a left ventricular papillary muscle and a right ventricular strip were dissected free, and set up in a modified Krebs solution in organ baths maintained at 37°C and aerated with 95% O_2 / 5% CO_2. Preparations were suspended under a resting tension of 1g, paced at 1Hz with square wave pulses of 5ms duration at twice threshold voltage and tension was measured isometrically. After equilibration for 1 h, the effective refractory period (ERP) was measured by interpolation of extrastimuli. Mefloquine (3μM) was added and contractility and ERP were measured 5, 10, 20, 30, 40 and 50 min later. Preparations were then washed and the next concentration of mefloquine (10μM) was added immediately after the 60 min measurements. This sequence was repeated with 30 and 100μM mefloquine. Time-matched control experiments were carried out in separate tissues which received the vehicle, propylene glycol (final concentration, 1% v/v).

In ventricular preparations, mefloquine tended to decrease ERP, e.g. from 144±9 at 0 min to 130±7ms at 230 min in papillary muscles (P=0.066, Wilcoxon test) whereas in left atria the apparent increase in ERP (from 48±3 to 55±4ms) was not significant (P=0.292). The vehicle had no effect on ERP in atrial or ventricular preparations and gradual decreases in resting and developed tension were seen with time. Mefloquine, however, had marked effects on contractile function at the higher concentrations (see Figure 1).

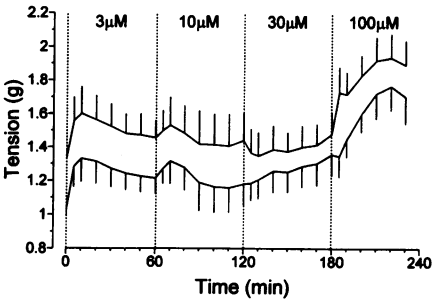

Figure 1. Effects of mefloquine on peak tension (upper line) and resting tension (lower line) in left papillary muscles. Values are mean ± s.e. mean. (n=6).

Resting tension increased in a concentration dependent manner. Developed tension (i.e. peak minus resting tension) was not altered by 3 or 10μM mefloquine but decreased in the presence of 30μM mefloquine (from 0.26±0.05g at 120 min to 0.11±0.01g at 170 min, P=0.016). However, within 5 min of addition of 100μM mefloquine, developed tension increased from 0.12±0.01 to 0.38±0.08g (P=0.016, Wilcoxon test) but this was not sustained. Similar effects were observed in right ventricular strips whereas in left atria only decreases in developed tension were recorded along with the increases in resting tension. These results suggest that while mefloquine has minor effects on effective refractory period, higher concentrations may cause serious impairment of cardiac contractile function. Further experiments are necessary to establish whether similar effects occur *in vivo*.

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96P MECHANISM OF POSITIVE INOTROPY BY PHENAMIL IN CANINE VENTRICULAR MUSCLE: POSSIBLE INDIRECT EFFECT ON THE Na^+-Ca^{2+} EXCHANGER

R. Bose, A. Guia & D. Bose, Departments of Pharmacology & Therapeutics, Anesthesia & Internal Medicine, University of Manitoba, Winnipeg, Canada, R3E 0W3.

Background: Phenamil is an amiloride derivative that inhibits epithelial type Na^+ channels and also blocks inwardly rectifying cardiac potassium channels (I_{K1}) at concentrations that produce positive inotropy in canine right ventricle. Phenamil prolongs phase 3 of the action potential (AP) and inhibits I_{K1} without affecting L-type calcium channels or outwardly rectifying potassium channels (Guia *et al*, 1995).

Aim: The goal of this study was to find the mechanism of positive inotropy by phenamil.

Methods: Right ventricular trabecula were obtained from pentobarbital anesthetised dogs. Isometric contractions were recorded in response to either electrical stimulation or rapid cooling (37°C to 1°C in < 1s). Rapid cooling releases Ca^{2+} from the sarcoplasmic reticulum. In pairs of rapid cooling contractures (RCC), separated by a period of rewarming for 1 min, the second RCC was smaller due to Ca^{2+} extrusion by the Na^+-Ca^{2+} exchanger during rewarming (Hryshko *et al*, 1989). Action potentials were recorded with glass microelectrodes. All experiments were repeated in tissues from 4-7 animals.

Results: Phenamil (60 μM) caused positive inotropy of 175±25% (P<0.05). This was associated with prolongation of action potential duration (APD_{90} from 207±6 to 262±12 ms (P<0.05). Prolongation of APD_{50}, even though significant at P<0.05, was smaller (172±5 increased to 212±11 ms). Paired RCC experiments showed that phenamil did not directly affect Na^+-Ca^{2+} exchange. The magnitude of decline of the second RCC was not reduced by phenamil (57±18% vs 62±1%; P>0.5). Under similar conditions, the Na pump inhibitor, ouabagenin (3 μM) reduced the magnitude of decline of the second RCC from 60% to 10%) (probably by preventing Ca^{2+} extrusion through the Na^+-Ca^{2+} exchange system. Since Na^+-Ca^{2+} exchange is dependent on the repolarization phase of the action potential for its electrochemical driving force, the prolongation of this phase by phenamil ought to reduce the driving force for the exchanger resulting in reduced removal of cytosolic Ca^{2+} and in positive inotropy. With depolarization of the trabecula (approximately 30 mV) by elevated extracellular K^+ (14.6 mM), the second RCC was larger than in the presence of normal extracellular K^+ (34% vs 64% of the first RCC).

Conclusion: The data support the possibility that slowing of terminal repolarization by phenamil may indirectly decrease Ca^{2+} extrusion via the Na^+-Ca^{2+} exchange process. Intracellular Ca^{2+} accumulation resulting from this may be the cause of the inotropy.

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97P DIACYLGLYCEROL ANALOGUE AND ANGIOTENSIN II ACTIVATION OF PKC DECREASES DELAYED RECTIFIER K^+ CURRENT IN RABBIT PORTAL VEIN

O. Clément-Chomienne, E.A Aiello[1], M.P. Walsh & W.C. Cole. Smooth Muscle Research Group, University of Calgary, Canada, T2N 4N1 and [1] Centro de Investigaciones Cardiovasculares, UNLP, La Plata, Argentina.

K^+ channels play an important role in the control of vascular muscle tone by influencing the level of membrane potential. In this study, the effect of protein kinase C (PKC) activation on 4-aminopyridine (4AP)-sensitive, delayed rectifier K^+ current (K_{dr}) in rabbit vascular myocytes was studied by whole-cell patch-clamp. Myocytes dialysed with 10 mM BAPTA, 1 mM GTP and 5 mM ATP were exposed either to direct activators of PKC, such as the diacylglycerol analogue 1,2-diC$_8$ or to the vasoconstrictor angiotensin II (ANG) known to activate PKC in vascular smooth muscle (Dixon *et al.*, 1994). At 20°C, 1,2-diC$_8$ (10 μM) caused a 85 % inhibition of K_{dr} (n=8, $p<0.01$) but the inactive diacylglycerol analogue 1,3-diC$_8$ did not affect K_{dr} (n=5, $p>0.05$). Myocytes exposed to ANG (100 nM) showed decline in K_{dr} which was reversible upon washout. Tail current recorded after 250 ms pulses to +30 mV and repolarization to -40 mV was reduced from 3.9 ± 0.7 pA/pF to 2.5 ± 0.5 pA/pF at 20°C (n=6, $p<0.05$) and from 4.5 ± 0.5 pA/pF to 3.13 ± 0.4 pA/pF at 30°C (n=17, $p<0.05$). The reduction in K_{dr} induced by ANG was blocked by a specific antagonist of AT_1 receptors, losartan (1 μM, n=5). Dialysis for 20 min with the specific inhibitors of PKC, calphostin C (1 μM, n=8) and chelerythrine (50 μM, n=6), reduced by 70% the inhibition of K_{dr} by 1,2-diC$_8$. The same inhibitors completely prevent the inhibition of K_{dr} by ANG, supporting the involvement of PKC. Replacement of external Ca^{2+} with Mg^{2+} along with the internal perfusion of the cells with 10 mM BAPTA did not affect inhibition of K_{dr} by 1,2-diC$_8$ or ANG, suggesting the participation of a Ca^{2+}-independent isoform of PKC. PKC isoenzymes expressed in rabbit portal vein were identified by Western blot analysis using isoenzyme-specific antibodies. α, ε and ζ isoenzymes were demonstrated to be present in this tissue.

The lack of requirement for Ca^{2+} (10 mM BAPTA and replacement of external Ca^{2+} by Mg^{2+}) and sensitivity of both 1,2-diC$_8$ and ANG treatment to chelerythrine (PKCζ is unaffected by chelerythrine, Clément-Chomienne & Walsh, 1996), suggest the involvement of the Ca^{2+}-independent PKC isoenzyme ε in the signal transduction pathway responsible for K_{dr} inhibition by 1,2-diC$_8$ and ANG. Based on experiments employing 4AP to selectively block K_{dr}, this conductance would appear to contribute to action potential repolarization and resting membrane potential in portal vein (Hara, Kitamura & Kuriyama, 1980). Therefore, the inhibition of K_{dr} observed in this study would be consistent with slower repolarization, enhanced Ca^{2+} influx and contraction provoked by action potentials in the portal vein. (Support to WCC and MPW from MRC & AHSF and OC-C from HSFC & AHFMR).

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98P EFFECT OF K^+ CHANNEL BLOCKERS AND INHIBITORS OF CYTOCHROME P450 ON ENDOTHELIUM-DEPENDENT RELAXATION OF RABBIT CAROTID ARTERY

G.J. Waldron, H. Dong, W.C. Cole and C.R.Triggle. Smooth Muscle Research Group, Department of Pharmacology and Therapeutics, The University of Calgary, 3330 Hospital Drive NW., Calgary, Alberta, Canada. T2N 4N1.

The acetylcholine-induced, endothelium-dependent relaxation of rabbit carotid artery that is resistant to high concentrations of nitric oxide synthase (NOS) inhibitors has been postulated to be due to incomplete blockade of NOS, and not release of EDHF, with the residual NO acting via K^+ channels (Najibi and Cohen, 1995). We sought to determine the effect of selective calcium-activated K^+ (K_{Ca}) channel blockers and inhibitors of cytochrome P450 on this phenomenon.

New Zealand White rabbits were killed with an overdose of pentobarbital (180 mgkg^{-1}) and carotid arterial rings were placed on platinum wires and suspended in 10 ml organ baths containing Krebs' solution under resting tension of 7g. The endothelium of some arteries was removed mechanically. Cumulative dose-response curves to acetylcholine were performed in the presence of 10 μM indomethacin. Data are presented as mean ± s.e.mean and statistically analysed by Student's t-test.

In endothelium-intact vessels acetylcholine induced a concentration-dependent relaxation of phenylephrine (1 μM)-induced tone that was largely resistant to inhibition by N^G-nitro-L-arginine methyl ester (L-NAME, 30 μM; maximum relaxation of 99 ± 1%, pD_2 7.03 ± 0.12 vs. 67 ± 3% and 6.48 ± 0.1; n=8; $P<0.01$ for both parameters). The relatively non-selective K^+ channel blockers tetrabutylammonium (1 mM) and charybdotoxin (100 nM) abolished the L-NAME-resistant relaxation, whereas the more selective K_{Ca} channel blockers apamin (3 μM) and iberiotoxin (100 nM) only partially inhibited the relaxation (maximum relaxations 38 ± 5% and 37 ± 6%; n=6 and 8 respectively; $P<0.01$ for both).

Endothelium-denuded vessels showed a greatly reduced response to acetylcholine (maximum relaxation 8 ± 1%, n=5). However, when these vessels were sandwiched next to vessels with endothelium intact, a transferable relaxation was observed (maximum relaxation 70 ± 8%; n=5). This relaxation was reduced, but not abolished by 30 μM L-NAME (maximum relaxation 51 ± 2%). In the presence of L-NAME and tetrabutylammonium the transferable relaxation was further reduced to 14 ± 1% (n=5).

In the presence of 30 μM L-NAME, the cytochrome P450 inhibitors proadifen (1 and 10 μM), 17-octadecynoic acid (3 μM) and clotrimazole (1 μM) also reduced the maximal relaxations (to 35 ± 7%, 14 ± 5%, 25 ± 7% and 22 ± 3% respectively; n=5-7; $P<0.01$ for each).

Therefore, inhibition of L-NAME-resistant relaxation, whether due to EDHF or NO, involves more than one subtype of K_{Ca} channel. Cytochrome P450 inhibitors also inhibit this relaxation, data which supports the notion that the factor responsible for the L-NAME-resistant relaxation requires the action of this enzyme for its synthesis/action.

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99P CONTRACTIONS INITIATED BY THE CARDIAC VOLTAGE SENSITIVE RELEASE MECHANISM ARE SELECTIVELY DEPRESSED IN MYOCYTES FROM YOUNG CARDIOMYOPATHIC HAMSTERS

Susan E. Howlett and Cindy Mapplebeck, Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, B3H 4H7 Canada.

We recently described a novel voltage sensitive release mechanism (VSRM) that triggers contraction in heart (Ferrier and Howlett, 1995). Contractions initiated by the VSRM are not abolished by blockade of sarcolemmal sodium (Na^+) or calcium (Ca^{2+}) channels but are inhibited by ryanodine, an agent that depletes Ca^{2+} stores in the sarcoplasmic reticulum (SR; Ferrier and Howlett, 1995). In this study we determined whether contractions attributable to the VSRM might be altered in cardiomyopathic (CM) hamster hearts, where an increased density of cardiac ryanodine receptors has been described in SR (Sapp and Howlett, 1994).

We used ventricular myocytes isolated from the hearts of young (80-100 days of age) normal and CM hamsters. In CM hearts, this stage corresponds to the stage of active necrosis and precedes the onset of hypertrophy and congestive heart failure. Hamsters were anesthetized with Na^+ pentobarbital (80 mg/kg) and myocytes were isolated by enzymatic dissociation with techniques described previously (Li *et al.*, 1995). Studies were conducted at 37°C, with discontinuous single electrode voltage clamp techniques and high resistance (16-22 MΩ) microelectrodes. Contractions were monitored with a video edge detector. Lidocaine (200 μM) and 4-aminopyridine (2 mM) were used to block Na^+ and transient outward currents, respectively. Contractions were initiated by voltage steps from post-conditioning potentials (V_{PC}'s) of -65 and -40 mV.

In normal cells, the contraction-voltage relationship determined from a V_{PC} of -65 mV was sigmoidal. The threshold for contraction was near -60 mV and the contraction-voltage relation reached a peak near -20 mV. By contrast, the threshold for contraction in CM myocytes was shifted approximately +15 mV. Also, the magnitudes of contractions were significantly depressed compared with those of normals at all potentials (e.g. contractions measured at -5 mV were 4.5 ± 0.4 μm, n=20 normal myocytes and 2.6 ± 0.4 μm, n=13 CM myocytes; mean ± s.e. mean, p=0.004). When the VSRM was inactivated by a V_{PC} of -40 mV, contractions initiated by L-type Ca^{2+} current were observed. The contraction-voltage relation for current-induced contractions was bell-shaped and of similar magnitude in myocytes from normal and CM hearts (e.g. contractions measured at -10 mV were 1.7 ± 0.3 μm, n=19 normal myocytes and 1.3 ± 0.3 μm, n=10 CM myocytes; n.s.). Amplitudes of inward Ca^{2+} currents initiated from V_{PC}'s of -65 or -40 mV also were not significantly different in myocytes from normal and CM hearts.

These results demonstrate that the component of cardiac contraction attributable to the VSRM is depressed selectively in myocytes from young CM hamsters when compared to normal. Thus, depression of the VSRM might contribute to development of contractile dysfunction in this model of cardiomyopathy and congestive heart failure.

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100P ENDOTHELIN-1 ON ARTERIAL AND VENOUS RESISTANCES IN ANAESTHETIZED RATS

Catherine C.Y. Pang, Su Lin Lim & Beatriz Palacios, Dept. of Pharmacology & Therapeutics, Faculty of Medicine, The University of British Columbia, Vancouver, B.C., Canada. V6T 1Z3

Endothelin-1, a potent vasoconstrictor, is well-known to reduce cardiac output (CO). Arterial as well as venous resistances are major determinants of CO. The aim of this study was to determine if endothelin-1 (ET-1) reduced CO in anaesthetized rats *via* elevations in arterial resistance (R_a) and venous resistance (R_v). We have previously reported that i.v. injection of a high dose of ET-1 in conscious rats causes a small increase in mean circulatory filling pressure (MCFP), the driving pressure of venous return (Waite & Pang, 1990). A second part of this study examined if the effects of ET-1 on arterial and venous resistances are antagonized by phentolamine.

Male Sprague-Dawley rats (400-500 g) were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.) and randomly divided into three groups (n = 6-7). One group was given i.v. injection of the vehicle (0.9% NaCl) and two groups were given i.v. bolus ET-1 (0.5, 1 and 2 nmol/kg) either in the absence or presence of phentolamine (300 μg kg^{-1} min^{-1}). Catheters were inserted into iliac arteries for the measurement of mean arterial pressure (MAP) and withdrawal of blood, the right iliac vein for the administration of drugs, and the inferior vena cava *via* the left iliac vein for the measurement of central venous pressure (CVP). A saline-filled, balloon-tipped catheter was inserted *via* the right external jugular vein into the right atrium to transiently stop circulation for the measurement of MCFP (Wang *et al.*, 1995). CO was measured by repeated injections of ^{57}Co-labelled microspheres whereas R_a and R_v were calculated by the formulae: R_a = MAP/CO; R_v = (MCFP - CVP)/CO (Wang *et al.*, 1995). All data are presented as mean±s.e.mean (n = 6-7) and were analyzed by ANOVA followed by Duncan's multiple range test at $P < 0.05$.

I.v. bolus of ET-1, at 0.5, 1 and 2 nmol/kg, increased MAP by 22±5, 34±4 and 40±4 mmHg, R_a by 33±12, 93±17 and 122±14% of control, MCFP by 1.0±0.3, 1.7±0.3 and 1.8±0.3 mmHg and R_v by 40±12, 117±15 and 143±22%, and decreased CO by -6±6, -28±6 and -35±6% of control, respectively. The vehicle did not significantly alter any of these variables. The dose-response effects of ET-1 on all variables were not significantly affected by pretreatment with phentolamine.

Our results show that ET-1 increased MAP in a dose-dependent manner *via* increments in R_a, as CO was reduced. ET-1 slightly increased MCFP but markedly increased venous resistance. Therefore, the reduction of CO by ET-1 was due to constrictions of arterial as well as venous resistance vessels. None of the measured variables in the anaesthetized rats were significantly altered by phentolamine suggesting that they were not mediated *via* modulation of sympathetic nerve activity.

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101P AMINOGLYCOSIDE ANTIBIOTICS REVERSE THE EFFECTS OF OXYHAEMOGLOBIN IN CEREBROVASCULAR SMOOTH MUSCLE

D.A. Cook, M. Gergawy & B. Vollrath. Dept. of Pharmacology, University of Alberta, Edmonton, Alberta, CANADA, T6G 2H7

Oxyhaemoglobin (OxyHb) is believed to be a major causative factor in the development of the cerebrovascular spasm which occurs 3-5 days after subarachnoid haemorrhage. OxyHb probably owes its activity to its ability to participate in free-radical reactions, and the products may activate phospholipases either directly or by causing release of the potent vasoconstrictor, endothelin (ET-1), whose effects are, at least in part, mediated by activation of phospholipase C (PLC). Thus, if activation of phospholipases is a key step in the development of vasospasm, inhibitors of PLC might reduce or abolish the effects of endogenous spasmogens. We have thus investigated the action of neomycin, a known inhibitor of PLC, and some of its analogues, on the contraction of ring preparations of basilar artery produced by OxyHb and ET-1. We have also tested the inhibitors of PLC on the prolonged elevation of intracellular calcium which is observed in cerebrovascular smooth muscle cells in culture, after exposure to OxyHb.

Isometric tension development in rings of canine basilar artery was measured using a standard organ bath procedure, as described previously (Vollrath *et al.*, 1994). IC_{50} values against the contractions produced by OxyHb and ET-1 were then calculated for the PLC inhibitors. Intracellular calcium concentrations ($[Ca^{2+}]_i$) were measured in primate cerebrovascular cells in culture, using the fluorescent dye Fura-2. Cells were incubated in the presence of OxyHb for 24 h, and the PLC inhibitors were added 3 h after the incubation began, to simulate the inevitable delay in administering prophylactic treatment of clinical vasospasm. Significance was determined by an ANOVA.

The IC_{50} in mM (mean and SE) for contractile responses against OxyHb for neomycin was 0.46 ± 0.10 and for gentamicin was 0.55 ± 0.11. When ET-1 was used as an agonist, the values were 0.52 ± 0.06 and 0.54 ± 0.05 respectively (N = 8-11). Preliminary studies with kanamycin suggest that this compound is substantially less potent with an IC_{50} against OxyHb of 3.88 ± 0.46 (N = 6)

OxyHb causes a significant increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) of 520 ± 20% when incubated for 24 h with cultured smooth muscle cells. In cells also incubated with neomycin, gentamicin, kanamycin or streptomycin, the increase in ($[Ca^{2+}]_i$) was significantly reduced to 52.6 ± 5.4%, 34.8 ± 12.2%, 30.3 ± 5.9% and 38.9 ± 2.9% respectively. In cells incubated with OxyHb for 72 h similar results were obtained.

Thus, inhibition of PLC reduces the effects of OxyHb, although there is also some evidence that the aminoglycosides may owe some of their activity to effects other than PLC inhibition. Although the compounds are not very potent, they retain their effects even after prolonged exposure to OxyHb, and thus warrant further investigation.

This work was supported by the MRC and the Upjohn Co.

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102P CONTRACTILE EFFECT OF BIG ENDOTHELIN-1 IN RABBIT CEREBRAL ARTERIES

J. Petersson, G.C. Hanson, B.F. Lindberg & E.D. Högestätt (introduced by A.H. Weston), Department of Clinical Pharmacology, Institute of Laboratory Medicine, Lund University Hospital, S-221 85 Lund, Sweden

Endothelin-1 (ET-1) is a powerful vasoconstrictor of cerebral arteries. Elevated levels of ET-1 have been measured in the cerebospinal fluid after subarachnoid haemorrhage in man (Suzuki *et al.*, 1990) and in cerebral ischaemic infarction (Barone *et al.*, 1994). Endothelin-1 is formed by proteolytic cleavage of the precursor peptide big endothelin-1 (big ET-1).

The aim of the study was to characterize the contractile effects of big ET-1 and the enzymatic conversion of big ET-1 to ET-1 in rabbit cerebral arteries. Isometric tension recordings from isolated ring preparations of rabbit basilar arteries were performed in conventional organ bath experiments (Högestätt *et al.*, 1983). Formation of ET-1 in membrane fractions of rabbit cerebral arteries (from 14 rabbits) after incubation with big ET-1 (20 µM; 2 h) was measured by high pressure liquid chromatography followed by radioimmunoassay (Lindberg *et al.*, 1994).

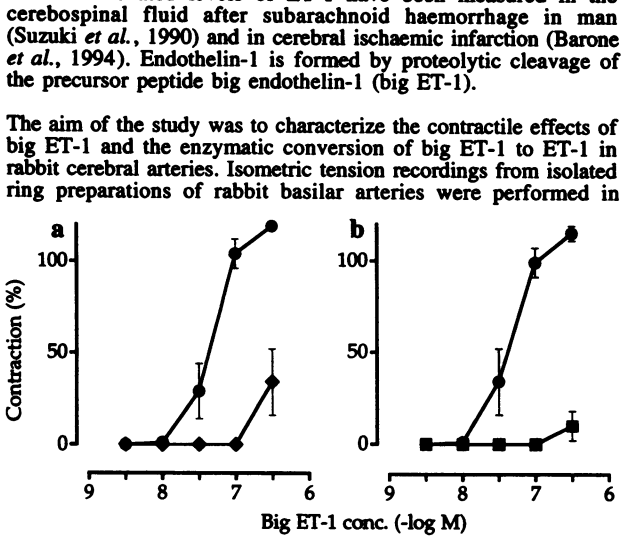

Figure 1. Concentration-response curves for big endothelin-1 in rabbit basilar arteries with endothelium after treatment with 30 µM phosphoramidon (a, ◆) or 3 µM BQ 123 (b, ■) and in controls (●). Levels of contraction are expressed in per cent of an initial contraction induced by an isotonic salt solution containing 60 mM potassium. Data are presented as means ± SEM (n = 5 - 6).

Big ET-1 induced concentration-dependent contractions in isolated segments of the basilar artery. Big ET-1 (pEC_{50} = 7.3 ± 0.1; n = 9) was approximately one order of magnitude less potent than ET-1 (pEC_{50} = 8.2 ± 0.1; n = 8). Removal of the endothelium did not attenuate the big ET-1-induced contraction (n = 6). The metalloprotease inhibitor phosphoramidon (30 µM) almost abolished the contractile response to big ET-1 (Figure 1a), whereas the ET-1-induced contraction was unaffected (n = 4). The contractile effects of big ET-1 (Figure 1b) and ET-1 (pEC_{50} = 7.4 ± 0.1 vs 8.3 ± 0.1 in controls; n = 6) were inhibited by the ET_A receptor antagonist BQ 123 (3 µM), but not by the ET_B receptor antagonist IRL 1038 (3 µM; n = 4 - 5).

The amount of ET-1 formed in the membrane fraction of rabbit cerebral arteries after incubation with big ET-1 was estimated as 50 pM (mean of two separate incubations) compared to 0.5 pM in a control incubation in the absence of big ET-1.

These results suggest that externally-applied big ET-1 is converted to ET-1 by a phosphoramidon-sensitive "endothelin converting enzyme" present in the vascular smooth muscle cells, and that ET-1 in turn mediates the big ET-1-induced contraction by activation of mainly ET_A receptors.

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103P VASOCONSTRICTOR ENDOTHELIN RECEPTORS IN HUMAN SMALL CORONARY ARTERIES *IN VITRO*

L.N.Pierre & A.P.Davenport. Clinical Pharmacology Unit, University of Cambridge, Box 110, Addenbrooke's Hospital, Cambridge, CB2 2QQ, UK.

Endothelin (ET) has been implicated in the pathogenesis of coronary vasospasm. ET is a potent constrictor of human conduit coronary arteries, acting predominantly via ET_A receptors with a small and variable ET_B component (Maguire and Davenport, 1995). However, the contribution of ET_B receptors to ET-induced constriction in small resistance coronary arteries is unclear. We have investigated the nature of ET receptors present in human resistance coronary arteries using heart apices obtained from 28 patients undergoing cardiac transplantation.

Autoradiography: Slide mounted cryostat sections (10μm) of apex were incubated for 2h with buffer containing 0.1nM of [^{125}I]PD151242 (ET_A selective) or 0.3nM [^{125}I]BQ3020 (ET_B selective) (Davenport *et al.*, 1994) both 2000Ci mmol^{-1}.

In vitro pharmacology: Epicardial arteries were dissected from the apex and 1-2 mm arterial rings were set up in a wire-myograph containing oxygenated Krebs-Henseleit solution (37°C). Each preparation was set to 0.9 of the internal diameter (i.d.) the vessel would have if relaxed and under a transmural pressure of 100mmHg. Following this normalisation procedure the vessels were grouped according to i.d. group A >400μm (577.7±31.5μm); group B <400μm (328.5±10.9μm) (mean±s.e.mean).

Following two stimulations with potassium rich solution (95 mM) a robust contraction to either 5-hydroxytryptamine or U46619 (thromboxane A_2 mimetic) was obtained. On plateau of the contraction bradykinin was administered (100nM) to test for a functional endothelium. Cumulative concentration response curves were constructed to either ET-1 (non-selective), or the ET_B receptor selective agonists ET-3 and sarafotoxin 6c (S6c). One curve was constructed per preparation. Agonist responses were expressed as a percent of the contraction to potassium.

Specific high density binding of [^{125}I]PD151242 was observed on the vascular smooth muscle of small intramyocardial vessels. In contrast, little or no specific [^{125}I]BQ3020 binding was identified (n=5).

All vessels had an intact endothelium. ET-1 potently contracted epicardial vessels in both groups with EC_{50} values of 2.5nM (0.8-7.0nM; n=5) and 1.5nM (0.4-5.0nM; n=5) (geometric mean, 95%CI), respectively. ET-1 was more potent than ET-3 in both groups. In group A, ET-3 yielded an EC_{50} of 51nM (16-160nM; n=11) and interestingly elicited a biphasic curve in 3 out of the 11 vessels tested. In group B, responses to ET-3 were obtained in 4 out of 9 vessels. The ET_B selective agonist S6c elicited contractions, with threshold concentrations between 1 and 100nM, in 3 out of 6 vessels from group A. However, these responses were rapidly reversed by the selective ET_A receptor antagonist FR139317 (1μM). S6c had little or no activity in the vessels tested from group B (n=4).

The autoradiographical results suggest that intramyocardial coronary arteries express mainly ET_A receptors on their vascular smooth muscle cells. Similarly, the preliminary *in vitro* pharmacology studies indicate that ET-mediated vasoconstriction in human small epicardial coronary arteries is elicited predominantly via the ET_A receptor subtype with a small ET_B constrictor component detectable in some patients. Additional studies using selective antagonists are required to determine the relative importance of these receptor subtypes in human small coronary artery vasoconstriction.

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104P SILICONE-INDUCED GRANULATION TISSUE: THE CONTRACTILE EFFECTIVENESS OF ANGIOTENSIN AND MEPYRAMINE

M.H. Pipelzadeh, J.J.R. Kirkpatrick and I. Naylor, Postgraduate Studies in Pharmacology, School of Pharmacy, University of Bradford, Richmond Road, Bradford, West Yorkshire, BD7 1DP.

In 1971 Gabiani *et al* coined the term 'myofibroblast' to distinguish a cell found in granulation tissue from the structurally similar fibroblast. They had a distinctive electron microscopic appearance and were thought responsible for wound closure. The agonist(s) responsible for inducing the contractile process in vivo is still debatable and attempts at elucidation have featured in a limited number of pharmacological studies. In this study the responses of 1 and 2 week granulation tissue formed around subcutaneously placed silicone implants (C) is compared with normal superficial fascia (SF). The *in vitro* reactivity of both SF and C to mepyramine (MPY) and angiotensin II (ANG II) was determined.

