EVIDENCE FOR DIFFERENTIAL SYMPATHOINHIBITORY ACTIONS OF 8-OH-DPAT AND CLONIDINE IN ANAESTHETISED CATS

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8-OH-DPAT causes only moderate thoracic sympathoinhibition in anaesthetised cats (Ramage & Fozard, 1987). This is surprising as the hypotensive action of 8-OH-DPAT is considered to be mediated entirely by the central nervous system (Fozard et al., 1987). One explanation for this lack of correlation between the thoracic sympathoinhibitory action and the hypotensive action of 8-OH-DPAT is that 8-OH-DPAT could be having a more potent sympathoinhibitory action at a different level or levels of sympathetic outflow. For clonidine also, it has been suggested that its sympathoinhibitory action may vary from one regional sympathetic outflow to another (Schmitt, 1975). Hence a comparison of the effect of 8-OH-DPAT and clonidine on three different sympathetic outflows was carried out in anaesthetised cats.

Cats were anaesthetised with a mixture of α-chloralose (70 mg kg^{-1}) and pentobarbitone sodium (12 mg). Simultaneous recordings were made from the cardiac, splanchnic and renal nerves along with brachial arterial pressure and heart rate as described previously (Ramage, 1987). A cumulative (cum.) dose response curve for 8-OH-DPAT (0.5-128 μg kg^{-1}) and clonidine (0.3-10 μg kg^{-1}) was produced for each drug with injections given into the jugular vein.

Both 8-OH-DPAT (n=6) and clonidine (n=5) caused a dose related fall in blood pressure and heart rate reaching maxima of -76±5 (s.e. mean) and -56±8 mmHg and -28±12 and -68±11 beats min^{-1}, respectively at the highest dose. These decreases in both blood pressure and heart rate were associated with sympathoinhibition in all three nerves. However, the degree of sympathoinhibition differed between the nerves. This is shown below for one dose, in table I.

Table I

Drug	Cum. Dose μg kg^{-1}	BP mmHg	HR beats min^{-1}	CNA Δ%	SNA Δ%	RNA Δ%
8-OH-DPAT	8	-46±3	-5±9	-37±10*	-53±10	-76±8*
Clonidine	3	-31±6	-42±6	-70±8#φ	-36±10φ	-25±12#

CNA, SNA and RNA - cardiac, splanchnic and renal nerve activity.
* # φ P < 0.05: (least sig. diff. two way analysis of variance)

These results show that different regional sympathetic outflows have varying sensitivities to sympathoinhibition caused by activation of central $5\text{-}HT_{1A}$ or α_2-adrenoceptors. It is therefore suggested that in the control of renal sympathetic outflow, central serotonergic pathways may play a more important role than noradrenergic pathways whereas the reverse control operates for cardiac sympathetic outflow.

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TRANSMITTER RELEASE FROM THE BASILAR ARTERY

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Cerebral blood vessels are densely innervated. In vitro studies have shown that the contractile responses to transmural nerve stimulation (TNS) cannot be blocked by the use of adrenoceptor antagonists, although tetrodotoxin (TTX) can abolish the response. This finding suggests a neurotransmitter other than noradrenaline (NA) may be involved. Serotonin (5HT) is strongly implicated in the aetiology of several cerebrovascular diseases. Histochemical and uptake studies have suggested adrenergic and serotoninergic innervation of pial arteries (L. Edvinsson et al., 1983; T.J. Verbeuren et al., 1983).

Using HPLC with electrochemical detection (LCED) we studied the release of endogenous noradrenaline, adrenaline, dopamine, and 5HT, in response to TNS of the cerebral arteries. Spiral preparations of the rabbit basilar and internal carotid arteries were superfused with oxygenated Krebs at 37°C, containing adrenergic and serotoninergic uptake inhibitors, and a β-blocker. Samples were collected into tubes containing sodium metabisulphite. 5HT was extracted using cation exchange chromatography while catecholamines were extracted using acid alumina. The amines were assayed using HPLC with electrochemical detection. The chromatographical conditions were varied to ensure correct identification of the substances being analysed. The rabbit internal carotid artery was used as a peripheral vessel for comparison, and the sheep basilar artery to examine any species variation.

LCED assay showed the presence of both NA and 5HT in the superfusate collected under control conditions and there was a significant increase in the release of both substances on TNS of these vessels (10 and 15 Hz). No detectable amounts of adrenaline, or dopamine were present in the samples. (See Table below).

	Rabbit Basilar Artery		Rabbit Internal Carotid Artery		Sheep Basilar Artery	
	Basal	Stimulated	Basal	Stimulated	Basal	Stimulated
5HT	545 ± 303 (n = 5)	2671 ± 895	1300 ± 514 (n = 5)	3509 ± 1400	239 ± 25 (n = 5)	680 ± 100
NA	5 ± 2 (n = 5)	28 ± 5	ND (n = 5)	ND	7 ± 2 (n = 5)	52 ± 9

ND = Not Detectable. Results - mean ± s.e.mean as pM/sample.

Our results have demonstrated both noradrenaline and 5HT are released on transmural nerve stimulation of the basilar artery.

Supported by the Joint Research Board of St. Bartholomew's Hospital.

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A COMPARISON OF THE EFFECTS OF R 56865 ON K^+-INDUCED CONTRACTION AND INCREASE IN SLOWLY EXCHANGING ^{45}Ca IN THE RAT AORTA

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The compound R 56865 (N-[1-[4-(4-fluorophenoxy)butyl]-4-piperidinyl]-N-methyl-2-benzothiazolamine) decreases the toxicity of cardiac glycosides and acts anti-ischaemic in the heart. Since in both cardiac glycoside toxicity and ischaemia calcium-overload plays a role we studied the effects of R 56865 in two models used for the measurement of calcium entry, the K^+-induced contraction and the K^+-induced increase in slowly exchanging ^{45}Ca in the rat aorta. The effects of R 56865 were compared with the actions of the calcium-entry blockers nifedipine, diltiazem and flunarizine.

Aortic rings from Wistar rats (200-300 g) were suspended in a Tyrode-solution containing 0.9 mM Ca^{2+} gassed with 95% O_2 and 5% CO_2 at a resting tension of 1 g and a temperature of 37°C. K^+-induced isometric contractions were determined by cumulative dose-response curves without or after pretreatment for 30 minutes with R 56865 or the calcium-entry blockers. The rings contained no functional endothelium. For measurement of slowly exchanging ^{45}Ca rat aortic strips were suspended at a resting tension of 0.33 g, pretreated for 1 hour with the drug under investigation and incubated for 5 minutes in ^{45}Ca-containing (1 µCi/ml) normal or depolarizing Tyrode-solution (NaCl 48 and KCl 80 mM in contrast to NaCl 124 and KCl 4 mM). The ^{45}Ca remaining in the strips after washing for 45 minutes with a Tyrode-solution of 4°C was measured according to Wermelskirchen et al. (in press).

The K^+-induced contractions were strongly and dose-dependently inhibited by nifedipine (10^{-9} - 10^{-7} M), diltiazem (10^{-7} - 10^{-5} M) and flunarizine (10^{-7} - 10^{-5} M). In contrast maximal attainable inhibition by R 56865 (10^{-5}M) amounted to 45%. In a comparable concentration-range the K^+-induced increase in slowly exchanging ^{45}Ca was dose-dependently and completely inhibited by all four agents. The basal uptake of slowly exchanging ^{45}Ca was not affected.

In conclusion, in contrast to nifedipine, diltiazem, and flunarizine, R 56865 has a stronger effect on the K^+-induced increase in slowly exchanging ^{45}Ca than on the contraction. This may indicate that the effects of R 56865 in a concentration of 10^{-6} and 10^{-5} M can not be explained by calcium-entry blockade alone. Additionally R 56865 may decrease the storage of calcium in those slowly exchanging compartments which increase upon K^+-induced depolarization. In other words, measurement of the effects of R 56865 on the K^+-induced increase in slowly exchanging ^{45}Ca may overestimate its calcium-entry blocking activity.

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PHARMACOLOGICAL CHARACTERISATION OF K^+-INDUCED CONTRACTILE RESPONSES IN RAT AORTIC RINGS

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The K^+ concentration-response curve ($[K^+]_o$ = 0.1 - 50 mM) on rat aortic rings exhibited three distinct phases, a relaxation, followed by a plateau, then a contraction (Cavero *et al.*, 1987). We previously demonstrated that the relaxation phase was due to activation of Na,K-ATPase. The aim of this study was to characterize pharmacologically the K^+-induced contractile response in the rat aortic ring.

Aortic rings with functional endothelium from male Sprague-Dawley rats (300-350g) were suspended in a K^+-free physiological salt solution (PSS composition in mM : NaCl 117; $CaCl_2$ 1.25; NaH_2PO_4 1.0; $MgSO_4.7H_2O$ 1.2; glucose 11.5; $NaHCO_3$ 25). A tension of 2 g was applied to each tissue. A relaxant response to acetylcholine (2 μM) in noradrenaline (0.02μM) contracted preparations indicated the presence of a functional endothelium. Cumulative concentration- response curves to KCl (2-50 mM) were determined 20 min after exposing the preparations to ethanol (0.1 % : control), (-)-Bay k 8644 (0.3 μM) or (-)-Bay k 8644 plus either BRL 34915, nicorandil, nitrendipine, diltiazem, HA 1004, bucindolol, pinacidil, minoxidil sulphate or apamin. The magnitude of contractions produced by 5 (E_{C5}) and 50 mM KCl (E_{C50}) were calculated for each preparation and reported as means ± s.e. mean.

The K^+ concentration-contractile response curve ($[K^+]_o$ = 2.5-50 mM) exhibited two apparently distinct phases separated by a plateau at 4-8mM, obtained in PSS with 1.25 mM $CaCl_2$. The maximum of the first phase (produced by 4-5mM K^+) of the curve was reduced by increasing the extracellular calcium concentration (to 2.5 and 5 mM), and enhanced by (-)-Bay k 8644 (0.3μM) (E_{C5} values : with 1.25mM $[Ca^{2+}]_o$ = 0.67±0.18g, n=9 ; with 2.5mM $[Ca^{2+}]_o$ = 0.24±0.1g, n=8 ; with 5.0mM $[Ca^{2+}]_o$ = 0.08±0.04g, n=6 ; with 1.25mM $[Ca^{2+}]_o$ and (-)-Bay k 8644 = 1.25±0.14g, n=9). The contraction to 50 mM K^+ (second phase) (with 1.25mM $[Ca^{2+}]_o$ = 1.64±0.15g, n=9) was unchanged by (-)-Bay k 8644 or an increase in $[Ca^{2+}]_o$.

