INHIBITION OF AGONIST-INDUCED ACTIVATION RESPONSES BY GDPβS AND GDP IN INTACT PLATELETS BY A G PROTEIN-GTP UNRELATED MECHANISM

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The stimulation of 1,2-diacylglycerol formation and granule secretion by GTP and stable GTP analogues, and their inhibition by the GDP analogue, guanosine 5'-O (2-thiodiphosphate) (GDPβS), which prevents G protein-GTP interaction, in permeabilised platelets [Haslam & Davidson 1984; Knight & Scrutton 1985], has served to establish a role for GTP-binding (G) proteins in mediating agonist-induced activation processes in platelets. This has been further underlined by the work of Brass et al [1987], who have demonstrated an inhibition of thrombin and U46619-induced phosphoinositide breakdown and secretion by GDPβS in permeabilised platelets. The aim of this study was to examine the role of G proteins in collagen-induced platelet activation processes using GDPβS and saponin-permeabilised platelets.

All experiments were performed using washed, human platelets resuspended in a Hepes-Tyrode buffer (pH7.4, plus 1mM Ca^{2+} for experiments without saponin), and pre-loaded with [^{14}C]-5 hydroxytryptamine (5HT) (dense-granule marker), quin 2 (Ca^{2+} indicator) or ^{32}P-phosphate (to monitor 45Kd protein phosphorylation). Platelets were incubated with GDPβS (0.3-3mM) or GDPβS + saponin (10-15μg/ml) for 60 sec followed by addition of agonist, and reactions terminated 3 min later.

GDPβS (0.3-3mM) had significant inhibitory effects on platelet aggregation and 5HT secretion induced by collagen (20μg/ml), thrombin (0.2U/ml), U46619 (1μM) and 1,2-dioctanoylglycerol (diC_8, 60μM) in saponin-permeabilised platelets, and surprisingly, in intact platelets. Similar inhibitory effects in intact platelets were also observed with ATP (in similar concentration ranges) and with GDP and GTP (at 2- and 10-fold higher concentrations respectively). All 4 nucleotides also inhibited ADP (10uM)-induced platelet aggregation in indomethacin (10μM)-treated platelets, under conditions where no 5HT secretion or phosphoinositide breakdown occurred. Furthermore, the inhibition of thrombin-induced aggregation and 5HT secretion by GDPβS and ATP was accompanied by a reduction in the thrombin-induced rise in intracellular Ca^{2+}, $[Ca^{2+}]i$ levels and 45Kd protein phosphorylation, an indicator of protein kinase C activation, in intact platelets. As G proteins are believed to be located on the inner side of the plasma membrane or even in the cytoplasm of cells [Gilman 1984] and, as the nucleotides tested are membrane-impermeable, the inhibitory effects of these compounds in intact platelets are unlikely to be due to G protein-GTP inhibition, but mediated via an extracellular site of action and possibly due to inhibition of the effects of endogenous/released ADP. This would gain support from earlier findings on the inhibition of ADP-induced aggregation by nucleoside di and tri phosphates such as GDP, CDP and GTP, ATP [Packham et al 1974], as well as on the importance of endogenous/released ADP in mediating processes induced by other agonists [Colman 1986]. Our results therefore suggest a need for re-interpretation of earlier data [Brass et al 1987], where, merely by using GDPβS, a role for G proteins in U46619-induced platelet activation has been proposed. The usefulness of GDPβS as a tool in studying G protein-GTP interactions in platelets is also questionable.

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DIFFERENCES IN THE α_1-RECEPTORS MEDIATING INOSITOL PHOSPHOLIPID HYDROLYSIS AND POTENTIATION OF CYCLIC AMP FORMATION IN RAT BRAIN

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In rat cerebral cortex noradrenaline (NA) stimulates inositol phospholipid breakdown via an α_1 adrenoceptor (Kendall et al, 1985), and despite having no effect alone, α_1 adrenoceptor agonists potentiate the formation of cyclic AMP due to β-adrenoceptor stimulation (Perkins & Moore, 1973). In the light of recent evidence supporting the existence of α_1 adrenoceptor subtypes (Morrow & Creese, 1986) we have determined a pharmacological profile of two neurochemical responses to α_1 adrenoceptor stimulation.

Inositol phospholipid breakdown was monitored by measuring total ^{3}H-inositol phosphate accumulation in cerebral cortex slices from male Sprague/Dawley rats (Kendall et al, 1985). Cyclic AMP formation in response to NA, was measured by the ^{3}H-adenine prelabelling method of Shimizu et al (1969). The α_1 adrenoceptor potentiation was calculated as the difference between the maximum stimulation due to the β-agonist isoprenaline (Iso) (10^{-5}M) and that due to Iso + NA (10^{-4}M) (α + β stimulation). Antagonists were allowed to equilibrate for 15 min before agonist additions. Isoprenaline typically produced a 12-fold increase in ^{3}H-cyclic AMP accumulation which was increased a further 3-fold by the addition of NA. Inhibition curves were constructed using data obtained by adding increasing concentrations of antagonists, apparent inhibition constants (app. K_i) and Hill slopes (nH) were calculated. The results are shown in Table 1.

	cAMP Formation		Inositol phospholipid hydrolysis	
Antagonist	App. Ki (nM)	nH	App. Ki (nM)	nH
Dihydroergocryptine (DHE)	0.14±0.03	1.00±0.02	8.0± 1.9	0.99±0.09
Prazosin	2.8 ±2.0	0.60±0.06	1.4± 0.1	0.93±0.05
Phentolamine	3.5 ±1.5	1.17±0.08	16.4± 4.1	1.03±0.03
Indoramin	5.9 ±1.7	0.60±0.08	10.0± 3.0	0.93±0.06
WB4101	8.7 ±3.1	0.63±0.09	1.4± 0.1	0.77±0.05
Yohimbine	20.1 ±5.0	0.68±0.10	785± 85	0.95±0.08

Results represent mean ±s.e.m. of 3-5 separate experiments. Although low Hill slopes suggest more than one receptor site, only one value for apparent ki could be calculated from data available.