Dorsal normal fascia (SF, n=8), and 1 and 2 week silicone-implanted granulation tissue, were obtained from female, Hooded Lister rats (200-300g, Bradford strain). The surgical grade, cylindrical silicone implants (10 dia X 20mm length) were inserted via incision in the lower dorsum and then placed in the mid-dorsal region under isoflurane/nitrous oxide/ oxygen anaesthesia. The incision site was sutured. All animals were killed by pentobarbitone overdosage. Tissue strips, 20x10 mm, were mounted under a resting tension of 2g, for superfusion with Krebs' solution (37^{0}C) at 3ml/min. Bolus doses of ANG II (10^{-10} - 10^{-7} moles) and MPY (8 to 128 μmoles) were added to the superfusate and the change in the isometric tension recorded.

The results (Fig 1) show that the responses to ANG II, of silicone induced granulation tissue at both 1 and 2 week were significantly greater and more prolonged as compared with normal fascia. This suggests the presence of myofibroblasts in this type of granulation tissue. The comparatively weak responses to ANG II seen in normal SF may also suggest that myofibroblasts may be present in the unstimulated tissue which upon stimulation by the silicone implant are activated or develop different cytoskeletal features (Sappino *et al.* 1990) or express receptors which are more sensitive to ANG II. Similar responses were observed with MPY, e.g. the responses to 128 μmole bolus dose were significantly greater (P>0.01, n=8) in C (180±39 mg) than in SF (85±13 mg).

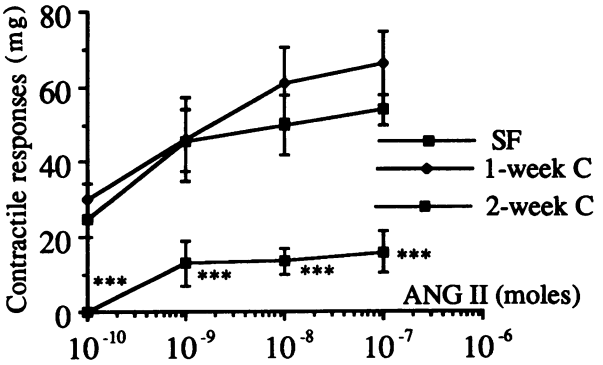

Fig 1: Angiotensin II dose response curves of normal superficial fascia (SF) and 1 & 2 week silicone implant capsule (C). Each point showing the mean ± sem of contractile responses, for the capsules was significantly greater, relative to normal fascia. (*** indicates statistical significance P<0.001, n=8, Student's t-test, unpaired).

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105P ANALYSIS OF COMPLEXITY IN THE POTENTIATING INTERACTION BETWEEN ANGIOTENSIN II AND B-HT 933 IN RABBIT SAPHENOUS VEIN

N.J. Welsh, N.P. Shankley, J.W. Black, Analytical Pharmacology, KCSMD, London SE5 9NU, UK

Angiotensin II (AII) has been reported to have a facilitatory effect on α_2-adrenoceptor-mediated responses in rabbit saphenous vein (Dunn *et al.*, 1991; Schumann & Lues, 1983). Previously, we have reported that although AII potentiated the effect of noradrenaline (NA) in the rabbit femoral artery, the opposite was not true, that is, NA did not potentiate responses to AII (Welsh *et al.*, 1996). Therefore, we have investigated the interaction between AII and B-HT 933, a selective α_2-adrenoceptor agonist, in the rabbit saphenous vein to determine, firstly, whether synergistic interactions are dependent on the order of agonist incubation in this assay and, secondly, whether the interaction data fits the '2-receptor-1-transducer' model of synergy developed by Leff (1987).

Endothelium denuded rings of saphenous vein (4mm) were obtained from rabbits (NZ White, 2.5-3.0kg) killed by pentobarbitone sodium (>60mg/kg i.v.). These were mounted between wire hooks with 2g loading force for isometric force recording in 20ml organ baths containing Krebs-Henseleit solution (including 0.3μM desipramine, 30μM corticosterone and 1μM timolol) maintained at 37°C and gassed with 95%0_2/5%CO_2. Antagonists were incubated for 60min.

AII behaved as a partial agonist (max.=1.23±0.13g; p[A]$_{50}$=7.76±0.06; n_H=0.97±0.08; n=6) in the system with respect to B-HT 933 (max.=2.88±0.25g; p[A]$_{50}$=5.28±0.14; n_H=1.30±0.04; n=6). Concentration-effect (E/[A]) curves to AII were shifted to the right by 30nM losartan (pA_2=9.00±0.24; 5 d.f.), consistent with an action at AT_1 receptors. B-HT 933 E/[A] curves were shifted by 30 and 100 nM rauwolscine (pK_B'=8.00± 0.22; 15 d.f.) and 0.3μM prazosin had no effect on the B-HT 933 E/[A] curve location (student's t-test: t=2.10; 7 d.f.), consistent with activation of α_2-adrenoceptors.

The B-HT 933 E/[A] curves were leftward shifted by preincubation with AII (3, 10, 30nM) with no change in the maximum response. Response to higher concentrations of AII were not well maintained and the maximum stable pretreatment response (0.37±0.09g; n=8) was achieved at 3nM AII which produced a log dose-ratio of -0.53 ±0.11. In contrast, preincubation with 1 and 3μM B-HT 933, which produced responses of 0.41±0.12g and 1.17±0.06g (n=6) respectively, had no effect on the AII E/[A] curve location (max. log dose ratio=-0.09±0.16) and the effect of the two agonists appeared to be additive.

The Leff (1987) model, in which the two agonist stimuli add and are mapped through a common transducer function, 'predicts mutual potentation' and that 'potentiation should be accompanied by a significant change in the curve threshold' which was not true for these data. In order to account for these data it was possible to extend the model by allowing one of the stimuli (AII) to have a secondary action to increase the efficiency of the combined-stimulus effector coupling.

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106P INFLUENCE OF CAPTOPRIL AND LOSARTAN ON THE HAEMODYNAMIC RESPONSES TO ENDOTOXAEMIA IN CONSCIOUS RATS

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

Inhibition of angiotensin-converting enzyme (ACE; kininase II) may exert effects due to loss of the action of angiotensin II and enhancement of the action of bradykinin. Since the latter peptide has been implicated in the cardiovascular responses to lipopolysaccharide (LPS), we compared the influence of the ACE inhibitor, captopril, with that of the AT_1-receptor antagonist, losartan, on the responses to LPS in conscious rats. Animals (male, Long Evans rats, 350-450 g) were chronically instrumented with pulsed Doppler probes and intravascular catheters to allow monitoring of renal, mesenteric and hindquarters haemodynamics; all surgery was under anaesthesia (sodium methohexitone, 40-60 mg kg^{-1} i.p., supplemented as required). Three groups of rats were studied:- Group a (n = 10) received captopril (2 mg kg^{-1} bolus, 2 mg kg^{-1} h^{-1} infusion) beginning 1 h before LPS (150 μg kg^{-1} h^{-1}) for 23 h; Group b (n = 9) received losartan (10 mg kg^{-1} bolus, 10 mg kg^{-1} h^{-1} infusion) beginning 1 h before LPS for 23 h; Group c (n = 8) received captopril and losartan beginning 1 h before LPS for 23 h. Resting cardiovascular variables in Group a, b, and c, respectively were:- heart rate (HR), 332 ± 5, 336 ± 7, and 328 ± 8 beats min^{-1} (mean ± s.e. mean); mean arterial blood pressure (MAP), 103 ± 2, 103 ± 1, and 102 ± 1 mm Hg; renal vascular conductance (RVC; [kHz mm Hg^{-1}]10^3), 65 ±7, 64 ± 6, and 57 ± 3; mesenteric vascular conductance (MVC), 57 ± 5, 66 ± 6, and 57 ± 5; hindquarters vascular conductance (HVC), 49 ± 4, 42 ± 3, and 55 ± 4. Cardiovascular changes at 1, 2 and 24 h during the experiments are summarised in Table 1.

By 1 h after the onset of LPS infusion (i.e., 2 h after the beginning of the experiment), the fall in MAP and the increases in RVC and HVC were significantly greater in the presence of losartan than in the presence of captopril. By 24 h, these differences were no longer apparent. In the presence of captopril and losartan, the haemodynamic effects of LPS were not different from those seen in the presence of losartan. Hence, the differences between the effects of LPS in the presence of captopril or losartan, alone, are likely to have been due to less effective suppression of the actions of angiotensin II by captopril than by losartan, rather than an involvement of bradykinin in the effects of the former.

Table 1. Cardiovascular changes in conscious, Long Evans rats. Values are mean ± s.e. mean; * P < 0.05 for change (Friedman's test); superscripts indicate P < 0.05 versus change in corresponding group (Kruskal-Wallis test).

Time after start		1h	2h	24h
Time after onset of LPS		0	1h	23h
	a	27 ± 10*	29 ± 11*	129 ± 12*
ΔHR	b	56 ± 10*	41 ± 14*	127 ± 8*
(beats min^{-1})	c	43 ± 9*	44 ± 11*	118 ± 9*
	a	-5 ± 2*	-27 ± 3*	-26 ± 3*
ΔMAP	b	-6 ± 2*	-41 ± 2*[a]	-28 ± 3*
(mm Hg)	c	-9 ± 2*	-39 ± 4*[a]	-27 ± 4*
	a	15 ± 3*	81 ± 9*	152 ± 24*
ΔRVC	b	27 ± 4*[a]	122 ± 16*[a]	164 ± 19*
(%)	c	38 ± 4*[a]	135 ± 26*[a]	181 ± 24*
	a	13 ± 5*	42 ± 12*	54 ± 9*
ΔMVC	b	14 ± 6*	68 ± 9*	40 ± 15*
(%)	c	25 ± 7*	87 ± 17*[a]	76 ± 15*
	a	-6 ± 4	10 ± 9	93 ± 15*
ΔHVC	b	18 ± 7	58 ± 12*[a]	111 ± 21*
(%)	c	6 ± 10	41 ± 16*	96 ± 27*

107P DISSOCIATION OF CHANGES IN PRESSOR AND CONSTRICTOR EFFECTS OF ANGIOTENSIN II AND OF VASOPRESSIN IN CONSCIOUS, ENDOTOXAEMIC RATS

S.B. Tarpey, T. Bennett and S.M. Gardiner. Department of Physiology and Pharmacology, Queen's Medical Centre, Nottingham NG7 2UH.

An increase in blood pressure can be due to a reduction in peripheral conductance, and/or a rise in cardiac output. It has been reported that angiotensin II (AII) and vasopressin (AVP) have reduced pressor effects in endotoxaemic rats (Schaller *et al.*, 1985), but it is not known if this is due, simply, to reduced constrictor action, so we investigated this possibility.

Experiments were carried out on conscious male, Long Evans rats (350-450g), chronically instrumented with pulsed Doppler probes (renal, mesenteric, and hindquarters) and intravascular catheters (all surgery under sodium methohexitone anaesthesia, 40-60 mg kg^{-1} i.p., supplemented as required). Animals were given 24 h infusions of saline (0.4 ml h^{-1}, n = 9) or lipopolysaccharide (LPS, 150 µg kg^{-1} h^{-1}; *E coli* serotype 0127:B8; n = 9). Before, and starting 2, 6 and 24 h into infusion, each animal was given alternating, increasing bolus doses of AII (5, 10 and 50 pmol) and AVP (0.5, 1 and 5 pmol), at 15 min intervals.

Some of the results are summarised in Table 1. Twenty four h after the onset of infusion, rats receiving LPS showed hypotension and increases in heart rate and renal and hindquarters vascular conductance relative to those receiving saline (see Gardiner *et al.*, 1995). AII at 10 pmol and AVP at 5 pmol had similar pressor effects in the presence of saline, although AII caused comparable reductions in renal and mesenteric vascular conductance, whereas AVP had a lesser effect in the kidney. In the presence of LPS, it is clear that for AII and AVP, the peak pressor effect can be reduced when the maximum renal constriction is increased and the change in mesenteric vascular conductance is unaffected. These results indicate there must be substantial differences in the changes in cardiac output elicited by AII and by AVP in saline-infused and LPS-infused rats, and signal the need for caution when interpreting changes in the pressor effects of vasoconstrictor agents as changes in vascular sensitivity.

Table 1. Resting variables and peak (at 0.5 min) responses to AII and AVP, 24 h after onset of saline (S) or LPS (L) infusion. Values are mean ± s.e. mean; * $P < 0.05$ for change (Wilcoxon test); † $P < 0.05$ versus saline group (Mann-Whitney test). HR = heart rate (beats min^{-1}); MAP = mean arterial blood pressure (mm Hg); RVC, MVC, HVC = renal, mesenteric and hindquarters vascular conductance, respectively ([kHz mm Hg^{-1}]10^3).

		Resting	AII (10 pmol)	AVP (5 pmol)
HR	S	357 ± 14	-63 ± 18*	-92 ± 9*
	L	444 ± 11†	-43 ± 6*	-67 ± 17*
MAP	S	110 ± 2	34 ± 4*	38 ± 2*
	L	98 ± 2†	19 ± 3*†	19 ± 2*†
RVC	S	57 ± 9	-49 ± 9*	-26 ± 4*
	L	125 ± 9†	-84 ± 7*†	-68 ± 6*†
MVC	S	68 ± 6	-51 ± 6*	-44 ± 4*
	L	66 ± 4	-39 ± 4*	-37 ± 3*
HVC	S	31 ± 2	-4 ± 2	16 ± 2*
	L	52 ± 4†	-1 ± 3	-8 ± 3

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

108P VASCULAR ACTIVITIES OF ANGIOTENSIN II AND VASOPRESSIN IN ISOLATED PERFUSED MESENTERIC ARTERIAL BEDS FROM ENDOTOXAEMIC RATS

S.B. Tarpey & M.D. Randall, Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

Recent interest has been focused on the ability of lipopolysaccaride (LPS) to modulate vascular responses and the possible involvement of nitric oxide (Fleming *et al.*, 1990). We have recently investigated the vasoconstrictor activities of angiotensin II (AII) and arginine vasopressin (AVP) in conscious rats subject to a 24h infusion of LPS (Tarpey *et al*, 1996). In this study we have investigated the vasoconstrictor activities of these agents in the isolated perfused superior mesenteric arterial bed from the same rats. In view of the involvement of nitric oxide (NO) in modulating vascular responsiveness in endotoxaemia we have investigated the effects of the NO synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME) on these vasoconstrictor responses.

Male, Long Evans rats (350-480g) from Tarpey *et al.* (1996) 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 anaesthetized with sodium pentobarbitone (44mg kg^{-1} i.v.) and the mesenteric arterial bed was cannulated and perfused with oxygenated Krebs-Henseleit solution at 5ml min^{-1}. Following 30 min equilibration, with normal buffer solution, perfusion pressure was raised by perfusion with high KCl (25mM) K-H buffer, and the responses to AII (0.1, 1.0 and 10.0nmol; 1 h apart; n=6) were determined. Between these doses, the mesentery was perfused with normal K-H buffer and a dose-response curve for AVP (0.3pmol-1.0nmol, 12 min apart; n=9) was obtained. Dose-response curves were then constructed in the presence of 100µM L-NAME.

Basal perfusion pressures for the saline (n=9) and LPS (n=9) groups were comparable, (14.4±2.0mmHg (mean ±s.e.mean) and 15.2±1.3mmHg respectively). Both peptides caused increases in perfusion pressure in the LPS and saline groups. AVP was significantly ($P<0.05$, ANOVA with Bonferroni's post-hoc test) more potent in the LPS treated (ED50= 20.4±4.8pmol) compared to saline (ED50=56.9±15.0 pmol) preparations. There were no significant differences in the maximal responses (Rmax), which were 165±8mmHg (LPS) and 153±9mmHg (saline). The responsiveness to both AII and KCl did not differ between groups. Addition of L-NAME significantly ($P<0.05$) increased both the potency of AVP in the saline group (ED50=20.0±4.0pmol) and the Rmax to AVP in the LPS group (from 165±8mmHg to 191±5mmHg). Responses to AII (1.0nmol) were also enhanced ($P<0.05$) in the presence of L-NAME (17.3±3.8 mmHg v. 60.1±18.1mmHg, saline; 10.2±1.6mmHg v. 23.5± 2.7mmHg, LPS).

The results show that in endotoxaemia the potency of AVP, but not AII, is enhanced. This finding is in agreement with a report of increased vasoconstrictor sensitivity in the isolated mesentery after LPS (Mitchell *et al.*,1993). However, our results contrast the *in vivo* haemodynamic study (Tarpey *et al.*, 1996) where mesenteric responses to AVP and AII were unaffected after 24h LPS. In the present study both peptides were modulated by NO to similar degrees in both LPS and saline groups and therefore NO is not responsible for mediating any changes between the groups.

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

Fleming, I., Gray, G.A., Jolou-Schaeffer, G. *et al.* (1990). Biochem. Biophys. Res. Commun., 171, 562-568.
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109P COMPARISON OF THE CARDIOVASCULAR EFFECTS OF SB 209509 (VML 251) AND SUMATRIPTAN IN DOGS

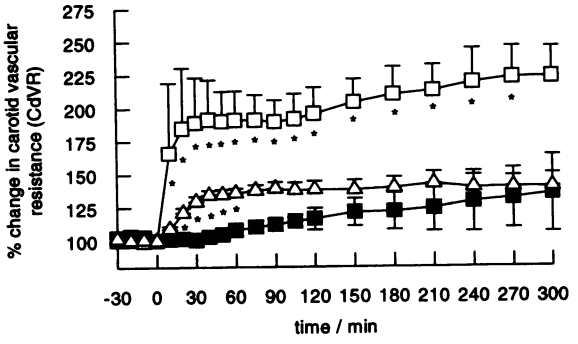
A.A. Parsons, S.G. Parker, P. Raval, C.A. Campbell, A.J. Hunter, T.C. Hamilton, & F.D. King. SmithKline Beecham Pharmaceuticals, Third Avenue, Harlow, Essex.

SB 209509 (VML 251) has been shown to constrict isolated cerebral arteries and to have high affinity for human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors (Brown *et al.*, 1996). The aim of the present study was to compare the cardiovascular effects of SB 209509 with sumatriptan in anaesthetised dogs.

Male or female beagle dogs (10-19kg) were anaesthetised by intravenous sodium pentobarbitone. Coronary (Co) or carotid (Cd) blood flow was recorded in open chest dogs from an electromagnetic flow probe following intravenous (iv) administration of SB 209509 or sumatriptan (100ng/kg to 1mg/kg). In a separate series of experiments, changes in Cd blood flow were recorded for up to 5 hours following intraduodenal (id) bolus administration of 0.3mg/kg SB 209509 or sumatriptan. In all experiments, arterial blood pressure and derived heart rate were recorded. Co or Cd resistance (VR) was calculated (arterial blood pressure/flow).

SB 209509 (iv) and sumatriptan (iv) produced dose-related increases in CdVR with maximal changes (mean ± sem) in CdVR of 188 ± 15.4% (n=5) and 224 ± 21.5% (n=5) and calculated ED50 values of 0.38 ug/kg and 34 ug/kg respectively. SB 209509 (< 300ug/kg) and sumatriptan (up to 1mg/kg) had no effect on coronary vascular resistance. However, high doses of SB 209509 (> 300ug/kg) produced a reduction (25%) in CoVR. Both SB 209509 and sumatriptan had little effect on blood pressure or heart rate. Following id administration, both SB 209509 and sumatriptan produced rapid increases in CdVR. In addition, SB 209509 produced increases in carotid vascular resistance for at least 4.5 hours (figure 1).

Figure 1: Effects of bolus id administration of SB 209509 (0.3mg/kg, n=3), open squares, sumatriptan (0.3mg/kg n=6, open triangles) or vehicle (n=3, solid squares) on carotid vascular resistance in dogs (* $p < 0.05$).



These data show that SB 209509 is at least 10 fold more potent than sumatriptan in increasing CdVR and has a long duration of action. High doses of SB 209509 produce a decrease in CoVR.

Brown A.M., Parsons, A.A., Raval, P. *et al.*, This meeting

110P SB 209509 (VML 251), A POTENT CONSTRICTOR OF RABBIT BASILAR ARTERY WITH HIGH AFFINITY AND SELECTIVITY FOR HUMAN 5-HT_{1D} RECEPTORS

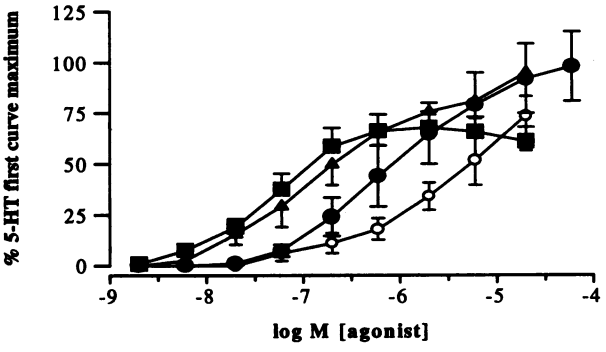
A.M.Brown[1], A.A.Parsons[2], P. Raval[2], R. Porter[3], N.S. Tilford[2], T.L. Gager[1], G.W. Price[1], M.D. Wood[1], A.J. Kaumann[4], R.A. Young[3], K. Rana[3], B.H. Warrington[3] & F.D. King[3]. Departments of [1]Psychiatry Research, [2]Neurology Research and [3]Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, UK, and [4]Babraham Institute, Cambridge, UK.

We have previously shown that (±) BRL 56905 is a potent 5-HT_{1D} agonist and constrictor of the rabbit isolated basilar artery (King et al, 1993). In the present study we have characterised the N-methyl derivative of BRL 56905, SB 205184 ((±)-3-methylamino-6-carboxamido-1,2,3,4-tetrahydrocarbazole), and its (R)(+) and (S)(-) enantiomers, SB 209509 (VML 251) and SB 209510 respectively.

The effects of SB 205184, SB 209509 and SB 209510 were compared with that of sumatriptan in basilar arteries isolated from New Zealand White rabbits, a preparation containing 5-HT_1-like (5-HT_{1D}) receptors (Parsons & Whalley, 1989) under conditions described previously (King *et al.*, 1993). Mean EC_{50}s were calculated as the mid-point location parameters of curves for individual data sets fitted to the Hill equation. The radioligand binding profile of SB 209509 was also investigated on a range of human recombinant receptors (5 $HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_7, Dopamine D_1, D_2, D_3), on rat α_1-adrenergic, 5-HT_{1A} and 5-HT_3 receptors and on guinea pig histamine H_1 receptors.

All compounds produced concentration -related contractions of rabbit basilar artery (Figure 1). Mean pEC_{50} ± s.e.mean [n] values were 7.2 ± 0.1 [7], 6.6 ± 0.2 [6] and < 5.7 [4] for SB 209509, SB 205184 and SB 209510 respectively. Sumatriptan produced marked contraction but was less potent (pEC_{50} 6.0 ± 0.2) than SB 209509 and SB 205184. SB 209509 had high affinity for both 5-$HT_{1D\alpha}$ (pKi =8.4) and 5-$HT_{1D\beta}$ (pKi =8.6) receptors, moderate affinity for 5-HT_{1A} (pKi = 7.3), 5-HT_{1F} (pKi=7.0) and 5-HT_7 (pKi = 6.7) receptors and low affinity for 5-HT_3 (pKi<6) and 5-HT_{1E} (pKi < 6) receptors, 5-HT_{2A} or 5-HT_{2C} (pKi < 5.3), and dopamine, histamine and α_1-adrenergic receptors (pKi < 5).

Figure 1. Contractile effects of SB 209509 (■), SB 205184 (▲), SB 209510 (○) and sumatriptan (●) in rabbit isolated basilar artery. Data are mean ± s.e.mean of 4-7 determinations.



We conclude that SB 209509 is a potent 5-HT_{1-like} receptor agonist in rabbit isolated basilar arteries with good selectivity for 5-HT_{1D} receptors over a number of other receptor subtypes including 5-HT_{1F}.

King , F.D. Brown, A.M., Gaster, L.M. *et al.* (1993) J. Med. Chem **36**, 1918-1919.

Parsons, A.A. and Whalley, E.T. (1989) Eur. J. Pharmacol. **174**, 189-196.

111P A COMPARISON OF THE AGONIST PROFILE OF SB 209509 (VML 251) AND SUMATRIPTAN IN HUMAN ISOLATED BASILAR AND CORONARY ARTERIES

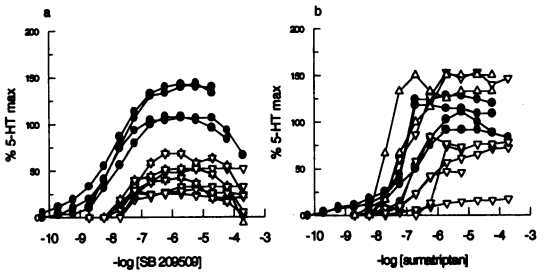
P. Raval[1], N.S. Tilford[1], S.J. Smith[1], R. Porter[2], F.D. King[2], A.J. Kaumann[3], A.J. Huner[1] & A.A. Parsons[1]. Neurology Research Department[1], Medicinal Chemistry[2], SmithKline Beecham Pharmaceuticals,UK, Addenbrookes Hospital[3], Cambridge, UK.

The contractile actions of sumatriptan in human basilar (BA) (Parsons *et al.*, 1988) and coronary (CA) arteries (Chester *et al.*, 1990; Kaumann *et al.*, 1994) are mediated via 5-HT_{1D} receptors. In this study, we have compared the effects of SB209509 (VML 251) (Brown *et al.*, 1996) and sumatriptan in human isolated BA and CA.

BA arteries were obtained <72 hours *post-mortem* and CA were obtained, from hearts of either recipient (CAr) or donor (CAd) patients, during heart transplant operations. The endothelium-denuded ring segments were set up for isometric recording of tension (resting tension 5-10mN) in oxygenated modified Krebs solution, at 37°C (Kaumann et al., 1994). Cerebral arteries were also incubated with indomethacin 2.8 μM to inhibit generation of spontaneous tone. Following challenge with 90mM KCl, the effects of SB 209509 or sumatriptan were determined as a percentage of the maximum response to 5-HT.

SB209509 and sumatriptan produced contraction of the BA and CA (Figure 1) with mean pEC50 ± s.e.mean (intrinsic activity, % 5-HT max. ± s.e.mean, n=rings/patients) of 7.86 ± 0.07 (1.25 ± 0.10, n=4/3) and 6.93 ± 0.09 (1.11 ± 0.08, n=4/3) in BA and 7.38 ± 0.12 (0.42 ± 0.06, n=7/4) and 6.57 ± 0.13 (0.79 ± 0.27, n=6/5) in CAr and 7.81 ± 0.20 (0.40 ± 0.09, n=3/2) and 7.35 (1.41, n=2/1) in CAd respectively (Figure 1). SB 209509, but not sumatriptan, exhibited a bell shaped concentration-response relationship which was marked in CA. The maximum response to SB 209509 in CA was significantly lower (ANOVA; Tukey multiple range: $p<0.05$) than the response in BA. No significant differences in the maximum responses to sumatriptan were present between arteries. SB 209509 was 3-9 fold more potent than sumatriptan in human arteries.

Figure. 1 Responses from individual subjects showing the effects of SB209509 or sumatriptan in human basilar (closed circle) and coronary arteries (CAr, inverted triangle and CAd triangle).



These data show that SB 209509 is a potent agonist in BA and CA and the response suggests a functional selectivity to the cerebral circulation.

Brown A.M., Parsons, A.A., Raval, P. et al., 1996 This meeting
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Kaumann A.J. , Frenken, M., Posival, H. *et al.*, (1994) Circulation, **90**, 1141-1153.
Parsons A.A., Whalley, E.T., Feniuk, W. *et al.*, (1988) Br. J. Pharmacol., **96**, 434-440.

112P *IN VITRO* ASSESSMENT OF THE VASCULAR EFFECTS OF 5-HT_{1D}-RECEPTOR AGONISTS: RELATIONSHIPS WITH 5-$HT_{1D\alpha}$- OR 5-$HT_{1D\beta}$- RECEPTOR BINDING AFFINITY

D Shaw, JA Stanton, MA Heald, MS Beer, F Sternfeld, L Street, RG Hill, E Cooke, J Longmore. Merck Sharp & Dohme Research Laboratories, Eastwick Road, Harlow, Essex, CM20 2QR.

The pharmacological characterisation of the 5-HT_{1D}-receptor subtype mediating vasoconstriction and sympatho-inhibition has been hampered by the lack of selective antagonists. Distinct species specific 5-HT_{1D}-receptor pharmacologies has added to the confusion. An alternative approach, used in this study, is to compare the functional effects of 5-HT_{1D}-receptor agonists in isolated blood vessels with their affinities at the cloned 5-$HT_{1D\alpha}$- or 5-$HT_{1D\beta}$- receptors of the corresponding species.

Vasoconstrictor and sympatho-inhibitory effects in isolated saphenous vein from dogs (Beagle, male/female, 15-25kg) were measured as described by Feniuk et al., 1979. [^{3}H]-5HT (2nM) displacement studies (10μM 5-HT used to define non-specific binding) and agonist-induced [^{35}S]GTPγS binding studies were carried out on membranes prepared from stable CHO cell lines expressing dog and human 5-$HT_{1D\alpha}$- and 5-$HT_{1D\beta}$-receptors. The [^{35}S]GTPγS binding studies were carried out as described by Lazareno & Birdsall (1993) using 100μM and 30μM GDP for the 5-$HT_{1D\alpha}$- and 5-$HT_{1D\beta}$-receptor assays respectively. Correlations were made using linear regression analysis.

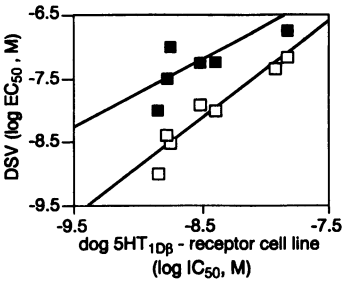
None of the compounds showed binding subtype selectivity (>10-fold) for dog or human 5-$HT_{1D\alpha}$– over 5-$HT_{1D\beta}$-receptors (see Table 1). Significant correlations were found for both sympatho-inhibition and direct contraction versus dog 5-$HT_{1D\beta}$-receptor affinity (see Figure 1) but not with dog 5-$HT_{1D\alpha}$-receptor affinity. Significant correlations were seen between effects in dog saphenous vein versus human 5-$HT_{1D\beta}$-receptor potency but not affinity. No other significant correlations ($r<0.7$) were seen.