The first phase of the K^+ curve, in the presence of (-)-Bay k 8644 was attenuated by BRL 34915 (0.3-1 μM), pinacidil (1 μM), minoxidil sulphate (10 μM) and nicorandil (10 μM), but was not modified by bucindolol (3 μM). While the response to 50 mM K^+ was only inhibited by bucindolol. Nitrendipine (0.01-0.1 μM) and diltiazem (1 μM) inhibited both phases of the K^+ curve. HA 1004 (1 μM), a purported inhibitor of protein kinase C (Hidaka *et al.*, 1984) only inhibited the first phase of the K^+ curve, in the presence of (-)-Bay k 8644 ; however 10 μM HA 1004 attenuated both phases. Finally, apamin (0.1 μM) failed to modify the contractile responses to K^+.

In conclusion, the contractile responses of the rat isolated aorta to 2-10 mM K^+ were enhanced by (-)-Bay k 8644 ; this effect was antagonised by BRL 34915, which can induce vascular hyperpolarisation via K^+ channel activation (Weir and Weston, 1986). Therefore, in this preparation, it is possible that a BRL 34915-sensitive K^+ channel is responsible for maintenance of vascular tone. Finally, part of the vasorelaxant activity to HA 1004 could be due to K^+ channel activation.

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PERTUSSIS TOXIN DOES NOT INHIBIT THE EFFECTS OF BRL 34915 ON RAT ISOLATED CARDIAC AND VASCULAR TISSUE

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In a variety of mammalian smooth muscles, BRL 34915 produces relaxation by opening $^{86}Rb^+$ permeable K^+ channels leading to membrane hyperpolarisation (Weir & Weston, 1986a,b; Quast, 1987; Allen et al, 1986). The nature of the K^+ channels opened by BRL 34915 is as yet unknown, although they do not appear to require Ca^{2+} entry through dihydropyridine-sensitive channels nor are they sensitive to apamin. In the present study, we have investigated whether pertussis toxin (PTx), an inhibitor of certain G-proteins (Dunlap et al, 1987) modifies the effects of BRL 34915 in rat isolated cardiac and vascular smooth muscles

Male Wistar rats (200-250g) were killed 40hr after the i.v. infusion of PTx (25µg in 1ml saline) or vehicle. Under isometric conditions, carbamylcholine (carb) produced a decrease in heart rate and contractile force of the isolated driven atria removed from saline-treated rats. Measurements of the action potential (see Scholtysik, 1987) showed that this was accompanied by a decrease in the AP duration and an increase in the rate of repolarisation. In atria preloaded with $^{86}Rb^+$ (5µCi/ml) for 90 min, carb (0.3-10µM) produced a concentration-dependent increase in the efflux rate constant K^+. These effects of carb were completely abolished in the atria of PTx-treated rats, demonstrating the efficacy of the toxin. BRL 34915 (300µM) increased the $^{86}Rb^+$ efflux rate constant by 35% ± 3 (n=6) in atria removed from PTx-treated rats, an effect which was not significantly different from control atria.

In portal veins removed from PTx-treated rats, BRL 34915 (30nM) reduced the frequency of spontaneous contractions without affecting their amplitude. At higher concentrations, the spontaneous mechanical activity was abolished and the simultaneous measurement of $^{86}Rb^+$ efflux (Quast, 1987) demonstrated that this was accompanied by an increase in the efflux rate constant. In aortic rings from the same rats, BRL 34915 (0.1-10µM) caused a concentration-dependent reduction of the contractions to noradrenaline, effects which saturated at 1µM, producing approximately 50% inhibition. Furthermore, the responses to low but not high concentrations of KCl were inhibited by BRL 34915. When aortic rings preloaded with $^{86}Rb^+$ for 90 min were challenged with BRL 34915 (0.3-10µM) for 20 min, a significant increase in efflux rate constant was observed. These effects of BRL 34915 in the portal vein and aorta were not significantly different from control tissues removed from saline-treated rats.

It is concluded that PTx treatment does not inhibit the effects of BRL 34915 in the rat isolated atria, portal vein and aorta. This suggests that BRL 34915 does not act through a PTx-sensitive G-protein in order to open K^+ channels in these tissues. However, these results do not exclude the possibility that BRL 34915 may act directly on a G-protein sensitive K^+ channel, thereby overruling the normal regulatory coupling mechanism of the channel.

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IN VIVO AND IN VITRO STUDIES OF CROMAKALIM (BRL 34915) AND GLIBENCLAMIDE IN THE RAT

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The vasorelaxant activity of the anti-hypertensive drug cromakalim (BRL 34915), which results from increased K^+ conductance (Hamilton et al., 1986), can be inhibited by K^+ channel blocking agents (Wilson, 1987). It has been proposed that blockade of ATP-sensitive K^+ channels is the mechanism by which glibenclamide and other sulphonylureas promote insulin secretion in pancreatic B-cells (Sturgess et al., 1985) to lower blood glucose levels. The possible interaction between cromakalim and glibenclamide on blood pressure, vasorelaxation in vitro, and insulin secretion both in vivo and in vitro was therefore investigated in male Sprague-Dawley rats.

In conscious rats pre-treated (2h) with glibenclamide (100mg/kg p.o.), cromakalim (0.075mg/kg i.v.) produced a peak fall in mean arterial pressure of 25.0±4.3mmHg (direct recording; n=7). In vehicle pre-treated controls (n=8), the corresponding fall, 22.5±2.9mmHg, was not significantly different ($p>0.05$). Isometric tension was recorded (Wilson et al., 1987) from rings of isolated thoracic aorta pre-contracted with noradrenaline (10^{-6}M) (n=5). Cromakalim produced concentration-related relaxation (max. 79±3%) with an IC_{40} value of $4.4(2.4\text{-}7.9)\times10^{-7}$M. In the presence of glibenclamide 10^{-6}, 3×10^{-6} and 10^{-5}M, the IC_{40} values for cromakalim were $2.7(1.8\text{-}4.0)\times10^{-6}$, $1.1(0.7\text{-}1.7)\times10^{-5}$ and $2.2(1.0\text{-}4.7)\times10^{-5}$M respectively. In separate experiments, the inhibitory effect of cromakalim (10^{-5}M) on noradrenaline concentration-response curves (a reduction to 38.3±7.8% of max. contraction) was completely reversed by glibenclamide (3.3×10^{-6}M). In batch incubated pancreatic islets cromakalim (10^{-5}M) neither reduced insulin secretion nor inhibited the increase (128%) to glibenclamide (5×10^{-5}M). Plasma insulin levels were determined during a glucose tolerance test (Sennitt et al., 1985) in conscious, 24h fasted rats (n=5). Cromakalim (0.3mg/kg p.o.) neither reduced plasma insulin nor inhibited the increase (81%) to glibenclamide (2.5mg/kg p.o.).

The results demonstrate that although glibenclamide is an inhibitor of the vasorelaxant effect of cromakalim in isolated vasculature, it does not inhibit the anti-hypertensive effect of cromakalim in vivo. In addition, cromakalim does not decrease insulin secretion in vivo or in vitro, nor does it inhibit the increase produced by glibenclamide. The significance of these results is under further investigation.

The authors acknowledge the technical assistance of Miss C. Peppard.

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PROFILE OF ADIBENDAN, A NEW POSITIVE INOTROPIC AND VASODILATING AGENT

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Adibendan (BM 14.478 = 7,7-dimethyl-2-(4-pyridyl)-6,7-dihydro-3H,5H-pyrrolo[2,3-f]benzimidazol-6-one) is a newly developed compound. It has been demonstrated to cause positive inotropic and vasodilating effects in a variety of experimental models (Müller-Beckmann, B. et al., 1987). Some of the data obtained with adibendan will be discussed.

Effects in vivo: Haemodynamic effects were investigated in anaesthetized rats, open-chest cats (pretreated with 0.3 mg/kg of the ß-blocking agent desacetylmetipranolol i.v.), and in conscious dogs. All animals were prepared for recording left ventricular pressure (LVP), LV dp/dt, blood pressure (BP) heart rate (HR) and ECG. After intravenous injection of incremental doses, LV dp/dt was increased over a dose range of 0.01 to 0.1 mg/kg in rats (7,200 $\pm$ 300 to 10,700 $\pm$ 500 mmHg/s), 0.001 to 0.3 mg/kg in cats (2,800 $\pm$ 200 to 4,500 $\pm$ 100 mmHg/s) and 0.01 to 3.0 mg/kg in dogs (2,400 $\pm$ 100 to 4,400 $\pm$ 500 mmHg/s). BP decreased markedly in the same dose ranges. HR increased in rats and dogs, but not in the ß-blocked cats. On the basis of equieffective doses ($ED_{1.5}$ = doses which increased LV dp/dt by 1,500 mmHg/s) adibendan was about 5 to 18 fold more effective than milrinone. In conscious dogs, chronically instrumented with right atrial catheters and electromagnetic flow probes around the aortic root, adibendan increased at doses of 0.01 to 10 mg/kg i.v. cardiac output by 39 $\pm$ 10 % and stroke volume by 17 $\pm$ 7 %, while it reduced total peripheral resistance by 45 $\pm$ 5 % and right atrial pressure by 3.0 $\pm$ 0.3 mmHg. In dogs, chronically instrumented with Konigsberg manometers, the positive inotropic effect of adibendan (1.0 mg/kg p.o.) lasted longer than the observation period of 6.5 h. The duration of action was definitely longer than that of milrinone. No signs of development of tolerance were observed in the same dogs after chronic administration of 1.0 mg/kg p.o. b.i.d. for 10 days.

Effects in vitro: Direct positive inotropic effects were achieved in papillary muscles (10^{-6} to 5 x 10^{-4} M) and electrically driven atria (10^{-8} to 5 x 10^{-4} M) from guinea-pig hearts. These effects were not influenced by propranolol (4 x 10^{-5} M), cimetidine (10^{-4} M) or tetrodotoxin (10^{-5} M), but diminished concentration dependently by carbachol (10^{-7} to 3 x 10^{-6} M). In contrast to isoprenaline there was only a slight chronotropic activity in spontanously beating guinea-pig atria (10^{-7} to 10^{-4} M). Atrial cAMP was increased from 1.46 $\pm$ 0.06 to 1.97 $\pm$ 0.03 pmol/mg wet wt. at 3 x 10^{-6} M, probably due to an inhibition of cardiac phosphodiesterase. IC_{50} of bovine PDE was 7.2 x 10^{-5} M. Calcium activated force of membrane-free heart muscle preparations was increased in chemically skinned porcine heart muscle fibres to 43 $\pm$ 7 % (10^{-7} to 10^{-4} M) and in skinned fibres of guinea-pig papillary muscles to 181 $\pm$ 32 % (10^{-9} to 5 x 10^{-4} M). Rat aortas were relaxed maximally by about 75 % (10^{-7} to 10^{-4} M).