The affinities observed for the antagonists are consistent with the involvement of α_1 adrenoceptors in both responses. However differences in the rank orders of affinities and in the absolute values for all of the antagonists except indoramin suggest that the recognition sites are not identical. Also the low Hill slopes for antagonism due to prazosin, yohimbine, WB4101 and indoramin suggest the involvement of more than one receptor in mediation of cAMP response. The data are therefore consistent with a proposal for the existence of α_1 adrenoceptor subtypes in rat cerebral cortex.

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GR 32191 , A NOVEL THROMBOXANE RECEPTOR BLOCKING DRUG: EFFECTS UPON PLATELETS AND VASCULAR AND AIRWAYS SMOOTH MUSCLE IN VIVO

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GR 32191 ([1R-[1α(Z),2ß,3ß,5α]]-(+)-7-[5-[[(1,1'-biphenyl)-4-yl]methoxy]-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptenoic acid, hydrochloride) has been shown in vitro to potently antagonise (pA_2 > 8.0) the actions of thromboxane (Tx) A_2 or its mimetics upon platelets and vascular smooth muscle from man and a variety of animal species (Lumley et al., 1987). We now describe the action of the compound in vivo upon platelets and vascular and airways smooth muscle in the guinea-pig and dog.

In the anaesthetised guinea-pig, collagen (0.1 mg/kg i.v.) produces thrombocytopenia, vasoconstriction and bronchoconstriction, effects which are associated with a rise in plasma TxA_2. Aspirin (15 mg/kg i.v.) prevents this rise in TxA_2 and virtually abolishes the bronchoconstrictor and vasoconstrictor response to collagen and partially blocks the thrombocytopenia (mean, n=5 (95% confidence interval) inhibition of 69 (59-79)%). The residual thrombocytopenia can be abolished by prostacyclin (1 µg/kg/min i.v.). GR 32191 (3-300 µg/kg) administered intravenously as a single dose to each animal (n=4-6 animals per dosage group), produced a dose-dependent inhibition of the collagen-induced thrombocytopenia with an ID_{50} of 19 (12-30) µg/kg i.v. and a maximum inhibitory effect of 80 (74-86)%, which was significantly greater ($P<0.05$) than that of aspirin. Both the vasoconstrictor and bronchoconstrictor effects of collagen were similarly antagonised by GR 32191. Its potency on vascular and airways smooth muscle in the anaesthetised guinea-pig was further analysed using the TxA_2 mimetic U-46619. Intravenously administered GR 32191 antagonised the vasoconstrictor effects of U-46619 producing parallel rightward displacements of the U-46619 dose-response curve with a DR_{10} value (dose to produce a 10-fold rightward displacement of the agonist dose-effect curve) of 41 (27-81) µg/kg. In contrast to the effect upon the vasoconstrictor responses to U-46619, GR 32191 produced non-parallel rightward displacements of the U-46619 bronchoconstrictor dose-response curve with marked suppression of the maximum effect. Whilst a DR_{10} value could not be calculated, GR 32191 was extremely potent, a mean DR of 3.9 (2.5-6.0, n=4) being obtained with a dose of 3 µg/kg.

In the barbitone anaesthetised dog GR 32191 antagonised collagen-induced platelet aggregation in whole blood ex vivo and U-46619-induced constriction of the mesenteric arterial bed in vivo. Mean (n=4-6) DR_{10} values of 37 (18-73) and 60 (36-101) µg/kg were obtained upon platelets and vascular smooth muscle respectively. Thus the effective blocking doses of GR 32191 upon platelets and vascular smooth muscle were similar in both species. Up to the highest dose tested the compound lacked any direct effect itself upon any of the indices measured and was specific. For example, ex vivo platelet aggregation to ADP and vasoconstriction to phenylephrine in the anaesthetised dog were unaffected at doses of GR 32191 which were 25 and 5 fold in excess of the corresponding DR_{10} dose respectively.

The profile of action of GR 32191 in vitro has shown it to be that of a potent and specific thromboxane receptor blocking drug (Lumley et al., 1987). The present study has confirmed this profile in vivo. The compound is orally active and long lasting in man, (Thomas et al., 1987) and clinical studies are underway.

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RECOVERY OF LEUKOTRIENES FROM ISOLATED RAT LUNGS

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Bronchoalveolar lavage is used commonly to assess experimentally-induced inflammatory changes within the lungs of small laboratory animals. Leukotrienes (LT's) are considered to play a significant role in various lung disorders (Leitch, 1984) and attempts have been made to measure these mediators in bronchoalveolar lavage fluid (BALF) obtained from experimental models of pulmonary inflammation (Shopp et al., 1986; Taniguchi, et al., 1986). We have studied the recovery of leukotrienes from BALF and from lung perfusates and homogenates following intratracheal instillation into rat lungs.

Aqueous samples (approximately 10ml) were prepared on preconditioned C_{18} AASP BOND ELUT cartridges and were analysed for lipoxygenase products by HPLC with ultra-violet detection at 280nm, 270nm and 236nm. Gradient elution (40-95% organic phase over 22.0 min) of a NOVAPAK C_{18} 4μM RADIAL PAK column with NaH_2PO_4 0.05M/methanol: acetonitrile 50:50 resolved the lipoxygenase products in a 22.0 minute run.

Initial experiments were carried out to determine the stability of leukotrienes in BALF. Lungs from healthy, male Sprague Dawley rats (250-300g) were lavaged with 2 x 5ml phosphate buffered saline (PBS) and the lavages pooled. One ml aliquots of BALF were incubated at 37°C for 5, 10, 20, 30 and 60 min with 10ng LTC_4 and 10ng LTB_4 or with 10ng LTE_4 and 50ng 5-HETE. There was no evidence of metabolism in any sample and recoveries were greater than 75% for LTB_4, LTE_4 and 5-HETE. LTC_4 was recovered by about 60% in each incubate.

Isolated rat lungs were perfused through the pulmonary artery with Tyrodes solution at 2ml min^{-1} and the effluent collected for assay. After 5 min perfusion, a bolus injection of 100ng LTC_4 and 100ng LTB_4 in 0.2ml PBS was delivered to the lungs via the trachea. The effluent was collected for a further 15 min in 10ml fractions. Perfusion was stopped and the lungs were lavaged with 2 x 5ml PBS. Finally, the lungs were homogenised in 10ml methanol and the tissue pellet remaining after centrifugation was washed once with 5ml methanol. The pooled methanol supernatants were concentrated and diluted to 20% organic phase prior to loading on C_{18} cartridges.