In conclusion, the results indicate that both the vasoconstrictor and sympatho-inhibitory effects in dog isolated saphenous vein are mediated by 5-$HT_{1D\beta}$-receptor activation. Ongoing studies indicate the use of functional assays providing measurements of efficacy and affinity for both dog and human cloned receptors are more appropriate in defining α/β receptor selectivity. Finally, the lack of significant correlations between functional effects in dog saphenous vein and measurements of affinity at cloned human 5-$HT_{1D\alpha}$- or 5-$HT_{1D\beta}$-receptors further illustrates the difficulties in interpreting data across species.

Table 1: Human 5-HT_{1D}-receptor cell line data; *Street et al 1995

	Human 5-$HT_{1D\alpha}$ (nM)		Human 5-$HT_{1D\beta}$ (nM)	
	Affinity-IC_{50}	Potency-EC_{50}	Affinity-IC_{50}	Potency-EC_{50}
5-HT	6.6	7.0	6.1	33
5-CT	1.9	2.6	2.3	12
Sumatriptan	7.3	9.7	9.3	150
CP122,288	6.1	13	8.1	140
Zolmitriptan	1.3	0.9	6.2	51
L-741,519*	1.2	1.8	3.4	17
L-741,604*	0.3	0.4	1.4	11

Figure 1: effects in dog isolated saphenous vein (DSV) versus dog 5-$HT_{1D\beta}$-receptor affinity. Closed squares - direct contraction ($r=0.82$); open squares-sympatho-inhibition ($r=0.96$).



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Feniuk et al (1979) Br. J. Pharmacol., 67, 247-254.
Street et al (1995) Am. Chem. Soc. Meeting (Chicago), MEDI 140.

113P THE EFFECT OF MELATONIN AGAINST AGONIST-INDUCED AND NEUROGENIC CONTRACTIONS OF TAIL ARTERIES FROM JUVENILE WISTAR RATS

K. N. Ting., E Scalbert*, P. Delagrange* and V.G. Wilson. Department of Physiology and Pharmacology, The Queen's Medical Centre, Nottingham NG7 2UH. *IRIS, 6 place des Pleiades, 92415 Courbevoie Cedex, France

Several studies have reported that melatonin, can produce either direct vasoconstriction (Evans *et al.*, 1992) or enhance agonist-induced and electrically-evoked contractions of the rat isolated tail artery (Viswanathan *et al.*, 1990; Krause *et al.*, 1995) Direct comparison of the potency of melatonin in each of these studies is difficult, however, because of differences in the age and strain of the rats, the detection method adopted and the experimental condtions employed. In the present study we have examined the effect of melatonin against agonist-induced and neurogenic contractions of tail arteries from juvenile Wistar rats.

Male Wistar rats (65-120 g wt; housed in 12 hour light/dark cycle, lights on 8.00 am) were killed by decapitation and 2-3mm proximal and distal segments of the tail artery mounted in a wire myograph containing Krebs-Henseleit saline (34°C; 95% O_2/5% CO_2). All vessels were placed under 0.5 g wt resting tension, exposed to 60 mM KCl, and then stimulated with either 0.3 μM phenylephrine (PE), or a 10 sec train of electrical pulses (1-2 Hz, 80 volts, 0.3 ms pulse width) at 3-4 min intervals (neurogenic responses; Krause *et al.*, 1995). Once stable contractions were established, melatonin (0.1nM-1μM) was added cumulatively (against both responses) or non-cumulatively (against neurogenic contractions only). Responses to melatonin have been calculated as a percentage of pre-melatonin-induced tone, shown as the mean ± s.e.mean and used to determine the negative logarithm of the concentration producing 50% of the maximum response (pD_2). Differences between mean values have been compared using an unpaired Student's t-test ($p<0.05$)

PE (0.3μM) produced a sustained contraction in both proximal (20.2±5.3% of the KCl response; 1.49±0.15g wt, n=12) and distal (66.2±7.8% of the KCl response; 1.03±0.14 g wt, n=12) segments of the tail artery. Cumulative addition of melatonin caused concentration-dependent contractions in 6/12 proximal and 6/12 distal segments with similar potency (pD_2: 9.20±0.26 and 9.48±0.13 (n=6), respectively) and maximum response (65.6±23.6% (n=6) and 32.6±4.6% (n=6), respectively, of the PE-induced tone). Cumulative addition of melatonin to proximal segments caused a concentration-dependent enhancement of neurogenic responses in 4/7 preparations. Relative to the neurogenic contraction (36.3±4.2% of the KCl response), the maximum enhancement was 171±35.5% (n=4), with a pD_2 of 8.7±0.22 (n=4). Enhancement of neurogenic responses by high concentrations of melatonin (> 3nM) were transient, often declining by 50% within 10 min of exposure, while those to low concentrations were sustained. Non-cumulative application of melatonin (10 min exposure, 25 min interval between responses) also potentiated neurogenic contractions (maximum effect of 178±30.3% and pD_2 - 8.5±0.1, n=7) but was active in all 7 preparations examined.

Our data indicates that melatonin can enhance agonist-induced and neurogenic contractions in rat isolated tail artery; the former effect apparent in both distal and proximal segments. Differences in the number of melatonin-responsive preparations, magnitude of the maximum effect observed, and perhaps the estimated potency of melatonin, may be related to the development of receptor desensitization. We recommend non-cumulative application of melatonin for the study of vasoconstrictor responses to this hormone.

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Krause, D.N., Barrios, V.E. and Duckles, S.P. (1995). *Eur. J. Pharmacol.* **276**, 207-213.
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114P MIXED BLOCK OF K^+ AND Na^+ CURRENTS BY KC8851, A STRUCTURAL ANALOGUE OF TEDISAMIL: *IN VITRO* AND *IN VIVO* STUDIES

J. McLarnon, L. Xu, S. Abraham and M.J.A. Walker, Dept. of Pharmacology & Therapeutics, Faculty of Medicine, The University of British Columbia, Vancouver, B.C., Canada, V6T 1Z3

Tedisamil (KC8857) is a known bradycardic agent with predominant actions on K^+ channels (Dukes et al., 1990). We now report that KC8851, a structural analogue of tedisamil, produces mixed block of both K^+ and Na^+ currents both in vitro and in vivo. The whole-cell patch clamp method, using protocols previously described in studies on rat ventricular cells (McLarnon and Xu, 1995), has been used to determine interactions of KC8851 with outward transient K^+ current (Ito) and inward INa (McLarnon and Xu, 1995). As an in vivo index of electrophysiological actions of KC8851, an electrical stimulation technique has been used in pentobarbital anaesthetised rats as described previously (Walker & Beatch, 1990).

In isolated ventricular myocytes KC8851 diminished the inactivation time course of the transient outward K^+ current Ito with an EC_{50} value of 2.0 ± 0.4 μM (n=8). The drug actions were not due to changes in the voltage dependence of channel activation or inactivation but instead were consistent with blockade of open channels. The application of an open channel blockade model yielded blocking and unblocking rate constants of 9.3 x 10^6 M^{-1} s^{-1} and 19.3 s^{-1}, respectively, which were not dependent on potential. KC8851 was also found to inhibit peak sodium current (INa) with an estimated EC_{50} value of 4.2 ± 0.4 μM (n=5). The effects of KC8851 to block INa were enhanced in a use-dependent manner. These results show KC8851 to be a potent mixed blocker of both Ito and INa in rat ventricle.

In vivo (n=6 rats/dose), KC8851 widened the Q-T interval of the ECG (ED_{50} of 0.24 ± 0.2 μmol kg^{-1}) and prolonged the effective refractory period (ED_{50} of 0.19 ± 0.2 μmol kg^{-1}). These in vivo actions were consistent with KC8851 effects to block K^+ current. In addition KC8851 had actions on Na^+ currents. The compound increased the following; threshold current for capture (iT, ED_{50} = 1.7 ± 0.3 μmol kg^{-1}), threshold for ventricular fibrillation (VFt, ED_{50} = 0.13 ± 0.2 μmol kg^{-1}) and the sensitive measure of Na^+ blockade (RSh, ED_{50} = 0.49 ± 0.3 μmol kg^{-1}).

The combined results of the in vitro and in vivo studies show that KC8851 is a potent mixed blocker of both Ito and INa in rat ventricular myocytes and in whole animals. These results differ from those previously documented for the structural analogue of KC8851, tedisamil, since that compound was found to be a selective blocker of K^+ currents with negligible blockade of Na^+ current (Dukes et al., 1990).

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McLarnon, J. & Xu, R. (1995) J. Pharmacol. Exp. Thera. 275, 389-396.
Walker, M.J.A. & Beatch, G.N. (1990) Proc. West. Pharmacol. Soc. 33, 123-127.

115P ATYPICAL DOSE RESPONSE CURVES FOR ANTIARRHYTHMIC DRUGS

T.D. Barrett, S. Abraham, E.S. Hayes, S.L. Yong, M.L. Walker, M.J.A. Walker, Dept of Pharmacology and Therapeutics, UBC, Vancouver, B.C., Canada, V6T 1Z3.

For some antiarrhythmics a classic sigmoid antiarrhythmic dose response (DR) curve is seen but for others (e.g., quinidine and flecainide, Barrett et al., 1995) a bimodal relationship is found. Bimodal curves may be due to an interplay of proarrhythmic and antiarrhythmic actions for experimental Class I drugs. We report here two experimental Class I compounds, RSD944 and RSD995, which further illustrate that DR relationships for Class I compounds may not be well described by a sigmoidal dose response curve.

Ischaemic arrhythmias were induced in male, pentobarbital anaesthetized rats by occlusion of the left anterior descending artery (Barrett et al., 1995). Rats were artificially ventilated with oxygen at a rate of 60 breaths/minute at 10 ml/kg and test compounds were infused continuously starting five minutes before occlusion. Serum [K^+] and occluded zone size were similar for all groups. An arrhythmia score was used to summarise the arrhythmic history of each animal. The arrhythmia score for vehicle treated rats was 5.5 ± 0.5 (mean ± SEM, n=40) and data in the present study is given as percent decrease from this value due to various doses of compounds. RSD995 ((±)-trans-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl] 3,4-dichlorocinnamide hydrochloride) and RSD944 ((±) trans-N-[2-(1-pyrrolidinyl)cyclohexyl]benzo[b]thiophene-3-acetamide hydrochloride) were synthesised by RSD Ltd.

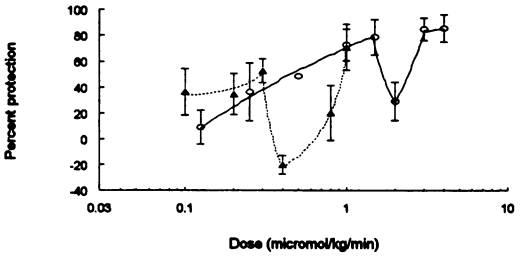

Figure 1. Antiarrhythmic dose response curve for RSD995 (circles/solid line) and RSD944 (triangles/broken line). Data is presented as the mean ± SEM and expressed as percent protection. Group sizes for each dose were n=6 for RSD995 and n=5 for RSD944.

Figure 1 shows the antiarrhythmic DR curve for RSD995 and RSD944. The curves for RSD995 and RSD944 were not sigmoidal. The eye line of best fit, shown in Figure 1, suggests a bimodal curve which may reflect an interplay of pro- and antiarrhythmic effects. At some doses sodium channel blockade prevents re-entry whereas at others it promotes re-entry.

RSD Ltd. (a subsidiary of Nortan Pharmaceuticals Inc.) is thanked for compounds and funds.

Barrett T.D., Hayes E.S., Walker M.J.A (1995) Eur. J. Pharmacol. 285, 229-238.

116P GLIBENCLAMIDE POSSESSES TRANSIENT, ISCHAEMIA SELECTIVE CLASS III ANTIARRHYTHMIC ACTIONS BUT DOES NOT PREVENT ISCHAEMIC ARRHYTHMIAS

T.D. Barrett & M.J.A. Walker, Dept. of Pharmacology & Therapeutics, UBC Vancouver, B.C., Canada, V6T 1Z3.

In vitro studies suggest that activation of the ATP dependent potassium current ($IK_{(ATP)}$) is responsible for shortening action potential duration during myocardial ischaemia (Wilde & Janse, 1994). Blockade of $IK_{(ATP)}$ may be antiarrhythmic by selectively prolonging action potential duration in the ischaemic myocardium (Wilde & Janse, 1994). In the present study we assessed the ischaemia selective Class III actions of glibenclamide and simultaneously assessed its antiarrhythmic actions in intact rabbits subject to acute myocardial ischaemia.

Male rabbits (2-3 kg) were anaesthetized with pentobarbital 50 mg/kg i.v. and artificially ventilated with oxygen. Body temperature was maintained at 37°C. Monophasic action potentials (MAPs) were recorded with Ag/AgCl contact electrodes from the epicardium. Glibenclamide or vehicle (5% DMSO/95% PEG-400) was given 20 min before occlusion. Blood pressure, lead II ECG, MAPs and the occurrence of arrhythmias were observed for 30 min after occlusion. Serum K+ and blood glucose concentration were measured before and after occlusion.

In vehicle treated rabbits blood glucose increased by 60% from pre-occlusion values of 5.0±0.4 mM (mean±SEM, n=14). The highest dose of glibenclamide prevented this time dependent increase. Serum K^+ was similar for all groups. Table 1 shows that glibenclamide had no antiarrhythmic effects. Effects of glibenclamide on MAPs was confined to a reduction in the maximum rate of action potential shortening induced by ischaemia. The magnitude of the ischaemia induced action potential shortening (measured 5 min after occlusion) was not influenced by glibenclamide at any of the doses tested. The lack of effect of glibenclamide on ischaemia induced arrhythmias may be attributed to a lack of electrophysiological effects in ischaemic tissue when arrhythmias occurred.

Wilde, A.A.M. & Janse M.J. (1994) *Cardiovasc. Res.* 28, 16-24.

Table I. Influence of glibenclamide on ischaemia induced arrhythmias and MAP duration changes. APD@5' is the action potential duration 5 min after occlusion as a percent of control, dAPD/dt is the maximum rate of action potential shortening, PVC, VT and VF indicate the incidence of premature ventricular contractions, ventricular tachycardia and fibrillation. Data are presented as the mean±s.e.mean (n=14 for vehicle and n=7 for treated groups). * equals statistical significance at $p<0.05$ (ANOVA with Turkey test for differences).

Dose	APD@5'	dAPD/dt	PVC	VT	VF
Vehicle	72±3%	2.4±0.4	13/14	5/14	8/14
3 mg/kg	69±6%	1.8±0.3	7/7	3/7	1/7
6 mg/kg	75±5%	1.5±0.5	5/7	3/7	1/7
12 mg/kg	58±11%	1.2±0.3	7/7	5/7	3/7
24 mg/kg	73±6%	0.9±0.2*	6/7	3/7	2/7

117P SAR EVIDENCE THAT ANTIARRHYTHMIC ACTIVITY IS UNRELATED TO OPIOID KAPPA AGONIST ACTIVITY

S.L. Yong, S. Abraham, M.K. Pugsley, E.S. Hayes, A.B. Zolotoy[1] & M.J.A. Walker, Dept of Pharmacology & Therapeutics, UBC, Vancouver, B.C., Canada, V6T 1Z3 and [1]RSD Ltd, Vancouver, B.C., Canada, V6S 2L2

We have previously reported that several opioid agonists ((-) PD129,290 and (+)PD129,289) confer antiarrhythmic actions unrelated to κ agonism (Pugsley et al., 1993). To further show that antiarrhythmic activity is independent of κ agonism, we present an SAR study of 10 arylbenzacetamides.

Production of arrhythmias in pentobarbitone-anaesthetised male rats were similar to those of Pugsley et al. (1993). Drugs were infused 5 mins before coronary-artery ligation and 3 mins before electrical stimulation measurements. Indices for detecting sodium (P-R, QRS, R-S height (Penz et al., 1992), iT-current threshold for induction of extrasystoles, Vft-current threshold for induction of ventricular fibrillo-flutter) and potassium (Q-T and ERP) current blockade were measured. These were calculated as D25% values (doses producing 25% change from control) and averaged into single values, Na^+ and K^+ (±s.e.mean)), respectively. Analgesia was studied in conscious mice using the tail-pinch method (Bianchi, 1952) 5 and 10 minutes after i.v. tail vein injection.

Results are listed in Table 1 and compounds are ranked most to least potent antiarrhythmic (AA_{50}). All compounds increased indices reflecting sodium and potassium channel blockade. Compounds 1-5 were equipotent in their effects, whereas 6-10 were less potent. Compounds 3, 4 and 7 were devoid of analgesic actions.

It is concluded that antiarrhythmic potency did not correlate with analgesic actions of the agents studied.

Funding and compounds provided by RSD Ltd. (a subsidiary of Nortran Pharmaceuticals Inc.).

Pugsley, M.K., Saint, D.A., Penz, M.P., Walker, M.J.A., (1993) *Br. J. Pharmacol.*, 110, 1579-1585.

Penz, M.P., Pugsley, M.K., Hsieh, M.Z., Walker, M.J.A. (1992) *J. Pharmacol. Methods.*, 27, 51-58.

Bianchi, C. (1952) *Br. J. Pharmacol.*, 11; 104-106.

Table 1: Pharmacological properties of 10 arylbenzacetamides. All values (n=5) are expressed in μmol/kg/min except for analgesia[a] (μmol/kg). *R-enantiomer; all others are racemates.

Compound	A	B	C	AA_{50}	ED_{50}[a]	Na^+	K^+
1	H	(-CH-CH-CH-CH-)		0.2	0.7	3.6±0.5	1.5±0.2
2	H	(-S-CH=CH-)		0.6	0.3	0.7±0.0	2.3±0.3
3*	H	(-S-CH=CH-)		0.6	none	1.6±0.2	2.3±0.4
4	3-benzothiophene			0.8	none	1.0±0.2	2.4±0.3
5	CF_3	H	H	0.9	2	2.8±0.3	4.0±0.6
6	H	H	H	2	14	6.9±1.1	6.7±1.4
7	OMe	OMe	H	2	none	9.7±0.5	10.7±0.8
8	NO_2	H	H	2	1.2	12.5±0.9	4.2±0.6
9	Br	H	H	2	1.2	11.4±1.0	13±1.2
10	NH_2	H	H	6	44	15.2±2.0	14.4±1.3

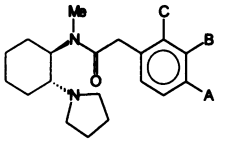

Figure 1: General Pharmacophore.

118P INCREASED ELECTROPHYSIOLOGICAL ACTIVITY IN RAISED K^+ AND LOW pH IMPROVES ANTIARRHYTHMIC EFFICACY FOR A GROUP OF MORPHOLINOCYCLOHEXYL DERIVATIVES

M.L. Walker, S. Abraham, S.L. Yong, A. Zolotoy[1], T.D. Barrett, & M.J.A. Walker, Dept of Pharmacology and Therapeutics, The University of British Columbia, Vancouver, B.C., Canada V6T 1Z3 and [1]RSD Ltd., Vancouver, B.C., Canada V6S 2L2

Antiarrhythmics whose actions are enhanced under conditions of ischaemia may show superior protection against arrhythmias due to myocardial ischaemia. For a group of morpholinocyclohexyl derivatives we have related antiarrhythmic effectiveness *in vivo* with ischaemic enhancement in isolated hearts. Due to the technical difficulties associated with determining drug potency in true ischaemia, we used a perfusate containing elevated K^+ and H^+ concentrations to model the conditions of ischaemia.

The Langendorff technique was used for hearts from male Sprague-Dawley rats (200-300g). The perfusate contained (in mM) NaCl, 121; KCl, 3.4; $MgSO_4$, 1.2; PIPES, 13.9; glucose, 11.1; $CaCl_2$, 2.5; and pH=7.4. In the test perfusate K^+ was 10 mM and pH=6.4. The ECG was measured and dose-response curves were constructed for effects in both solutions. A ratio of potencies (test/normal, or T/N) was calculated on the basis of D25% (dose required to produce a 25% change from control). The lower the value of T/N, the more potent the drug in high K^+ and low pH. For the *in vivo* experiments male Sprague-Dawley rats (200-300g) were anaesthetized with pentobarbitone and occlusion of the left anterior descending coronary artery was carried out as described in Barrett et al (1995). ED_{50} values for antiarrhythmic activity (AA) and depression of blood pressure were determined for each drug and the therapeutic index (T.I.) was calculated.

The structures and results are shown in Table 1. The data suggest that drugs whose activity is enhanced by certain conditions of ischaemia (in this case hyperkalemia and acidosis) show superior antiarrhythmic potency against coronary artery occlusion-induced arrhythmias and have a greater therapeutic index.

RSD Ltd./Nortran is thanked for compounds and funding.

Barrett, T.D., Hayes, E.S., & Walker, M.J.A. (1995) *Eur. J. Pharmacol.* 285, 229

Compound	-R	T/N	ED_{50}±SEM AA (μmol/kg/min)	ED_{50}±SEM BP (mmHg)	T.I.
RSD1019	*p*-bromophenyl	0.01	2.5±0.3	71±1	0.04
RSD1018	7-benzo[b]thiophene	0.03	3.7±0.1	37±6	0.10
RSD1009	2-naphthalene	0.15	5.7±2.1	70±16	0.08
RSD1010	phenyl	0.26	7.1±1.1	34±1	0.21
RSD1014	*p*-nitrophenyl	1.0	NE*	36±7	–

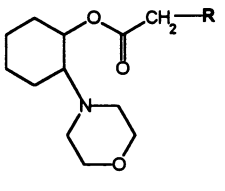

Table 1: Comparison of potency ratio *in vitro* (T/N) with antiarrhythmic activity, blood pressure lowering, and therapeutic index. *NE = not estimable due to poorly defined dose-response curve

119P RSD1000: A NOVEL ANTIARRHYTHMIC AGENT WITH AN IMPROVED THERAPEUTIC INDEX

S.L. Yong, S. Abraham, M. Walker, & M.J.A. Walker, Dept of Pharmacology & Therapeutics, UBC, Vancouver, BC, Canada, V6T 1Z3

We are studying antiarrhythmic drugs which have selective actions in ischaemic cardiac tissue with the aim of improving therapeutic indices. This study reports one such agent, RSD1000[(±)-trans-[2-(4-morpholinyl)cyclohexyl]naphthalene -1-acetate.HCl].

Antiarrhythmic action was assessed in intact, pentobarbitone-anaesthetised male rats (n=5) subjected to either electrical stimulation of the left ventricle or coronary artery occlusion. Drug treatment was a continuous infusion given 5 minutes prior to electrical stimulation and pre-occlusion. In isolated rat hearts (n=5), ECG changes induced by RSD1000 in a normal buffer and in a buffer with raised $[H^+]$ and $[K^+]$ were measured. The time between drug concentrations was 3 minutes. Effective concentrations (μM) producing 25% change of P-R interval from control in "ischaemic"(T) and normal (N) buffers, was expressed as T/N.

An arrhythmia score (AS) was used to summarize the arrhythmia history of each animal in the occlusion study. Figure 1 shows that RSD1000 had a dose-related antiarrhythmic activity. Higher doses were required to protect against electrically-induced ventricular fibrillation (VFt) or decreased blood pressure (↓BP) (Table 1). This was a better profile of activity than standard antiarrhythmics, see Barrett et al, (1995).

Figure 1: Antiarrhythmic Dose-Response Curve for RSD1000

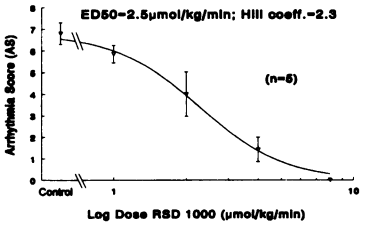

Table 1: Comparison between RSD1000 and Class I Agents.

	AA_{50}	T/N	$\downarrow BP_{ED50}$	$T.I._{BP}$	$\uparrow VFt_{ED50}$	$T.I._{VFt}$
RSD1000	2.5	0.0±0.7	10.2±2.0	0.3	8.8±2.2	0.3
Lidocaine	5.4	0.0±0.5	5.4±1.0	1.4	4.6±1.3	1.1
Quinidine	5.8	0.7±1.1	2.4±0.6	2.4	1.5±0.4	3.9
Flecainide	10.0	0.7±0.9	1.5±0.5	6.7	1.4±0.3	7.1

RSD1000 had a better therapeutic index (T.I.) than the standard agents. This may be related to the fact that RSD1000 is 100 times more potent in ischaemia-like conditions. Possibly, RSD1000's actions in the ischaemic zone selectively prevents arrhythmias without depressing blood pressure or VFt.

Funding and compound provided by RSD Ltd. (a subsidiary of Nortran Pharmaceuticals Inc.).

Barrett, T.D., Hayes, E.S., Walker, M.J.A., (1995) *Eur. J. Pharmacol.*, 285, 229-238.

120P ANTIHISTAMINE-INDUCED VENTRICULAR ARRHYTHMIAS

G.N. Beatch[1,2] (intr. by M.J.A. Walker), [1]University of Ottawa Heart Institute and [2]Bureau of Drug Research, Drugs Directorate, Health Protection Branch, Ottawa, Canada K1A 0L2

Terfenadine has been associated with prolongation of QT intervals and *torsades de pointes* (Davies *et al.*, 1989) which may be related to blockade of outward potassium currents underlying cardiac repolarization (Woosely *et al.*, 1993). The proarrhythmic potential of the antihistamines, terfenadine, fexofenadine, and diphenhydramine, were compared to vehicle and dofetilide, a class III antiarrhythmic drug, in a rabbit model of drug-induced *torsades de pointes* (Carlsson *et al.*, 1990). Methods & Results: Sixty-eight male NZW rabbits (2.5-3.5 kg) were used. Anaesthesia was induced with methohexital (5 mg/kg IV), and maintained with α-chloralose (90 mg/kg + 10 mg/kg/h IV). Rabbits were artificially ventilated with 50% O_2 in medical air. Lead II surface ECG, and carotid arterial blood pressure were recorded digitally at 1000 samples/s. Initial studies determined the chance of inducing ventricular tachycardia (VT) during IV infusion of $Terf_{LOW}$ (terfenadine, 4-40 nmol/kg), Fex_{LOW} (fexofenadine, 40-400 nmol/kg) or $Diph_{LOW}$ (diphenhydramine, 40-400 nmol/kg) compared to vehicle (1-10% DMSO in PEG200), with n=7 per group. Subsequently, the risk for VT induction was assessed for infusions of $Terf_{HIGH}$ (terfenadine, 0.1-1 μmol/kg), Fex_{HIGH} (fexofenadine, 1-10 μmol/kg) or $Diph_{HIGH}$ (diphenhydramine, 0.1-10 μmol/kg) compared to Dofetilide (0.02-0.2 μmol/kg), with n=10 per group. During 30 min IV infusions in combination with the α_1-agonist, methoxamine (20 μg/kg/min), Fex_{LOW}, $Diph_{LOW}$ and vehicle each induced VT in 1/7 rabbits. Dofetilide induced VT in 7/10 rabbits, $Terf_{HIGH}$ and $Diph_{HIGH}$ induced VT in 1/10 rabbits each, while Fex_{HIGH} induced VT in 3/10 rabbits. In these rabbits in which VT occurred, the dose of drug delivered at the time of VT was: $Terf_{HIGH}$, 0.9 μmol/kg (plasma level 160 ng/ml); Fex_{HIGH}, 0.7, 5.2 and 1.8 μmol/kg (plasma levels 0.8, 3.2 and 2.3 μg/ml); $Diph_{HIGH}$, 0.06 μmol/kg; and dofetilide 0.013±0.06 μmol/kg. At the time of VT, QTc intervals ($QTc=QT/\sqrt{RR}$) were: $Terf_{HIGH}$, 350 $ms/s^{-½}$; Fex_{HIGH}, 340 $ms/s^{-½}$; $Diph_{HIGH}$, 370 $ms/s^{-½}$; and dofetilide 430±72 $ms/s^{-½}$. Conclusions: These preliminary results suggest that fexofenadine may be associated with an increased incidence of proarrhythmia in this model compared with terfenadine and diphenhydramine, but that this risk is relatively small compared with dofetilide.

Carlsson, L., Almgren, O., Duker, G. (1990) *J. Cardiovasc. Pharmacol.* 16, 276-285.
Davies, A.J., Harinda, V., McEwan, A. *et al.* (1989) *Br. Med. J.* 298, 325.
Woosely, R.L., Chen, Y., Frieman, J.P. *et al.* (1993) *JAMA* 269, 1532-1536.

121P THE EFFECTS OF HALOFANTRINE ON HAEMODYNAMICS AND ECG INTERVALS IN THE ANAESTHETIZED GUINEA-PIG

A.J. Batey, J.P. Lambert, G. Edwards & S.J. Coker, Department of Pharmacology & Therapeutics, University of Liverpool, Ashton Street, Liverpool L69 3BX.

There has been recent concern over the potential cardiac side effects of halofantrine (Nosten *et al.*, 1993). Adverse effects can include QT interval prolongation and torsade de pointes arrhythmias. We have investigated the effects of halofantrine *in vitro* (Lightbown *et al.*, 1996) and this study was designed to examine the effects of halofantrine in an *in vivo* animal model. Male Dunkin-Hartley guinea pigs (375 to 540 g) were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.p.) and a carotid artery and jugular vein were cannulated, for blood pressure measurement and drug administration, respectively. The ECG was recorded from a lead I or II signal. The trachea was cannulated for ventilation with room air. Halofantrine was given as an i.v. formulation in dimethyl acetamide (40%)/propylene glycol (60%) v/v, diluted as required in 5% w/v glucose solution, and administered as five consecutive bolus doses (0.3, 1, 3, 10 and 30 mg kg^{-1}). A separate control group received equivalent volumes of vehicle. Animals were allowed to stabilize for 20 min before the first dose of halofantrine or vehicle was given. Twenty min after each dose of drug, approximately 200μl of arterial blood was withdrawn for later analysis of halofantrine levels by h.p.l.c. (Mberu *et al.*, 1993). Subsequent doses of halofantrine were administered 25 minutes after the previous dose. Data for each parameter were recorded for pre-drug values, and at 5, 10, 15 and 20 min post drug. ECG traces were also studied qualitatively for changes in morphology.