We conclude that adibendan is a compound which acts as a positive inotropic and vasorelaxing agent via an increase in intracellular cAMP content (due to inhibition of PDE). A calcium-sensitizing effect on contractile proteins may contribute to its inotropic effect.

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THE DA_2 AGONIST, SK&F 101468-A IS HYPOTENSIVE IN SPONTANEOUSLY HYPERTENSIVE RATS

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SK&F 101468-A is a selective, dopamine DA_2 agonist (Gallagher et al., 1985). This study investigates the effect of SK&F 101468-A on blood pressure and heart rate in anaesthetised and conscious spontaneously hypertensive rats (SHR). Rats, anaesthetised with dial-urethane, were infused i.v. with SK&F 101468-A at doses of 0.025, 0.05, 0.1, 0.2 or 0.5 mg/kg, over 6 minutes. Blood pressure and heart rate were measured before, during and for 55 minutes after the infusion. In addition SHR were cannulated at the femoral artery and vein, under halothane anaesthesia, a minimum of 48 hours prior to use. Blood pressure and heart rate were measured 15 and 5 minutes before and for 5 hours after dosing. Doses of SK&F 101468-A used were 0.5, 2.5, or 5.0 mg/kg i.v. bolus, or 10, 15, 20, or 40 mg/kg p.o.

Table 1 Changes in blood pressure and heart rate in anaesthetised rats following an infusion of SK&F 101468-A. (Mean ± S.E.M. n=5)

	Dose of SK&F 101468-A infused (mg/kg)					
	Saline	0.025	0.05	0.1	0.2	0.5
Blood Pressure (mmHg)	-3±1	-35±9*	-39±8*	-54±8*	-49±11*	-73±8*
Heart Rate (bpm)	-6±2	-11±6	-14±5	-41±15*	-41±4*	-64±13*

* Statistically significantly different from control value, $p<0.05$ (Dunnett's multicompare)

Table 2 Peak change in blood pressure seen in conscious SHR within 60 minutes of dosing with SK&F 101468-A, and concomitant changes in heart rate. (Mean ± S.E.M. n=6)

Dose (mg/kg)	Saline	0.5 i.v.	2.5 i.v.	5.0 i.v.
Blood pressure (mmHg)	-7±4	-11±8	-25±4*	-28±6*
Heart rate (bpm)	5±8	3±29	-32±12*	42±13*

Dose (mg/kg)	Saline	10 p.o.	15 p.o.	20 p.o.	40 p.o.
Blood pressure (mmHg)	6±6	-5±5	-12±10	-19±7*	-25±5*
Heart rate (bpm)	6±19	35±12*	41±35	5±15	-3±11

* Statistically significantly different from predose values (95% confidence intervals, Student's t-test)

In anaesthetised and conscious SHR, SK&F 101468-A caused a dose related fall in blood pressure. In anaesthetised SHR the response was maintained throughout the observation period whereas heart rate values recovered towards saline control values. In the conscious SHR blood pressure returned towards, but did not reach, predose values, by 5 hours post dose. Changes in heart rate were variable and not considered to be drug related.

Administration of the dopamine DA_2 antagonists L-sulpiride (0.5mg/kg i.v.) or domperidone (1mg/kg i.v.) 60 minutes after oral administration of SK&F 101468-A (20mg/kg) completely reversed the hypotension.

Some rats treated with SK&F 101468-A were observed to exhibit stereotyped sniffing and ptosis, but these activities were not quantified.

These results demonstrate that SK&F 101468-A reduces blood pressure in the SHR in a dose related manner. Evidence is provided that this effect is mediated by DA_2 dopamine receptor agonist activity.

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ACTIONS OF BRADYKININ AND RELATED PEPTIDES ON RABBIT COELIAC ARTERY RINGS AND BLOOD PRESSURE

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Bradykinin relaxes rabbit coeliac artery rings. This response does not require intact endothelium and is prostaglandin-mediated (Förstermann et al., 1986). There are varying reports as to whether the hypotensive action of bradykinin in the rabbit is antagonised by cyclo-oxygenase inhibitors (reviewed by Nasjletti & Malik, 1979). To characterise these responses further, we used: a) analogues of bradykinin that are relatively selective for B_1 or B_2 receptors (Regoli et al., 1986); and b) 5-(N,N-hexamethylene) amiloride, a more potent inhibitor of Na^+/H^+ exchange than amiloride (Simchowitz & Cragoe 1986). This compound inhibits Ca^{2+}-stimulated prostacyclin synthesis in rat aorta (Ritter et al., 1987). New Zealand white rabbits of either sex (2.5-5.8 kg) were used. For experiments in vitro, animals were killed with pentobarbitone (120 mg kg^{-1} iv). The coeliac artery was removed rapidly and cut into 2 mm rings. These were mounted on hooks connected to an isometric force transducer and suspended in Krebs' solution at 37°C at a basal tension of 2 g. Phenylephrine (usually $3x10^{-6}$ M) was added to the organ bath to contract the arterial rings before adding bradykinin or other relaxant drugs. For experiments in vivo, rabbits were anaesthetised with pentobarbitone, and the jugular vein and carotid artery cannulated for administration of drugs and measurement of blood pressure respectively. The concentration of bradykinin that caused 50% relaxation of the arterial rings (EC_{50}) was 6.1 ± 2.8 $x10^{-7}$ M (mean ± S.E. mean, n = 22) and the relaxation was reversibly antagonised by ibuprofen (5×10^{-7} - 5×10^{-6} M, n = 13). Increasing the concentration of bradykinin in the presence of ibuprofen resulted in a parallel shift to the right of the log dose-response relation without reduction of the maximum response, indicating the presence of spare receptors. $DesArg^{10}$-kallidin was approximately 100 times more potent than bradykinin (EC_{50}=6.4 ± 2.8 x 10^{-9} M, n = 8). Relaxation caused by either agonist was reversibly antagonised by $[Leu^9]$-$desArg^{10}$- kallidin and, less potently, by $[Thi^{6,9},D\text{-}Phe^8]$-kallidin. In each case antagonism was surmountable by increasing the concentration of bradykinin. These antagonists did not influence relaxations caused by prostacyclin. 5-(N,N-hexamethylene) amiloride (10^{-6} M) also inhibited relaxation caused by bradykinin but not by prostacyclin. However, unlike ibuprofen and the peptide antagonists, it caused flattening of the log concentration-response relation of bradykinin. Its effect was slowly reversible. Amiloride (10^{-6} M) had little or no effect on the response to bradykinin. In anaesthetised animals, bradykinin (1-3 µg iv bolus) caused a fall in arterial pressure which was little, if at all, affected by ibuprofen (5 mg kg^{-1} iv, n = 5). $DesArg^{10}$-kallidin (1-3 µg iv bolus) was without effect. The hypotensive effect of bradykinin was not affected by $[Leu^9]$-$desArg^{10}$-kallidin (2-20 µg iv immediately before bradykinin). We conclude that, in the rabbit, bradykinin relaxes coeliac artery rings by combining with a B_1 receptor which may be closely linked to a Na^+/H^+ exchanger that stimulates synthesis of a vasodilator cyclo-oxygenase product. In contrast, the hypotensive response to intravenous bradykinin is probably initiated by B_2 receptors and not prostaglandin mediated.

Supported by the BHF and MRC. Peptides were provided by Prof D. Regoli.

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A RE-EVALUATION OF THE TOPOGRAPHY OF BEHAVIOUR INDUCED BY SELECTIVE D-2 DOPAMINE RECEPTOR STIMULATION: STUDIES WITH LY 163502

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The prominent behavioural effects of selective D-1 dopamine receptor antagonists, many of them similar to those of typical neuroleptics and D-2 antagonists (Waddington, 1986), have challenged the earlier view that D-2 receptors play a prepotent role in the regulation of behaviour (Seeman, 1980). This has prompted us to re-evaluate the behavioural consequences of selective D-2 receptor stimulation with LY 163502, a new highly potent and selective D-2 agonist (Bymaster et al, 1986), in comparison with the classical non-selective dopaminergic agonist apomorphine.

Male Sprague-Dawley rats were injected s.c. with LY 163502, apomorphine or vehicle. They were observed using a rapid time-sampling behavioural check list procedure, which generates 'counts' for each individual behaviour evident, and a conventional 0-6 stereotypy rating scale (Molloy & Waddington, 1987).

mg/kg	Stereotypy	Sniffing	Locomotion	Still
Vehicle	0.7±0.1	11.7±1.7	3.1±0.8	22.1±1.2
LY 0.006	0.5±0.1	4.6±1.0*	0.2±0.2*	26.2±1.4
0.05	1.9±0.2**	22.3±1.8**	5.9±1.9	12.6±2.2**
10.0	1.8±0.2**	23.0±1.6**	10.5±1.9**	10.2±2.9**

Means ± s.e. mean, n = 5-13. **, $p < 0.01$; *, $p < 0.05$

Over a 1h period, LY 163502 initially decreased (0.003-0.006 mg/kg) and then increased (0.012-0.05 mg/kg) counts for sniffing and locomotion though it failed to induce classical stereotyped behaviour; the presence of repeated periods of stillness confirmed that no continuous, compulsive stereotypy was evident. Up to a 200-fold higher dose of LY 163502 (0.1-10.0 mg/kg) failed to increase further counts for sniffing or stereotypy scores, or to reduce further stillness, with only some modest increase in locomotion being evident. Conversely, apomorphine initially decreased (0.06-0.25 mg/kg) and then markedly stimulated (1.0-4.0 mg/kg) behaviour; a classical stereotypy syndrome was induced by the highest dose (score 3.6±0.2; n=6), consisting of sniffing, locomotion and some licking, with periods of stillness essentially abolished.

Like selective stimulation of D-1 receptors with SK&F 38393 (Molloy & Waddington, 1987) or its new high potency analogue SK&F 77434 (O'Boyle & Waddington, 1987; Murray & Waddington, 1988), selective stimulation of D-2 receptors with LY 163502 appears insufficient to induce classical stereotyped behaviour. This is consistent with evidence that co-stimulation of both D-1 and D-2 receptors is necessary for full expression of compulsive stereotypy (Mashurano & Waddington, 1986). Rather, selective stimulation of D-1 or D-2 receptors each induces a fragmented activation of behaviour, with some elements in common and some which are topographically distinct.

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THE D-1 AGONIST CY 208-243 REVERSES MPTP-INDUCED MOTOR DEFICITS IN THE COMMON MARMOSET

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The D-1 agonist SKF 38393 (2,3,4,5-tetrahydro-7,8 di-hydroxy-1-phenyl-1H-3-benzazepine) does not reverse MPTP-induced motor deficits in the common marmoset but inhibits the antiparkinsonian activity of the D-2 agonist quinpirole hydrochloride (Nomoto et al 1985,1987). We now report that another D-1 agonist the benzergoline CY 208-243 ((-)-(6aR)(12bR)-4,6,6a,7,8,12b-hexahydro-7-methylindolo [4,3-ab]-phenanthridine) (Markstein et al 1987) is effective in this model.