Recovery from perfusates was - LTC_4 0-5%; LTB_4 10-25%; from BALF - LTC_4 0-28%; LTB_4 0-15%; and from lung homogenates - LTC_4 30-55%; LTB_4 50-70%. Similar findings have been reported by Harper et al (1984) using radiolabelled leukotrienes and these results suggest that lung homogenates may offer a better estimate of leukotriene content within lungs than analysis of BALF.

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SOME OBSERVATIONS ON THE SPECIFICITY OF THE POTENT THROMBOXANE RECEPTOR AGONIST EP171

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We have previously reported to the Society (Jones et al, 1985) that the 16-p-fluorophenoxy prostanoid EP171 is a highly active thromboxane receptor agonist with a slow onset and offset of action. Its contractile action on the pig pulmonary artery is typical - EC_{50} = 0.07nM; equipotent molar ratio = 0.0093 (U-46619 = 1.0); pA_2 for EP092 (Armstrong et al, 1986) antagonism = 8.1. We now describe attempts to determine the specificity of EP171 with respect to other prostanoid receptors linked to contractile smooth muscle systems.

On the dog iris sphincter preparation, which appears to contain only PGF receptors (Kennedy et al, 1983), EP171 behaves as a fast onset/fast offset full agonist (EC_{50} = 150nM) and is 44 ± 4 (s.e.m., n = 6) times less active than $PGF_{2\alpha}$. U-46619 is 196 ± 12 (n = 4) times less active than $PGF_{2\alpha}$. The actions of all three prostanoids are not blocked by EP092 (1μM). In this situation the specificity (TXA/PGF) of EP171 is superior to that of U-46619.

The guinea-pig stomach fundic strip is a PGE-sensitive preparation thought to be useful for prostanoid receptor characterisation owing to its lack of thromboxane and PGF receptors (Kennedy et al, 1983). Our studies (isometric recording on ventral strips, indomethacin 1μM, atropine 20nM) show EP171 and U-46619 to have biphasic log concentration-response curves. The more sensitive (thromboxane-like) contractile component has a maximum about 35% of the PGE_2 maximum. EC_{20} values for PGE_2, EP171 and U-46619 are 0.38, 0.55 and 33nM respectively. Submaximal responses to EP171 and U-46619 are abolished by pretreatment with 1μM EP092 whereas responses to PGE_2 are unaffected. In contrast, submaximal responses to EP171 and U-46619 are unaffected by the PGE receptor antagonists SC19220 (30μM) and SC25191 (10μM) (Sanner et al, 1973) whereas matching responses to PGE_2 are abolished. These findings appear to be at variance with those of Kennedy and colleagues, who found that SC19220 blocked responses to PGE_2 and U-46619 equally. It is likely that SC19220 (alone or in combination with the solvent) is non-selective at the concentration used (300μM).

With increasing concentrations of EP171 above 10nM, fast onset/fast offset (PGE-like) responses are elicited on the fundic strip. Comparison of PGE_2, EP171 and U-46619 in the presence of 'tone' induced by 10nM EP171 gives EC_{70} values of 2.9, 530 and 6300nM respectively and EPMR of 1.0, 181 ± 25 (n = 4) and 2150 (n = 2) respectively. Again the specificity (TXA/PGE) of EP171 is high.

Further study of the specificity of EP171 will be facilitated by the use of preparations containing a single prostanoid receptor population.

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CATIONIC FACTORS AFFECTING PHOSPHOLIPASE A_2 ACTIVITIES IN EXTRACTS OF HUMAN LUNG

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We have described phospholipase A_2 (PLA_2) activities in cell-free extracts of human lung, liberating free arachidonic acid (AA) from phosphatidylcholine (PC) and phosphatidylethanolamine (PE) labelled with ^{14}C-AA in the 2-position (Bakhle & Yeats, 1986). The highest specific activity was found in a light particulate subcellular fraction, sedimenting at 100,000x g, although there was also PLA_2 activity in a heavier particulate fraction. We have now studied the effects of varying cationic composition on the PLA_2 activity of human lung using the light particulate fraction.

The standard incubation conditions (pH7.4, 5mM Ca^{++}, 15uM substrate, 40min incubation time at 37°C), gave 432±52 pmoles AA (n=14; 5 lungs) hydrolysed from PC and 6.54±0.55 nmoles AA (n=12; 5 lungs) from PE per mg of lung protein (mean ± SEM). In all the subsequent work, assays were performed in duplicate, at least twice, with protein from three different lungs.

Hydrolysis of AA from either PC or PE required the presence of Ca^{++}; at 9.5mM, PC hydrolysis was 141±15% of that at 5 mM but was not further increased by raising Ca^{++} to 20 mM, whereas PE hydrolysis was maximal at 5 mM. Addition of EDTA (25mM) or EGTA (10mM) decreased PE hydrolysis more effectively (8±2% and 18±4% respectively) than PC hydrolysis (38+11% and 42+11%). However, with 2:2-dipyridyl, o-phenanthroline or 8-hydroxyquinoline, chelating agents for transition metal ions, PLA_2 activity was increased and no inhibition was noted at concentrations of up to 1mM of these chelating agents. At 10uM no effects were seen but, at 100uM, hydrolysis of PE was maximally stimulated to about 250%. Maximal stimulation of PC hydrolysis was achieved at 1mM of these three chelating agents, the most effective being 8-hydroxyquinoline (281±27%). Desferrioxamine did not affect PLA_2 activity at concentrations up to 1mM.

The PLA_2 activity in the heavier particulate fraction, sedimenting at 10,000x g and with mitochondrial marker enzymes, responded almost identically to changes in cation concentrations. However, the chelating agents for transition metal ions were generally less effective stimulators of either PC or PE hydrolysis, their maximum effect being about 150%, but inhibition was never seen.