Blood pressure, PR interval, and QRS duration were all unchanged by halofantrine. A progressive bradycardia was seen, which became significant after 10 mg kg^{-1} (33$\pm$11 beats min^{-1} compared with 3$\pm$8 beats min^{-1} in controls, $P<0.05$). Significant changes in the rate corrected QT interval (QTc) were evident after 1 mg kg^{-1} halofantrine (Figure 1). QRS complex changes and AV block were seen in all animals at high doses. Typically, this took the form of second degree type I Mobitz (Wenkebach) block. After 0.3, 1, 3, 10 and 30 mg kg^{-1} halofantrine concentrations were 137$\pm$92, 152$\pm$94, 254$\pm$188, 422$\pm$145 and 1500$\pm$468 ng ml^{-1} respectively.

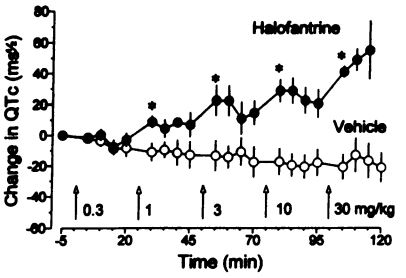

Figure 1. Change in QTc interval (mean $\pm$ s.e. mean, n=6 per group). *$P<0.05$, compared to vehicle, Kruskal-Wallis analysis of values measured 5 min after each dose of drug only.

The blood halofantrine levels are similar to those reported clinically (Karbwang *et al.*, 1993), and AV block has been reported in patients receiving halofantrine (Nosten *et al.*, 1993). The low heart rates associated with AV block can be a risk factor for ventricular tachycardias (e.g. torsade de pointes). This study suggests that the guinea pig may be a suitable model for studying the cardiotoxicity of halofantrine.

Supported by SmithKline Beecham Pharmaceuticals Ltd.

Lightbown, I.D., Batey, A.J. & Coker, S.J. (1996) This meeting.
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Mberu, E.K., Muhia, D.K. & Watkins, W.M. (1992) *J. Chromatogr.* 581, 156-160.
Nosten, F., ter Kuile, F.O., Luxemburger, C., *et al.*, (1993) *Lancet*, 341, 1054-1056.

122P EFFECTS OF HALOFANTRINE ON THE EFFECTIVE REFRACTORY PERIOD IN GUINEA-PIG ISOLATED CARDIAC TISSUES

I.D. Lightbown, A.J. Batey & S.J. Coker, Department of Pharmacology and Therapeutics, University of Liverpool, Ashton Street, Liverpool L69 3BX

Treatment with the antimalarial, halofantrine has been associated with a prolonged QTc interval (Nosten *et al.*, 1993) and the potentially lethal cardiac arrhythmia torsade de pointes (Monlun *et al.*, 1993). Since the mechanisms underlying these cardiac effects of halofantrine are not understood clearly at present, we have examined whether halofantrine has any direct actions on cardiac tissues. Male Dunkin-Hartley guinea-pigs (285 to 665g) were killed by a blow to the head followed by exsanguination. Isolated left atria, left papillary muscles and right ventricular strips were dissected out and suspended under a resting tension of 1g in modified Krebs solution at 37°C, gassed with 95%O_2/CO_2. Tissues were stimulated at 1Hz, 5ms pulse width, twice threshold voltage and tension was measured isometrically. The effective refractory period (ERP) was measured by the interpolation of extra stimuli. In the first study, increasing concentrations of halofantrine (1, 3 and 10μM), dissolved in DMSO, were added to the same tissues which were washed between addition of each concentration at 1h intervals. To overcome solubility problems with halofantrine, a modified vehicle (0.1% Tween 80 in DMSO) was used in a second study on halofantrine (10, 30 and 100μM). In Study 2, each preparation was exposed to only one concentration of halofantrine and a time-matched vehicle control group was included. In all experiments in both studies the final concentration of DMSO was 1%.

The pre-drug ERP measured from left papillary muscles in Study 1 was 163 $\pm$ 3ms, and 50 min after addition of halofantrine (1, 3 and 10μM) ERP increased to 168 $\pm$ 4*, 173 $\pm$ 5* and 173 $\pm$ 5*ms, respectively; *$P<0.05$, compared to pre-drug value, Wilcoxon test. Similar increases in ERP were seen with higher concentrations of halofantrine in study 2 but the vehicle also increased ERP significantly (Table 1). In right ventricular strips, similar increases in ERP were observed with halofantrine in both studies, and with vehicle in study 2. Neither halofantrine nor vehicle altered ERP in left atria.

Table 1. ERP values (ms) measured from left papillary muscles before and 60 min after addition of vehicle or halofantrine.

	Vehicle	*10μM*	*30μM*	*100μM*
Pre	164 $\pm$ 5	161 $\pm$ 4	156 $\pm$ 8	165 $\pm$ 6
Post	173 $\pm$ 6*	180 $\pm$ 3*	174 $\pm$ 6*	179 $\pm$ 5**

Values are mean $\pm$ s.e.mean (n=6-10 per group) *$P<0.05$, **$P<0.01$ compared to pre-treatment values, Wilcoxon test. There were no significant differences between treatment groups at either time point, Kruskal-Wallis analysis.

These studies suggest that the increases in ventricular ERP observed in the presence of halofantrine, may be vehicle or time-dependent effects. Although we have shown that halofantrine (but not vehicle) caused significant QTc prolongation *in vivo* (Batey *et al.*, 1996) the above data suggests that corresponding changes in ERP are difficult to separate from vehicle or time-dependent effects *in vitro*. Thus future studies on the mechanisms underlying the cardiac toxicity of halofantrine will utilise *in vivo* models.

Supported by SmithKline-Beecham Pharmaceuticals Ltd.

Batey, A.J., Lambert, J.P., Edwards, G. & Coker, S.J. (1996). This meeting.
Monlun, E., Pillet, O., Cochard, J.F. *et al.*, (1993). *Lancet* 341, 1541-1542
Nosten, F., ter Kuile, F.O., Luxemburger, C., *et al.*, (1993). *Lancet*, 341, 1054-1056.

123P AN AUTORADIOGRAPHIC STUDY OF [^{125}I]-DIPHENYLENEIODONIUM SULFATE LABELLING OF RAT AORTIC PROTEINS INVOLVED IN THE BIOTRANSFORMATION OF GLYCERYL TRINITRATE

J.J. McGuire & B.M. Bennett. Department of Pharmacology & Toxicology, Queen's University, Kingston, Ontario, Canada. K7L 3N6

We have reported previously that the flavoprotein inhibitor diphenyleneiodonium sulfate (DPI) inhibits glyceryl trinitrate (GTN)-induced relaxation in isolated rat aorta by inhibiting the metabolic activation of GTN to NO (or an NO-like compound) in a manner that is partially irreversible. We hypothesized that this irreversible inhibition may have occurred by formation of a DPI-adduct with vascular NADPH-cytochrome P450 reductase. Our experiments using cloned, expressed and purified rat liver NADPH-cytochrome P450 reductase and using aortic microsomes have demonstrated that DPI causes a time-dependent and concentration-dependent inhibition of NADPH-cytochrome P450 reductase activity.

In the current study we used SDS PAGE and autoradiography to examine the formation of ^{125}I-DPI adducts with proteins obtained from endothelium denuded isolated rat aorta exposed to 1 μM ^{125}I-DPI. We observed the consistent appearance of 8 radiolabelled proteins from the 105,000xg pellet of homogenized rat aorta (M_r= 10, 15, 25, 37, 52, 66, 79, 83 kDa). We also observed an NADPH-dependent formation of ^{125}I-DPI adducts with two proteins (M_r = 50 and 79 kDa) after exposure of aortic microsomes to 0.3-10 μM ^{125}I-DPI. Radioisotope labelling of a 79 kDa protein would be consistent with the formation of an ^{125}I-DPI adduct with NADPH-cytochrome P450 reductase. Using cloned, expressed and purified rat liver NADPH-cytochrome P450 reductase (kindly supplied by Dr. B.S.S. Masters, University of Texas at San Antonio) we observed an NADPH-dependent formation of ^{125}I-DPI protein adducts. Given that the 79 kDa radiolabelled aortic microsomal protein comigrates with ^{125}I-DPI labelled NADPH-cytochrome P450 reductase, and immunoreacts with rabbit anti-rat liver NADPH-cytochrome P450 reductase antibody as determined by immunoblotting techniques, we conclude that the 79 kDa band is NADPH-cytochrome P450 reductase. Although the identity of the 50 kDa microsomal protein is unknown, its size is consistent with the cytochromes P450.

We have assessed whether aortic microsomes or purified cytochrome P450 reductase biotransform GTN by measuring the formation of its dinitrate metabolites glyceryl-1,2-dinitrate (1,2-GDN) and glyceryl-1,3-dinitrate (1,3-GDN) using megabore capillary column gas liquid chromatography. Under anaerobic conditions, incubation of 0.2 μM GTN and 1 mM NADPH with 5 μg/ml purified enzyme for 1 hr resulted in the formation of 35 ± 12 nM 1,2-GDN and 31 ± 23 nM 1,3-GDN. Incubation of enzyme with 100 μM NADPH and 10 μM DPI for 15 min prior to exposure to 0.2 μM GTN resulted in complete inhibition of GTN biotransformation (n=4, $p<0.05$, one-way ANOVA, Newman-Keuls post-hoc test). Similar results were obtained using aortic microsomes. Incubation of 0.2 μM GTN and 1 mM NADPH with 0.5 mg/ml microsomal protein for 1 hr resulted in the formation of 31± 16 nM 1,2-GDN and 19 ± 8 nM 1,3-GDN. Pretreatment of microsomes with 100 μM NADPH and 10 μM DPI for 30 min resulted in complete inhibition of GTN biotransformation (n=4, $p<0.05$, Student's t test for paired data).

Taken together, these data are consistent with the hypothesis that the mechanism of action of DPI with respect to inhibition of GTN-induced relaxation is due, at least in part, to covalent modification of NADPH-cytochrome P450 reductase, resulting in inhibition of enzyme activity.

(Supported by the Heart and Stroke Foundation of Ontario and the Pharmacological Society of Canada).

124P ENANTIOSELECTIVE INHIBITION OF THE BIOTRANSFORMATION AND PHARMACOLOGICAL ACTIONS OF ISOIDIDE DINITRATE BY DIPHENYLENEIODONIUM SULFATE

J.D. Ratz and B.M. Bennett. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, CANADA. K7L 3N6

Biotransformation of organic nitrates to an activator of guanylyl cyclase (presumably NO or some closely related species) appears to be prerequisite for their vasodilator activity. We have shown previously that the D- and L-enantiomers of isoidide dinitrate (D-IIDN and L-IIDN) exhibit a potency difference for relaxation and cyclic GMP accumulation in isolated rat aorta and that this is related to preferential biotransformation of the more potent enantiomer (D-IIDN) (Bennett *et al.* 1986). Other studies have suggested a role for the cytochromes P450-NADPH-cytochrome P450 reductase system in the biotransformation of organic nitrates; the flavoprotein inhibitor, diphenyleneiodonium sulfate (DPI), inhibits the biotransformation and pharmacological actions of glyceryl trinitrate (McGuire *et al.* 1994), and inhibits aortic NADPH-cytochrome P450 reductase activity.

The objective of the current study was to examine the effect of DPI on the enantioselectivity of IIDN action. In isolated rat aortic strip preparations, exposure to 0.3μM DPI resulted in a 3.6-fold increase in the EC_{50} value for D-IIDN-induced relaxation ($P<0.001$, One-way ANOVA), but had no effect on the EC_{50} value for L-IIDN-induced relaxation ($P>0.05$, One-way ANOVA). The EC_{50} values for D- and L-IIDN-induced relaxation in the presence of DPI were not different. Vascular biotransformation of IIDN to its mononitrate metabolite, isoidide mononitrate (IIMN), was assessed using megabore capillary column gas-liquid chromatography. In control aortic strip preparations incubated with 2μM D- or L-IIDN for 5 minutes, there was significantly more D-IIMN formed (5.0 ± 1.5 pmol/mg protein) than L-IIMN (2.1 ± 0.7 pmol/mg protein). Exposure to 0.3μM DPI resulted in a significant 2.2-fold decrease in D-IIMN formation to 2.3 ± 0.6 pmol/mg protein, but had no effect on L-IIMN formation. IIDN-induced cyclic GMP accumulation was assessed at a concentration of each enantiomer that resulted in 60-80% relaxation of the isolated rat aortic strip preparation (2μM D-IIDN and 10μM L-IIDN). Under these conditions, cyclic GMP accumulation was increased 2.0-fold and 1.8-fold for D- and L-IIDN, respectively. Consistent with both the relaxation and biotransformation data, treatment of tissues with 0.3μM DPI significantly inhibited D-IIDN-induced cyclic GMP accumulation, but had no effect on L-IIDN-induced cyclic GMP accumulation.

These data suggest that the basis for the potency difference for relaxation by the two enantiomers is preferential biotransformation of D-IIDN to NO, by an enzyme that is inhibited by DPI. Given that DPI binds to and inhibits rat aortic NADPH-cytochrome P450 reductase, the data are consistent with a role for the cytochromes P450-NADPH-cytochrome P450 reductase system in this enantioselective biotransformation process. The data also suggest that other metabolic activation processes for organic nitrates exist, since biotransformation, relaxation and cyclic GMP accumulation by L-IIDN were unaffected by DPI.

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125P NITRIC OXIDE (NO) DOES NOT MEDIATE THE WITHDRAWAL-INDUCED ANTIHYPERTENSIVE EFFECT OF VASOPRESSIN (AVP) IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

R. Tatchum Talom & J.R. McNeill, Dept of Pharmacology & C.R.F.R.U., Univ. of Saskatchewan, Saskatoon, Sk., Canada.

We reported previously that cessation of a 3 hour infusion of AVP induces a dramatic fall in blood pressure (BP) below pre-infusion control values in SHR, but not in normotensive rats (Wang & McNeill, 1994). This withdrawal-induced antihypertensive phenomenon (WAP) appears to be specific to AVP as it is not observed with other vasoconstrictrors (Chiu & McNeill, 1985). However, the mechanisms by which the WAP occurs remain unclear. The present study investigated the contribution of NO in the WAP.

Male SHR (16-20 week-old) were treated with (1) L-Arginine (SHR/L-Arg; 1.25 g l^{-1}, 2 weeks), (2) N^{w}-nitro-L-arginine (SHR/L-NNA; 0.05 g l^{-1}, 1 week), (3) Methylene blue (SHR/MB; 0.05 g l^{-1}, 2 weeks), or (4) indomethacin (SHR/Indo; 0.065 g l^{-1}, 1 week). All drugs were given P.O. in drinking water. Control SHR (SHR/CON) and Wistar Kyoto (WKY) rats received tap water.

At the end of each treatment and under ether anaesthesia, PE 50 catheters were implanted into the left femoral artery (for BP measurement) and vein (for AVP infusion). Twenty four hours later, changes in BP (Δ BP, mmHg) before (I), during (II) and after (III) a 3 h infusion of AVP (20 ng kg^{-1} min^{-1}) were recorded in conscious unrestrained rats.

Results are expressed as mean ± s.e.mean (*; $ = $p < 0.05$, ANOVA and Scheffe test; n = 7/group).

Table 1. Basal BP values prior to AVP (BP, mmHg; I) and maximal changes in BP (Δ BP, mmHg) during (II) and after (III) AVP infusion (* = $p < 0.05$, *vs* WKY; $ = $p < 0.05$, *vs* SHR/CON; ANOVA and Scheffe test).

	BP (mmHg)	Δ BP (mmHg)	
	(I)	(II)	(III)
WKY	97.0±4.0	+ 37.0±4.6	- 19.0±4.8
SHR/CON	160.0±3.9*	+ 46.3±3.4*	- 47.5±6.3*
SHR/L-Arg	161.0±7.7*	+ 43.8±4.5	- 48.1±7.5*
SHR/L-NNA	195.0±7.2*$	+ 24.4±3.5$	- 67.2±7.3*$
SHR/MB	156.9±3.4*	+ 38.8±2.6	- 43.7±7.5*
SHR/Indo	155.0±4.5*	+ 38.6±3.6	- 41.6±5.4*

Cessation of three hour infusion of AVP was followed by a large fall in BP in SHR, but not in WKY. L-Arg, MB, or Indo did not affect the WAP. L-NNA exaggerated the WAP. Because the amplitude of the WAP depends on the pre-infusion BP level (Chiu & McNeill, 1989), we suggest that the L-NNA-induced exaggeration of the WAP is due to the high pre-infusion BP. In summary, our data suggest that NO does not mediate the WAP in SHR.

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126P THE EFFECTS OF 3-MORPHOLINOSYDNONIMINE UPON LOCAL CEREBRAL BLOOD FLOW IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

I.P. Fouyas, P.A.T. Kelly, I.M. Ritchie & I.R. Whittle, Department of Clinical Neurosciences, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU.

Essential hypertension is associated with attenuated endothelium-dependent vasodilatation and reduced nitric oxide (NO) bioactivity (Calver et al., 1992). However there is evidence of heterogeneity in the responsiveness of blood vessels from different vascular beds. In the present study we have examined the effects of an NO donor, 3-morpholino-sydnonimine (SIN-1), upon local cerebral blood flow (LCBF) in normotensive and spontaneously hypertensive rats (SHR).

Experiments were performed on conscious, lightly restrained, age-matched SHR (n=8) and normotensive rats (n=17). Normotensive rats were infused (i.v.) with SIN-1 (1.8 or 3.6mg.kg^{-1}.h^{-1} n = 6 and 5 respectively), or an equal volume of saline vehicle (40µl.min^{-1}, n = 6). After 20min infusion LCBF was measured using the quantitative [^{14}C]-iodoantipyrine autoradiographic technique (Sakurada et al., 1978). SHR's were infused with either 1.8mg.kg^{-1}.h^{-1} SIN-1 (n = 4), or saline (n = 4). Mean arterial blood pressure (MABP) was measured throughout the experiments, and blood gases were measured prior to any treatment, and again immediately before the measurement of LCBF. Data (presented as mean ± s.e.m.) were analysed using t-test with the Bonferroni correction for multiple comparisons. Acceptable levels of significance were set at $P<0.05$.

In normotensive rats, the lower dose of SIN-1 produced a small (-11%), but significant decrease in MABP, from 131 ± 3mmHg to 117 ± 3mmHg, and LCBF was increased significantly in 11 out of the 12 brain cerebellar, hippocampal and neocortical areas examined. Increases were most marked (+69%) in the paramedian lobule of the cerebellum (from 80 ± 4 to 135 ± 9ml.$100g^{-1}$.min^{-1}) and least marked (+23%) in white matter tracts (from 40 ± 2 to 49 ± 2ml.$100g^{-1}$.min^{-1}). A smaller (+15%) increase in striatum was not significant.

In the SHR group, the same dose of SIN-1 produced a 35% reduction in MABP (from 159 ± 12 to 104 ± 5mmHg), which was greater than that in normotensive rats. However, within the cerebrovascular bed, SIN-1 had no significant effect upon LCBF.

In normotensive rats, the higher dose of SIN-1 reduced MABP to levels (101 ± 2mmHg) comparable with the SIN-1-treated SHR group. There were no significant differences in LCBF between the two doses of SIN-1 in either cerebellar or neocortical areas, but in hippocampus the increases observed at the lower dose in dentate (from 82 ± 5 to 106 ± 6ml. $100g^{-1}$.min^{-1}; +29%) and CA2/3 (from 77 ± 5 to 97 ± 3ml.$100g^{-1}$.min^{-1}; +26%) were enhanced (+61 and + 38% from control values respectively). Again, in striatum, there was no significant difference from control (+9%).

These results indicate that whilst exogenous NO acts upon peripheral blood vessels to effect reductions in MABP which are greater in SHR than in normotensive rats, the response of cerebral blood vessels to SIN-1 is attenuated in SHR. This study confirms that NO-induced vasodilatatory capacity is not uniform in different vascular beds of SHR.

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127P ALPHA-PRESSOR RESPONSES IN PITHED RATS AFTER THE INHIBITION OF NITRIC OXIDE SYNTHESIS

M.A. Aleixandre, V. López-Miranda, M. Puerro, & A. Ortega (Introduced by J. Tamargo) Dpto. Farmacología. Fac. Medicina, U. Complutense, 28040 Madrid, España.

According to Zanzinger et al. (1994) vasodilation by nitric oxide (NO) may involve specific actions on α-adrenergic constrictor mechanisms, and Maclean et al. (1994) have recently shown that acute administration of the inhibitor of NO synthase N$^{\omega}$-nitro-L-arginine methylester (L-NAME) induces a potentiation of sympathetic vasoconstriction evoked by electrical nerve stimulation in pithed rats.

We study the effect of the selective α_1- and the selective α_2-adrenoceptor agonists, methoxamine (10-3000 μg/kg) and B-HT 920 (3-1000 μg/kg), on control, acutely (100 mg/kg), and chronically (100 mg/kg/day in drinking water for 7 or 21 days) L-NAME-treated pithed rats. The animals were pithed according to the method of Shipley and Tilden (1947), and the increases in the systolic (SBP) and the diastolic (DBP) arterial blood pressure were measured using a Panlab 4C Datasystem. Results are expressed as mean values ± s.e. mean for 6-8 rats. α-Adrenoceptor agonist dose-response curves were constructed, and the effect of the treatments was expressed as the area under each dose-response curve (AUC), taking the AUC for the control mean values as 100. For comparison of the areas Student's t-test was used (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

Methoxamine and B-HT 920 increased the SBP and the DBP in a dose-dependent manner in pithed rats. The increases were similar for both agonists in control and acutely L-NAME-treated rats. However, the chronic treatment with L-NAME caused a clear reduction of the pressor effect caused by both α-adrenoceptor agonists, and their dose-response curves were shifted to the right in a non-parallel manner. In the case of B-HT 920 the shift was clearly greater when the treatment was longer (see table 1). Our results suggest that the acute inhibition of NO synthesis did not alter the vasoconstrictor effect mediated by the stimulation of vascular α_1- and α_2-adrenoceptors, but the continuous modification of vascular tone caused by a chronic inhibition of its synthesis could provoke a compensatory decrease in the vasoconstrictory mechanisms elicited by the stimulation of such receptors.

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TABLE 1. AUC of the increases in SBP (AUC-SBP) and DBP (AUC-DBP) caused by α-adrenoceptor agonists in L-NAME-treated pithed rats.

		100 mg/kg L-NAME		
	Control	Acute	7 days	21 days
Methoxamine				
AUC-SBP	100.0 ± 4.1	95.1 ± 2.3	57.6 ± 1.9***	55.1 ± 1.4***
AUC-DBP	100.0 ± 5.6	111.0 ± 4.2	63.4 ± 3.6***	56.8 ± 2.4***
B-HT 920				
AUC-SBP	100.0 ± 6.8	112.7 ± 6.4	68.8 ± 7.3**	33.7 ± 6.7***
AUC-DBP	100.0 ± 7.8	125.6 ± 10.1	73.1 ± 7.0*	32.7 ± 8.5***

128P REGULATION OF ENDOGENOUS NITRIC OXIDE VASODILATOR FUNCTION BY INSULIN *IN VITRO*

D.W. Laight, A.V. Kaw, M.J. Carrier & E.E. Ånggård
The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London, EC1M 6BQ.

Vasodilation to insulin *in vivo* may contribute to the metabolic responsiveness to this hormone by enhancing glucose delivery to insulin-sensitive tissues (Baron, 1994). Indeed, a defect at the level of insulin-mediated vascular function could more fully account for insulin resistance *in vivo* (Wiernsperger, 1994). This regulation of vascular tone by insulin may be nitric oxide (NO)-dependent (Steinberg *et al.*, 1994; Sobrevia *et al.*, 1996). Our aim was to assess the role of insulin in the regulation of both agonist-stimulated and basal endogenous NO vasodilator function in the rat aorta *in vitro*.

Male Wistar rats (250-300 g) were anaesthetised with pentobarbitone sodium (60 mg/kg i.p.), euthanised and thoracic aortic rings mounted under isometric conditions in Krebs-Henseleit solution (composition in mM: NaCl 133; KCl 4.7; NaH_2PO_4 1.35; $NaHCO_3$ 16.3; $MgSO_4$ 0.61; $CaCl_2$ 2.52; glucose 7.8) gassed with carbogen and warmed to 37°C. Rings were exposed to human insulin (100 nM) or vehicle (0.1% (w/v) bovine serum albumin) for 20 min and then either (i) precontracted with noradrenaline (100 nM) to assess vasorelaxation to acetylcholine (ACh, 1 nM-1 μM) or sodium nitroprusside (SNP, 1-300 nM) or (ii) contracted with phenylephrine (PEP, 1 nM-10 μM) with or without a 10 min pretreatment with N^{G}-nitro-L-arginine methyl ester (L-NAME, 0.3 mM) to assess modulation by basal NO. Data are mean±s.e. mean (compared using Student's paired t-test).

Insulin increased the area under the concentration-response curve (AUC) for ACh from 109.1±12.2 (n=8) to 135.3±13.0 (n=8) ($P<0.01$). In addition, the pD_2 value for ACh was increased from 7.26±0.13 (n=8) to 7.45±0.21 (n=8) ($P<0.05$). In contrast, vasorelaxation to SNP was not significantly different in the control group (AUC=162.4±15.6; pD_2=7.68±0.18, n=6) and insulin-treated group (AUC=178.2±9.6; pD_2=7.79±0.11, n=6) ($P>0.05$). L-NAME elicited a leftward shift in the PEP concentration-response curve and increased the maximal contraction in both groups. The changes in contractility to PEP due to L-NAME were similar in the control group (ΔpD_2=0.65±0.13; ΔE_{max}=0.52±0.14 g, n=5) and insulin-treated group (ΔpD_2=0.59±0.14; ΔE_{max}=0.56±0.20 g, n=4) ($P>0.05$). In addition, maximal vasorelaxation to ACh (1 μM; 65.9±5.1 % in the control group, n=5) during maximal contraction to PEP (10μM) was abolished by L-NAME.

The data demonstrate that human insulin acutely enhances ACh-stimulated, NO-dependent vasorelaxation in the rat aorta *in vitro* in the absence of an effect on basal endogenous NO vasodilator function. The potentiation of vasorelaxation to ACh by insulin is probably unrelated to a non-specific increase in vasodilator reactivity to NO since responses to SNP were unaffected. This finding accords well with a recent report by Taddei *et al.* (1995) that insulin potentiates vasodilation to ACh, but not SNP, *in vivo*. The physiological relevance of these vasoactive actions of insulin *in vivo* in both health and disease, remains to be established.

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129P VASORELAXATION TO THE NOVEL PHOSPHODIESTERASE TYPE V INHIBITOR, ONO-1505, IN THE CAROTID ARTERY OF THE CHOLESTEROL-FED RABBIT

D.W. Laight, M. Konneh, M.J. Carrier & E.E. Änggård
The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London, EC1M 6BQ.

Agonist-stimulated, endogenous nitric oxide (NO)-dependent vasorelaxation *in vitro* can be demonstrated to be reduced in the carotid artery of the cholesterol-fed rabbit, (Matz *et al.*, 1994; Stewart-Lee *et al.*, 1994; 1995; Laight *et al.*, 1996), a widely employed experimental model of atherosclerosis. We have now attempted to assess basal endogenous NO vasodilator function in the isolated carotid artery of the cholesterol-fed rabbit by examining endothelium-, NO-dependent vasorelaxation to a novel phosphodiesterase (PDE) type V inhibitor (see Komas *et al.*, 1991), ONO-1505 (4-[2-(2-hydroxyethoxy)ethylamino]-2-(1*H*-imidazol-1-yl)-6-methoxyquinazoline methanesulfonate).

Male New Zealand White rabbits were maintained for 10 weeks on a standard chow diet with or without cholesterol (1% w/w) before sacrifice with an overdose of pentobarbitone sodium (120 mg/kg i.v.). Carotid artery rings were mounted under isometric conditions in Krebs-Henseleit solution (composition in mM: NaCl 133; KCl 4.7; NaH_2PO_4 1.35; $NaHCO_3$ 16.3; $MgSO_4$ 0.61; $CaCl_2$ 2.52; glucose, 7.8) containing indomethacin (5.6μM), gassed with carbogen and warmed to 37°C. After precontraction with noradrenaline (10μM), maximal vasorelaxation to acetylcholine (ACh, 1μM) was assessed. Rings were then washed and precontracted on a second occasion with NA (10μM) to assess vasorelaxation to ONO-1505 (0.1-300μM). Some preparations were pretreated for 10 min with N^G-nitro-L-arginine methyl ester (L-NAME, 300μM) to verify the component of vasorelaxation due to NO. Data are mean±s.e. mean (compared using Student's unpaired t-test).

Vasorelaxation to ACh was reduced from 53.2±3.9% (n=4) in the control group (n=4) to 35.4±5.6% in the cholesterol-fed group (n=5) ($P<0.05$). ONO-1505 elicited 100% vasorelaxation in the control and cholesterol-fed groups with pD_2 values of 4.35±0.07 (n=4) and 4.07±0.08 (n=5), respectively ($P<0.05$). L-NAME abolished the initial phase of vasorelaxation to ONO-1505 (≤10 μM) while 100% vasorelaxation could still achieved by 300μM ONO-1505. Unlike the case in the absence of NO synthase inhibition, the pD_2 values for ONO-1505 in the presence of L-NAME were similar in control (3.82±0.03, n=4) and cholesterol-fed (3.80±0.02, n=5) groups ($P>0.05$).

The reduced potency of vasorelaxation to ONO-1505 observed after cholesterol-feeding, was not preserved in the presence of L-NAME, indicating that altered non-specific vasodilator reactivity and/or drug access was not responsible. This suggests a selective deficit in endothelium-, NO-dependent vasorelaxation to a PDE type V inhibitor which may reflect defects in basal endogenous NO vascular function (see Laight *et al.*, 1996) and/or an altered sensitivity of PDE type V to ONO-1505 in hypercholesterolaemia. This is in addition to a perturbation in the agonist-stimulated, endothelium-, NO-dependent response to ACh.