Common marmosets of either sex (290-390 g) were rendered parkinsonian with MPTP (2 mg/kg sc for 5 days) and studied three months later. The administration of L-DOPA (12.5 mg/kg ip) plus benzerazide (12.5 mg/kg po) and the D-2 agonist (+)-PHNO ((+)-4-propyl-9-hydroxynapthoxazine) (4 µg/kg sc) reversed their motor deficits and increased locomotor activity. In contrast administration of SKF 38393 (10 mg/kg sc) had no effect on motor deficits or on motor activity. The subsequent administration of CY 208-243 (0.5 - 1.25 mg/kg sc) produced a dose related reversal of motor deficits and an increase in activity (Table 1). The effect was apparent within 10 minutes and lasted up to 180 minutes. The effect of CY 208-243 (1.0 mg/kg sc) was almost totally inhibited by prior treatment with SCH 23390 (1-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzapine-7-ol) (2 mg/kg sc;30 minutes previously) but sulpiride (20 mg/kg sc; 60 minutes previously) produced only a partial inhibition. In another group of naive normal marmosets (260-365 g) CY 208-243 (1.0 mg/kg sc) produced no alteration in behaviour or activity. These animals were then given MPTP (2 mg/kg/day sc for 8-10 days) rendering them parkinsonian but without exposure to other drugs. Subsequent administration of CY 208-243 (1.0 mg/kg sc) reversed the motor deficits and increased locomotor activity (Table 1).

Table 1: Activity counts in 3 hours following treatment with CY 208-243

Experiment	Drug treatment				
	Dose of CY 208-243 (mg/kg sc)				
	0 (vehicle)	0.5	0.75	1.0	1.25
MPTP treated	98 ± 46	1099 ± 284*	2403 ± 943*	2360 ± 614*	3545 ± 1243*
MPTP plus	Vehicle		Sulpiride		SCH 23390
CY 208-243 (1 mg/kg sc)	2360 ± 614		1172 ± 363*		430 ± 498*
	Naive animals		MPTP treated		
CY 208-243	Vehicle	CY 208-243	Vehicle	CY 208-243	
(1 mg/kg sc)	228 ± 116	643 ± 176 N/S	350 ± 111	2158 ± 1085*	

* $p < 0.05$ (n = 6-8 animals) Two tailed Student's t-test

The D-1 agonist CY 208-243 reverses motor deficits in MPTP treated marmosets but is without effect in normal animals. The effect of CY 208-243 is apparent in drug naive MPTP animals and does not require D-2 agonist priming.

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JAT is a Medical Research Council (R.S.A.) Post Doctoral Fellow.

AGONIST AND ANTAGONIST PROPERTIES OF 1-PHENYL-3-BENZAZEPINE ANALOGUES AT THE D-1 DOPAMINE RECEPTOR

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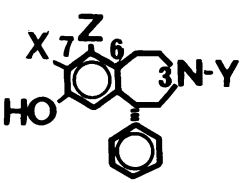
Studies using a series of 1-phenyl-3-benzazepine analogues of SKF 38393 and SCH 23390 have investigated the structural and stereochemical requirements for high affinity, selective interaction with the D-1 dopamine (DA) receptor (O'Boyle & Waddington, 1985; 1987). Agonist and antagonist properties of these compounds, and two 3-N-allyl derivatives, have now been studied by assessing their effects on dopamine-sensitive adenylate cyclase.

Adenylate cyclase activity in rat striatal homogenates was measured by the method of Kebabian et al (1972). Antagonism was measured as IC_{50} for antagonising the increase in cAMP caused by 100 µmDA; agonist effects were assessed in the absence of DA and were calculated as a % of the maximal cAMP response to DA.



Compound	7-X	3-Y	6-Z	Antagonist IC_{50} µM	Agonist Intrinsic Activity % 100 µM DA	N
SKF 38393	OH	H	H		41.4	3
SKF 77174	H	H	H	1.95 ± .94		4
SKF 85257	CH_3	H	H	1.21 ± .71		4
SKF 75670	OH	CH_3	H	.29 ± .14		4
SKF 83692	H	CH_3	H	1.52 ± .35		4
SKF 23390	Cl	CH_3	H	.004 ± .001		3
SKF 83566 R	Br	CH_3	H	.028 ± .01		3
S	Br	CH_3	H	3.96		2
SKF 77434	OH	C_3H_5	H		43.1	4
SKF 82958	OH	C_3H_5	Cl		133.0	5

± means S.E.M.

3 benzazepines, all having a 7-OH group, stimulated the D-1 receptor. SKF 38393 and SKF 77434 acted as partial agonists producing 40% of the stimulatory effect of 100 µM DA. SKF 82958, the 6-Cl-3N-allyl analogue, acted as a full agonist. The remaining benzazepines inhibited the stimulation of adenylate cyclase by DA. Compounds having a halogen (Cl or Br) at position 7 were the most potent, while the least active had H or CH_3 at this position. Biochemical activity of these compounds resided in the R-enantiomer since the 7-Br derivative R-SKF 83566 was >100 times more potent than its S-antipode.

These results emphasise that the substituent at position 7 of the benzazepine molecule has a critical effect on both D-1 affinity and intrinsic activity.

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AN INVESTIGATION OF CLONIDINE-INDUCED MYDRIASIS IN CONSCIOUS MICE AS A MODEL OF CENTRAL α_2-ADRENOCEPTOR FUNCTION

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Low doses of clonidine induce mydriasis (pupillary dilatation) and this has been shown to be mediated by α_2-adrenoceptors in the brains of cats (Koss and Christensen, 1979) and rats (Hey et al, 1985). However, the response is routinely measured in anaesthetised animals and this precludes other behavioural evaluations and may distort any biochemical parameters which are concomittently monitored. In view of this, we have developed a method for studying α_2-adrenoceptor mediated mydriasis in conscious mice.

Adult male C57/Bl/60la mice (Olac) weighing 15-35g were used. Drugs were administered intraperitoneally (i.p.) or intracerebroventricularly (i.c.v.) dissolved in 0.9% NaCl solution. Pupil diameters were measured in artificial light at 10 min intervals for up to 60 min after drug injection. A Wild Ml binocular microscope with a graticule scale in one eyepiece was used. Results were analysed using Student's 't'-test either paired or unpaired.

The pupil diameter ± s.e. mean of untreated control mice was 0.38 ± 0.02 mm (n=6). A time-course measured after clonidine (100 µg/kg i.p.) showed that the increase in pupil diameter was maximal (33%; $P<0.01$) 10 min after injection and returned to control values by 60 min. Clonidine (0.3-3,000 µg/kg i.p.) dose-dependently induced mydriasis 10 min later. The increase was maximal (69%; $P<0.01$) at 500 µg/kg and the ED50 was 40 µg/kg. Pupil diameter was reduced by 36% ($P<0.01$) and 29% ($P<0.01$), respectively, 10 min after injection of the α_2-adrenoceptor antagonists, idazoxan (1 mg/kg i.p.) and yohimbine (2 mg/kg i.p.). In addition, pretreatment with these doses of the α_2-adrenoceptor antagonists 30 min earlier markedly inhibited the mydriatic effect of clonidine (100 µg/kg i.p.). However, the α_1-adrenoceptor antagonist, prazosin (1 mg/kg i.p.) and the β-adrenoceptor antagonist, pindolol (1 mg/kg i.p.) had no effect on either pupil diameter or the mydriatic actions of clonidine (100 µg/kg i.p.). Clonidine (0.25 µg in 2 µl saline), equivalent to the ineffective dose of 10 µg/kg injected i.p., was injected i.c.v. using the stereotaxic apparatus described by Heal (1984). This produced a 28% ($P<0.01$) increase in pupil diameter 10 min later and suggests that the mydriasis was centrally mediated. This hypothesis was strengthened by the findings that the mydriasis induced by clonidine (100 µg/kg i.p.) was inhibited by simultaneous i.c.v. injection of idazoxan (2.5 µg) and yohimbine (2.5 µg), but not by prazosin (2.5 µg) or pindolol (2.5 µg).

In conclusion, the results show that clonidine-induced mydriasis can be measured in conscious mice. Furthermore, this response is probably specifically mediated via α_2-adrenoceptors in the CNS and this may, therefore, provide a useful model of the function of these receptors. However, it is unclear, at present, whether the α_2-adrenoceptors involved are located on pre- or postsynaptic sites and further work to clarify this issue is in progress.

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THE EFFECT OF GALLAMINE ON THE RAT ISOLATED SUPERIOR CERVICAL GANGLION

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Although Brown et al (1980) reported that muscarine depolarised rat isolated superior cervical ganglion with a uniphasic log concentration-effect curve, we found using a greater range of concentrations that both muscarine and bethanechol produced biphasic curves. Six preparations were examined on each day. An initial serial concentration-effect curve was obtained on each (10^{-8}-10^{-6}M muscarine or $3x10^{-7}$-$3x10^{-5}$M bethanechol). They were then equilibrated for 1 hour with gallamine (0, 10^{-6}, $3x10^{-6}$, 10^{-5}, $3x10^{-5}$ or 10^{-4}M). A full semi-cumulative concentration-response curve (10^{-8}-10^{-4}M muscarine or $3x10^{-7}$-$3x10^{-2}$M bethanechol) was then obtained as described by Brown et al (1980).

In the absence of gallamine the semi-cumulative log concentration-effect curves to both muscarine or bethanechol were biphasic. Low concentrations of gallamine (10^{-6}-$3x10^{-6}$M) converted these biphasic curves into uniphasic curves. Higher concentrations of gallamine (10^{-5}M-10^{-4}M) then displaced these uniphasic curves to the right in a parallel fashion. In order to quantify the shift produced by 10^{-5}M gallamine, concentration ratios were measured at a response level of half of that produced by 10^{-6}M muscarine or $3x10^{-5}$M bethanechol in the initial serial curve. The concentration ratios for bethanechol and muscarine were 3.5±0.2 (4) and 2.8±0.2 (3) respectively (mean ± s.e.m.(n)).

These changes may be attributable to the presence of two types of muscarinic receptor in this preparation. Stimulation of only the receptor mediating the depolarisation would be expected to produce a uniphasic log concentration-effect curve. Stimulation of the other receptor is believed to have an inhibitory effect as a result of which the observed curves with bethanechol and muscarine are biphasic. This latter receptor appears to be more sensitive to gallamine than that mediating the depolarisation. It may therefore correspond to the gallamine-sensitive receptor mediating the hyperpolarisation observed by Newberry et al (1985) and others with muscarinic agonists.