Although the effects of Ca^{++} on PLA_2 activity were comparable to those already described (Irvine, 1982), the results with the transition metal chelators were unexpected. Recently, zinc ions have been shown to inhibit PLA_2 activity derived from macrophages (Finnen & Flower, 1987). However, our results do not need to reflect a direct involvement of transition metals in PLA_2-catalysed hydrolysis but may be linked with other inhibitory substances or enzymes in our heterogenous cell membrane fraction. Our results also suggest that there may be differences in the cationic requirements for hydrolysis of PC and PE by PLA_2.

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ON THE FORMATION OF 9-HYDROXY-LINOLEIC ACID BY GUINEA PIG PULMONARY MACROPHAGES

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As an open entry to the outside world, the pulmonary airways are continually exposed to potentially noxious agents, including inhaled viruses and bacteria. The pulmonary macrophage (PM) is an important determinant of the lungs' ability to prevent deleterious effects of airborne particles on pulmonary tissues. The PM possesses various mechanisms by which it fulfils its defensive role. Thus, phagocytosis, secretion of lysosomal enzymes, and the release of reactive oxygen species (ROS) all directly play a role in the clearance of inhaled foreign particles. The formation and release of arachidonic acid-derived mediators, such as prostaglandins and leukotrienes, often represents an indirect mechanism in the defense reaction, as some of these mediators propagate the inflammatory reaction by recruting other inflammatory cells. We recently reported that PM are also able to metabolize linoleic acid into a product which might function as a mediator in inflammatory reactions (Engels et al., 1986; see also Henricks et al., 1988). In the present study we investigated how the production of this metabolite, i.e. 9-hydroxy-linoleic acid (9-HODE), can be influenced. In this way we hope to obtain more insight in the physiological significance of 9-HODE in inflammatory reactions in the pulmonary airways.

Guinea pig PM were obtained by lung lavage in situ. Incubations of PM were carried out at 37°C for 15 min in Krebs-bicarbonate solution. The experiments were performed either in the presence or absence of exogenous radiolabeled arachidonic acid ([^{3}H]-AA) and linoleic acid ([^{14}C]-LA), or after prelabeling the PM with [^{3}H]-AA and [^{14}C]-LA. Prelabeling of PM (20×10^{6}/ml) was carried out by incubation for 45 min in the presence of 2 µCi/ml [^{3}H]-AA and 1 µCi/ml [^{14}C]-LA, followed by a washing procedure to remove non-incorporated radiolabel. After the incubations PM were pelleted and supernatant fractions were subjected to extraction and fractionation procedures as described previously (Engels et al., 1986). Fatty acid metabolites were analyzed by reverse phase high performance liquid chromatography (HPLC) and by bioassay for peptide leukotrienes (LTC_4, LTD_4), using the guinea pig ileum strip. With HPLC analysis, products were detected by UV absorption or by counting radioactivity in the eluate.

Non-stimulated PM produced 9-HODE and thromboxane B_2 (TxB_2). 13-HODE was also formed but only in minor amounts. No leukotrienes or other lipoxygenase products could be detected by HPLC or bioassay analysis. Stimulation with opsonized zymosan increased 9-HODE production 2-4 fold. When exogenous [^{3}H]-AA and [^{14}C]-LA (8 nM and 17µM, respectively) were present, products could only be measured as radioactivity, but not by UV absorbance. This indicates that the exogenous substrate fatty acids inhibited the formation of 9-HODE and TxB_2 from endogenous pools. The present experiments revealed that 9-HODE formation could be inhibited by indomethacin (1 µM), indicating that it is formed through a cyclooxygenase-catalyzed reaction. Interestingly, thiourea (25mM) almost totally inhibited the formation of 9-HODE, whereas catalase (5000 U/ml) did not. This suggests that ROS, possibly the hydroxyl radical, are important in the formation of 9-HODE. Considering the PM's ability to produce ROS, 9-HODE production may be regulated by the PM itself. Since 9-HODE influences the formation of ROS by PM (Henricks et al., 1988), there seems to be a complex interplay between ROS generation and the formation of fatty acid mediators by PM.

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EFFECTS OF 9-HYDROXY-LINOLEIC ACID ON GUINEA-PIG PULMONARY MACROPHAGE ACTIVITY

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Pulmonary macrophages (PM) are the primary defenders in the lung against inhaled particles. These cells exhibit a variety of biological activities, like phagocytosis and killing of microorganisms and secretion of enzymes, reactive oxygen metabolites, prostaglandins, leukotrienes and other mediators. The activities of phagocytic cells are not always beneficial to the host because reactive oxygen species and enzymes may also inflict considerable damage to host tissues. Recently, we demonstrated that guinea pig PM synthesize substantial amounts of the linoleic acid metabolite, 9-hydroxy-linoleic acid (9-HODE) under non-stimulated conditions (Engels et al., 1986). In the present study, we investigated the effects of 9-HODE and its precursor 9-hydroperoxy-linoleic acid (9-HPODE) on the phagocytic and metabolic activity of guinea pig PM.

The PM were obtained by lavage of the lungs of male guinea pigs. The cell suspensions were washed, differentiated and brought to a concentration of $5x10^6$ PM/ml. To quantitate phagocytic cell activity, radiolabeled, preopsonized bacteria were used (Henricks et al., 1987). Superoxide production was assayed by measuring the superoxide dismutase-sensitive reduction of ferricytochrome c and hydrogen peroxide release was determinated using the horseradish peroxidase-mediated oxidation of phenol red by hydrogen peroxide (Henricks et al., 1987). Generation of chemiluminescence was monitored in a type 1251 LKB Wallac luminometer in the presence of 0.5 mM lucigen. Measurements were made every 60 sec over a 30 min period at 37°C. Phorbol myristate acetate (PMA) was used as stimulus in the assays to measure the metabolic responsiveness of PM.

9-HODE in concentrations up to 1 μM did not influence the phagocytic capacity of PM. Both control and 9-HODE-incubated PM phagocytized about 40% of the added bacteria during a 30 min incubation period. 9-HODE and 9-HPODE were able to inhibit the amount of chemiluminescence generated by PMA-stimulated PM (see Table 1). This inhibition was both time- and concentration dependent. No effects of 9-HODE and 9-HPODE were observed on the release of superoxide and hydrogen peroxide by the PM (see Table 1). Similar results were observed when opsonized zymosan particles were used instead of PMA as stimulus. Since 9-HODE and 9-HPODE also inhibited the chemiluminescence response of a cell-free enzyme system (xanthine-xanthine oxidase) which produces reactive oxygen metabolites (38±2 and 30±3% inhibition, respectively), we suggest that 9-HODE possesses scavenging properties towards a reactive oxygen metabolite which is not superoxide or hydrogen peroxide. These results suggest that the formation of 9-HODE and 9-HPODE by PM may represent a regulatory and protecting mechanism towards the cell's own released reactive oxygen metabolites.