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130P CHARACTERISATION OF VASORELAXATION TO THE NOVEL PHOSPHODIESTERASE TYPE V INHIBITOR, ONO-1505, IN THE RAT ISOLATED AORTA

D.W. Laight, M.J. Carrier & E. E. Änggård
The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London, EC1M 6BQ.

Inhibitors of phosphodiesterase (PDE) type V (see Nicholson, 1991) relax vascular smooth muscle by enhancing intracellular levels of cyclic guanylyl monophosphate (cGMP) (Komas *et al.*, 1991; Lindgren *et al.*, 1991; Murad *et al.*, 1985). We assessed vasodilation to ONO-1505 (4-[2-(2-hydroxyethoxy)ethylamino]-2-(1*H*-imidazol-1-yl)-6-methoxyquinazoline methanesulfonate), a novel dual inhibitor of PDE type V and thromboxane synthase, in the rat aorta *in vitro*.

Male Wistar rats (250-300g) were anaesthetised with pentobarbitone sodium (60 mg/kg i.p.), euthanised and thoracic aortic rings prepared and mounted under isometric conditions in Krebs-Henseleit solution (composition in mM: NaCl 133; KCl 4.7; NaH_2PO_4 16.3; $MgSO_4$ 0.61; $CaCl_2$ 2.52; glucose 7.8) gassed with carbogen and warmed to 37°C. Some rings were denuded of endothelium by intimal rubbing or pretreated (10 min) with enzyme inhibitors. Rings were then precontracted with noradrenaline (100 nM) and ONO-1505 added in a cumulative manner. Data are mean±s.e. mean.

ONO-1505 (0.1-300μM) elicited vasorelaxation (Figure 1) (pD_2=4.80±0.16; E_{max}=100±0%, n=12) which was unaffected by indomethacin (10μM, n=11) and the thromboxane synthase inhibitor, furegrelate (10μM, n=11). In contrast, the initial phase of vasorelaxation to ONO-1505 (≤10μM; pD_2'=5.71±0.14; E_{max}'=45.2±6.6%, n=12) was abolished by methylene blue (10μM, n=4), N^G-nitro-L-arginine methyl ester (L-NAME, 300μM, n=11) and endothelial denudation (n=4) while 100% relaxation (pD_2<4) could still be achieved by 300μM ONO-1505.

The data suggests that vasorelaxation of rat aorta to ≤10μM ONO-1505 is endothelium- and nitric oxide (NO)-dependent and secondary to the activity of soluble guanylyl cyclase. This is consistent with the potentiation of the vasodilator effect of endothelium-derived NO by the inhibition of PDE type V. However, at higher concentrations of ONO-1505 (>10μM), vasorelaxation is increasingly mediated by a pathway independent of the guanylyl cyclase system which does not concern the inhibition of thromboxane synthase.

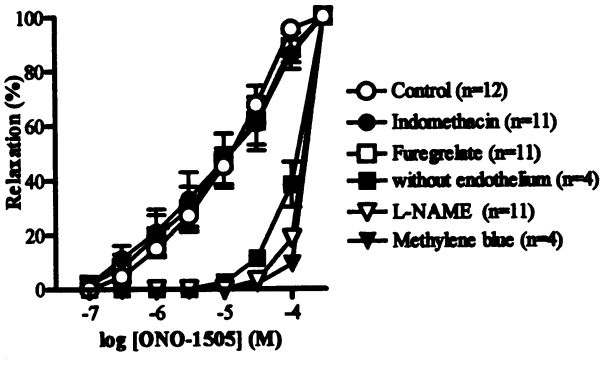

Figure 1. Vasorelaxation to ONO-1505 in rat aorta *in vitro*.

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131P EFFECTS OF Cu^+ CHELATION OR GLUTATHIONE ON NITROSO-GLUTATHIONE-INDUCED RELAXATION OF RAT AORTA

C. Cheung, J. Collier and R.J. MacAllister, Department of Pharmacology and Clinical Pharmacology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE.

Endogenous nitric oxide (NO) contributes to the regulation of arterial vascular tone by providing background vasodilatation. S-nitrosothiols are adducts of NO, some of which, including S-nitrosoglutathione (GSNO) occur endogenously. Certain S-nitrosothiols cause vasodilatation by releasing NO and have been implicated as endogenous modulators of the action of NO within the vasculature (Stamler *et al.*, 1992). Elucidation of the pathway(s) whereby S-nitrosothiols are biotransformed to release NO might enable their physiological role to be determined. Previous studies have implicated glutathione (Radomski *et al.*, 1992) and copper ions (Cu^+; Gordge *et al.*, 1995) in the biotransformation of GSNO to NO by platelets. In this study we investigated the effects of glutathione supplementation or Cu^+ chelation (using bathocuproine disulphonate; BCS) on the potency of GSNO, endogenous NO and exogenous NO donors on rat aorta.

Endothelium-intact or -denuded ring segments of rat aorta were mounted under isometric conditions (basal tone 1g) in Krebs' buffer and following preconstriction with phenylephrine (PE; 0.1μM), concentration-response curves were constructed to GSNO (1nM-10μM), carbachol (1nM-3μM), sodium nitroprusside (SNP; 1nM-1μM) or glyceryltrinitrate (GTN; 1nM-3μM) alone; after washout, vessels were incubated with glutathione (1mM) or BCS (10μM) for 15-60 min, preconstricted to the original starting tension with PE and concentration-response curves repeated in the presence of glutathione or BCS. Relaxations were analysed by comparing the negative log of the EC_{50} values (pD_2; expressed as mean$\pm$s.e.mean) using Student's *t* test for paired or unpaired samples as appropriate ($P<0.05$ is considered statistically significant).

GSNO relaxed endothelium-intact vessels (pD_2 6.7$\pm$0.1) and was more potent in the presence of glutathione following 15 min incubation (pD_2 7.2$\pm$0.1; n=5; $P<0.05$) and 60 min incubation (pD_2 6.9$\pm$0.1 in control and 7.2$\pm$0.1 in the presence of glutathione; n=5; $P<0.05$). The pD_2 values in endothelium-intact vessels for carbachol (n=4), SNP (n=5) and GTN (n=6) were 6.7$\pm$0.1, 8.4$\pm$0.1 or 7.9$\pm$0.2 respectively and were unchanged in the presence of glutathione (15 min incubation; 6.7$\pm$0.2, 8.4$\pm$0.1 and 8.0$\pm$0.1 respectively; $P>0.5$ compared to control). In endothelium-denuded vessels the pD_2 values for GSNO (n=10) and SNP (n=5) alone were 6.4$\pm$0.1 and 8.0$\pm$0.04 respectively; following incubation (15 min) with glutathione, GSNO and SNP were more potent (pD_2 7.1$\pm$0.1 and 8.5$\pm$0.1 respectively; $P<0.01$). In the presence of BCS (15 min incubation) the potency of GSNO was unchanged in endothelium-intact vessels (pD_2 values of 6.8$\pm$0.2 and 6.6$\pm$0.1 respectively; n=4; $P>0.05$). After incubation with BCS for 60 min the potency of GSNO was reduced (pD_2 values of 6.9$\pm$0.1 and 6.6$\pm$0.05 respectively; n=5; $P<0.01$) but the potency of SNP was unchanged (pD_2 values of 8.3$\pm$0.2 and 8.4$\pm$0.2 respectively; n=4; $P>0.05$). These results suggest that in rat aorta, glutathione increases whereas Cu^+ chelation reduces the potency of GSNO, although some of the effects of glutathione were not specific for GSNO. The effects of glutathione or Cu^+ chelation on the potency of GSNO are consistent with altered biotransformation of GSNO to NO.

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132P PROSTACYCLIN, NITRIC OXIDE AND CYCLIC NUCLEOTIDE SYNTHESIS IN STENTED AND UNSTENTED PORCINE VEIN GRAFTS

D. Mehta, J.Y. Jeremy, M.B. Izzat, A.J. Bryan & G.D. Angelini. Bristol Heart Institute, Bristol Royal Infirmary, Bristol, BS 2 8HW, UK

We have recently demonstrated that external stenting of saphenous vein-carotid artery interposition grafts in pigs reduces early intimal thickening (Izzat *et al.*, 1996), although the mechanisms underlying this effect are unknown. We have also demonstrated that the synthesis of prostacyclin (PGI_2), cAMP and cGMP is depressed in unstented porcine vein grafts whereas nitric oxide synthase (NOS) was induced (Jeremy *et al.*, 1996). Since the PGI_2-cAMP and NO-cGMP axes inhibit cell proliferation we suggested that these alterations may contribute to the pathophysiology of intimal thickening. In order to investigate whether stents elicit their beneficial effects through modulation of these influential systems, we measured PGI_2, cAMP and cGMP synthesis by porcine stented vein graft, unstented vein graft and ungrafted saphenous vein.

Bilateral saphenous vein-carotid artery interposition graft surgery was carried out as previously described (Izzat *et al.*, 1996). A non-restrictive, external, Dacron velour stent was applied to one vein graft and the other left unstented. One month after surgery, the vessels were excised and PGI_2, cAMP and cGMP measured by radioimmunossay following in vitro incubation with specific stimulators: PGI_2: Ca^{2+} ionophore A23187 [A23187]; cAMP: forskolin [FK]; cGMP: sodium nitroprusside [SNP] and A23187. There were no significant differences between PGI_2, cAMP and cGMP (SNP-stimulated) synthesis by stented vein graft and ungrafted saphenous veins whereas all were significantly reduced in unstented vein grafts (table 1). In contrast, cGMP synthesis in response to A23187 was significantly greater in stented vein grafts compared to control saphenous veins but not to unstented vein grafts (Table 1), indicating that NOS is up-regulated in both stented and unstented vein grafts. This is probably due to shear stress and arterial pressure to which both stented and unstented vein graft are subjected.

It is concluded that external stents normalise the down-regulation of PGI_2, cAMP and cGMP synthesis without affecting the induction of NOS. Since the PGI_2-cAMP and NO-cGMP axes inhibit platelet and leukocyte adhesion and vascular smooth muscle cell migration and proliferation, it is reasonable to suggest that the efficacy of the stent is due, at least in part, to the maintenance of these systems.

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Table 1. Maximal synthesis of PGI_2, cAMP and cGMP (mean $\pm$ SEM; n = 8; all data below are related to release / μg DNA) Stimulators are shown in parentheses. * $p< 0.001$ (ANOVA; relative to ungrafted saphenous vein)

	pg 6-keto-$PGF_{1\alpha}$ (A23187)	*pmoles cAMP (FK)*	*fmoles cGMP (SNP)*	*fmoles cGMP (A23187)*
ungrafted saphenous vein	73 $\pm$ 9	3.4 $\pm$ 0.5	410 $\pm$ 40	165 $\pm$ 20
stented vein graft	62 $\pm$ 4	3.3 $\pm$ 0.6	390 $\pm$ 30	280 $\pm$ 26*
unstented vein graft	22 $\pm$ 5*	2.6 $\pm$ 0.4*	280 $\pm$ 25*	265 $\pm$ 24*

133P EFFECT OF *IN VITRO* ANGIOPLASTY ON VASCULAR DEFENCE MECHANISMS IN RAT CULTURED AORTAE

J. Milnes, F. Sandeman, D. Barrett, S. Birkett, J.L. Johnson, G.D. Angelini, & J.Y. Jeremy, Bristol Heart Institute, Bristol BS2 8HW, UK.

Restenosis is a common complication following coronary artery balloon angioplasty (BA) which involves proliferation of vascular smooth muscle cells (VSMCs) and neointima formation. Blood vessels posess systems that may counter restenosis, viz. the nitric oxide (NO)-cGMP and prostacyclin (PGI_2)-cAMP axes (Jeremy *et al.*, 1996). *In vivo*, balloon angioplasty in the rat results in neointima formation within 14 days. Human saphenous vein in organ culture also forms a neointima in 14 days (George *et al.*, 1996). Therefore, in order to investigate the impact of BA on these vascular defence mechanisms we explored the use of an organ culture model of the rat aorta following *in vitro* angioplasty.

Male Sprague Dawley rats (250g) were decapitated, aortae excised and subjected to *in vitro* angioplasty using an angioplasty balloon (3 French) inflated x 3 for 30 seconds. Aortae were then cultured for 14 days. Sections of tissues were fixed for histology and scanning electron microscopy (EM) or were used for biochemical analysis. PGI_2, cAMP and cGMP werw measured by radioimmunossay following incubation with stimulators: PGI_2: adrenaline, phorbol ester (PE), calcium ionophore A23187 [A23187], cAMP: PGE_1 and forskolin [FK], cGMP: sodium nitroprusside [SNP] and A23187.

Histology and EM showed that at day zero, BA caused structural damage to the media and removal of the endothelium (confirmed by effect on A23187-stimulated cGMP synthesis; table 1) which mimics what happens in vivo. All tissues appeared viable after 14 days in culture.There was no neointima formation, no endothelial regrowth and also loss of endothelium in controls. At day 0, there were significant decreases in PGI_2, cAMP and cGMP synthesis in BA-treated tissues (table 1). After 14 days culture, there were significant reductions in BA-treated aortae in: 1) PGI_2 synthesis stimulated with adrenaline and PE (but not A23187), 2) PGE_1 (but not FK) stimulated cAMP synthesis and 3) A23187-(but not SNP) stimulated cGMP synthesis and also in controls (table 1).

These data indicate that: 1) blood borne factors are required for neointima formation in cultured rat aorta (e.g platelets, leukocytes, growth factors), 2) angioplasty elicits a direct negative effect on the PGI_2 - cAMP axis, possibly mediated by protein kinase C. In turn this may render the vessel susceptible to restenosis since PGI_2 inhibits VSMC proliferation and platelet and leukocyte adhesion.

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Table 1. PGI_2, cAMP and cGMP synthesis by cultured rat aortae in response to various stimulators (mean ± SEM; n = 8). All data are related to release / mg tissue / min. C = control, A = angioplasty-treated. * $p < 0.05$ (ANOVA).

	pg 6-keto-$PGF_{1\alpha}$						pmoles cAMP				fmoles cGMP			
	10μM A32187		1μM PE		10μM adrenaline		10μM forskolin		10μM PGE_1		10μM A23187		10μM SNP	
	C	A	C	A	C	A	C	A	C	A	C	A	C	A
DAY 0	82±8	65±7*	62±7	45±4*	70±7	60±6*	2.9±0.2	1.6±0.3*	2.5±0.2	1.8±0.1*	20±2	0±0*	390±40	310±31*
DAY 14	70±8	60±6	58±6	12±1*	60±6	10±1*	2.6±0.2	2.2±0.2	2.3±0.1	0.4±0.1*	1±0.1	0±0	320±30	230±20*

134P L-ARGININE ADMINISTRATION REDUCES BALLOON ANGIOPLASTY-INDUCED INTIMAL HYPERPLASIA IN THE FROXFIELD HERITABLE HYPERLIPIDAEMIC RABBIT

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

Angioplasty-induced vascular intimal injury is characterised by prolonged reduction of endothelial cell nitric oxide release (Azuma et al.,1990) which may contribute to the development of an intimal hyperplasia and smooth muscle cell proliferation and mitogenesis which characterises restenosis. McNamara et al., (1993) demonstrated that stimulation of the endogenous NO pathway by supplementation of dietary L-arginine reduced intimal hyperplasia following angioplasty in normocholesterolaemic rabbits, however there have been no comparable studies in heritable hyperlipidaemic rabbits. The aim of the present study was to elucidate the effect of L-arginine supplementation in the Froxfield Heritable Hyperlipidaemic (FHH) rabbit.

Nineteen male FHH rabbits (16 weeks old, 3.0-4.0kg) were anaesthetised with halothane/nitrous oxide and subjected to balloon injury of the left subclavian artery as described previously (Hadoke et al, 1995). Test animals were administered L-arginine 2 days prior to and for either 2 (n=6) or 4 (n=6) weeks post-angioplasty in their drinking water (5g/l). All animals were sacrificed 28 days after surgery. To assess the effect of balloon injury and L-arginine on the development of an intimal hyperplasia, the left and right subclavian arteries were removed and cut into 3-4 mm rings which were fixed in neutral buffered saline and processed for morphological analysis. The vessels were embedded in paraffin wax and cut into 5μm sections and stained with the Masson-Goldner Trichrome stain and photomicrographs of the stained sections were taken. Computerized planimetry was used to quantify the medial, luminal and neointimal areas for individual vessels which were measured as the % area within the external elastic lamina.

In non-treated rabbits 28 days after angioplasty, the injured vessel had developed a significant intimal hyperplasia (Table 1). The neointimal hyperplasia was devoid of lipid and typically abundant in smooth muscle cells with complete re-endothelialization of the injured area. Neointimal development was not evident in the non-treated vessel. The luminal and medial areas were smaller, although not significantly, in the injured compared to the non-injured vessels. L-arginine administration for 4 weeks, but not 2 weeks, significantly reduced the extent of the intimal hyperplasia compared to the injured vessels from untreated rabbits. Luminal and medial areas of the injured vessels did not differ from the non-injured vessels, although following 2 weeks administration of L-arginine the medial area was significantly greater in the injured vessel compared to the untreated group (Table 1).

Table 1 **Values as a % of area within external elastic lamina**

	LUMEN	MEDIA	NEOINTIMA
CONTROL			
Non-injured	68.67±3.2	32.63±4.0	0
Injured	57.64±10.9	24.15±8.2	18.84±3.6
2 WEEKS L-Arg			
Non-injured	65.22±2.7	36.45±5.4	0
Injured	51.40±13.1	34.98±9.5*	13.56±6.9
4 WEEKS L-Arg			
Non-injured	70.38±4.7	29.48±4.7	0
Injured	63.87±5.6	29.08±4.2	7.18±3.9*

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

These results illustrate that L-arginine administration as a method for elevating endogenous nitric oxide production reduces the extent of intimal hyperplasia which occurs in the FHH rabbit following balloon angioplasty.

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135P THE MODULATION OF EDHF ACTIVITY BY NITRIC OXIDE IN THE RAT ISOLATED SUPERIOR MESENTERIC ARTERIAL BED

A.I. McCulloch & M.D. Randall, Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH.

We have previously shown that nitric oxide (via cyclic guanosine monophosphate; cGMP) may modulate the activity of potassium channel activating agents (McCulloch & Randall, 1996). The putative endothelium-derived hyperpolarising factor (EDHF) is thought to act via the opening of potassium channels (Chen & Suzuki, 1989). Therefore the present investigation examined the effects of 8-bromo cyclic guanosine monophosphate (8-bromo cGMP) on the nitric oxide synthase inhibitor-insensitive component of endothelium-dependent relaxations to carbachol, which is thought to be mediated via EDHF acting on potassium channels.

Male Wistar rats (270-340g) were anaesthetised (60mg kg^{-1}, sodium pentobarbitone; i.p.) and the mesenteric arterial bed was cannulated and perfused at 5ml min^{-1} with oxygenated Krebs-Henseleit solution (McCulloch & Randall, 1996). A pressure transducer was placed close to the inflow cannula to continuously monitor perfusion pressure. After 30min equilibration, the perfusion pressure was raised (ca. 50mmHg) by the addition of methoxamine (5-7μM) to the buffer and dose-response curves were constructed for the endothelium-dependent vasorelaxant carbachol, in absence and presence of 100μM N^G-nitro-L-arginine methyl ester (L-NAME; Parsons *et al.*, 1994) with some experiments performed in the additional presence of 6μM 8-bromo cGMP.

Basal perfusion pressure was 23.7±1.4mmHg (mean±s.e. mean, n=8) and was increased by 53.1±4.4 mmHg following the addition of methoxamine. Carbachol produced dose-related reductions of established tone, described by an ED_{50} of 0.11±0.01nmol and a maximum relaxation (R_{max}) of 82.7±2.3%. Addition of L-NAME significantly ($P<0.001$; ANOVA with Bonferroni's *post-hoc* test) reduced the potency of carbachol (ED_{50}=0.52±0.11nmol) whereas the R_{max} was unaffected (82.1±2.4%, n=5). 8-bromo cGMP had no effect on vasorelaxation to carbachol alone (ED_{50}=0.24±0.13nmol, R_{max}=74.8±1.5%, n=3). However, in the presence of L-NAME, addition of 8-bromo cGMP caused a significant reduction in the potency of carbachol (ED_{50}=1.42±0.28nmol, $P<0.001$) and in the maximal relaxation (R_{max}=59.1±1.7%, $P<0.001$, n=7).

The results of the present investigation clearly show that in the rat mesenteric arterial bed there is a substantial nitric oxide-independent component of endothelium-dependent relaxations to carbachol. The magnitude of this nitric oxide-independent component (which is thought to be mediated via EDHF) was significantly and selectivity reduced by the addition of 8-bromo cGMP. These results imply that cGMP may modulate the EDHF component of endothelium-dependent vasorelaxation. Thus on loss of basal nitric oxide (and therefore a reduction in basal cGMP) the magnitude of the EDHF response is increased. Taken together these results suggest that there is 'cross-talk' between nitric oxide and EDHF which may be mediated via cGMP-dependent control of K^+ channels.

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136P RESTORATION OF NORMAL ENDOTHELIAL FUNCTION *IN VIVO* IN CHOLESTEROL-FED RABBITS BY THE NOVEL PHOSPHODIESTERASE V INHIBITOR, ONO 1505

M. Konneh, A. Stewart-Lee, D.W. Laight, M.J. Carrier & E.E. Änggård
The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London, EC1M 6BQ.

Endothelial dysfunction is a well established pathological complication associated with atherosclerosis (Flavahan & Vanhoutte, 1995). Impaired responses to endothelium-dependent vasodilators have been shown in arteries and arterioles from cholesterol fed animals and from humans with hypercholesterolaemia and atherosclerosis. One of the manifestations of increased cholesterol levels is an impairment of nitric oxide (NO) vascular function (Laight *et al.*, 1996). This defect can be seen as a loss of arterial compliance which can be detected as an inability to lower the dicrotic notch (DN) on a peripheral pulse curve, measured by a photoplethysmograph. The use of digital pulse photoplethysmography (PPG) provides a valuable, non-invasive, index of arterial dilation, and hence endothelial dysfunction. The response in the DN to the endothelium-dependent vasodilator acetylcholine (ACh), is depressed in cholesterol-fed rabbits (Klemsdal *et al.*, 1994). We examined the effect of a novel phosphodiesterase (PDE) type V inhibitor, ONO-1505 (4-[2-(2-hydroxyethoxy)ethylamino]-2-(1*H*-imidazol-1-yl)-6-methoxyquinazoline methanesulfonate), on this endothelial dysfunction *in vivo* using PPG.

Male New Zealand White rabbits (2-2.5kg) were maintained for 10 weeks on a standard chow diet (control) with or without cholesterol (1% w/w). Animals were slightly sedated with hypnorm (0.1ml/kg, i.m. containing 0.315mg/ml fentanyl citrate and 10mg/ml fluanisone). A PPG probe was placed on a shaved rabbit ear while the contralateral ear vein was cannulated for drug administration. ACh (0.4-2 μg/kg/min i.v.) was infused before and after a bolus dose of ONO-1505 (10 mg/kg i.v.). Four baseline recordings were made prior to ACh infusion. Pulse curve analyses were averaged over at least 20 cycles.

Figure 1 shows that dose-dependent decreases in the relative height of the DN elicited by ACh, were impaired in cholesterol-fed rabbits (2-way-ANOVA, $P<0.05$), e.g., control, E_{max}=32.8±7.7% (n=5) and cholesterol-fed, E_{max}=16.4±10.4 (n=6,) (Fig. 1a). ONO-1505 augmented the response to ACh (2-way-ANOVA, $P<0.05$) in both groups, such that endothelium-dependent vasodilation in the cholesterol-fed group treated with ONO-1505 (E_{max}=29.5±8.6%, n=6) was comparable to that in untreated controls (E_{max}=32.8±7.7%, n=5) (Fig. 1). However, responses to ACh in cholesterol-fed rabbits treated with ONO-1505 (E_{max}=29.5±8.6, n=6,) remained impaired relative to treated controls (E_{max}=45.5±5.7, n=5) (Fig. 1b).

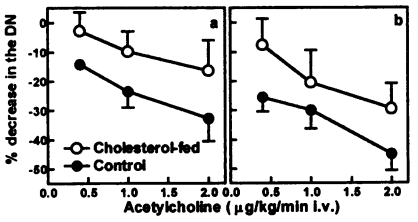

Figure 1. Change in the relative height of the DN. (a) Before ONO-1505. (b) After ONO-1505. The data are mean±s.e. mean. Drug effects are expressed as % change, in the relative height of the DN, from baseline.

Our data confirm defects in the endothelium-dependent regulation of arterial compliance in hypercholesterolaemia (Klemsdal *et al.*, 1994) and further suggest that, while endothelial dysfunction is not reversed, normal function may be restored by PDE type V inhibition *in vivo*, presumably by the accumulation of cGMP.

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137P USE OF SIN-1 TO ANALYSE INTERACTIONS BETWEEN NITRIC OXIDE AND SUPEROXIDE

L.A. Quine, M.J. Carrier, E.E. Änggård, William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London. EC1M 6BQ.

The balance between nitric oxide (NO·) and superoxide ($O_2^{\cdot-}$) may be important for vascular reactivity under normal physiological conditions (Darley-Usmar *et al.*, 1995). $O_2^{\cdot-}$ may also be involved in accelerating NO· oxidation under pathological conditions where oxidative stress is a factor, such as atherosclerosis, rheumatoid arthritis and diabetes. In addition production of NO· and $O_2^{\cdot-}$ can result in the formation of toxic peroxynitrite. We have used 3-morpholinosydnonimine (SIN-1), which generates $O_2^{\cdot-}$ and NO· spontaneously, *in vitro*, as a model to investigate the interaction of these two radical ion species. In this study we have measured the production of nitrite and nitrate (both oxidation products of NO·) following the release of NO· and $O_2^{\cdot-}$ from SIN-1.

Nitrite and nitrate were released from SIN-1 (20mM; pH 5.5) in a time-dependent manner. All data is expressed as mean ± s.e.mean. Production increased linearly for 8h and remained constant for at least 90h (maximum production was 20±0.6μM nitrite and 100±2.1μM nitrate; n=3). In all experiments SIN-1 was incubated at 37°C for 2h, samples were then removed for nitrite and nitrate analysis. Nitrite was assessed by addition of the Griess reagent (0.5% sulphanilamide, 0.05% N-naphthylethylene diamine dichloride and 2.5% phosphoric acid). Nitrate was converted to nitrite by addition of nitrate reductase (1U/ml), NADPH (500μM) and FAD (50μM) 30min prior to nitrite analysis. NADPH was removed by addition of lactate dehydrogenase (100U/ml) and pyruvic acid (100mM). SIN-1 produced 9.5±0.7μM (n=10) nitrite and 96.4±2.4μM (n=4) nitrate after 2h incubation. Incubation of SIN-1 with superoxide dismutase (SOD; 400U/ml) was associated with a 2-fold increase in nitrite (16.2±0.8μM, n=4) and a 35% decrease in nitrate (65.0±1.1μM, n=4). Incubation of SIN-1 with catalase (500U/ml) resulted in a 9-fold increase (78.0±0.4μM, n=4) in nitrite formation but no change in nitrate release. Bovine serum albumin had no effect on nitrite production demonstrating that this was not a non-specific effect of protein. Increasing the pH of the incubation medium from 5.5 to 10 significantly increased nitrite release (>250μM). Incubation with the catalase inhibitor aminotriazole (20mM) prevented the catalase-dependent increase in nitrite release. Incubation of SIN-1 with Iron (II) sulphate (1-100μM) dose-dependently increased nitrite release (n=3); which was completely reversed by addition of deferoxamine (Fe^{3+} chelator; 100μM) or phenanthroline (Fe^{2+} chelator; 1mM). Oxidation of NO· to nitrite and nitrate was completely inhibited by incubation with vitamin C (5mM). Incubation with trolox C, N-acetylcysteine or H_2O_2 (n=3) dose-dependently decreased nitrite (IC_{50}'s were about 100μM, 50μM and 2mM respectively) and had no effect on nitrate release.

We have demonstrated that production of NO· derived oxidation products from SIN-1 can be modulated by agents known to catalyse the destruction of $O_2^{\cdot-}$.The degradation of SIN-1 provides an interesting model system for the analysis of NO· oxidation and the interaction of $O_2^{\cdot-}$ with NO·, processes increasingly important in the regulation of NO·-dependent vasorelaxation. This model may be used for the assessment of novel anti-oxidant compounds designed to block $O_2^{\cdot-}$ thus increasing available NO· for biological activity.

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138P ALMOST COMPLETE INHIBITION OF THE NEO-INTIMAL RESPONSE TO BALLOON CATHETER INJURY IN THE RAT CAROTID BY A COMBINATION OF ANTIBODIES TO PDGF-BB AND bFGF

C. Rutherford, W. Martin, M.J. Carrier, G.A.A. Ferns* & E.E. Änggård The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London, EC1M 6BQ. *Division of Metabolic & Molecular Medicine, School of Biological Sciences, University of Surrey, Guildford, Surrey, GU2 5XH.

Percutaneous transluminal angioplasty is often used in the treatment of coronary artery disease. However up to 40% of vessels reocclude within 12 months (RITA trial participants, 1993). This restenosis is believed to be the result of uncontrolled cellular proliferation, migration and extracellular matrix synthesis (Clowes *et al.*, 1983). Factors involved include platelet derived growth factor-BB (PDGF-BB) and basic fibroblast growth factor (bFGF). The use of antibodies as therapeutic agents is increasing (Colburn, 1994), particularly where blocking cytokine/growth factor-receptor interactions may be beneficial. We examined the effects of combinations of purified sheep IgG polyclonal antibodies against PDGF-BB (α-P) and bFGF (α-F) on neo-intimal development in the balloon-catheterized rat carotid artery.