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RELATIVE EFFICACIES OF MUSCARINIC AGONISTS FOR INHIBITION OF ADENYLATE CYCLASE ACTIVITY IN RAT STRIATAL AND MYOCARDIAL MEMBRANES

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In principle the use of agonists for receptor characterisation has great potential, as two receptor (or receptor-effector complex) specific parameters are involved, affinity and efficacy, rather than just one, affinity, in the case of antagonists. However, whereas antagonist affinity is relatively easily determined, agonist affinity and efficacy have proved more intractable. It has recently been shown that dose-response curves for the inhibition of adenylate cyclase by muscarinic agonists in membranes from the rat corpus striatum (Keen & Nahorski, 1987) and rabbit myocardium (Ehlert, 1987) can be simply related to the agonist binding curves obtained under identical conditions in the same membrane preparations. In the present study the dose-response and occupancy curves for three muscarinic agonists have been compared in membranes from rat striatum and myocardium, to allow an estimation of the occupancy-response relationships in these tissues.

Adenylate cyclase activity was measured by a modification of the method used by Olianas *et al* (1983). Agonist binding was measured under the same assay conditions (i.e. in the presence of 10^{-4} M GTP) by inhibition of the atropine (1 μM) displaceable binding of ^{3}H-N-methylscopolamine.

The dose-response and binding curves obtained under these conditions, and analysed by computer-assisted fitting, approximated to simple Langmuir isotherms. The maximum responses (% inhibition of basal activity), EC_{50} values and apparent binding constants (K) obtained are summarized in the table below (mean ± SEM, n = 3-5). The relative efficacies of the agonists in the different tissues were obtained from the calculated occupancy-response relationships, as described by Furchgott & Bursztyn (1967).

Table	Log EC_{50}	E_{max}	Log K	Efficacy (relative to CCh)
Striatum				
Carbachol	-5.19 ± 0.12	23.0 ± 3.9	-3.62 ± 0.08	-
Arecoline	-4.82 ± 0.20	24.1 ± 2.8	-4.21 ± 0.27	0.13
Pilocarpine	-4.55 ± 0.91	17.9 ± 1.9	-5.17 ± 0.24	0.03
Heart				
Carbachol	-4.39 ± 0.19	24.6 ± 1.4	-4.45 ± 0.06	-
Arecoline	-4.89 ± 0.07	24.7 ± 0.6	-4.61 ± 0.08	~1
Pilocarpine	-5.10 ± 0.10	10.8 ± 1.2	-5.00 ± 0.25	0.45

It is widely assumed that most muscarinic agonists exhibit little selectivity between any of the possible muscarinic receptor subtypes. However, the present results show clear differences in the relative efficacies of the agonists studied between striatum and heart. This is strong evidence for differences between the muscarinic receptors linked to inhibition of adenylate cyclase in these tissues.

MK is a Merck Sharp & Dohme Research Fellow.

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THE EFFECT OF MUSCARINIC ANTAGONISTS ON SPONTANEOUS ALTERNATION IN MICE

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Rats and mice permitted to explore a T-maze will tend to enter the arm least recently visited, i.e. they will spontaneously alternate (Tolman, 1925). Although the muscarinic antagonist scopolamine has been reported to impair alternation behaviour (Squire, 1969) few other muscarinic antagonists have been studied. We have therefore compared the activities of brain penetrating (scopolamine, benzhexol, benztropine) and non-brain penetrating (N-methyl scopolamine, pirenzepine) muscarinic antagonists on this task.

CDI mice (20-30g, Charles River) were tested in a perspex T-maze based on that of Galey et al (1985). Each mouse was only tested once, each test consisting of two trials separated by an interval of 8 minutes. In the first trial the mouse was placed in the start box for 30 seconds and then the door opened to allow it to investigate the central corridor and one of the side arms chosen at random, the door to the alternate arm being closed. In the second trial, both arms were open and the arm chosen by the animal recorded. Drugs were administered 20 minutes before the first trial.

Scopolamine ($0.8mg.kg^{-1}$ s.c.), benzhexol ($10mg.kg^{-1}$ s.c.) and benztropine ($3mg.kg^{-1}$ s.c.) all caused an impairment in alternation whereas N-methyl scopolamine ($0.8mg.kg^{-1}$ s.c.) did not (see Table). A lower dose of benzhexol ($1mg.kg^{-1}$) had no effect. Scopolamine ($10\mu g.5\mu l.^{-1}$ per mouse), but not pirenzepine ($30\mu g.5\mu l.^{-1}$ per mouse), impaired alternation when given by intracerebroventricular injection. The percentage of mice alternating were: controls (5µl Krebs solution) 79% (n=52), scopolamine 56% (54) and pirenzepine 69% (55). A higher dose of pirenzepine ($60\mu g.kg^{-1}$ per mouse) was also used but some mice died following administration of this dose.

Table 1. The effect of muscarinic antagonists on spontaneous alternation

The doses used are indicated in the text. In each experiment animals were treated with the test antagonist, scopolamine or saline.

Test Antagonist	% of mice alternating ± s.e. (n)		
		Scopolamine	Saline
N-methyl scopolamine	90 ± 2% (41)	31 ± 3%** (39)	78 ± 3% (45)
Benzhexol	58 ± 4%* (78)	48 ± 4%** (71)	74 ± 4% (76)
Benztropine	55 ± 1%** (47)	36 ± 3%** (44)	82 ± 3% (44)

* $p<0.05$; ** $p<0.01$ (compared with control) Mantel-Haenszel test

It can be concluded that muscarinic antagonists act centrally to impair spontaneous alternation in mice.

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DEVELOPMENTAL ASPECTS OF MUSCARINIC-INDUCED INOSITOL POLYPHOSPHATE ACCUMULATION IN RAT CEREBRAL CORTEX

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The immature brain with its developing innervation and receptor density provides a useful model for an investigation into the relationship between receptor activation and phosphoinositide responsiveness. In a previous report we have shown that by assaying total ^{3}H-inositol phosphates (^{3}H-InsP) carbachol produces supramaximal responses within the first week of development which decline to adult values at >21 days (Nahorski & Rooney, 1987). There is also no observable change in the EC_{50} value for carbachol-induced ^{3}H-InsP accumulation. In the present study we have extended these initial observations by investigating the dose-response relationship to carbachol and lithium for individual inositol polyphosphates during development.

350 x 350 μM slices of rat cerebral cortex were prepared and preincubated with ^{3}H-inositol in the presence or absence of lithium. Incubations were terminated by addition of 7% (w/v) perchloric acid and ^{3}H-InsPs or ^{3}H-glycerophosphoinositol derivatives separated by Dowex or hplc anion exchange chromatography.

Lithium (5 mM) enhances carbachol-stimulated ^{3}H-InsP$_1$ and ^{3}H-InsP$_2$ accumulations at every age tested but the enhancement on ^{3}H-InsP$_1$ (9.1-fold) at 7 days was greater than that at 40 days (4.3-fold). Lithium also enhances ^{3}H-InsP$_2$ accumulations but by a similar extent at all ages tested. A marked inhibitory effect on ^{3}H-InsP$_3$ and ^{3}H-InsP$_4$ accumulations i.e. 29-33% and 76-79% respectively is also produced by lithium at every age tested. The effects of lithium on carbachol-induced ^{3}H-InsP accumulations also seem to be time-dependent. Whereas effects on ^{3}H-InsP$_1$ and ^{3}H-InsP$_2$ were observed at 5 and 30 min, the inhibitory effect on ^{3}H-InsP$_3$ and ^{3}H-InsP$_4$ were only seen at the later time point. Negligible changes in the EC_{50} values for carbachol-induced polyphosphate accumulations were observed either in the presence or absence of lithium. The time-dependent inhibitory effects of lithium on ^{3}H-InsP$_3$ were also observed on the separated isomeric forms. Thus, lithium inhibited both ^{3}H-Ins-1,3,4-P$_3$ and ^{3}H-Ins-1,4,5-P$_3$ by 29-38%.

The potentiation of ^{3}H-InsP$_1$ accumulation produced by lithium in 7 and 40 day old rats is also dose-related with a maximal and half-maximal effect at 3-5 mM and 0.6 mM respectively at both ages. The inhibitory effect of lithium on ^{3}H-InsP$_3$ and ^{3}H-InsP$_4$ was also seen at very similar concentrations. The enhanced ^{3}H-InsP$_2$ accumulations were less sensitive to lithium with maximal and half-maximal effects at 15-30 mM and 4-6 mM respectively at both ages. A higher degree of labelling in phosphatidylinositol-4,5-bisphosphate is also observed in younger rats.

It is clear from these results that the supramaximal responses produced in young rats cannot be explained by a differential sensitivity to lithium. However, the extent to which the various pathways of inositol polyphosphate dephosphorylation are blocked by lithium may vary during development. Future studies should be focussed on a more detailed analysis of the effects of lithium on the isomeric forms of inositol phosphates produced during development.

This work was supported by the S.E.R.C.

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DOWN REGULATION OF MUSCARINIC BINDING SITES IN NG108-15 CELLS FOLLOWING CHRONIC TREATMENT WITH AGONISTS

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Muscarinic receptors in cultured cells are reported to be down-regulated by the administration of full agonists such as carbachol. It has been suggested that this phenomenon is related to efficacy (Cioffi and El-Fakahany, 1986). The present study has examined the relationship between efficacy as defined by the NMS/Oxo-M binding ratio in rat cerebral cortex (Freedman et al, 1987) and muscarinic receptor down regulation as measured by [3H]-N-methylscopolamine binding in lysed NG108-15 cells.

Neuroblastoma x glioma hybrid cells, NG108-15, were grown as described previously (Freedman et al, 1984). Cells were harvested in assay buffer, then lysed in 5mM Hepes buffer pH 7.4 on ice. [^{3}H]NMS binding was measured in 20mM Hepes-Krebs buffer pH 7.4 for 60 min at 30°C and filtration through GF/B filters. [^{3}H]NMS bound saturably (K_D = 0.098nM; Bmax 170 fmol/mg) with a pharmacological profile (Table 1) similar to that seen in rat cerebral cortex (Freedman et al, 1987). Cells were exposed to agonists for 3.25h in 20mM Hepes Krebs buffer pH 7.4 and washed after lysing to remove drug. Receptor number was determined by saturation analysis to detect true receptor loss rather than drug occlusion. Agonist concentrations calculated to give a receptor occupancy of 80% were used for these studies.