Table 1 % superoxide, hydrogen peroxide and chemiluminesence generated by PMA-stimulated PM in the absence or presence of 1 μM 9-HODE or 9-HPODE.

	Superoxide	hydrogen peroxide	chemiluminescence
Control	100	100	100
9-HODE	98 ± 5	100 ± 9	61 ± 3
9-HPODE	111 ± 3	103 ± 4	58 ± 3

Engels, F., Willems, H. & Nijkamp, F.P. (1986) FEBS Lett. 209, 249-253.
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EPITHELIUM-DERIVED PGE_2 MAY PLAY A ROLE IN THE ENDOTOXIN-INDUCED RESPIRATORY AIRWAY HYPERRACTIVITY OF THE GUINEA-PIG

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Damage of pulmonary epithelium and bronchial hyperreactivity are phenomena which both can be observed in asthmatic patients (Laitinen *et al.*, 1985). Previously, we showed that bronchial hyperreactivity could be induced in the guinea-pig after i.p. administration of the Gram-negative bacterium *Haemophilus influenzae* (Folkerts & Nijkamp 1985). The endotoxin of Gram-negative bacteria seemed to be the responsible part, since 4 days after an i.p. injection with endotoxin (LPS; *Escherichia coli* $O_{111}:B_4$, 1 mg/kg body weight) an increased sensitivity to arecoline and histamine was observed *in vivo* and *in vitro* (manuscripts in preparation). Since in the isolated guinea-pig trachea prostaglandins are released during contractions which may influence smooth muscle tone (Orehek *et al.*, 1975) and we recently showed that arachidonic acid-induced relaxation is epithelium-dependent (Nijkamp & Folkerts, 1987) we now investigated the role of the epithelium and epithelium-derived prostaglandins in the endotoxin-induced bronchial hyperreactivity.

First the reactivity to arecoline and histamine of isolated guinea-pig tracheae from saline and endotoxin-treated guinea-pigs were compared with spirals of which the epithelium had been removed from both experimental groups. Intact tracheae from endotoxin-treated animals showed a 27% increased contraction ($P<0.01$) to arecoline and a 69% increased contraction ($P<0.01$) to histamine as compared to the intact controls. A similar increased responsiveness in both groups was observed in epithelium-denuded spirals. No additive responses were observed in the epithelium-denuded endotoxin preparations. The initial tone of the tracheae was not changed after epithelium removal. After preincubation with the cyclo-oxygenase inhibitor indomethacin [10 μM] all preparations became hyperreactive to histamine. No differences in the maximal responses between the four groups were observed pointing to a role for prostaglandins in the endotoxin-induced hyperreactivity.

In additional experiments PGE_2 production in the organ bath was measured (RIA) in histamine (0.1 mM) stimulated intact and epithelium-denuded spirals from saline and endotoxin-treated guinea-pigs. The basal PGE_2-values (in pg PGE_2/100 μl bath fluid, N=6-9) of the intact saline and endotoxin preparations were 38.3 ± 5.6 and 25.1 ± 4.8 respectively. In the epithelium-denuded tracheae the values were significantly reduced 15.0 ± 2.3 and 14.6 ± 2.2 ($P<0.01$) respectively. After histamine stimulation the PGE_2-level was significantly increased in all groups. The PGE_2-value in the intact control preparations amounted 123.7 ± 15.9. In the epithelium-denuded control and endotoxin preparations the PGE_2-content was ca 60% less ($P<0.01$). Interestingly, the PGE_2-content in tracheae from endotoxin-treated guinea pigs was also 34% less ($P<0.05$) as compared to the intact stimulated control group.

From this study it is concluded that epithelium-derived PGE_2 may play a pivotal role in the endotoxin-induced respiratory airway hyperreactivity, and in the increased responsiveness observed in epithelium-denuded tracheal spirals.

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GRANULOCYTE-DEPENDENT ENHANCEMENT OF LEUKOTRIENE RELEASE IN PRIMATE BRONCHOALVEOLAR MACROPHAGES

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The release of inflammatory mediators from cells in the airway lumen may be an important event in lung pathophysiology. In this study we have investigated the opsonized zymosan (OPZ) and *Ascaris suum* (AS) dependent release of immunoreactive leukotriene C_4 (LTC_4) from cells obtained by bronchoalveolar lavage (BAL) of stump-tailed monkeys (*Macaca arctoides*). Experiments were performed on normal and AS-sensitized animals (Wells *et al.*, 1986). Cells recovered by BAL in non-sensitized animals had the following composition: macrophages 40 ± 3%; epithelial cells 26 ± 8%; eosinophils 17 ± 4%; mast cells 8 ± 4% small monocytes 6 ± 1% and neutrophils 0.5 ± 0.2%, n=26). In contrast, monkeys recently infected with AS had larger proportions of mast cells (31 ± 3%) and eosinophils (38 ± 4%, n=24).

Cells were separated by centrifugation at 450 x g (20 min 20°C) on 4-step discontinuous gradients of Percoll (1.06-1.09 g ml^{-1}). Interface fractions were harvested, washed and challenged with either 1.5 mg ml^{-1} OPZ or 1 μg ml^{-1} AS for 45 min; conditions previously established to elicit maximum release of LTC_4. Release induced by AS occurred only in mast cell containing fractions from sensitized animals. There was a significant correlation between LTC_4 release and numbers of mast cells ($r=0.75$, $n=34$, $p<0.001$). In contrast, OPZ-induced release occurred in macrophage rich fractions in both animal groups ($r=0.84$, $n=58$, $p<0.001$), but was greater in cells from sensitized animals. This was confirmed in unfractionated cells, which from AS-sensitive animals released 11.1 ± 0.8 ng per 10^6 macrophages (n=5) compared with 4.6 ± 0.9 ng in unsensitized animals ($p<0.05$, n=5).