Male Wistar rats (400-450g) received doses (3mg/0.5ml/rat i.p.) of non-immune (α-NI, n=9), α-P (n=9), α-F (n=8) or both α-P/F (n=8), on the night prior to surgery, immediately after surgery, and then daily for 8 days. Rats were anaesthetized with xylazine (40mg/kg) and ketamine (10mg/kg), the left common carotid artery was then injured with a 2F Fogarty balloon catheter. 24h before killing, rats received bromodeoxyuridine (BrdU) (25mg/kg) and deoxycytidine (18.75mg/kg) to label proliferating cells. Eight days post-operatively the rats were killed by anaesthetic overdose and their arteries perfusion fixed with 4% paraformaldehyde. 5μm sections were cut and stained with Verhoeff-Van Geisen stain for elastin, adjacent sections were immunostained for BrdU-positive cells. Intimal and medial area and intimal cellular composition were determined by computerised morphometry. Fig.1 shows a 91% ($p<0.01$) reduction in area together with an 82% increase in cell density ($p<0.025$) in rats treated with both antibodies (α-P/F). Combined antibody treatment had no significant effect on intimal (22.2±2.5) or medial cell (2.0±0.9) proliferation compared to α-NI (intimal: 26.5±4.8, medial: 2.2±0.9). Data refers to % BrdU positive cells expressed as mean±s.e. mean.

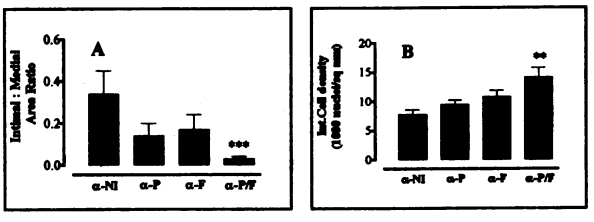

Figure 1. The effects of antibodies on; (A) intima : media cross-sectional area, and (B) intimal cell density. ** $p<0.025$, *** $p<0.01$ (Kruskal-Wallis test with Dunn correction).

Our data suggest that PDGF-BB and bFGF are major contributors to neo-intimal development. Cell migration and extracellular matrix synthesis may be of greater importance than cell proliferation in determining the neo-intimal response, possibly operating via independent pathways. Identification of these key cytokines could lead to the development of therapeutic agents which may be useful in the treatment of restenosis.

This work was funded by ONO Pharmaceutical Co., Ltd., Osaka Japan, and the British Heart Foundation.

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139P EFFECTS OF ONO-1505, A NOVEL PHOSPHODIESTERASE TYPE V AND THROMBOXANE SYNTHASE INHIBITOR, ON REDUCED NERVE BLOOD FLOW AND CONDUCTION VELOCITY IN DIABETIC RATS

N. Omawari, M. Dewhurst, P. Vo & D. R. Tomlinson, Department of Pharmacology, St. Bartholomew and the Royal London School of Medicine and Dentistry, Queen Mary & Westfield College, Mile End, London E1 4NS

Deficient nerve blood flow contributes to early conduction defects in experimental diabetic neuropathy. This may be caused by defects in the release or action of vasoactive mediators (Kamata *et al.*, 1989; Omawari *et al.*, 1996). This study therefore investigated the effects of treatment with ONO-1505 (4-[2-(2-hydroxyethoxy)ethylamino]-2-(1H-imidazol-1-yl)-6-methoxyquinazoline methanesulfonate), a combined inhibitor of phosphodiesterase type V and thromboxane A_2 synthase, on the deficient nerve blood flow of diabetic rats. The role of nitric oxide was examined by microinjecting L-NAME followed by L-arginine or sodium nitroprusside (SNP) into the sciatic nerve endoneurium. Age-matched control, diabetic (55 mg kg^{-1} i.p. streptozotocin) and ONO-1505 (100 mg $kg^{-1}day^{-1}$ p.o.) treated diabetic rats (male Wistar) were studied. After 5-7 weeks rats were anesthetised with Na pentobarbitone (50 mg kg^{-1}) and diazepam (2 mg kg^{-1}) and sciatic nerve laser Doppler flux (LDF), mean arterial pressures (MAP) and motor nerve conduction velocity (MNCV) measured as described previously (Karasu *et al.*, 1995). A glass micropipette (10-15 μm tip), positioned 1-2 mm proximal to the LDF probe was used to deliver drugs into endoneurium. Significance levels were derived either by paired t tests or for between group comparison, by ANOVA with Duncan's multiple range tests ($P<0.01$).

Basal LDF and MNCV were decreased ($P<0.01$) in diabetic animals compared to controls. ONO-1505 prevented ($P<0.01$) diabetes-induced reductions in LDF and MNCV. Microinjecting L-NAME reduced LDF to a greater extent in control and ONO-1505 treated, than untreated diabetic rats. The subsequent administration of L-arginine or SNP returned LDF to basal levels for controls, whereas the responses in diabetic rats were less significant (Table 1).

These data suggest that ONO-1505 may be useful in the prevention of peripheral nerve ischaemia in streptozotocin diabetic rats.

This study was supported by a grant to the William Harvey Research Institute from Ono Pharmaceutical Co. Ltd.

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Table 1. MNCV, MAP and LDF of control, diabetic and ONO-1505 treated diabetic rats. Effects of L-NAME, followed by L-arginine or SNP on LDF. All data mean±1S.D, ND = Not determined, NS = Not significant, number of rats in brackets.

			Sciatic nerve laser Doppler flux (Arbitrary units)						
Group	MNCV (m/s)	MAP (mmHg)	Basal	L-NAME	*P*-value	L-arginine	*P*-value	SNP	*P*-value
Control	53.5±7.8	114±13	209±45	75±30 (12)	<0.001	153±74 (6)	<0.05	242±115 (5)	NS
Diabetic	42.5±5.6	101±14	81±36	45±13 (11)	<0.01	76±38 (6)	NS	196±89 (7)	<0.006
Diabetic+ONO-1505	50.0±7.7	110±8	145±57	73±19 (6)	<0.03	N.D.		205±39 (4)	<0.02

140P ALTERED COX-1 mRNA LEVELS IN NERVE, AORTA, KIDNEY AND RETINA OF STZ-DIABETIC RATS: EFFECTS OF EVENING PRIMROSE OIL (EPO) OR AN ALDOSE REDUCTASE INHIBITOR

F. Cai, Z.Y. Jiang and D.R. Tomlinson, Department of Pharmacology, St Bartholomew's & The London Hospitals School of Medicine and Dentistry, QMW, London E1 4NS, U.K.

Altered prostanoid metabolism may participate in the pathogenesis of diabetic complications. The rate-limiting enzyme in prostanoid synthesis is cyclo-oxygenase (COX) and its constitutive form is COX-1. It is not clear whether altered prostanoid metabolism results from changes in COX-1 mRNA in critical tissues in diabetes. This study quantified the contents of COX-1 mRNA in sciatic nerve, thoracic aorta, retina and kidney from control and streptozotocin (STZ; 55mg/kg i.p.)-diabetic rats (male Wistar), by reverse transcriptase-competitive polymerase chain reaction (RT-cPCR). RNA was extracted as described elsewhere (Chomczynski & Sacchi, 1987) and reverse transcribed using a primer specific for COX-1. A competitive DNA mimic, with 3' and 5' flanking regions identical to the physiological COX-1 cDNA was added and PCR reactions run for 23-30 cycles. The precise amplification was calculated by interpolation in a standard curve derived from the mimic and the endogenous signals (measured by ethidium bromide staining and scanning of slab gels). Tissue contents of COX-1 mRNA are in the Table.

Diabetes reduced COX-1 mRNA in aorta and sciatic nerve, with a non-significant increase in the retina. EPO treatment (5 % w:w via powdered diet) increased COX-1 mRNA levels in diabetic sciatic nerve and retina, but did not increase COX-1 mRNA levels in aorta or kidney from diabetic rats. The aldose reductase inhibitor WAY-121509 (2-[(4-bromo-2-fluorophenyl)methyl]-6-fluorospiro[isoquinoloine-4(1H),3'-pyrrolidine]-1,2',3,5'(2H)-tetrone; 10 mg $kg^{-1}day^{-1}$ via powdered diet) did not affect COX-1 mRNA levels in any of the examined diabetic tissues. These data correlate well with our measurements of prostacyclin production under similar conditions (Stevens *et al.*, 1993), demonstrating that altered prostanoid metabolism can be in part, explained by altered COX-1 mRNA contents in diabetic rats. Apart from providing substrate for arachidonate, EPO appears to stimulate COX-1 mRNA expression.

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Group	Sciatic nerve	Aorta	Kidney	Retina	Number
	(attomol μg $tRNA^{-1}$)				
Control	0.89 ± 0.32[a]	8.80 ± 2.37[a]	0.50 ± 0.33	0.031 ± 0.02[a]	8,8,8,6
Diabetic	0.55 ± 0.20[b]	3.99 ± 1.67[b]	0.51 ± 0.20	0.049 ± 0.024	8,9,8,6
Diabetic-ARI	0.50 ± 0.26[b]	4.10 ± 1.11[b]		0.043 ± 0.019	6,5,5
Diabetic-EPO	1.21 ± 0.28[c]	4.75 ± 2.72[b]	0.54 ± 0.25	0.065 ± 0.017[b]	6,6,8,5

Data are mean ± SD. $p < 0.05$ (a *vs.* b or c, b *vs.* c) when compared groups of the same tissue by one-way ANOVA

141P HAEMODYNAMIC EFFECTS OF 17α-ETHYNYLESTRADIOL ON ENDOTOXAEMIC RATS

Beatriz Palacios & Catherine C. Y. Pang, Department of Pharmacology & Therapeutics, Faculty of Medicine, The University of British Columbia, Vancouver, B.C., Canada. V6T 1Z3

Oestrogen pretreatment has been reported to protect rats from death induced by endotoxin (Nolan, 1967). Moreover, estradiol-17β increased cardiac output in ovariectomized sheep *via* peripheral vasodilatation (Magness & Rosenfeld, 1989). The aim of this study was to investigate the effect of posttreatment with 17α-ethynylestradiol (17α-EE) on mean arterial pressure (MAP), cardiac output (CO), total peripheral resistance (TPR) and survival in rats challenged with *E. coli* lipopolysaccharide (LPS).

Five groups (n = 6-7) of male Sprague-Dawley rats (400-470 g) were anaesthetized with thiobutabarbitone (100 mg kg^{-1} i.p.), intubated and surgically-prepared for repeated injections (150 μl) of ^{57}Co-labeled-microspheres for measurement of CO (Pang, 1983). At 30 min after the completion of surgery, one group was i.v. injected with saline (0.9% NaCl) and four groups were injected with LPS (1 mg kg^{-1} over 2 min). After another hour, the LPS-treated rats were i.v. injected with 17α-EE (0.25, 0.5 or 1 mg kg^{-1}) or the vehicle (80% v/v ethanol). Haemodynamic parameters were measured at 1, 2.5, 4, 5 and 6 h after LPS injection. All data are presented as mean±s.e.mean and analyzed by ANOVA, followed by Duncan's test at $P < 0.05$.

There was no significant difference in baseline MAP (97-101 mmHg), CO (101-110 ml min^{-1}) and TPR (0.91-1.00 mmHg min ml^{-1}) among the groups. There was a small, significant decline of MAP (-10±3 mmHg) but no change in CO or TPR in the saline-treated time-control rats at the end of the 6-h observation period. LPS progressively reduced MAP and CO but increased TPR during the observation period. At 6 h after injection of LPS, MAP (-27±8 mmHg) and CO (-52±6 ml min^{-1}) were significantly reduced while TPR (+0.33±0.1 mmHg min ml^{-1}) was significantly increased relative to the corresponding time-controls. Whereas none of the 7 time-control rats died (0% mortality), 4 of 11 LPS-treated rats died (36% mortality).

The two low doses (0.25 and 0.5 mg kg^{-1}) of 17α-EE completely and the highest dose (1 mg kg^{-1}) partially (44%) reversed hypotension at 4-6 h after injection of LPS. CO was partially restored by all doses of 17α-EE at 4 and 5 h after injection of LPS. At 6 h after injection of LPS, CO was completely restored by 0.25 and 0.5 mg kg^{-1} and partially restored by 1 mg kg^{-1} (59%) of 17α-EE. 17α-EE, at 0.5 and 1 mg kg^{-1} but not 0.25 mg kg^{-1}, completely reversed the increase in TPR at 5 or 6 h after injection of LPS. Three out of twenty one 17α-EE treated rats died (one in each dose group).

Our findings show that treatment with 17α-EE did not significantly increase survival (4/11 *vs* 3/21, P>0.05, Fisher's Exact Probability) in anaesthetized endotoxaemic rats even though it increased CO and MAP.

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142P DOES THE RENAL RESPONSIVENESS TO VASOPRESSIN CHANGE DURING PREGNANCY AND LACTATION IN THE RAT?

D. E. Hartley, G. Stafford, Z. Zaman & M. L. Forsling, Department of Physiology, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH

Vasopressin concentrations are reduced during the third trimester of pregnancy (Davison *et al.*, 1988), but excretion of a water load is impaired, suggesting that renal responsiveness to the hormone is altered. A study has therefore been performed on the renal response to vasopressin during pregnancy and lactation in the rat.

Female Sprague Dawley rats (approx. 300 g) at a gestational age of about 12 days were each implanted with a jugular vein cannula under fentanyl citrate (i.m.), fluanisone (i.m.) and diazepam (i.p.) anaesthesia (0.32, 10 & 2.5 mg kg^{-1} bw respectively). They were left at least 48 h to recover, placed in a metabolism cage and 0.077M NaCl infused at 150 μl min^{-1}. Following an equilibration period of not less than 2.5 h, each spontaneous urination was timed and collected. After a control period of at least 45 min, the animals were infused with vasopressin at 0 or 20 μu min^{-1} for 60 min followed by a 90 min recovery period. Urinary volume, osmolality and electrolyte concentrations were measured. Observations were made on a daily basis during pregnancy and the first 12 days of lactation.

During the control infusion, rates of salt and water excretion matched the rates of administration. There was no significant change in the magnitude of the antidiuresis produced by vasopressin over the period of time studied (ANOVA). The mean antidiuretic response during pregnancy was 44 ± 2.4 % and 48 ± 2.8 % (s.e. mean, n=23) during lactation, compared to 41 ± 3 % (n=36) during the oestrous cycle (Forsling *et al.*, 1996). By contrast the natriuretic response was 23 ± 4.2 % during pregnancy and 41 ± 5.3 % during lactation ($p<0.01$, t test), compared to 24 ± 5% during the oestrous cycle. Infusion of vasopressin produced no significant change in potassium excretion. It appears, therefore, that the natriuretic, but not the antidiuretic response to vasopressin increases during lactation in the rat. This could be due to the oxytocin released during suckling as oxytocin and vasopressin may act synergistically to pomote sodium excretion (Windle *et al.*, 1995).

Financial assistance from Tommy's Campaign is gratefully acknowledged.

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143P INFLUENCE OF RILMENIDINE ON REFLEX ACTIVATION OF THE RENAL SYMPATHETIC NERVES IN ANAESTHETISED WISTAR RATS

T. Zhang & E. J. Johns, Department of Physiology, Medical School, Birmingham B15 2TT

Rilmenidine is a compound which is thought to act on putative imidazoline receptors on the rostral venterolateral medulla to cause a decrease in sympathetic nerve traffic to most organs including that to the kidney (Ernzberger et al, 1990). What has not been clarified is whether activation of these receptors could in any way interfere with the renal sympatho-excitation which occurs during stimulation of somatosensory receptors (Davis & Johns, 1995). This was tested in the present study using brachial nerve stimulation (BNS) to examine how both the magnitude and pattern of the renal nerve responses might be changed by rilmenidine.

Male Wistar rats (300-315g) were anaesthetised with N2O/O2/fluothane, a femoral vein, was cannulated, a saline infusion at 3ml/h was begun and thereafter the gaseous anaesthetic was replaced with iv chloralose/urethane (initially 12/180mg). Mean blood pressure (MBP) was measured via a femoral artery. Both brachial plexi were placed on stimulating electrodes while the renal nerves were exposed via a flank incision and sealed in place with Wacker Sil Gel 604. Integrated renal nerve activity (RNA) was amplified (x100 thousand) and high and low pass filters set at 0.1 and 1 kHz. MBP and RNA were recorded in blocks of 3.5 min before and during brachial nerve stimulation (BNS) at 0.8, 1.6 and 3.2Hz (0.2ms, 15V) and in the last minute data was collected at high speed to generate power spectra. Two groups of rats were studied, one was given saline (n=8) and the second injected with 100 and then 200 μg/kg rilmenidine iv (n=6) 15 min before measurement. Means ± s.e.m were compared using ANOVA with significance taken at $P<0.05$.

MBP at 101±4mmHg in the vehicle group, was lower in the animals given rilmenidine (89±5 and 75±7 mmHg at the low and high doses, both $P<0.05$), while heart rate (HR) was decreased (vehicle, 435± 14 b/min versus 409±17 and 369±20b/min at the low and high doses of rilmenidine, both $P<0.05$). RNA was 4.9±0.8, 4.5±0.7 and 3.8±0.7 mv/s in the presence of vehicle, 100 and 200 μg/kg rilmenidine while in the power spectra, total power, at 5.1±1.3 in vehicle group, was decreased by both doses rilmenidine ($P<0.01$-0.001), the %power at HR frequency, at 17.3±3, was lower only at the high dose drug ($P<0.01$). BNS caused frequency related increases in BP, HR and RNA reaching between 5%-35% ($P<0.05$) at 3.2Hz in the absence and presence of both doses of rilmenidine, the magnitudes of which were similar. At the same time total power was increased, by 110-190% at 3.2Hz ($P<0.01$ to 0.001) which was the same in all groups. However, the %power at HR was decreased between 50-60% at all stimulation frequencies in the vehicle and 100μg/kg dose ($P<0.05$ to 0.01) but with the 200μg/kg dose, no change was evident because of the low basal values with this dose.

These data shows that rilmenidine caused a dose related suppression of MBP and HR and to a lesser extent in the RNA and the power spectral parameters. The renal nerve responses to BNS were marginally altered at the high dose of drug. These results suggest that therapeutic doses of rilmenidine would not markedly alter the reflex neural control of kidney function.

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144P THE ACTION OF RILMENIDINE ON SOMATOSENSORY-INDUCED RENAL SYMPATHOEXCITATION IN ANAESTHETISED HYPERTENSIVE RATS

T. Zhang & E. J. Johns, Department of Physiology, Medical School, Birmingham B15 2TT

Rilmenidine has potent vasodepressor and bradycardic activities and consequently the compound has use as an antihypertensive drug (Dubar & Pillion, 1995). One of its actions appears to be at the putative imidazoline receptor of the rostral venterolateral medulla which causes a depression of the sympathetic nervous system. In genetic rat models of hypertension and in hypertensive patients there is evidence that the sympathetic outflow to the kidney is enhanced and may be more susceptible to inhibition by rilmenidine. Furthermore, it is not yet clear whether rilmenidine can interfere with reflex activation of the renal nerves. This was investigated in the present study by stimulating somatosensory receptors in the periphery and determining whether rilmenidine modulated either the magnitude or pattern of the subsequent activation of the renal nerves.

Male stroke-prone spontaneously hypertensive rats (SHRSP, 290-310g) were anaesthetised with N2O/O2/fluothane which was gradually replaced with chloralose/urethane,12/180mg via the femoral vein. The femoral artery was cannulated to measure mean blood pressure (MBP), both brachial plexi placed on stimulating electrodes, while the left kidney was exposed via a flank incision and its nerves placed on recording electrodes and sealed in place with Wacker Sil Gel 604. The first group of rats (n=7) received vehicle, the second (n=5) was given rilmenidine 100μg/kg followed by second dose 200μg/kg and measurements taken 15 min after each dose. The brachial nerves were stimulated (BNS) at 0.8, 1.6 and 3.2Hz at 15V, 0.2ms for 3.5min periods and during the last minute of recording the data was collected at high speed for later off-line power spectral analysis. The BNS protocol was undertaken 15 min after the drug administration. Data were compared using ANOVA with significance taken at $P<0.05$.

Basal values of MBP in the vehicle group, at 158±6 mmHg and HR, at 370±11 b/min were lower in the animals given the low and high doses of rilmenidine (MBP, 137±9 and 114±13 mmHg and HR, 318±16 and 298±23b/min, respectively, all $P<0.05$). The basal level of RNA was 10.96±1.70mV/s in the vehicle animals but was lower following 200μg/kg rilmenidine, at 8.27±1.74 mV/s ($P<0.001$) while total power (TP), at 7.5±1.8, and % power at HR frequency, at 24±4, were reduced in the presence of both doses of rilmenidine (TP 5.5±1.4 and 4.1±1.3; % power at HR 14±3 and 8±3, $P<0.05$-0.001). BNS caused frequency related increases in MBP, HR and RNA of between 5-25% ($P<0.05$ to 0.001) at 3.2Hz in the absence and presence of both doses of drug and the magnitudes of responses were the same. In the vehicle group and following both doses of rilmenidine, BNS increased TP, between 80-200% and %power at HR frequency, between 60-200% at all frequencies of stimulation and the magnitudes of the responses were all virtually identical.

These data demonstrate that in this rat model of genetic hypertension, rilmenidine caused a depression of the basal levels of MBP, HR, RNA and power spectral characteristics. However, the magnitudes of the reflexly induced responses in RNA and the power spectra parameters were unaffected even at the highest dose of drug. It is possible to conclude that rilmenidine does not interfere with the somatosensory mediated reflex activation of the renal nerves.

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145P EVALUATION OF BICARBONATE EFFECTS ON THE RENAL CLEARANCE OF AMANTADINE AND KYNURENIC ACID IN THE UNINEPHRECTOMIZED RAT

K.B. Goralski, D.D. Smyth and D.S. Sitar, Dept. of Pharmacol. and Ther., Univ. of Manitoba, Winnipeg, Canada R3E OW3.

We have demonstrated that physiological levels of bicarbonate increase the rate of amantadine (Escobar et al. 1994) but not kynurenic acid accumulation into isolated rat renal proximal and distal tubules. However, both are extensively secreted. The objective of this study was to evaluate the *in vivo* functional importance of bicarbonate levels, on the renal elimination of amantadine and kynurenic acid in an anaesthetized rat model. We hypothesize that changes in amantadine (organic cation) accumulation observed in isolated renal tubules would be reflected as changes in its renal elimination.

Uninephrectomized male Sprague Dawley rats (270-340)g were anaesthetized with sodium pentobarbital (60mg/kg ip.) and placed on a heating pad (37°C). After surgery (tracheotomy, and insertion of cannulae into the left carotid artery, left jugular vein and the remaining ureter) all rats were given 5 i.u heparin/ 0.9%saline iv. at 97µl/min. Rats were allowed to stabilize for 45 min and were given a bolus dose (3mg/kg iv.) at t=0 of [^{3}H]-amantadine (n = 12) or [^{3}H]-kynurenic acid (n = 8). Beginning at t=5 min, urine samples were collected at twenty min intervals and arterial blood samples (100µl) were withdrawn at the beginning and end of each urine collection. At t=25 min treatment rats were infused with $NaHCO_3$ (5mmol/kg iv.) at 111µl/min (approximately 12 min). During this period the heparin/saline infusion was stopped. Control rats in each group were maintained from t=0-125 min on heparin/saline (97µl/min, iv.). Amantadine and kynurenic acid in urine and in plasma were measured by liquid scintillation counting and their interval renal clearance was calculated. Creatinine was measured by the alkaline picrate assay.

Table 1 shows that after the addition of bicarbonate, the amantadine/creatinine clearance ratio decreased compared to control values. The kynurenic acid/creatinine clearance ratio increased above the control values. The mean amantadine/ creatinine clearance ratio decrease approached significance and the mean kynurenic acid/creatinine clearance ratio did not change. We conclude that the *in vitro* bicarbonate effect on amantadine renal tubule uptake appears to be associated with decreased renal clearance of amantadine. This model may be useful to test for other *in vivo* consequences of our *in vitro* observations.

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Table1. Amant/creat and kyn/creat clearance ratios in response to bicarbonate infusion. Results are expressed as the mean clearance ratio ± SD. * P<0.05; $^+$ P<0.1 compared to control (unpaired t-test). P<0.05 was considered significant.

	Urine collection periods 1-6 (min)						
Group	(5-25)	(25-45)	(45-65)	(65-85)	(85-105)	(105-125)	Mean ratio[1]
$Clr_{amant/creat}$, control (n=5)	2.56 ± .84	2.31 ± .68	2.38 ± .90	3.3 ± 2.0	2.43 ± .62	2.60 ± 1.1	2.60 ± .98
$Clr_{amant/creat}$, bic (n=7)	2.07 ± .45	1.78 ± .53	1.65 ± .30$^+$	1.78 ± .29$^+$	1.77 ± .36*	1.64 ± .50	1.75 ± .39$^+$
$Clr_{kyn/creat}$ control (n=4)	3.3 ± 1.0	2.74 ± .37	2.19 ± .24	2.02 ± .25	1.55 ± .23	1.30 ± .31	2.37 ± .61
$Clr_{kyn/creat}$, bic (n=4)	3.33 ± .43	1.80 ± .88$^+$	2.63 ± .52	2.53 ± .39$^+$	1.90 ± .17*	1.68 ± .14$^+$	2.1 ± 1.0

[1] The mean ratio represents the mean Clr ratio for the time period following bicarbonate infusion (t = 25-125 min).
Abbreviations: Clr = clearance, bic = bicarbonate, amant = amantadine, kyn = kynurenic acid, creat = creatinine

146P BINDING OF [^{125}I]-AB-MECA TO LOW AFFINITY SITES IN HUMAN KIDNEY

F.D. Russell & A.P. Davenport, Clinical Pharmacology Unit, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2QQ, U.K.

Adenosine A_1 and A_{2a} receptors regulate human renal function. A recent study showed that porcine proximal convoluted tubules express the A_3 receptor subtype (Blanco *et al.*, 1992) and there is now evidence for the coexistance of adenotin-like binding proteins and adenosine receptors in mammalian tissues (Work *et al.*, 1991). The function of the binding proteins is at present unknown. The aim of this study was to characterise the binding of ^{125}I-4-aminobenzyl-5'-N-methylcarboxamido adenosine ([^{125}I]-AB-MECA), a ligand with high affinity for adenosine A_1, A_{2a} and A_3 receptors, in human kidney.

Histologically normal human kidney (male, 33-74 years, n=7) was obtained from patients undergoing nephrectomy for hypernephroma. The tissues were frozen immediately in liquid N_2 and sections (10 µm) were cut on a cryostat at -22°C. Sections were labelled with [^{125}I]-AB-MECA (0.5 nM, 30 min, 23°C) in Tris buffer (Tris 50 mM, $MgCl_2$ 10 mM, EDTA 5 mM; pH 7.4) containing 1 mM PMSF. Non-specific binding was determined using N^6-3-iodobenzyl-5'-N-methylcarbamoyl adenosine (IB-MECA; 100 µM). Sections were washed in Tris buffer (3x1 min, 4°C) and either wiped from the slide and counted in a gamma counter for ligand binding studies or dried and apposed to Hyperfilm-ßmax (Amersham) for autoradiography.

Specific [^{125}I]-AB-MECA binding was linear over the concentration range used in saturation analysis (0.04-20 nM). Unlabelled AB-MECA inhibited binding of [^{125}I]-AB-MECA by 30% at 100 µM, indicating the presence of sites with lower affinity for the ligand than adenosine A_1 (K_D=3.4 nM, Olah *et al.*, 1994), A_{2a} (K_D=25.1 nM, Olah *et al.*, 1994) or A_3 receptors (K_D=1.5 nM, Patel *et al.*, 1996). The binding sites were resistant to blockade by A_1 (DPCPX, 30% inhibition at 300 µM) and A_{2a} selective ligands (CGS21680, 10% inhibition at 300 µM; 8-(3-chlorostyryl)caffeine, 12% inhibition at 100 µM). Although 8-(p-sulfophenyl)theophylline (0.1-300 µM) failed to inhibit [^{125}I]-AB-MECA binding, as expected for the presence of the A_3 subtype, the affinity of the binding sites for IB-MECA (pIC_{50}=5.3, nH=1.2, n=3) and 9-chloro-2-(2-furyl)[1,2,4] triazolo[1,5-c]quinazolin-5-amine (CGS15943, 25% inhibition at 100 µM) was lower than the affinity of expressed human A_3 receptors for these ligands (pKi=8.0 and 8.1, respectively; Patel *et al.*, 1996). Competition of IB-MECA was not altered by inclusion of 1 mM GTP in the labelling solution (pIC_{50}=5.3, nH=1.3, n=3). 5'-N-ethylcarboxamidoadenosine (NECA; 10 nM-300 µM) failed to compete for [^{125}I]-AB-MECA binding sites and no specific binding was observed with 20 nM [^{3}H]-NECA. Autoradiography showed that binding of [^{125}I]-AB-MECA was predominantly located in proximal convoluted tubules.

We conclude that [^{125}I]-AB-MECA detects low affinity sites in sections of human kidney and that these differ to adenotin-like binding proteins that have low affinity for [^{3}H]-NECA in human platelets and placenta (Work *et al.*, 1991). The [^{125}I]-AB-MECA binding sites were present in high density in human renal proximal convoluted tubules.

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147P EFFECTS OF THE SELECTIVE ADENOSINE A_2 AGONIST, CGS 21680, ON VENOUS TONE

R. Tabrizchi (introduced by C.L. Wainwright), Department of Pharmacology & Therapeutics, The University of British Columbia, Vancouver, B.C., V6T 1Z3, Canada

Adenosine is believed to vasodilate through interactions with its membrane-bound receptors. We examined the effects of CGS 21680 on mean circulatory filling pressure (MCFP), an index of body venous tone, in male Sprague-Dawley rats (420-460g). The rats were anaesthetized with pentobarbitone (65 mg kg^{-1}, i.p.) and maintained at 37±1° C. Catheters were inserted into iliac arteries and veins for measurement of blood pressure (BP), venous pressure, blood withdrawal and infusion of chemicals. A catheter was inserted into the left ventricle via the right carotid artery for repeated cardiac output (CO) measurements using ^{57}Co-labeled microspheres. A balloon-tipped catheter was inserted into the right atrium to transiently stop circulation following inflation as required for MCFP measurements (Pang & Tabrizchi, 1986). Measurement of CO and MCFP were made prior to and after 12-14 min infusions of the vehicle (0.02 M HCl; 4.5, 13.5 and 39 µl min^{-1}) or CGS 21680 (0.1, 0.3 and 0.9 µg kg^{-1} min^{-1}) in intact animals as well as rats pretreated with a combination of mecamylamine (M) (5 mg kg^{-1}) and atropine (A) (1 mg kg^{-1}). In intact rats, CGS 21680 reduced BP and TPR, and increased CO at two highest doses. It also reduced MCFP at the lowest dose and increased HR at the highest dose (Table 1). In ganglion-blocked rats, CGS 21680 reduced CO when compared to control values prior to ganglion-blockade. In addition, it further reduced TPR and MCFP relative to the corresponding values following ganglion blockade (Table 1). The results show that stimulation of vascular A_2 receptors in intact animals causes greater vasodilatation of arterial than venous blood vessels due to opposing influence of sympathetic nerve activation as a result of CGS 21680-induced hypotension.