Table 1 Relationship between muscarinic agonist efficacy and receptor down-regulation

Compound	NG108-15 Cells			Rat cerebral cortex
	K(app) (μM)	Test Conc. (μM)	% Control Bmax	NMS/Oxo-M Ratio
Carbachol	12	1000	30 ± 6	4100
Methylfurmethide	3.3	200	66 ± 15	1400
Oxotremorine	1.2	100	79 ± 11	860
RS86	6.7	300	101 ± 4	130
Pilocarpine	2.6	200	104 ± 9	100
Atropine	0.00055	0.03	99 ± 18	2.1

K(app) = apparent affinity constant corrected for ligand occupancy. Mean values for 3 - 4 determinations ± S.E.M.

As the NMS/Oxo-M ratio increased from antagonist to partial agonist and full agonist a significantly greater down regulation of muscarinic receptors was observed, the largest effect (70% decrease in Bmax) being caused by the full agonist carbachol.

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STIMULUS-SPECIFIC INHIBITION OF HUMAN NEUTROPHIL H_2O_2 PRODUCTION BY HUMAN RECOMBINANT LIPOCORTIN 1

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Release of active oxygen metabolites from polymorphonuclear leukocytes (PMN's) may be implicated in the pathogenesis of tissue damage in inflammatory diseases. Glucocorticoids are potent anti-inflammatory drugs in vivo and have been reported to inhibit the respiratory burst in PMN's (Nelson and Wennhold, 1978). At least some of the actions of steroids are mediated by the steroid-inducible phospholipase inhibitory protein, lipocortin (Flower, 1985). We report here the effects of human recombinant lipocortin 1 (r-LC1, Wallner et al, 1986) upon spontaneous and stimulated H_2O_2 production by human neutrophils and compare its effects with those of PLA_2 inhibitors pBPB and mepacrine.

Human neutrophils were isolated from whole blood using Percoll density gradient centrifugation and H_2O_2 was measured by horseradish peroxidase-dependent oxidation of phenol red. Cells were incubated in phosphate buffered saline containing 9 mM glucose with or without PLA_2 inhibitors for 15 minutes prior to stimulation with PBS (spontaneous) FMLP (5 μM), heat aggregated IgG (HAGG 200 μg/ml) or PMA (10 ng/ml). Maximum release in the absence of inhibitors was 2.4 $\pm$ 0.6 μM H_2O_2 without simulation and 7.1 $\pm$ 0.7, 8.7 $\pm$ 0.4 and 32.6 $\pm$ 2.2 μM H_2O_2 in response to FMLP, HAGG and PMA respectively (mean $\pm$ s.e.m., n=9).

Preincubation of PMN with r-LC (0.5-10 μM) inhibited spontaneous and FMLP-stimulated H_2O_2 production with little effect on HAGG and PMA stimulated responses (Table 1). 'Sham' lipocortin, prepared from E.coli without the LC gene had no effect upon H_2O_2 production. pBPB inhibited spontaneous and FMLP-stimulated H_2O_2 production at lower concentrations than HAGG-stimulated release. The effects of mepacrine were inconsistent, but spontaneous release of H_2O_2 was much more sensitive to inhibition than was FMLP, HAGG or PMA-stimulated release.

Table 1 Inhibition of H_2O_2 production. (*$p<0.05$)

TREATMENT		(%) INHIBITION (mean $\pm$ s.e.m.; n$\geq$3)			
		spontaneous	FMLP	HAGG	PMA
rLC	M				
	5×10^{-7}	4.7$\pm$1.1	11.2$\pm$4.7	2.7$\pm$1.4	0
	1×10^{-6}	15.4$\pm$1.6*	34.5$\pm$2.6*	0	0
	5×10^{-6}	61.3$\pm$5.7*	45.5$\pm$4.3*	3.7$\pm$2.5	0
	1×10^{-5}	83.4$\pm$8.1*	53.6$\pm$8.5*	14.6$\pm$6.2	4.9$\pm$3.4
pBPB	5×10^{-6}	65.3$\pm$4.1*	57.0$\pm$5.1*	26.2$\pm$4.2*	10.4$\pm$2.0*
mepacrine	5×10^{-5}	93.0$\pm$5.6*	54.4$\pm$10.6*	34.5$\pm$3.5*	41.3$\pm$3.3*

The effects of lipocortin suggest that it could mediate the effects of steroids upon H_2O_2 release. The differing sensitivities of stimuli to inhibition by putative inhibitors of phospholipase A_2 suggest that different mechanisms of cell activation leading to H_2O_2 release exist, and that phospholipase A_2 may be involved in some of these.

We thank Biogen Research for gifts of r-LC. TRJS and ALD were supported by SERC.

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5-LIPOXYGENASE INHIBITION : A MECHANISM FOR THE ANTIGASTRIC ULCER ACTIONS OF SULPHASALAZINE AND PHCL28A?

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We have shown (Berry et al, 1988) that sulphasalazine (SZP), PhCL28A (2-hydroxy -5-(3,5-dimethoxycarbonyl-benzoyl)benzene acetic acid) and carbenoxolone (CBX) are potent inhibitors of gastric mucosal peptido-leukotriene (LT) synthesis in vitro, and have suggested that this effect may explain the antiulcer properties of these compounds. Given that CBX pre-treatment prevents both ulcer formation and the increased gastric mucosal LTC_4 in the rat ethanol- ulcer model (Peskar et al, 1986), we have validated our in vitro findings using this model.

Fasted (22 hrs) female Wistar rats (200-220g, 6 per group) received either vehicle, SZP ($100mg.kg^{-1}$, i.p.), PhCL28A ($30mg.kg^{-1}$, i.p.), CBX ($150mg.kg^{-1}$, p.o.) or BW755C ($100mg.kg^{-1}$, p.o.) 30 minutes before oral injection of 50% ethanol (1ml per rat). One hour later the animals were killed, the stomachs excised and the ulcers scored. Gastric mucosal fragments were then incubated and directly assayed for peptido-LTs (Amersham RIA kits) as in Peskar et al, 1986.

Ethanol (50%) caused marked gastric ulceration which was accompanied by significant increases in LTC_4 formation (being the major peptido-LT formed, >95%, verified by RIA of reverse phase HPLC fractions after Sep-Pak sample clean up). All four compounds markedly inhibited ulcer formation (Table), and at the same time the increase in LTC_4 synthesis was no longer seen, with BW755C and PhCL28A depressing LTC_4 release below that of the vehicle-treated controls.

Table. LTC_4 Formation in Gastric Mucosa (pg/mg, dry weight).

Compound	Controls	Ethanol Alone	Ethanol + Drug	Ulcer Inhibition
SZP	409 ± 34	646 ± 67[2]	398 ± 22[5]	70%
PhCL28A	574 ± 54	873 ± 105[1]	359 ± 44[1,6]	98%
CBX	251 ± 30	381 ± 30[1]	239 ± 26[4]	72%
BW755C	324 ± 29	602 ± 55[3]	121 ± 28[3,6]	92%

[1] $p<0.05$, [2] $p<0.01$, [3] $p<0.001$ vs controls.
[4] $p<0.05$, [5] $p<0.01$, [6] $p<0.001$ vs ethanol alone. Student's unpaired t-test.

Our results obtained with CBX are in agreement with those of Peskar et al, 1986 and support the suggestion that BW755C may owe its antiulcer properties to 5-lipoxygenase inhibition (Wallace and Whittle, 1985). Finally, we conclude that there is now considerable evidence to suggest that SZP and PhCL28A inhibit gastric ulceration by 5-lipoxygenase inhibition.

We thank the Wellcome Foundation for their generous gift of BW755C.

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INHIBITION OF 5-LIPOXYGENASE IN VITRO BY SULPHASALAZINE (SZP), PhCL28A AND CARBENOXOLONE (CBX)

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Leukotrienes (LTs), particularly LTC_4, are becoming increasingly important in the pathology of gastric lesions. Topical LTC_4 causes vasoconstriction and stasis of mucosal blood flow (Whittle et al, 1985), the LT antagonist, FPL55712, protects rats against stress ulcers (Ogle & Cho, 1986) and the increased gastric mucosal LTC_4 formation in rat ethanol ulcers is reversed by antiulcer doses of CBX (Peskar et al, 1986). We therefore explored the effects of SZP, PhCL28A (both antiulcer in the rat, Berry & Lloyd, 1987) and CBX in vitro on rat peritoneal cell and rat gastric mucosal LT synthesis.

Cells obtained by lavage of rat (Male, Sprague Dawley, 200-250g) peritoneal cavities with Ca^{++} and Mg^{++} free phosphate-buffered saline (PBS) plus heparin (1µg/ml). After centrifugation (10min at 400g), the pellets were resuspended in PBS containing Ca^{++}, Mg^{++} and GSH (3mM) and preincubated (1ml containing 50µl Na_2CO_3, 0.5%) with the test compounds or their vehicles for 10min at 37°C before the addition of arachidonic acid (10µg/ml) and a further 10min incubation. After stopping the reactions by centrifugation (400g) for 10min at 4°C, the supernatants were assayed for LTB_4 using Amersham RIA kits. Fragments of gastric mucosa (50-100mg wet weight) were taken from female Wistar rats (200-220g) and preincubated in 1ml Tyrode's solution (pH7.4, containing drug or vehicle) for 30min at 4°C. The fragments were transferred to fresh Tyrode's solution (1ml) containing the drug (or vehicle), 3mM GSH and 50µl Na_2CO_3 (0.5%) and incubated for 30min at 37°C, whereupon the fragments were removed, dried overnight at 50°C and weighed. The peptido-LTs in the incubates were assayed, after being boiled for 2min, with Amersham $LTC_4/D_4/E_4$ RIA kits. IC_{50} values for each compound were estimated from three-point log-dose-inhibition curves from at least three separate experiments.

The peritoneal cells (1.1-1.6 x 10^6 cell/ml) generated 10-16 ng/ml immunoreactive LTB_4 in the control samples, and peptido-LT formation by the mucosal fragments ranged from 300-800pg LT/mg dry weight. The table shows that all four compounds inhibit lipoxygenase activity, with gastric mucosal LT formation being considerably more sensitive to inhibition by SZP and CBX than that of the peritoneal cells.

Table: In vitro lipoxygenase inhibition by four antiulcer compounds.

	Inhibitory Activity (Mean IC_{50} ± S.E.M. (n))	
Compound	Rat Peritoneal Cells	Gastric Mucosal Fragments
Sulphasalazine	201 ± 24µM (3)	30 ± 11.5µM (3) **
PhCL28A	51 ± 18µM (3)	23.3 ± 8.8µM (3)
Carbenoxolone	39.3 ± 5.1µM (4)	3.7 ± 2.3µM (3) **
BW755C	3.3 ± 0.5µM (7)	11.9 ± 10µM (3)

** $p<0.01$ vs rat peritoneal cells. Students t-test for unpaired samples.

We conclude from these results that: 1) 5-lipoxygenase inhibition may be a common mechanism for the antiulcer properties of the four compounds, 2) this study highlights the importance of an in vitro system derived from the organ where the pathology is observed, and 3) 5-lipoxygenase inhibitors may be a rationale for novel antiulcer drugs.