In further studies, addition of a high density (HD) cell fraction (composition: mast cells 60%, eosinophils 36%) from AS-sensitive animals to unfractionated cells from non-sensitized animals increased OPZ-induced LTC_4 release from 6 ± 0.5 to a maximum 11.3 ± 1.1 ng 10^6 macrophages ($p<0.05$), despite the fact that OPZ did not cause release from the HD fraction alone (n=7). There were significant correlations between the percentage enhancement of LTC_4 release and the numbers of eosinophils and total granulocytes, but not the numbers of mast cells added ($r=0.92$ $p<0.001$; $r=0.53$ $p<0.001$ and $r=0.05$ $p>0.05$ respectively, n=39 observations).

These experiments suggest that potentially important cellular interactions occur between alveolar macrophages and granulocytes which have effects on the regulation of mediator production. These interactions may be of potential importance in pulmonary pathophysiology.

AMC is an SERC scholar.

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PROTAGLANDIN (PG) D_2 SYNTHESIS BY HUMAN BRONCHOALVEOLAR MAST CELLS

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Human mast cells enzymatically dispersed from lung parenchyma generate substantial amounts of the bronchoconstrictor prostanoid PGD_2 (Holgate et al., 1984). In this study we have investigated the capacity of cells obtained by lavage of the bronchoalveolar lumen to generate PGD_2 upon immunological and calcium-dependent activation and have attempted to localise its cellular origin. Thirteen patients undergoing clinically indicated fibreoptic bronchoscopy for interstitial lung disease had bronchoalveolar lavage (BAL) performed (sarcoidosis 3; asbestosis 1; lipid pneumonia 1; fibrosing alveolitis 8) (Agius et al, 1985). After lavage an aliquot of the sample was cytocentrifuged for staining with May Grunwald Giemsa, toluidine blue and alcian blue/safranin revealing a mast cell content of 0.07 - 0.6%. Aliquots of cells (min 0.5 x 10^6 cells ml^{-1}) were challenged either with IgG anti-human IgE (1/1000 - 1/10 dilution) or calcium ionophore A23187 (0.1 - 3μM). In 5 experiments BAL cells were subjected to density centrifugation on continuous gradients of Percoll, and each of four fractions challenged with either anti-IgE (1/10) or A23187 (3.0 uM). All incubations were carried out for 30 min at 37°C and terminated by rapid centrifugation at 300 g at 4°C. PGD_2 and histamine were assayed by a specific radioimmunoassay and radio-transfer enzymatic assay respectively.

Both A23187 and anti-IgE produced dose related releases of histamine and PGD_2 with maximum generation of PGD_2 occurring with 1.1 μM A23187 (286 $\pm$ 151 ng PGD_2 per 10^6 mast cells, n = 3) and 1/100 anti-human IgE (57.7 $\pm$ 9.0 ng PGD_2 per 10^6 mast cells, n = 3). A close correlation was found between PGD_2 generation and histamine release with intercepts that did not depart significantly from zero in the case of immunological ($r = 0.86$, $p<0.01$, n = 14 experiments); A23187 ($r = 0.75$, $p<0.01$ n = 19 experiments) and pooled challenges ($r = 0.76$, $p<0.001$, n = 33) respectively. The amount of PGD_2 released per ng of histamine was 86.1 $\pm$ 8.1 pg ng^{-1} (n = 19) in the case of A23187 and 42.6 $\pm$ 11.1 pg ng^{-1} (n = 14) in the case of anti-IgE. These results contrast with our previous observations in human lung parenchyma where immunological activation is a more effective secretagogue for PGD_2 than A23187 (Holgate et al., 1984). In only 2 of the 5 gradient experiments was sufficient PGD_2 released from cells in the fractions to be detected by RIA. In both experiments (1 A23187, 1 anti-IgE challenge) a close concordance was observed between the mast cell content, histamine release and PGD_2 generation in the fractions.

We conclude that cells recovered by BAL release substantial quantities of PGD_2 upon calcium- and IgE-dependent stimulation and that the major cellular source of this PG is the mast cell.

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AH 21-132 A NOVEL RELAXANT OF AIRWAY SMOOTH MUSCLE

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AH 21-132 is a benzonaphthyridine derivative (Markstein et al,1984) which relaxes human and guinea-pig airway smooth muscle in vitro and is more potent than aminophylline as a relaxant of guinea-pig tracheal rings, whether tension was generated spontaneously or by addition of carbachol (10^{-6} M) or histamine (10^{-4} M) (Table 1). Relaxation of guinea-pig trachea by AH 21-132 was independent of beta-adrenoceptor activation since addition of propranolol (10^{-6} M) was without effect (EC_{50}=1.6 uM; n=10). AH 21-132 was also a more potent relaxant of spontaneous tone in human bronchus (EC_{50}=4.7 uM; n=4) when compared with aminophylline (EC_{50} = 140 uM; n=4). Epithelial removal enhanced the relaxant action of AH 21-132 (EC_{50}=0.8 uM; n=10), but not of aminophylline (EC_{50}=89 uM, n=10).

Table 1. Relaxation of the guinea-pig tracheal ring (EC_{50} umol, n=10)

Treatment	Basal Tone	Carbachol	Histamine
AH 21-132	1.6	6.9	3.2
Aminophylline	78.4	225.0	131.0

In anaesthetised ventilated guinea-pigs, sustained bronchospasm induced by i.v. bombesin was reversed by administration of AH 21-132 or aminophylline intravenously (EC_{50}, 0.08 and 1.1 mg/kg respectively) or intra-duodenally (EC_{50}, 5.7 and 11.1 mg/kg respectively). Pretreatment (5 min) with AH 21-132 inhibited bronchospasm induced by a variety of constrictor agents (Table 2). AH 21-132 is also effective by inhalation, significantly increasing the collapse time of guinea-pigs (78±5 sec, n=20) exposed to an aerosol of acetylcholine (0.3%) at a dose of 1 ug/ml (116±10 sec, n=10) or greater. Comparable inhibition was observed with aminophylline at 1 mg/ml (119±12 sec, n=10).