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Table 1. Effects of vehicle or CGS 21680 on CO (ml min^{-1}), BP (mmHg), MCFP (mmHg), heart rate (HR) (beats min^{-1}) and total peripheral resistance (TPR) (mmHg min ml^{-1}) in intact (IT) or ganglion-blocked (GB) animals. Values are mean ± s.e.mean, (n = 6)

	Vehicle					CGS 21680				
IT	control	control	4.5	13.5	39	control	control	0.1	0.3	0.9
CO	81±5	91±2	87±4	84±5	93±6	79±3	83±2	82±5	93±3[a]	88 ± 3[a]
BP	111±2	110±3	110±2	108±2	109±2	111±2	109±3	91±2[a]	83±2[a]	73±2[a]
MCFP	5.5±0.2	5.6±0.2	5.5±0.1	5.6±0.1	5.5±0.1	5.2±0.2	5.2±0.2	4.6±0.4[a]	5.5±0.3	5.5±0.4
HR	353±8	350±8	357±9	358±9	352±9	380±17	380±14	380±14	391±15	400±17[a]
TPR	1.4±0.1	1.2±0.05	1.3±0.07	1.3±0.08	1.2±0.1	1.4±0.06	1.3±0.03	1.1±0.06[a]	0.8±0.04[a]	0.8±.04[a]
GB	control	M+A	4.5	13.5	39	control	M+A	0.1	0.3	0.9
CO	85±3	79±5	78±5	78±3	78±3	90±3	84±6	71±4[a]	75±3[a]	86±3
BP	111±2	76±3[a]	78±4[a]	80±4[a]	83±5[a]	109±3	74±2	57±2[ab]	48±2[ab]	45±2[ab]
MCFP	5.0±0.2	3.7±0.2[a]	3.6±0.2[a]	4.0±0.2[a]	4.0±0.3[a]	5.3±0.2	3.7±0.1[a]	3.1±0.2[ab]	3.0±0.2[ab]	3.2±0.2[ab]
HR	363±11	298±16[a]	297±16[a]	310±10[a]	320±12[a]	356±9	310±10[a]	315±6[a]	315±6[a]	321±5[a]
TPR	1.3±.05	0.9±0.06[a]	1.0±0.08[a]	1.0±0.07[a]	1.0±0.08[a]	1.2±0.04	0.9±0.05[a]	0.8±0.03[a]	0.6±0.01[ab]	0.5±0.03[ab]

Significantly different from control[a] or M+A[b]. Data were analyzed using ANOVA and Duncan's multiple range test ($P < 0.05$).

148P ENHANCED CARDIAC PRECONDITIONING IN ISOLATED PERFUSED HEARTS FROM TRANSGENIC ((mREN-2)27) HYPERTENSIVE RATS

M.D. Randall, Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH.

Recent interest has focused on the ability of short periods of ischaemia to protect the heart against prolonged ischaemia and this protective effect has been termed cardiac preconditioning (Murry *et al.*, 1986). In the present investigation, the effects of preconditioning were assessed in hypertension, which is a major risk factor for ischaemic heart disease. The effects of preconditioning were investigated in hearts isolated from heterozygous, transgenic ((mREN-2)27) hypertensive (TGH) rats (which harbour a mouse renin gene; Mullins *et al.*, 1990), and their normotensive controls (Hannover Sprague-Dawley; SD).

Male TGH and SD rats (386-550g) were heparinized (1,000U kg^{-1} i.p.) and anaesthetized with sodium pentobarbitone (60mg kg^{-1} i.p.). Following a thoracotomy, the hearts were rapidly excised and perfused in the Langendorff mode at constant flow (20 ml min^{-1}) with oxygenated Krebs-Henseleit buffer (Randall, 1995). A fluid-filled balloon catheter was inserted in to the left ventricle in order to measure developed left ventricular pressure (DLVP), from which heart rate was derived. Mechanical performance was assessed as the mathematical product of DLVP and heart rate (rate-pressure product, RPP). 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 (15 ml min^{-1}). The non-preconditioned hearts were continuously perfused prior to the 30min ischaemia.

The baseline RPPs were 21,557±853mmHg beats min^{-1} (mean±s.e.mean; Hannover Sprague-Dawley; n=14) and 20,136±773mmHg beats min^{-1} (TGH; n=18). In the absence of preconditioning, mechanical performance was substantially depressed on reperfusion, and there was no difference between TGH (area under the curve (AUC) for the RPP plot against time for the 60min of reperfusion (RPP_{0-60}) = (7.4±3.4)x10^4 mmHg beats; n=7) and SD (RPP_{0-60}=(5.48±4.51)x10^4 mmHg beats; n=7). Similarly the RPP after 60min of reperfusion ($RPP_{t=60}$) did not differ between groups (being 1,659±816mmHg beats min^{-1} in TGH and 891±693mmHg beats min^{-1} in SD). Cardiac preconditioning caused significant ($P<0.05$, ANOVA) protection in both groups, but this was significantly ($P<0.05$) greater in the TGH hearts (AUC for RPP_{0-60}=(8.58±1.64)x10^5 mmHg beats and $RPP_{t=60}$=14,500±2,840mmHg beats min^{-1}; n=11) compared to the SD hearts (AUC for RPP_{0-60}=(2.54±1.1)x10^5 mmHg beats and $RPP_{t=60}$=6,046±2,025mmHg beats min^{-1}; n=7). In both the TGH and SD hearts, preconditioning induced significant protection of diastolic function. The enhanced effects of preconditioning on mechanical performance in the TGH hearts was unaffected by the angiotensin AT_1-receptor antagonist, losartan (3µM).

The results of the present investigation clearly show that cardiac preconditioning is enhanced in hearts from TGH rats and the beneficial effects of preconditioning in TGH hearts occur independently of AT_1-receptor activation.

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149P EFFECT OF A DOPAMINE (D1) AGONIST UPON PLATELET ACCUMULATION IN THE RABBIT

M. Emerson, C.P. Page & W. Paul, Department of Pharmacology, King's College London, Manresa Road, London SW3 6LX, U.K.

We have shown previously (Emerson et al, 1996) that intracarotid (i.c.) infusion of dopamine (1mg/kg/min) significantly reduces thrombin-induced platelet accumulation in the cerebral vasculature of the rabbit. These results extended previous *in vitro* findings (Braunstein et al, 1977). It has been suggested that inhibitory effects of dopamine on platelets occur via D1-like receptors (De Keyser et al, 1988). Here we have studied the effects of a dopamine (D1) receptor agonist (Z10997A: (S)-N-propyl-N-[6-[2-(3,5-dihydroxyphenyl) ethylamino)hexyl]-5,6-dihydroxy-1,2,3,4-tetrahydro-2-napthylamine) *in vivo* in a model of thromboembolism using continuous monitoring of ^{111}Indium-labelled platelets in the cerebral and pulmonary vasculature of male NZW rabbits (May et al, 1990).

Animals were anaesthetised with diazepam (4mg/kg i.p.) followed 10 min later by Hypnorm (0.4ml/kg i.m.). Thrombin (90U/kg i.c.) induced a transient accumulation of labelled platelets in the pulmonary vasculature followed by sustained accumulation in the cerebral vasculature. Z10997A infusion was commenced 40 min prior to administration of thrombin and continued for the duration of the recording period. Platelet accumulation was expressed as mean ± s.e. mean (n=5) of the maximum % increase in counts above baseline values. Control and experimental values were compared using an unpaired t-test.

Pre-treatment with Z10997A (1-25μg/kg/min) had no significant effect ($P>0.05$) on subsequent platelet accumulation in the cerebral vasculature although accumulation was reduced from 91.6±7.9 (saline control) to 63.8±10.5 (non-significant) by the highest dose. However, infusion of Z10997A (25μg/kg/min) significantly ($P<0.05$) increased the initial maximum % increase in platelet counts in the lung following administration of thrombin from 21.0±4.6 (saline infusion) to 50.0±9.1.

These results do not support the hypothesis that dopamine exerts inhibitory effects upon platelets via D1-like receptors *in vivo*. Furthermore, the ability of D1-agonists to increase the retention of platelet aggregates in the pulmonary vasculature has important implications for their potential clinical use. In conclusion, further studies with agonists and antagonists specific for other dopamine receptor subtypes are indicated in order to define dopamine's site of action.

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150P EFFECT OF SALMETEROL ON MEDIATOR RELEASE FROM HUMAN LUNG MAST CELLS

L.K. Chong, E. Cooper, [1]C.J. Vardey & P.T. Peachell. Department of Medicine and Pharmacology, University of Sheffield, Royal Hallamshire Hospital, Floor L, Sheffield S10 2JF and [1]Glaxo/Wellcome, Gunnels Wood Road, Stevenage, Herts SG1 2NY (introduced by M.S. Lennard).

Salmeterol is a long-acting ß adrenoceptor agonist which is being used in the treatment of asthma. As well as direct bronchodilator effects, salmeterol may act in asthma to inhibit human lung mast cell (HLMC) responses. Several studies have shown that salmeterol can prevent the stimulated release of mediators from chopped human lung and HLMC (Butchers *et al.*, 1991; Lau *et al.*, 1994). Our main aim was to determine whether salmeterol acts to inhibit HLMC activity through ß adrenoceptor-mediated or ß adrenoceptor-independent mechanisms. The ß adrenoceptor agonists salmeterol and isoprenaline inhibited the anti-IgE-induced release of both histamine and sulphopeptidoleukotrienes (sLT) from HLMC in a dose-dependent manner. Both compounds were more potent and efficacious as inhibitors of sLT generation than histamine release (approx. EC_{50} values for the inhibition of histamine and sLT respectively, isoprenaline 10^{-9}M and 10^{-11}M; salmeterol 10^{-6}M and 10^{-12}M). Low (10^{-11}M) concentrations of salmeterol were particularly effective at inhibiting sLT generation (63±12% inhibition, mean±s.e.m., $p<0.05$) whereas alternative ß adrenoceptor agonists, salbutamol and terbutaline, were ineffective at inhibiting sLT generation at this concentration. Salmeterol (10^{-5}M) displayed a longer duration of action than isoprenaline (10^{-5}M) because the inhibitory activity of salmeterol on histamine release persisted following long-term incubation (16 h) of HLMC with salmeterol (inhibition after 16 h, 53±5%; after 30 min, 64±9%). The inhibitory effects of isoprenaline were lost following an identical long-term incubation (inhibition after 16 h, 5±4%; after 30 min, 48±8%). The ß adrenoceptor antagonists, propranolol (10^{-6}M) and ICI 118,551 (10^{-7} M) caused three hundred-fold and hundred-fold rightward shifts in the dose-response curve for the inhibition of histamine release by isoprenaline. The antagonism of salmeterol effects by both ß adrenoceptor antagonists was more complex. At lower (< 10^{-6}M) concentrations of salmeterol, both antagonists shifted the dose-response curve of salmeterol to the right. However, at higher concentrations (≥10^{-6}M) of salmeterol, both antagonists were ineffective. We have reported previously that long-term (4 h) pretreatment of HLMC with ß adrenoceptor agonists induces a functional desensitization to ß adrenoceptor agonists (Chong *et al.*, 1995). Incubation (4 h) of HLMC with isoprenaline (10^{-6}M) led to a subsequent significant ($p<0.001$) reduction in the ability of a second exposure of isoprenaline (10^{-5}M) to inhibit histamine release (22±3% inhibition in desensitized HLMC, 40±5% in untreated HLMC). Incubation (4 h) of HLMC with isoprenaline (10^{-6}M) had no effect on the salmeterol (10^{-5}M) inhibition of histamine release (30±4% inhibition in desensitized HLMC, 32±4% in untreated HLMC). Overall, these findings indicate that salmeterol is an effective inhibitor of the IgE-mediated release of mediators from HLMC. The present data also suggest that salmeterol may act to inhibit mediator release from HLMC by both ß adrenoceptor-mediated and ß adrenoceptor-independent mechanisms.

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151P SPECIES VARIATION IN THE RESPONSES OF RAT, GUINEA-PIG AND PIG CORONARY VESSELS TO METABOLIC ACIDOSIS

D.A. Wilson & B. Woodward, School Of Pharmacy & Pharmacology, University Of Bath, Bath BA2 7AY.

Metabolic acidosis (decrease in Na-bicarbonate concentration) has been shown to cause a nifedipine-sensitive vasoconstriction in the isolated rat heart, while eliciting a negative chronotropic and inotropic effect (Wilson & Woodward 1995). We were interested to investigate whether the acidotic vasoconstriction seen in the rat was dependent on the buffer used, and if a similar response was seen in coronary vessels of other species. We have therefore studied the effects of acidosis in Langendorff-perfused rat and guinea pig hearts and isolated porcine coronary artery rings.

Hearts from pentobarbital anaesthetised male Wistar rats or Dunkin-Hartley guinea pigs (300-350g) were perfused (10ml.min.$^{-1}$) with Krebs-Henseleit solution (K-H) or HEPES-buffered Tyrode solution (HBT; both 37°C; pH 7.4). Delevoped tension (DT) and heart rate (HR) were measured via a tension transducer attached to the apex of the heart and coronary perfusion pressure (CPP) was used as an index of coronary tone. Acidosis (pH 6.8) was applied for 5 minutes followed by a 10 minute recovery period. In experiments using porcine coronary artery rings, tissues were mounted isometically at a resting tension of 1 gram. Data expressed as mean±s.e.m., n=4. Statistical analysis performed using t-test except where stated otherwise.

Acidosis (pH6.8) had no effect on the basal tension of porcine coronary artery rings in K-H solution. Rings were therefore constricted up using potassium (42 mM) which elicited an increase in tension to 2.3±0.3g (approxiamately 70% of maximum). Following a 5 minute application of acidosis (Krebs-Henseleit solution, pH 6.8), tension decreased to 1.7±0.2 g ($p<0.05$). This effect reversed on return to normal pH.

In perfused rat and guinea pig hearts acidosis (K-H or HBT) reduced DT and HR. In contrast, acidosis increased CPP in rat hearts and decreased it in guinea pig hearts. This effect was seen with both K-H and HBTin the rat, but only with K-H in the guinea pig (Table 1). The increase in CPP in the rat heart observed with both buffers was significantly attenuated by the calcium antagonist 100nM nifedipine ($p<0.05$).

	K-H		HBT	
	pH 7.4	pH6.8	pH 7.4	pH 6.8
RAT CPP	112±12	160±5 *	95±10	183±11*
GUINEA PIG CPP	60±4	44±3*	43±3#	39±5

Table 1: Data from experiments comparing the acidotic responses of rat and guinea pig coronary circulations (* $p<0.05$ compared to pH7.4; # $p<0.05$ compared to K-H control (ONEWAY ANOVA)

This data shows species variation with respect to the effects of acidosis on the coronary circulation. Guinea pig and porcine coronary vessels dilate in response to acidosis while rat coronary vessels constrict. Results obtained in rat and guinea pig hearts using two buffer systems show that the vasoconstriction is independent of the buffer used, at least in the rat.

D.A.W. is a University of Bath research student.

Wilson, D.A. & Woodward, B. (1995). *Br. J. Pharmacol.*: **115:** pP73

152P EFFECTS OF DIAZEPAM ON CHLOROQUINE INTOXICATED, ANAESTHETIZED RATS

D.A. Hughes & S.J. Coker, Department of Pharmacology and Therapeutics, University of Liverpool, Ashton Street, Liverpool L69 3BX.

The role of diazepam in the treatment of chloroquine cardiotoxicity is unclear. Toxic doses of chloroquine are typically associated with hypotension, negative inotropy and broadening of the QRS complex (Hughes & Coker, 1994). Clinically, high doses of diazepam, together with artificial ventilation and administration of adrenaline are used successfully to reverse these effects (Riou *et al.*, 1988). To investigate whether this may be demonstrated in an animal model of chloroquine toxicity, cardiovascular responses in the presence of diazepam were examined in anaesthetized rats.

Male Wistar rats (300 - 390 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.), and cannulated for drug administration, recording of blood pressure (BP) and left ventricular (LV) pressure. A lead II ECG was monitored throughout. A thoracotomy was performed to facilitate artificial ventilation with air, and arterial blood gases were maintained within normal limits. Rats were assigned randomly to two groups : control, chloroquine 1 mg kg^{-1} min^{-1} for 1 hr with diazepam vehicle (a) (PEG 300 : ethanol : saline, 1:1:3) administered at 30 minutes; and chloroquine 1 mg kg^{-1} min^{-1} for 1 hr with diazepam 2 mg kg^{-1} at 30 minutes. In order to administer a higher dose of diazepam, an alternative vehicle (b) was required, (N,N - dimethylacetamide : Tween 80 : saline, 2:1:17). Thus, two further groups of rats were assigned randomly to either 10 mg kg^{-1} of diazepam or vehicle.

Chloroquine caused significant increases in the QRS interval in all but the high dose diazepam group, which were unchanged in the presence of either vehicle or diazepam (Table 1). Decreases in LV +dP/dt$_{max}$ were unaltered following the administration of diazepam. No differences were observed within studies at any corresponding time point (unpaired t test).

Table 1. Effects of diazepam (DZ) on QRS duration, left ventricular (LV) +dP/dt$_{max}$ and mean blood pressure (BP) in chloroquine treated rats.

	QRS (ms)	LV +dP/dt$_{max}$ (mmHgs^{-1})	Mean BP (mmHg)
Pre - drug	29 ± 1	5770 ± 220	93 ± 6
30 min post chloroquine	35 ± 0**	3650 ± 290**	68 ± 5*
15 min post vehicle (a)	34 ± 1	2750 ± 210	58 ± 4
Pre - drug	28 ± 1	5720 ± 160	93 ± 6
30 min post chloroquine	35 ± 1**	2920 ± 270**	64 ± 6**
15 min post DZ 2 mg kg^{-1}	34 ± 1	2970 ± 360	59 ± 5
Pre - drug	29 ± 1	6770 ± 510	95 ± 5
30 min post chloroquine	33 ± 2*	4260 ± 330**	78 ± 5
15 min post vehicle (b)	32 ± 2	4780 ± 320	83 ± 5
Pre - drug	28 ± 1	6020 ± 570	93 ± 4
30 min post chloroquine	31 ± 1	4060 ± 260*	81 ± 5
15 min post DZ 10 mg kg^{-1}	32 ± 1	4740 ± 280	81 ± 4

Values are expressed as mean ± s.e.mean, n=6 per group. * $P<0.05$; ** $P<0.01$ versus pre - drug value within group (paired Student's t test).

The results indicate that diazepam together with mechanical ventilation, but in the absence of inotropic support, failed to reverse chloroquine induced - cardiotoxicity in the pentobarbitone anaesthetized rat.

DAH is a recipient of a Wellcome Toxicology studentship.

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153P THE EFFECT OF EICOSAPENTAENOIC ACID AND DOCOSAHEXAENOIC ACID ON CONTRACTURES OF RAT AORTA

E.J. Cooper, A.G. Richardson, J.W. Smith & K.A. Wilson
Pharmaceutical Sciences Institute, Aston University, Birmingham B4 7ET.

We have previously reported the effect of eicosapentaenoic acid (EPA) on contractures of the isolated aortic rings from the rat (Bretherton *et al.*,1995). The aim of the present series of experiments was to compare the effects of EPA with docosahexaenoic acid (DHA) on KCl contractures, and to study the effect of a combination of the two fish oil fatty acids.

Male Wistar rats (200-250g) were stunned and killed by cervical dislocation. The thoracic aorta was removed and aortic rings, devoid of endothelium, were suspended in Krebs buffer containing propranolol (10^{-6}M), EDTA (10^{-5}M), and ascorbic acid ($5x10^{-5}$M) at 37°C, gassed with carbogen, under a resting tension of 2g. Isometric contractures were recorded. After 1hr equilibration, KCl (60mM) was added to the preparation. This was taken as the sensitising response (SR). Non-cumulative concentration-response curves were then constructed to KCl before and 30 mins after EPA ($5x10^{-5}$M, or $1x10^{-4}$M), DHA ($5x10^{-5}$M, or $1x10^{-4}$M), a combination of EPA and DHA (EPA $5x10^{-5}$M + DHA $5x10^{-5}$M), or ethanol (ETH, 1%), the vehicle.

KCl contractures were reduced by DHA at a concentration of $1x10^{-4}$M but not at $5x10^{-5}$M. DHA at $1x10^{-4}$M caused an increase in EC_{50} with no significant change in E_{max}, indicating a shift to the right of the concentration-response curve. In contrast, EPA reduced KCl contractures at both concentrations but had a greater effect at $5x10^{-5}$M. A combination of $5x10^{-5}$M EPA with $5x10^{-5}$M DHA had no effect on contractures induced at low concentrations of KCl, as indicated by the EC_{50} values, but significantly reduced E_{max} (see table 1).

Table 1 The effect of EPA or DHA alone or in combination on KCl contractures of rat aorta.

	KCl EC_{50} ($x10^{-3}$M)	E_{max}(% SR)
ETHANOL	15.4 + 1.6	108.4 + 9.7
DHA ($1X10^{-4}$M)	*27.2 ± 3.5	110.1 ± 8.4
DHA ($5X10^{-5}$M)	15.7 ± 2.6	92.7 ± 8.4
EPA ($1X10^{-4}$M)	*26.8 ± 4.3	94.9 ± 9.7
EPA ($5X10^{-5}$M)	*23.9 ± 2.2	*80.4 ± 5.1
DHA + EPA	17.2 ± 2.0	*82.0 ± 5.2

Values are mean ± s.e. mean. * indicates significant difference compared with ETH. $P< 0.05$ using Students' unpaired t-test (n>6).

The results of this study clearly indicate that DHA is only effective at reducing KCl contractures of rat aorta at higher concentrations than EPA. Furthermore DHA reduces the effect of EPA.

Bretherton, N., Richardson, A.G., Smith, J.W. *et al.* (1995) *Br. J. Pharmacol.* 116:150P

154P EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$ ON CONTRACTILITY IN THE RAT ISOLATED HEART AND CALCIUM TRANSIENTS IN THE CARDIAC MYOCYTE

S. F. Yew & B. Woodward, School of Pharmacy & Pharmacology, University of Bath, Bath BA2 7AY.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been shown to have a positive inotropic action in cardiac muscle but its mechanism of action is not clear. Otani et al. (1988) reported that inositol phosphates were increased in the presence of $PGF_{2\alpha}$ in rat ventricular papillary muscles. We examined the contractile effects of $PGF_{2\alpha}$, an FP receptor agonist, in perfused rat hearts and its effects on Ca^{2+} transients in rat ventricular myocytes.

Isolated hearts from pentobarbitone sodium anaesthetized male Wistar rats (280-300g) were perfused with Krebs-Henseleit buffer by a modified Langendorff technique at 10 ml/min, 37°C. Developed tension was measured with an isometric transducer attached to the apex of the left ventricle. Calcium tolerant ventricular myocytes were enzymatically dissociated and loaded with 5μM fura-2 AM for fluorescence microscopy studies of changes in intracellular Ca^{2+}. These myocytes were superfused with HEPES buffer (1mM Ca^{2+}) and electrically stimulated at 1Hz, 1 ms and 25°C. Myocytes were alternately excited with wavelengths of 340nm and 380nm and the fura-2 emmision wavelength at 510nm monitored.

Bolus doses of 0.01-300nmoles $PGF_{2\alpha}$ increased developed tension of whole heart in a dose dependent manner (n=6). The increase in developed tension was 9.52±2.73%. (mean±s.e.mean) with 0.1nmole $PGF_{2\alpha}$ and 18.25±4.55% with 1nmole $PGF_{2\alpha}$. The maximum value was not achieved due to limitations of the ethanol cosolvent, therefore the ED_{50} value was not obtained.

In fura-2 loaded cardiac myocytes, 3μM $PGF_{2\alpha}$ did not increase the calcium transient induced by electrical stimulation (fura 340/380 ratio amplitude = 0.62±0.08 after 3 min from predrug value of 0.66±0.09, n=9). However, these cells did respond to 300nM noradrenaline with an increase in the Ca^{2+} transient (maximum fura 340/380 ratio amplitude = 1.00±0.09 after 100s from 0.62±0.07, P<0.01, paired T-test, n=8). In some experiments, myocytes which did not respond to $PGF_{2\alpha}$ were subsequently treated with noradrenaline (n=3). In all of these myocytes, noradrenaline increased the calcium transients as before. The duration of the calcium transients as measured at 5% of the amplitude of the 340/380 ratio above baseline was unaffected by $PGF_{2\alpha}$ (duration = 612±27ms after 3 min from predrug value of 615±23ms). In contrast, noradrenaline shortened the duration of the Ca^{2+} transient from 543±19ms to 407±18ms after 100s (P<0.001, paired T-test).

Therefore, assuming that FP receptors are preserved on isolated myocytes, we conclude that $PGF_{2\alpha}$ does not increase intracellular Ca^{2+} as its mechanism of positive inotropy in rat hearts.

S.F. Yew is a Universiti Kebangsaan Malaysia scholar.

Otani, H., Otani, H. & Das, D.K. (1988) *J. Pharmacol. Exp. Ther.* 244, 844-851.

155P LIPOSOMAL CYCLOSPORINE: BIODISTRIBUTION IN THE RAT HEART TRANSPLANT MODEL

E. Choice, M. Meloche[1] & T.D. Madden (Introduced by C.Y. Pang) Department of Pharmacology & Therapeutics, Faculty of Medicine, University of British Columbia, Vancouver, B.C. V6T 1Z3 and [1]Department of Surgery, University Hospital, Faculty of Medicine, University of British Columbia, Vancouver, B.C. V6T 1B5

The goal of our research is to develop a liposomal drug delivery system for immunosuppressants for the post-operative management of transplant rejection. The current research focuses on the characterization of liposomal cyclosporine in a rat heart transplant model. The advantages that may be realized with this research include: decreasing cyclosporin A (CsA) nephrotoxicity, increasing the drug's potency by localizing its delivery to the site of action and increasing the drug's therapeutic index. The differential accumulation of liposomal cyclosporin A after intravenous tail injection was investigated in lung, liver, spleen, kidney and heart tissues and compared to that of an abdominal cardiac graft.

Groups consisted of isogeneic, allogeneic and allogeneic treated with cyclosporin A (10mg CsA/kg/day, i.p.) transplants. Seven days after transplantation, cyclosporine containing liposomes were administered i.v. ([^{3}H]CsA[^{14}C]cholesteryl hexadecyl ether radiolabels). The accumulation of liposomes (% recovered dose) in untreated allografts was 25-fold greater than in rats with isogeneic cardiac transplants (53 ± 15% versus 2 ± 2%, P<0.002). A corresponding decrease in liposome distribution to the liver (50%), when compared to the levels in livers of isogeneic transplant rats, was observed.

Interestingly, the daily administration of CsA to rats with allogeneic heart transplants resulted in similar levels of accumulation of liposomes (% recovered dose, corrected for plasma volume in the tissue) in the transplanted hearts of these rats when compared to levels found in isogeneic heart transplants of control rats (2.1 ± 2.4% and 1.0 ± 8.4%).

It was noted, however, that cyclosporin A did not remain associated with the liposomes as evidenced by dissimilar biodistribution compared to that of the carrier and lack of preferential accumulation (% recovered dose) of drug in transplanted versus internal control hearts (2.3 ± 0.9% and 1.7 ± 0.7%). We have previously described this behaviour (Ouyang *et al*, 1995 and Choice *et al*, 1995) and point out that although CsA delivery in cremophor and liposome vectors results in equivalent clearance and biodistribution of CsA, the latter may be a safer form of drug administration.

The results to date demonstrate that liposomes do show a preferential accumulation in heart allografts and have the potential, therefore, to be employed in the specific delivery of a drug to a transplanted organ such as the heart.

Choice, E., Masin, D., Bally, M.B. *et al.* (1995) Transplantation 60(9), 1006-1011.
Ouyang, C.,Choice, E., Holland J. *et al.* (1995) Transplantation 60(9), 999-1006.

156P A NOVEL METHOD FOR THE QUANTIFICATION OF TUMOUR NECROSIS FACTOR AND LYMPHOTOXIN IN COLLAGEN-INDUCED ARTHRITIS

M.H.Gascoigne, A.M.Nesbitt, R.Foulkes & P.S.Ward. Department of Pharmacology, Celltech Therapeutics Ltd.,216 Bath Road, Slough, SL1 4EN.

Tumour necrosis factor alpha (TNFα), is a proinflammatory pleiotropic cytokine found to play an important role in rheumatoid arthritis and in its murine models (Piguet *et al*, 1992). Lymphotoxin (LT) a closely related cytokine has been less well characterised in murine models of arthritis, mainly due to the lack of discriminatory reagents.

The aim of this experiment was to determine local production of TNFα and LT in murine collagen induced arthritis and to correlate this with severity of inflammation. TNFα is not detected systemically in these mice and as the foot is the primary site of inflammation, local cytokine measurements are more relevant.

Mice, male, DBA/1, 25g (Harlan U.K.) were immunised with chick sternal collagen to induce arthritis (Ward *et al*, 1995) On day 35, the feet were scored from 0 (normal) to 3 (maximal involvement). The mice were killed, hind feet removed and immediately snap frozen in liquid N_2. They were then homogenised (modified RPMI 1640, 3ml), centrifuged (1900g, 4°C, 40 minutes) and the supernatant removed. This was assayed for TNFα/LT using an L929 bioassay in the presence of 1μg.ml^{-1}of Actinomycin D which has a detection limit of 0.01ng.ml^{-1} This assay is based on cytotoxicity of TNFα to L929 cells. The amount of TNFα present is measured by using TN3 19.12, a specific antibody for murine TNFα, which does not neutralise LT. The soluble human p55 TNFα receptor which neutralises both murine TNFα and LT, is used to quantify the additional cell killing attributable to LT.