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ACCUMULATION OF POLYMORPHONUCLEAR LEUKOCYTES IN ALLERGIC INFLAMMATION AND INHIBITION BY MOAB 60.3

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The monoclonal antibody (MoAb) 60.3 recognises the polymorphonuclear leukocyte (PMNL) cell surface glycoprotein antigen designated as CD18. Intravenous injection of MoAb 60.3 has been shown to inhibit PMNL accumulation in response to chemoattractants in the rabbit (Arfors et al, 1987). We have investigated the possibility that preincubation of radiolabelled PMNL with this antibody inhibits their emigration in vivo. The aim of the study was to investigate the effects of MoAb 60.3 on PMNL accumulation in vivo and compare this with its effects on leukocyte adherence in vitro. PMNL accumulation was measured both in response to exogenous inflammatory mediators, zymosan-activated plasma (ZAP, source of C5a des Arg), FMLP, LTB_4, IL-1 and in response to endogenous mediators generated in a passive cutaneous anaphylactic reaction (PCA) and a reversed passive Arthus reaction (RPA) in rabbit skin.

Rabbit PMNL were separated from whole blood and labelled with ^{111}In (Rampart & Williams, 1987). Prior to the final wash ^{111}In-PMNL from each donor rabbit were divided into two aliquots one of which was treated with MoAb 60.3 (88μg/ml of cell suspension containing $2\text{-}5x10^7$ PMNL) for 20 min at 20°C. Cells were then washed and resuspended in autologous plasma before i.v. injection into two recipient rabbits. PMNL infiltration and oedema formation in rabbit skin were simultaneously measured as the local accumulation of i.v. injected ^{111}In-PMNL and ^{125}I-human serum albumin. Mediators were injected intradermally (i.d) in 0.1ml volumes. PCA reactions were induced by i.d. injection of IgE antibodies 48 hours prior to antigen challenge (bovine gamma globulin 5mg/kg i.v.). RPA reactions were induced by i.d. injection of IgG antibodies immediately before i.v. antigen challenge. At the end of the 4hr in vivo test period, blood was removed into heparin (3U/ml) and passed through nylon wool columns with or without pretreatment of the columns with ZAP or FMLP (final concentrations of 10% and $10^{-7}M$ respectively). Columns were counted to provide a measure of ^{111}In-PMNL adherence.

Treatment of ^{111}In-PMNL with MoAb 60.3 resulted in significant inhibition of cell accumulation in the skin in response to ZAP, LTB_4 ($5x10^{-11}$ - $5x10^{-10}$moles), FMLP ($5x10^{-12}$ - $5x10^{-11}$moles) and IL-1 (1.0-10 U), e.g. the response to ZAP was inhibited by 51±15% (n=5). Accumulation of ^{111}In-PMNL in the RPA and the PCA reaction were also significantly ($p<0.01$) reduced (numbers shown are ^{111}In-PMNL accumulating per site for control vs. MoAb 60.3 treated cells); RPA 2372±440 vs. 1408±172; PCA, 3319±257 vs. 1903±257 (mean±SEM n=5). At the end of the experiments, control ^{111}In-PMNL exhibited a basal adherence of 36.2±5.3% (mean±SEM n=7) to nylon wool columns. FMLP and ZAP significantly increased basal adherence by 91.4± 10.5 and 53.6±11.9% respectively. However, MoAb 60.3 treated ^{111}In-PMNL exhibited a lower basal level of adherence (22.0±4.7%) and failed to respond to either FMLP or ZAP ($p<0.1$). MoAb 60.3 had no effect on oedema formation (the labelled cells constitute about 3.0% of the recipient rabbit's circulating PMNL).

Our results demonstrate that the pretreatment of neutrophils with MoAb 60.3 results in an inhibition of their accumulation in vivo in response to exogenous mediators and endogenous mediators generated in allergic reactions. In addition, our in vitro results suggest that this inhibitory effect of MoAb 60.3 is as a result of inhibiting both the basal and the stimulated expression of the CD18 antigen.

This work was supported by the Medical Research Council, Pfizer Central Research, U.K. and U.S.A. and National Fund for Scientific Research (Belgium).

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EFFECTS OF DICLOFENAC AND INDOMETHACIN ON INTERLEUKIN-1α-MEDIATED CHANGES IN PGE_2 AND ARACHIDONIC ACID RELEASE IN SYNOVIAL CELLS

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Human synovial cells, when incubated with interleukin-1 (IL-1), release prostaglandin E_2 (PGE_2). The mechanism underlying this response involves phospholipase activation (Godfrey et al, 1987) and cyclo-oxygenase induction (O'Neill et al, 1987). We report here how two non-steroidal anti-inflammatory drugs affect IL-1-induced PGE_2 release and IL-1-induced changes in the distribution of arachidonic acid (AA)-associated radioactivity in membrane lipids.

Cells were prepared and PGE_2 estimated as previously described (O'Neill et al, 1987). Studies with [^{3}H]-AA involved incubating confluent cells (1.5×10^6 cells per $25cm^2$ flask) with 0.5 μCi of [5,6,8,9,11,12,14,15-^{3}H]-arachidonic acid (Amersham) for 24h. Cells were then washed 3 times and incubated for 4h with human recombinant (hr) IL-1α (0.5ng/ml), hrIL-1α plus either diclofenac (diclo) or indomethacin (indo) (0.1 to 10 μM) or with diclo or indo (10 μM) alone or media alone. Lipids were then extracted, separated and quantified using a method described by Emilsson and Sundler (1985).

Both diclo and indo inhibited hrIL-1α (0.5ng/ml)-induced PGE_2 release, with IC_{50} values of 1.6nM and 5.5nM respectively. However, at higher concentrations, both drugs (1-10 μM) reduced IL-1-induced increases in free AA levels (Table 1).

IL-1 caused a decrease in phospholipid-associated radioactivity concomitant with the increase in AA, indicating activation of phospholipase. Both diclo (10 μM) and indo (1-10 μM) significantly decreased the IL-1-induced elevation in AA and increased the radioactivity associated with phosphatidylethanolamine (PE) and triglyceride (TG) (Table 1). No significant changes were observed in other lipid species examined and neither drug had any effect on unstimulated cells (not shown).

Table 1

	Control (media)	+IL-1α 0.5ng/ml	+IL-1α 0.5ng/ml +diclo 10μM	Control (media)	+IL-1α 0.5ng/ml	+IL-1α 0.5ng/ml +Indo 1μM	10μM
PGE_2	125± 78	407± 37	-	207±129	673± 30	-	-
AA	509± 31	1723±156	705± 78*	841± 52	2848±259	2201±130*	1683±259*
PE	4231±470	2664±327	4546±156**	6990±776	4401±318	5566±129**	5437±103**
TG	1097±313	1254± 78	1724±158**	1812±518	2071±129	3107±258**	3106±517**

Results in dpm represent mean ± SEM from 3 separate experiments, each in duplicate. *denotes significant decrease ($p<0.05$), **denotes significant increase ($p<0.05$) compared to IL-1 alone. Separate controls were used for each drug and only concentrations showing significant effects are given.

The effect of diclo or indo on free AA levels could be the result of phospholipase inhibition, or an increased reacylation of AA into PE and TG. This effect might be responsible for effects of non-steroidal anti-inflammatory drugs not attributable to inhibition of cyclo-oxygenase.

We are grateful to Drs Stern and Lomedico (Hoffman-LaRoche, USA) for the gift of IL-1α. This work was partly supported by Ciba-Geigy, UK.

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144P

CYTOKINE-INDUCED INCEASES IN RABBIT SKIN VASCULAR PERMEABILITY

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Agents which are chemoattractant for neutrophils in vitro or in vivo are generally potent inducers of increased vascular permeability in rabbit skin (Wedmore and Williams, 1981). We have previously reported that whilst lacking direct chemokinetic activity for neutrophils, interleukin-1 (IL-1) induces the production of a neutrophil chemokinetic factor from cultured synovial fibroblasts (Watson et al, 1987). We have therefore examined the ability of such supernatant to increase vascular permeability and compared it with the direct effects of the recombinant cytokines IL-1, tumour necrosis factor (TNF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) in rabbit skin.

Oedema formation in rabbit skin was assessed as described by Wedmore and Williams, 1981. In these experiments, skin sites were injected with cytokines, supernatant or appropriate vehicles at different times before administration of a superimposed injection of prostaglandin E_2 (PGE_2, 300pmoles). ^{125}I-albumin was injected intravenously immediately before the PGE_2 and responses measured over 30 min, when animals were killed, a blood sample taken and skin samples punched out and radioactivity counted. Oedema (expressed as µl plasma exudated) was determined from 6 replicates in each of 3-8 animals.

None of the recombinant cytokines stimulated significant oedema over the 30 min immediately after coadministration with PGE_2. However IL-1 and GM-CSF increased vascular permeability peaking at 1 and 2 h respectively. At sites pretreated with IL-1 (84fmoles) for 1 h, significant ($p < 0.005$, paired t-test) plasma accumulation was detected compared to sites pretreated with the IL-1 vehicle, bovine serum albumin (BSA, 250µg) in the same experiments (25.12±1.49µl compared with 15.89±1.28µl respectively). The IL-1-induced increase in vascular permeability detected at 1 h was dose related between 8.4 and 280fmoles/site and IL-1α and IL-1ß were approximately equipotent. GM-CSF (10pmoles) stimulated significant vascular permeability ($p < 0.05$) at sites 2 h after administration (23.74±3.14µl compared with 15.84±1.79µl in BSA-treated sites), but TNF (84fmoles/site) did not increase permeability. None of the cytokines increased plasma accumulation without PGE_2. In similar experiments, bradykinin (400pmoles) or leukotriene B_4 (100pmoles) added together with PGE_2 stimulated a large and immediate response of 62.95±7.14µl and 65.66±5.33µl plasma/site respectively. In contrast to the delayed effects of IL-1 and GM-CSF, IL-1-stimulated synovial cell supernatant (0.1ml) caused an immediate oedema response when combined with PGE_2 (28.91±3.27µl compared with 14.32±1.97µl in sites treated with control synovial cell supernatant, p 0.05) and still increased permeability after 1 h (22.69±2.31µl compared with 13.67±1.27µl in control sites). No elevation of permeability was seen in sites pretreated for 2 or 4 h. Thus the above recombinant cytokines do not produce large changes in vascular permeability in rabbit skin compared with the well-established mediators bradykinin and leukotriene B_4. The changes observed with IL-1 may be related to its ability to elicit a neutrophil chemokinetic factor from fibroblasts.