Table 2. Inhibition of bronchospasm by AH 21-132 in guinea-pigs

Treatment	dose mg/kg	PAF (0.056)	Hist (10)	Ach (32)	Sub P (10)	LTC4 (5)
AH 21-132	1.0	97±1	93±2	82±5	46±8	100±0
	0.1	52±6	16±9	19±7	0±4	59±13
Aminophylline	3.2	81±3	52±7	14±9	43±7	67±14

Numbers in each column indicate % inhibition ± sem in groups of 5 animals, numbers in brackets indicate the dose of spasmogen used (ug/kg).

AH 21-132 can relax airway smooth muscle in vitro and in vivo whether given intravenously, orally or by inhalation. Use of AH 21-132 in asthma therapy merits consideration.

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PROPERTIES OF AH 21-132 THAT COULD CONTRIBUTE TO PROPHYLACTIC ACTIVITY IN ASTHMA

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Exposure of guinea-pigs to isoprenaline (Morley and Sanjar, 1987) induces an airway hyperreactivity that is comparable to that achieved by PAF (Mazzoni et al, 1985): however, the mechanisms underlying these responses can readily be distinguished since vagal section abrogates only isoprenaline-induced hyperreactivity and platelet depletion abrogates only PAF-induced hyperreactivity. Interestingly, prophylactic anti-asthma drugs may inhibit both types of hyperreactivity, hence ketotifen has been compared with AH 21-132 using these tests. Anaesthetised (100 mg/kg i.p. phenobarbitone and 30 mg/kg i.p. pentobarbitone), ventilated (1Hz, 10ml/kg) guinea-pigs were used to monitor airway resistance (R_L cm H_2O/l/sec), calculated from tracheal airflow and transpulmonary pressure, using a Buxco respiratory analyser. In such preparations airway hyper-reactivity was induced by intravenous infusion of PAF (600 ng/kg/h) or isoprenaline (100 ug/kg/h). Hyperreactivity was estimated as the difference between the effect upon R_L of intravenous bombesin (240 ng/kg) before, and 20 minutes after, infusion of PAF or isoprenaline. AH 21-132 (0.1-10 mg/kg) or ketotifen (0.1-1 mg/kg) were administered as a bolus injection before, and as an infusion, during exposure to isoprenaline or PAF. Both types of airway hyperreactivity were inhibited by AH 21-132 or ketotifen in a dose related fashion (table).

Treatment	Incremental Increase of R_L to Bombesin after Infusion of				
	n	PAF	n	Isoprenaline	
Saline	25	226±19	34	260±19	
AH 21-132					
0.1	10	138±34 *	10	228±40	NS
0.32	10	116±28 *	10	127±23	*
1.0	10	73±24 *	10	94±18	*
10.0	5	-58±24 *	5	-84±46	*
ketotifen					
0.1	10	153±24 *	10	225±34	NS
1.0	10	61±15 *	10	133±28	*

* Significant (P<0.01) inhibition by comparison with saline treatment

AH 21-132 shares with ketotifen, cromoglycate and theophylline the capacity to inhibit development of distinct forms of airway hyperreactivity, implying utility as a prophylactic anti-asthma drug.

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THE EFFECT OF AH 21-132 AND OTHER ANTI-ASTHAM DRUGS ON PAF-INDUCED EOSINOPHILIA IN THE GUINEA-PIG LUNG

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Exposure of guinea-pigs to an aerosol of platelet activating factor (PAF) induces a selective eosinophil accumulation within the airway lumen which is maximal at 48 hours (Aoki et al., 1987). The effect of AH 21-132 and established anti-asthma drugs on PAF-induced eosinophilia has been assessed.
Male Dunkin-Hartley guinea-pigs (450-600 g) were exposed to an aerosol (jet nebulizer, DeVilbiss) of PAF (10 ml of a 10 ug/ml solution of 0.25% BSA) for one hour. 48 hours after exposure, guinea-pigs were killed by i.p. injection of pentobarbitone (100 mg/kg) and the airway lumen was washed with 6x10 ml of Ca^{2+} and Mg^{2+} free Tyrode containing EDTA (20 mM). Bronchoalveolar lavage (BAL) fluid was centrifuged at 200g for 10 minutes and the cell pellet resuspended in 1 ml of Tyrode. Total cell counts were made using Turk's solution and differential cell counts were made from smears fixed in methanol (100%) and stained by Leishman's. Drugs were administered subcutaneously via an osmotic infusion pump (Alzet), for four days prior to PAF exposure and 2 days thereafter. Each treatment group comprises 10 animals and results are summarised in the table.

Treatment	Dose (mg/kg/day)	% eosinophilia (BAL)	P (t-test)
Saline	-	22 ± 2	-
AH 21-132	1	10 ± 1	0.0005
	0.1	18 ± 2	0.02
Ketotifen	1	11 ± 1	0.0005
	0.1	14 ± 1	0.0005
Aminophylline	10	10 ± 2	0.0005
Dexamethasone	1	9 ± 1	0.0005
DSCG	1	13 ± 2	0.002
Indomethacin	1	19 ± 1	NS
Mepyramine	2	20 ± 3	NS
Salbutamol	1	19 ± 2	NS

Previous studies have indicated that established prophylactic anti-asthma drugs inhibit PAF-induced airway hyperreactivity (Mazzoni et al, 1985). The present study further characterises this drug category to include inhibition of PAF-induced intra-pulmonary eosinophil accumulation. Inhibition of eosinophilia and eosinophil-induced pathology may be a necessary property of prophylactic anti-asthma drugs; thus, AH 21-132 may exhibit prophylactic activity in asthma.

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HYPERRESPONSIVENESS TO IRRITANT INDUCED COUGH BUT NOT BRONCHOCONSTRICTION IN GUINEA-PIGS EXPOSED TO CIGARETTE SMOKE FOR 2 WEEKS

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Exposure of airways to irritants may produce changes in airways responsiveness to bronchoconstrictor stimuli. However, also the possibility that such an exposure may affect the sensitivity to tussive stimuli should be examined, since cough and reflex bronchoconstriction (Bc) have been demonstrated to have a separate pharmacology. We have now examined cough and Bc induced by cig. smoke and nebulized citric acid (CA) and capsaicin (Caps), respectively, in guinea-pigs exposed to cig. smoke for 2 weeks.