Concentrations of TNFα and LT (Table 1) correlate with increasing severity of disease in foot homogenates (Spearman rank correlation p<0.0001 for total killing). This clearly demonstrates a link between severity of arthritis and both these cytokines.

Disease Score	Total Killing (pg.ml^{-1})	TNFα (pg.ml^{-1})	Lymphotoxin (pg.ml^{-1})
Normal	42.3±9.9•••	25.4±3.7	10.5±3.4
0	37.3±5.1 •••	20.0±3.8	12.6±1.6
1	67.0±9.4 •	n.a.	n.a.
2	104.6±5.9	n.a.	n.a.
3	94.3±6.0	46.1±3.0$^{\$\$}$	23.9±5.3$^{\$\$}$

Table 1. Data are mean±s.e.m. (n = 5-18, n.a.-not available). Data were compared using ANOVA and Tukey-Kramer Multiple comparisons test. * p<0.05 v.s. 0,2,3. *** p<0.001 v.s. 2,3. $\$\$$ p< 0.01 vs, normals,0.

The method described above has therefore been established as a way of measuring local concentrations of TNFα and LT in the feet of mice with collagen arthritis. It can also be used in future experiments to investigate local rather than systemic production of other mediators that may be involved in arthritis.

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Ward, P.S., Boden, T, Woodger, R *et al* (1995) *Br.J.Rheumatol* 34, 153P.

157P THE NITRIC OXIDE SYNTHASE INHIBITOR, 7-NITRO INDAZOLE, INHIBITS CARRAGEENAN-INDUCED HINDPAW OEDEMA IN THE RAT

Rachel L.C. Handy & P.K. Moore, Pharmacology Group, Biomedical Sciences Division, King's College, Manresa Road, London SW3 6LX.

Isoform non-selective inhibitors of nitric oxide synthase (NOS) such as L-N(G) nitro arginine methyl ester (L-NAME) reduce carrageenan-induced hindpaw oedema in the rat (Ialenti *et al.*, 1992). We have now compared the ability of L-NAME and 7-nitro indazole (7-NI; a relatively selective inhibitor of neuronal NOS *in vivo*, Moore *et al.*, 1993) to influence carrageenan-induced hindpaw oedema in the rat.

Rats (male, Wistar, 110-140g) were injected intraplantar with 150 µl carrageenan (1, 2 or 3% w/v). Oedema formation (30 min - 6 h) was measured as increase in hindpaw weight. Each hindpaw (to the level of the tibiotarsal joint) was immersed into a beaker of warm water placed on a balance and the increase in weight recorded. At the end of the experiment, animals were stunned by a blow to the head and killed by exsanguination. Both hindpaws were removed, homogenised in 5 volumes of 20 mM Tris HCl buffer (pH 7.4, containing 2 mM EDTA) and NOS activity determined by monitoring conversion of L-^{3}H arginine to L-^{3}H citrulline as described previously (Moore *et al.*, 1993) in the presence of $CaCl_2$ (0.75 mM) or EGTA (1 mM) to measure the activity of constitutive (i.e. neuronal and/or endothelial) and inducible isoforms of NOS respectively.

Intraplantar injection of carrageenan (2% w/v) resulted in an increase in hindpaw weight which was evident at 30 min (0.36 ± 0.06 g, n=6). The response to carrageenan was also dose-dependent (e.g. hindpaw weight at 2 h: 1%, 0.83 ± 0.13 g; 2%, 1.11 ± 0.09 g and 3%, 1.21 ± 0.08 g, n=6). Administration of 7-NI or L-NAME (5-25 mg kg^{-1}, i.p. 30 min prior to 2% w/v carrageenan injection) resulted in dose related inhibition of hindpaw oedema formation in the period 2-6 h (e.g. 2 h: 25 mg kg^{-1} 7-NI; 0.61 ± 0.06 g c.f. 0.94 ± 0.06 g in vehicle-injected control animals, n=6, P<0.01: 25 mg kg^{-1} L-NAME; 0.64 ± 0.07 g c.f. 1.06 ± 0.06 g, n=6, P<0.01). Intraplantar carrageenan (2% w/v) administration caused a significant increase in both Ca^{2+}-dependent (1.55 ± 0.27 pmol mg protein^{-1} 15 min^{-1} c.f. 0.05 ± 0.01 pmol mg protein^{-1} 15 min^{-1} in non-injected hindpaws, n=6, P<0.01) and Ca^{2+}-independent (0.67 ± 0.14 pmol mg protein^{-1} 15 min^{-1} c.f. 0.05 ± 0.03 pmol mg protein^{-1} 15 min^{-1} in non-injected hindpaws, n=6, P<0.01) NOS activity in hindpaws removed 6 h after injection. Administration of either 7-NI or L-NAME (e.g. 25 mg kg^{-1}, i.p.) significantly reduced hindpaw Ca^{2+}-dependent (7-NI: 75.4 ± 4.4 % inhibition, n=6, L-NAME; 86.3 ± 3.0 %, n=6) and totally abolished Ca^{2+}-independent NOS activity.

These results suggest that 7-NI exhibits anti-inflammatory activity in this model most probably by inhibiting Ca^{2+}-dependent and/or Ca^{2+}-independent NOS.

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158P CHARACTERISATION OF THE PROSTANOID RECEPTORS THAT MEDIATE INHIBITION OF LIPOPOLYSACCHARIDE-INDUCED TUMOUR NECROSIS FACTOR-α GENERATION FROM HUMAN MONOCYTES

Koremu Meja., Paul M. Seldon., Peter J. Barnes and Mark A. Giembycz. *Department of Thoracic Medicine, National Heart and Lung Institute (Imperial College), London.*

Exposure of human peripheral blood monocytes to lipopolysaccharide (LPS) results in the induction of a number of early response genes including those which encode pro-inflammatory cytokines such as interleukin-1β and tumour necrosis factor-α (TNFα). Although we have reported previously that prostaglandin (PG) E_2 inhibits the generation of TNFα from human monocytes (Seldon *et al.*, 1995), the prostanoid-receptor subtype(s) mediating this effect was not classified. In this abstract, therefore, we describe the results of a systematic study designed to identify and classify the receptor(s) which regulate TNFα production in human monocytes using naturally occurring and synthetic prostanoid agonists and antagonists.

Human monocytes were purified from the peripheral blood of normal individuals and cultured on 24-well plates in FCS-supplemented RPMI 1640. Cells were stimulated with *Salmonella enteritidis* (3 ng/ml; EC_{84}) in the absence and presence of the drugs under investigation and the TNFα released in to the culture medium was quantified at 18 h by a sandwich ELISA. Cyclic AMP and cyclic AMP-dependent protein kinase (PKA) activity were also measured in some experiments using well established techniques.

In human monocytes that were adherent to plastic culture plates, neither PGD_2, PGE_2, $PGF_{2\alpha}$, cicaprost nor U-46619 induced the expression of the TNFα gene. Similarly, PGD_2, $PGF_{2\alpha}$ and the thromboxane mimetic, U-46619, failed to affect the elaboration of TNFα in LPS-exposed cells at concentrations up to 10 µM. In contrast, PGE_1, PGE_2 and the stable prostacyclin analogue, cicaprost, inhibited TNFα generation in a concentration-dependent under identical experimental condition with pIC_{50} values of 7.14 ± 0.05 (n = 4), 7.01 ± 0.07 (n = 3) and 8.00 ± 0.22 (n = 4) respectively. With respect to PGE_2, the EP-receptor agonists, 16,16-dimethyl PGE_2 (non-selective), misoprostol (EP_2/EP_3-selective), 11-deoxy PGE_1 (EP_2-selective) and butaprost (EP_2-selective) were essentially full agonists (relative intrinsic activities > 0.9) as inhibitors of LPS-induced TNFα generation with pIC_{50} values of 6.21 ± 0.13 (n = 5), 6.02 ± 0.11 (n = 5), 5.67 ± 0.11 (n = 5) and 5.59 ± 0.12 (n = 5) respectively. EP-Receptor agonists which have selectivity for the EP_1-(17-phenyl-ω-trinor PGE_2) and EP_3-receptor (MB 28,767, sulprostone) were inactive or only weakly active when examined at concentrations up to 10 µM. Pre-treatment (30 min) of monocytes with the putative EP_4-receptor antagonist, AH 23848B (10 and 30 µM; Coleman *et al.*, 1994) failed to antagonise the inhibitory action of PGE_2. The ability of PGE_2 to suppress LPS-induced TNFα generation was potentiated by rolipram (pIC_{50} = 7.59 ± 0.09, n = 3), a selective inhibitor of the phosphodiesterase 4 isoenzyme family, implicating the cyclic AMP/PKA cascade in this effect. PGE_2 at a concentration (1 µM) that suppressed LPS-induced TNFα generation by 85.5 ± 2.2 % (n = 3) failed to elevate the intracellular cyclic AMP content of human monocytes. However, in the presence of rolipram (100 µM) a highly significant increase in cyclic AMP mass was observed (from 1.3 ± 6.4 ± 1 pmol/10^6 cells, n = 4). Similarly, PGE_2 (1 µM) increased the PKA activity ratio from 11.0 ± 1.6 to 21.8 ± 3.6 % (n = 4) which was increased further (to 72.1 ± 2.9%, n = 4) by a concentration of rolipram (100 µM) which, by itself, produced only a modest increase in PKA activity ratio (to 18.3 ± 3.6%, n = 4).

Collectively, these data suggest that human peripheral blood monocytes express at least two distinct populations of inhibitory prostanoid receptor that mediate inhibition of LPS-induced TNFα generation which have the pharmacological and second messenger characteristics of EP_2- and IP-receptors. No evidence for excitatory prostanoid receptors was obtained using TNFα generation as a measure of monocyte function.

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159P THE INHIBITORY EFFECT OF CYCLIC AMP-ELEVATING DRUGS ON LPS-INDUCED TNFα GENERATION FROM HUMAN MONOCYTES IS NOT MEDIATED BY IL-10

Paul M. Seldon., Peter J. Barnes and Mark A. Giembycz.
Department of Thoracic Medicine, National Heart and Lung Institute (Imperial College), London.

We have recently demonstrated that a range of cyclic AMP-elevating agents including phosphodiesterase inhibitors, β-adrenoceptor agonists and E-series prostaglandins effectively suppress the induction of a number of cytokines from human monocytes in response to bacterial lipopolysaccharide (LPS) including tumour necrosis factor-α (TNFα) (Seldon *et al.*, 1995). Similarly, LPS-induced liver injury in mice (Arai *et al.*, 1995) and LPS-induced TNFα generation by murine macrophages (Kambayashi *et al.*, 1995) are also inhibited by agents that increase cyclic AMP. The mechanism of action of cyclic AMP is unclear but, in the mouse, is believed to involve the generation of the anti-inflammatory cytokine, interleukin-10 (IL-10) (Arai *et al.*, 1995; Kambayashi *et al.*, 1995). In this abstract we report the results of studies designed to determine the extent to which IL-10 contributes to the suppression of TNFα generation from LPS-stimulated human monocytes.

Human monocytes were purified from the peripheral blood of normal individuals and cultured on 24-well plates in FCS-supplemented RPMI 1640. Cells were stimulated with *Salmonella enteritidis*,(3 ng/ml; EC_{84}) in the absence and presence of the drugs under investigation and the TNFα released in to the culture medium was quantified at 18 h by a sandwich ELISA.

After a lag of approximately 1 h, LPS evoked a time-dependent generation of TNFα ($t_{1/2}$ = 2.8 h) that was prevented in monocytes that had been treated with actinomycin D (5 μg/ml) and cycloheximide (10 μg/ml) indicating a requirement for new protein synthesis. The induction of the TNFα gene was suppressed by exogenous hrIL-10 (EC_{50} = 133.9 ± 41.4 pg/ml, n = 3) and by rolipram, 8-bromo cyclic AMP, prostaglandin E_2 (PGE_2) and salbutamol (Table 1). Pre-treatment of monocytes with an anti-IL-10 monoclonal antibody (IL-10 mAb; Pharmingen, clone JES3-9D7) at a concentration (100 ng/ml) that neutralised the effect of a maximally effective inhibitory concentration (1 ng/ml) of exogenous hrIL-10 significantly potentiated (by 11.8 ± 5.1%, n = 5) the elaboration of TNFα (from 2.7 ± 0.2 to 3.0 ± 0.4 ng/ml, n = 5, P < 0.05 - Student's paired *t*-test) in response to LPS. However, IL-10 mAb failed to affect the ability of rolipram, PGE_2, salbutamol and 8-bromo cyclic AMP to suppress LPS-induced TNFα generation with respect to both the EC_{50} and maximum inhibition (Table 1).

Table 1. Effect of an IL-10 mAb on the inhibition of LPS-induced TNFα release from human monocytes (n = 3 - 7)

	- IL-10 mAb		+ IL-10 mAb	
	EC_{50} (μM)	Inhibition (%)	EC_{50} (μM)	Inhibition (%)
Rolipram	0.42 ± 0.12	97.8 ± 1.1	0.60 ± 0.11	95.1 ± 0.2
Salbutamol	0.020 ± 0.003	38.0 ± 7.7	0.029 ± 0.002	28.8 ± 5.6
8-Br-cAMP	76.8 ± 19.8	100	78.8 ± 2.8	99.9 ± 0.1
PGE_2	0.015 ± 0.005	95.2 ± 2.5	0.034 ± 0.009	87.2 ± 3.9

Collectively, these data suggest that the elaboration of IL-10 from LPS-stimulated human monocytes acts in an autocrine manner to suppress (albeit modestly) the release of the pro-inflammatory cytokine, TNFα. However, in contrast to murine hepatocytes and macrophages, IL-10 does not, apparently, mediate the inhibitory effect of cyclic AMP-elevating drugs on TNFα gene expression.

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160P DEXAMETHASONE INHIBITS MONOCYTE RECRUITMENT DURING ACUTE INFLAMMATION VIA ENDOGENOUS LIPOCORTIN 1

Stephen J Getting, Roderick J Flower & Mauro Perretti.
Department of Biochemical Pharmacology, The William Harvey Research Institute, Bart's Medical College, Charterhouse Square, London EC1M 6BQ.

In previous studies we have focussed on the role played by endogenous lipocortin 1 (LC1) in the inhibitory action exerted by dexamethasone (DEX) on the recruitment of polymorphonuclear leucocytes (PMN) [1]. Using several anti-LC1 polyclonal sera we have now investigated whether endogenous LC1 played a similar role in DEX inhibition of monocyte recruitment in a murine model of acute inflammation.

Male Swiss Albino mice (28-32 g) were treated s.c with a control sheep serum (termed NSS), an anti-LC1 polyclonal serum (termed LCS3), or with two antisera raised against the LC1 N-terminus peptide Ac2-26 in sheep (termed LCPS1 and LCPS2) (50 μl in all cases) [2] 24 h before treatment with DEX (30 μg s.c.) or PBS (100 μl). Mice were then challenged with zymosan (1 mg in 0.5 ml saline, i.p.) and peritoneal cavitites washed with 3 ml of PBS (supplemented with 3 mM EDTA and 25 U/ml heparin) 4 h or 24 h later. Differential cell counts were performed using a Neubauer hæmatocytometer in Turk's staining solution. Monocyte and macrophage (termed Mono-Mø) influxes are reported as 10^6 cells per mouse (mean ± s.e.mean of n mice per group), and statistical differences were assessed by ANOVA. ß-glucuronidase activity was measured in 24 h lavage fluids using the specific enzyme substrate phenolpthalein mono-ß-glucuronic acid and reading absorbance at 550 nm (data are reported as U/ml).

Mono-Mø represented the major cell type in the peritoneal fluids 24 h post-zymosan injection as assessed in naive mice (12.4 ± 1.4 x10^6 cells per mouse, n=15). This cell influx was sensitive to DEX treatment. Table 1 shows that DEX was able to reduce Mono-Mø infiltration in NSS-pretreated mice (-53% of inhibition, $P<0.01$), whereas the steroid was no longer effective in LCS3-, LCPS1- and LCPS2-pretreated animals.

Table 1. Zymosan peritonitis: 24 h time-point.

Pretreatment (-24 h)	Treatment (-1 h)	Mono-Mø (10^6 per mouse)
NSS	PBS	11.3± 0.5 (12)
NSS	DEX	5.3 ± 0.5 (14) **
LCS3	PBS	8.9 ± 0.6 (15)
LCS3	DEX	8.0 ± 0.8 (16)
LCPS1	PBS	11.0 ± 1.1 (7)
LCPS1	DEX	10.7 ± 0.6 (16)
LCPS2	PBS	9.3 ± 1.2 (7)
LCPS2	DEX	9.7 ± 0.9 (16)

No differences in ß-glucuronidase activity in the 24 h lavage fluids were seen between control mice (1415±243 U/ml) and those treated with DEX (1367±275 U/ml). The effectiveness of the anti-LC1 antibodies on DEX inhibition of PMN recruitment 4 h post-zymosan administration was confirmed, such that the steroid exerted 69% inhibition of cell influx in NSS-pretreated animals, but only reductions of 14%, 14% and 3% of PMN accumulation were measured after DEX treatment in LCS3-, LCPS1- and LCPS2-pretreated mice (n=6).

In conclusion, we report here that endogenous LC1 plays a role in modulating DEX inhibition of monocyte accumulation into the mouse peritoneal cavity inflamed with zymosan.

This work was supported by a grant of the ONO Pharmaceutical Co. to the William Harvey Research Institute. RJF is a Principal Research Fellow of the Wellcome Trust.

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[2] Perretti M. *et al.*, (1996) Br. J. Pharmacol. **117**, 1145.

161P CELLULAR MECHANISMS CONTROLLING GM-CSF RELEASE FROM CULTURED HUMAN AIRWAY EPITHELIAL CELLS

H. Klapproth, K. Racké* & I. Wessler, Department of Pharmacology, Univ. Mainz, Obere Zahlbacher Str. 67, D-55101 Mainz, *Department of Pharmacology, Univ. Bonn, D-53113 Bonn, Germany.

It is known, that interactions within the cytokine network trigger the release of other cytokines. For example, IL1β has been shown to stimulate the release of GM-CSF from airway epithelial cells (Cromwell et al., 1992). Little is known about the second messenger systems that modulate the release of cytokines. Therefore, in the present study receptor mediated effects and second messenger systems controlling the release of GM-CSF were investigated. Primary cultures of human bronchial epithelium (HBE) were prepared by pronase (0.1%) treatment of bronchi obtained at thoracotomy from patients with lung cancer. After 10 days of culture epithelial cells were incubated for 24 h with test-substances and GM-CSF content was measured in the cell culture supernatant by a commercial ELISA kit.

Baseline release of GM-CSF was 60±13 ng/10^6 cells/24 h (n=11). The cytokines IL1β (0.1-10 ng/ml) and TNFα (1,10 ng/ml) stimulated GM-CSF release in cultured HBE cells in a concentration dependent way. For example, 10 ng/ml IL1β enhanced GM-CSF release more than 10-fold (n=7, p<0.01). The stimulatory effect of IL1β was time dependent showing a latency of 8 h. RT-PCR analysis indicated the induction of GM-CSF mRNA in HBE cells after stimulation with IL1β. The sphingomyelinase system with the production of ceramide represents a second messenger system which has been shown to be acivated by IL1β (Mathias et al., 1993). Applied C2-ceramide (10 μM) induced a GM-CSF release comparable to 1ng/ml IL1β (n=4, p<0.05).

Pretreatment of the cells with dexamethasone (10 μM) inhibited the stimulatory effect of IL1β (n=3, p<0.05). Also dichloroisocumarine (DCI, 50 μM), an inhibitor of the transcription factor NF-κB, supressed IL1β -induced GM-CSF release into the supernatant (n=4, p<0.05). Staurosporine (100 nM), a non-specific PKC-inhibitor, completely blocked IL1β-induced GM-CSF release (n=4, p<0.05), whereas phorbol 12,13-dibutyrate (100 nM) increased GM-CSF release and strongly enhanced IL1β-induced GM-CSF release (>300%, n=4, p<0.05). The inactive phorbol 12-myristate 13-acetate was ineffective(n=2). Furthermore the effect of β-adrenergic stimulation of the cells was analysed. Preincubation of the cells with isoprenaline (1 μM) inhibited IL1β-induced GM-CSF release by 40% (n=4, p<0.05). This inhibition was strongly intensified by IBMX (100 μM) resulting in a GM-CSF release of almost baseline levels (n=4, p<0.05).

Further to these cytokines bradykinin (BK, 100 nM, n=5) and histamine (100 μM, n=5) enhanced GM-CSF release threefold (p<0.05). Pretreatment of the cells with the B2 receptor antagonist icatibant (0.1 μM) prevented the effect of BK (n=5). Pretreatment of the cells with actinomycin D (1 μM), a transcription inhibitor, or with cycloheximide (10 μM) blocked histamine-induced GM-CSF release (n=3, p<0.05).

In conclusion, the macrophage derived cytokines TNFα and IL1β as well as the mast cell derived mediator histamine trigger GM-CSF release from human airway epithelial cells. The latency of the stimulatory effect, the experiments with actinomycin D and RT-PCR analysis suggest enhanced transcription of the respective gene and release of de-novo synthesized GM-CSF. Synthesis of GM-CSF is upregulated by PKC activity, ceramid production and the transcription factor NF-κB. Synthesis of GM-CSF is downreglated by glucocorticoids and cAMP-dependent activity .

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162P AXOTOMY INCREASES THE RESPONSE OF RAT SENSORY NEURONES TO Y_2 AGONISTS

F.A. Abdulla, W.F. Colmers & P.A. Smith. Dept. of Pharmacol., Univ. of Alberta, Edmonton, T6G 2H7, Canada.

When peripheral sensory nerves are injured, sympathetic fibres sprout into dorsal root ganglia (DRG). Noradrenaline from these fibres activates ectopic excitatory α-adrenoceptors on DRG cells; a response that may contribute to the aetiology of certain types of chronic pain (McLachlan *et al.* 1993). Because sympathetic fibres release neuropeptide Y (NPY; Lundberg *et al.*, 1983) and axotomy alters the expression of mRNA for NPY-receptor in DRG (Zhang *et al.*, 1994), we tested whether peripheral nerve injury altered the sensitivity of these neurones to NPY. The sciatic nerve of adult male rats (120-170g) was sectioned and a 10mm segment removed to prevent regeneration. 2-7 weeks later, DRG cells were dissociated and A-, H- and C-cells examined by whole-cell recording. Table 1 shows that NPY (1μM) increased the excitability of C-cells more than that of H- and A-cells and these effects were greater after axotomy. C-cell Ca^{2+}-current (I_{Ca}) was suppressed more by NPY than that of H- or A-cells. All cell types showed a greater response to the peptide after axotomy. Increased excitability and I_{Ca} suppression were also seen with the Y-2 agonist PYY[3-36](300 nM). NPY enhanced I_{Ca} in a few control C-cells (4/29) by 26.1±2.9% as did the Y-1 agonist, [Pro^{34}]-PYY (300 nM; in 9/24 C-cells by 23.5±3.8%). After axotomy [Pro^{34}]-PYY enhanced the current in only 2/22 cells (by 16.8±3.8%). This enhancement was occluded by 2μM nifedipine indicating L-channel ($I_{Ca,L}$) involvement. ω-conotoxin GVIA (1μM) occluded I_{Ca} suppression induced by the Y-2 agonist indicating N-channel ($I_{Ca,N}$) involvement. Because increases in excitability are mediated via Y-2 receptors, the axotomy-induced increase in Y-2 effects on $I_{Ca,N}$ and the decrease in Y-1 effects on $I_{Ca,L}$ may contribute to the appearance of injury-induced ectopic activity. This work therefore highlights the therapeutic potential of Y-2 antagonists.

Table 1. Effects of NPY and PYY[3-36] on DRG neurones (n = 9-11 rats in each group; * = P<0.05 Student's t-test or χ^2 test as appropriate).

	NPY excitability↑	NPY I_{Ca}↓	NPY (I_{Ca} %↓)	PYY[3-36]I_{Ca}↓	PYY[3-36] (I_{Ca}%↓)
Control A-cells	4/18 cells	3/25 cells	17.6±2.7%	4/26 cells	16.8±2.2%
Axot. A-cells	12/23 cells *	11/21 cells *	34.9±2.7% *	12/23 cells *	30.6±7.3% *
Control H-cells	4/16 cells	5/26 cells	20.7±4.3%	5/21 cells	17.5±2.7%
Axot. H-cells	11/19 cells *	13/24 cells *	35.7±4.4% *	12/22 cells *	29.8±3.0% *
Control C-cells	8/19 cells	13/29 cells	36.4±2.9%	11/25 cells	27.4±3.0%
Axot. C-cells	17/22 cells *	24/32 cells *	50.4±3.5% *	18/24 cells *	44.0±2.5% *

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163P M-CURRENT POTENTIATION BY LOW MUSCARINE CONCENTRATIONS IN BULLFROG SYMPATHETIC GANGLIA

D.E. Kurennyi, H. Chen[1] & P.A. Smith. Dept. of Pharmacology, University of Alberta, Edmonton, T6G 2H7 Canada and [1]National Research Institute of Chinese Medicine, Taipei Hsien, 23177, Taiwan.

The M-current (I_M) is a non-inactivating K^+ current that is suppressed by a variety of agonists that act through heptahelical, G-protein-coupled receptors (Adams *et al.*, 1982). Suppression of this current invokes marked increases in neuronal excitability. M-channel conductance (g_M) is increased as intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is increased from 'zero' to about 250nM but is decreased when $[Ca^{2+}]_i$ is further increased to 450nM or more (Yu *et al.*, 1994). Since it has been reported that the concentration of ACh required to elevate $[Ca^{2+}]_i$ in neuroblastoma cells is lower than that required to produce g_M suppression (Robbins *et al.*, 1993), we hypothesised that the effects of muscarine on g_M in B-cells of bullfrog sympathetic ganglion might be concentration dependent and that g_M potentiation might be seen with low agonist concentrations. This possibility was tested using standard whole-cell recording techniques (Selyanko *et al.*, 1990). The suppression of steady-state I_M at -30mV by relatively high concentrations of muscarine (≥200nM) was accompanied by a shift in the g_M activation curve to more positive potentials (up to +15mV shift with 10μM muscarine; n=8). The time constant for I_M activation (τ_a at -30mV) was reduced by about 15% (n=7) but this was not statistically significant. The time constant for deactivation (τ_d at -50mV) was increased by about 40% with 1μM muscarine (n=9). This contrasted with the effects of low concentrations of muscarine (≤30nM) that shifted the g_M activation curve to more negative potentials (up to -5mV shift with 10nM muscarine; n=8), increased both τ_a and τ_d by about 30% ($p<0.05$; n=7 for both) and *increased* steady-state I_M by as much as 30% in 5 out of 20 cells treated with 10nM muscarine. Since the changes in g_M kinetics seen with low concentrations of muscarine resemble those seen following modest elevation of $[Ca^{2+}]_i$ (Yu *et al.*, 1994), the increase in g_M may reflect submembrane changes in $[Ca^{2+}]_i$. Although more profound elevation of $[Ca^{2+}]_i$ to about 450nM is known to suppress g_M (Marrion *et al.*, 1991) this mechanism is unlikely to explain the suppression of g_M seen with higher agonist concentrations (*see* Kirkwood *et al.*, 1991; Selyanko & Brown, 1996) because the changes in g_M kinetics invoked by elevation of $[Ca^{2+}]_i$ to 450nM are quite different from those seen during muscarine-induced g_M suppression.

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164P INTRINSIC MEMBRANE PROPERTIES DETERMINE FIRING PATTERNS OF NEURONS IN THE RAT AUDITORY THALAMUS

F. Tennigkeit, D.W.F. Schwarz[1] & E. Puil
Department of Pharmacology & Therapeutics and [1]The Rotary Hearing Centre, Division of Otolaryngology, Department of Surgery, Faculty of Medicine, The University of British Columbia, Vancouver, B.C., V6T 1Z3, Canada

A principal function of neurons in the ventral medial geniculate body (MGBv) is the transformation of auditory signals. Also, thalamic neurons change their firing patterns according to different behavioral states. Thus, the responses to similar auditory stimuli in MGBv neurons are dramatically different during wakefulness, sleep and anesthesia (Creutzfeldt *et al.*, 1980; Zurita *et al.*, 1994).
Using whole-cell patch-clamp techniques in *in vitro* slices (21-25° C), we recorded from 78 MGBv neurons of 16-21 day-old Sprague-Dawley rats. The position and morphology of the neurons were verified by inclusion of neurobiotin in the recording pipette and with subsequent, immunohistological staining.
At depolarized membrane potentials, resembling those during wakefulness, we observed, after a voltage ramp like delay, tonic firing of action potentials in response to depolarizing current pulse injection. The delay to firing and the voltage ramp, itself, were sensitive to tetrodotoxin (TTX, 300 nM, n = 32) and 4-aminopyridine (4-AP, 100 μM, n = 11), implying an involvement of a persistent Na^+ and A-type K^+ currents.
In a hyperpolarized voltage range, as in thalamic neurons during deep sleep (Hirsch *et al.*, 1983), we evoked spike bursts on the crest of a prominent low-threshold spike (LTS). The current underlying the LTS was likely a T-type Ca^{2+} current, because it was sensitive to blockade by Ni^{2+} (500 μM, n = 5) or removal of Ca^{2+} from the extracellular medium (n = 7).
On the LTS, we observed action potentials, which were blocked by application of TTX (300 nM, n = 32), and high threshold Ca^{2+}spikes (HTS), which were blocked selectively by application of Cd^{2+} (50 μM, n = 4). The total blockade of the HTS left intact, the action potentials and the LTS.
The persistent Na^+ and 4-AP sensitive K^+currents seem to promote a coupling of the HTS to the LTS. The HTS was not observed on the LTS after application of TTX (300 nM, n = 32). A HTS could be evoked on the LTS on coapplication of TTX (300 nM) and 4-AP (100 μM, n = 5), but not after coapplication of TTX (300 nM) and tetraethylammonium chloride (2 mM, n = 5).
Conclusion: Using selective pharmacological blockade, we surmise that the contributions of specific ionic currents to complex tonic and burst firing patterns depend on the initial membrane voltage and may have an important role in auditory transmission during various behavioral states.

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