We are grateful to Drs Stern and Lomedico (Hoffman-La Roche,USA), Dr Muller (Ciba-Geigy, Switzerland), Dr Adolf (Boehringer Institute, Austria); and Dr Gillis (Immunex, USA) for the gifts of IL-1α, IL-1ß, TNF and GM-CSF respectively. We thank Ciba-Geigy USA and the MRC for financial support.

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RAT PAW HYPERALGESIA AND OEDEMA IN RESPONSE TO NMDA NAJA NAJA PHOSPHILIPASE A_2

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Inflammation following injection of phospholipase A_2 (PLA_2) from various sources into rat paws and rabbit skin has been previously described (Brain et al, 1977; Cirino and Sorrentino, 1986; Pruzanski et al, 1986). The responses observed included erythema, oedema and leukocyte infiltration. We now describe the development of hyperalgesia and oedema in response to subplantar injection in rats of PLA_2 from Naja naja venom.

Male Sprague Dawley rats (approx. 150g) were injected with PLA_2 (Sigma, P6139, 790 unitsmg^{-1}, right paw) and saline (0.1ml, left paw). Nociceptive pressure threshold and paw volume were measured as previously described (Haworth and Carey, 1985). Hyperalgesia did not develop until 1hr post-PLA_2 injection, was maximal at 2-3hr and had declined by 4hr. In contrast, oedema was maximal at 0.5hr and remained relatively constant up to 4hr. Hyperalgesia at 3hr post-100, 30 and 10μg PLA_2 was 140±30 ($p<0.01$), 100±19 ($p<0.01$) and 38±29gcm while oedema at 0.5hr was 0.59±0.05 ($p<0.001$), 0.60±0.05 ($p<0.001$) and 0.43±0.07ml ($p<0.01$) respectively (mean±sem, $n=6$). Injection of PLA_2 (100μg) preincubated with p-bromophenacyl bromide ($10^{-4}M$) resulted in inhibition of hyperalgesia (max. 99±17%, $p<0.001$) and oedema (50±11%, $p<0.01$) confirming the involvement of PLA_2 activity in the development of these responses. Measurement of immunoreactive PGE_2 and LTB_4 in paw exudate after injection of 100μg PLA_2 showed that PGE_2 was maximal at 3hr (4.0±0.3ngml^{-1}) while LTB_4 peaked at 0.5hr (5.0±0.2ngml^{-1}) correlating with maximal hyperalgesia and oedema respectively. Hyperalgesia but not oedema was inhibited by flurbiprofen, 2mgkg^{-1} p.o. (max. 107±20%, $p<0.01$ and 8±3% respectively). In contrast, BW755C, 100mgkg^{-1} s.c., reduced hyperalgesia and oedema (max. 70±7%, $p<0.001$ and 75±6%, $p<0.001$ respectively).

These results indicate that Naja naja PLA_2-induced hyperalgesia is mediated by cyclo-oxygenase metabolites while oedema may be mediated in part by lipoxygenase metabolites. PLA_2-induced paw inflammation may have utility in assessing the in vivo efficacy of novel inhibitors of eicosanoid biosynthesis.

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PROTECTION OF THE ISCHAEMIC MYOCARDIUM FROM REPERFUSION INJURY BY A THROMBOXANE (TX) RECEPTOR ANTAGONIST

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We have previously shown that inhibition of TX synthetase fails to exert any protective action on the reperfused ischaemic cat myocardium (Thiemermann & Schrör, 1984). In the present study, we demonstrate that the new thromboxane receptor antagonist BM 13.505 (4-[2-(4-Chlorobenzenesulfonylamino)ethyl]-benzene acetic acid = BM) exhibits cardioprotective actions which probably involve an inhibitory effect on polymorphonuclear cells (PMN).

Adult cats (body weight 2.5-3.3 kg) were anaesthetized with pentobarbital sodium (30 mg/kg i.v.) and received additionally 0.2 mg/kg alloferin. After midsternal thoracotomy, the left anterior descending coronary artery (LAD) was occluded at time 0 for 3 h, followed by 2 h of reperfusion. Mean arterial blood pressure (MABP), a standard lead ECG, the platelet count (PC) in right atrial blood and collagen induced platelet ATP-secretion were determined as previously described (Thiemermann & Schrör, 1984). The white blood cell count (WBC) was determined microscopically. The oxygen metabolism of PMN was measured in whole blood ex vivo by the myeloperoxidase (MPO) assay after stimulation of the PMN with opsonized cat zymosan. Cats were treated with BM 13.505 (BM) (20 mg/kg x h, i.v.) starting 30 min after LAD occlusion or physiological saline (VEH). At the end of the experiment, ie. time 5 h, the heart was removed and creatine phosphokinase (CK) was determined. The effectivity of TX receptor blockade was confirmed ex vivo in strip preparations of the cat aortas by demonstration of a severely suppressed contraction by the TX mimetic U-46.619 at unchanged responses to serotonin. The data are mean $\pm$ s.e. of 6-10 observations.

The initial MABP was 143 $\pm$ 5 mmHg in the VEH group and 143 $\pm$ 3 mmHg in the BM group. A 3 h, the MABP amounted to 134 $\pm$ 4 mmHg in the VEH group but to 152 $\pm$ 6 mmHg in the animals treated with BM ($P < 0.05$). Reperfusion was associated with a fall in MABP in both groups of cats. BM significantly attenuated the ischaemia induced increase in the ST-segments and largely prevented the development of a Q-wave during reperfusion, amounting to 0.18 $\pm$ 0.04 mV at 5 h in VEH and 0.05 $\pm$ 0.02 mV in BM-treated cats ($P < 0.05$). This was associated with a significant prevention of loss of CK-specific activity from the ischaemic myocardium, amounting to 12.1 $\pm$ 2.7 IU/mg protein in the ischaemic region of VEH cats (non-ischaemic 39.6 $\pm$ 5.0) but to 29.5 $\pm$ 2.1 IU/mg protein (non-ischaemic 45.5 $\pm$ 4.8) after treatment with BM ($P < 0.05$). BM only slightly antagonized the ischaemia induced fall in PC, amounting to 62 $\pm$ 1 and 61 $\pm$ 2% of control at 3 and 5 h in the VEH group and 69 $\pm$ 3 and 67 $\pm$ 4% of control in BM-treated animals ($P > 0.05$). However, BM did inhibit the collagen induced platelet ATP-secretion, amounting to 445 $\pm$ 120 x 10^3 counts at 3 h in the VEH group but only to 259 $\pm$ 43 x 10^3 counts in the BM group ($P < 0.05$). The WBC (65-75% PMN) amounted to 8,900 $\pm$ 1,300 at time 0 in the VEH group and to 7,400 $\pm$ 1,100 in the BM group. After LAD occlusion, there was a continuous rise in the VEH group, amounting to 26,700 $\pm$ 2,100 at 5 h in the VEH group but only to 15,700 $\pm$ 2,800 in the BM group ($P < 0.05$). Accordingly the MPO activity in VEH animals at 5 h amounted to 148 $\pm$ 24 x 10^3 counts in VEH animals but to 73 $\pm$ 10 x 10^3 counts in the BM cats ($P < 0.05$), suggesting that the treatment with BM reduced the MPO activity in whole blood primarily by reducing the rise in WBC count.

The data demonstrate beneficial effects of BM 13.505 on the reperfused ischaemic cat myocardium which might be mediated both by an antiplatelet and antineutrophil action.

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THE EFFECT OF TWO MONOCLONAL ANTIBODIES TO MAC 1 ON POLYMORPHONUCLEAR LEUKOCYTE (PMNL) ACCUMULATION IN VIVO

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The increased adherence of PMNL to venular endothelial cells (EC) is a key phase in the acute inflammatory response. Increased PMNL-EC adherence is initiated by chemoattractants generated extravascularly. In vitro studies suggest that chemotactic agents, such as C5a, FMLP and LTB_4 (but not interleukin 1, IL 1) promote PMNL adhesiveness by increasing the expression of a membrane glycoprotein complex (known as the Mac 1 antigen) on the surface of PMNL. This complex consists of 2 subunits, αM (or CD 11) and β (or CD 18), not-covalently linked together. Monoclonal antibodies to either subunit induce in vitro defects in PMNL spreading, adherence and chemotaxis. The present study was undertaken to investigate the effect of two monoclonal antibodies, Mo 60.3 and Mo 198 (recognizing the β and αM subunit respectively of the Mac 1 complex on rabbit PMNL) on PMNL accumulation in vivo.

Rabbit PMNLs (> 95 % pure) from donor animals were labelled with ^{111}In (Rampart and Williams 1987). Half of the ^{111}In-PMNL ($3-6 \times 10^7$ cells) was incubated then with 80 µg of monoclonal antibody for 10 min. Before being injected into the experimental animal, the cells were washed to remove the excess antibody. The other half of the ^{111}In-PMNL was treated with saline and injected into another rabbit (= control). Zymosan-activated plasma (ZAP, as a source of C5a), FMLP, LTB_4, IL 1, zymosan and saline were injected (in 0.1 ml volumes) in the clipped dorsal skin of these animals and local PMNL accumulation was measured over a 4 hour period. The results are summarized in Table 1.

Table 1. The effect of Mo 60.3 and Mo 198 pretreatment on ^{111}In-PMNL accumulation in rabbit skin.

Agent	Dose/site	Control	Mo 60.3	Control	Mo 198
ZAP	100 µl	3300 ± 280	750 ± 130	2940 ± 220	3080 ± 180
FMLP	5.10^{-11}moles	3130 ± 260	790 ± 160	2120 ± 160	2650 ± 240
LTB_4	10^{-10}moles	1430 ± 200	320 ± 70	1620 ± 180	2090 ± 180
IL 1	10 units	1980 ± 120	325 ± 75	1820 ± 180	1740 ± 120
Zymosan	100 µg	2210 ± 160	830 ± 120	2450 ± 220	2570 ± 200
Saline	100 µl	280 ± 30	300 ± 25	280 ± 20	310 ± 30

Results are number of ^{111}In-PMNL per site, mean ± SEM, n = 6.

Treatment of ^{111}In-PMNL with Mo 60.3 or Mo 198 did not reduce the number of cells circulating in the recipient animals. Mo 60.3 greatly suppressed accumulation of ^{111}In-PMNL in response to all agents tested, whereas cells treated with the antibody to the αM subunit responded normally. Interestingly, although in vitro studies have shown that IL 1 increases PMNL-EC adherence by inducing the expression of an adhesive molecule on the EC (and not on PMNL), Mo 60.3 also inhibited IL 1-induced PMNL accumulation in vivo.

We conclude that the β subunit (CD 18) of the Mac 1 membrane glycoprotein complex is essential for PMNL adherence and emigration in vivo.

This work was supported by the National Fund for Scientific Research (NFWO) Belgium, and Pfizer Central Research, U.K.

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