Guinea-pigs (275-400 g) were exposed in a smoking machine to either unfiltered cig. smoke (Kentucky 2R1 reference cig.) or room air during 1 h, twice daily for 2 weeks. At each 1 h session, 15 guinea-pigs received smoke from 8 cig. (35 puffs from each; puff time 5 s, air time 10 s). On the day after the last exposure (to smoke or room air) unanaesthetized animals were challenged with cig. smoke (185 s puff time = 1 cig.) or ultrasonically generated (Omron NEU 10) aerosols of CA (0.39 M) or Caps (30 μM) for up to 7 min. The number of coughs produced during the first 3 min of exposure was counted and the time to onset of Bc (development of a slow laboured breathing with exaggerated abdominal movements) recorded. Statistical differences were calculated by use of Student's t-test for unpaired observations.

The weight gain during the 2-week period was slightly smaller in the cig. smoke exposed than in the air exposed guinea-pigs. Between exposures some of the smoke exposed animals spontaneously sneezed and coughed. CA and Caps, but not cig. smoke, induced significantly more coughing in smoke exposed than in room air exposed guinea-pigs (Table 1). Caps and smoke induced BC was unaffected by 2 weeks' exposure to cig. smoke, whereas the time to onset of CA induced BC was significantly prolonged in such animals (Table 1).

Table 1. Effect of 2 weeks' exposure to cigarette smoke or room air on cough and time to bronchoconstriction (Bc) in conscious guinea-pigs (mean ± SEM, n=8 in each group).

	air-exposed		smoke-exposed			
	coughs	Bc (s)	coughs		Bc (s)	
CA	7.0±1.4	138±12	17.1±2.5	P<0.01	239±37	P<0.05
Caps	3.0±0.5	98±10	7.4±1.4	P<0.01	143±24	NS
Cig. smoke	7.4±0.7	222±45	7.9±2.0	NS	241±55	NS

The observation that the sensitivity to tussive but not to bronchoconstrictor stimuli was enhanced in cig. smoke exposed guinea-pigs supports the view that cough and Bc are separate airway reflexes. Furthermore, these data indicate that airway sensitivity to tussive and bronchoconstrictor stimuli may be controlled independently. Cough and Bc produced by CA and Caps in guinea-pigs have been demonstrated to be mediated by Caps-sensitive sensory neurons (Forsberg & Karlsson 1986), whereas cig. smoke, containing nicotine, particulate matter and a vapour phase, in addition may stimulate irritant receptors (Forsberg & Karlsson 1987). Thus, the hyperresponsiveness to CA and Caps, but not to smoke, appears to suggest an enhanced responsiveness in Caps-sensitive C-fiber endings mediating cough.

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IS THERE A NOVEL CONTRACTILE PROSTANOID RECEPTOR IN HUMAN LARGE AIRWAYS?

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Guinea-pig trachealis possesses contractile prostanoid receptor subtypes for prostaglandin(PG)-E and thromboxane (TXA_2) whereas only the latter receptor is present in guinea-pig and human lung strips (Gardiner et al 1987). We have now investigated whether contractile prostanoid receptor subtypes are also present in human large airways. In our study we used the TXA_2 mimetic U46619, the TXA_2 antagonists EP092 and AH23848, 16,16dimethylPGE$_2$ (DME_2) and an antagonist of the contractile PGE receptor AH6809. Human bronchiolar rings (HBR), 6-10mm diameter, were suspended in 10ml baths containing Tyrodes solution and flurbiprofen (8×10^{-6}M) at 37°C, and gassed with carbogen. Changes in tension were measured isometrically.

U46619 and DME_2 were potent contractile agonists of HBR producing maximum responses that were 85% and 68% of the KCl-induced maximum respectively. The EC_{50} of U46619 was 2.0 (95% confidence limits of 1.2-2.8) $\times 10^{-8}$M compared to 3.4(2.5-4.3) $\times 10^{-8}$M in the guinea-pig tracheal ring (GPTR). The EC_{50} of DME_2 was 8.0(6.4-9.6) $\times 10^{-8}$M compared to 5.2(3.4-7.0) $\times 10^{-9}$M in the GPTR. Results with the antagonists are given in Table 1.

TABLE 1 Antagonist pA_2 (or pK_B) values in human and guinea-pig airways (N $\geq$ 5)

PREPARATION	AGONIST	ANTAGONIST pA_2/pK_B* ± 1 SEM		
		EP092	AH23848	AH6809
HBR	U46619	6.8 ± 0.4*	6.8 ± 0.3*	< 6.0
	DME_2	< 6.0	< 6.0	< 6.0
GPTR	U46619	8.7 ± 0.2	8.5 ± 0.4*	< 6.0
	DME_2	< 6.0	< 6.0	7.2 ± 0.1

The ineffectiveness of AH6809 and the significantly lower potencies of EP092 and AH23848 suggest that human bronchioles do not contain AH6809-sensitive contractile PGE receptors or the typical TXA_2 receptor. It seems likely that either a subtype of the thromboxane receptor is present as has been provisionally suggested for the rabbit aorta (Jones et al, 1982) or that a novel contractile prostanoid receptor is present.

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THE FIRST HOME OFFICE REGISTRATION OF 'COMMERCIAL PREMISES': THE WELLCOME PHYSIOLOGICAL RESEARCH LABORATORIES

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The 1876 Cruelty to Animals Act required, amongst other conditions, that premises to be used for experiments on living animals had to be registered with the Home Office and open to random inspection by a Home Office Inspector. For the first few years of the Act, such registration was confined to laboratories associated with hospitals, Universities or medical corporations, although occasionally private premises belonging to medical practitioners were also registered. In 1900 the first application was made by a pharmaceutical manufacturer, Henry Wellcome, for the registration of his Physiological Research Laboratories. These laboratories, not ostensibly associated with the business side of Burroughs, Wellcome and Co., had been established initially to produce diphtheria anti-toxic serum. By the time of Wellcome's application they had moved to extensive premises in Brockwell Park, South London and Wellcome's ambitions for pharmaceutical development necessitated that experimental work be undertaken there. His application to the Home Office caused a considerable stir, and advisers such as the Laboratories Committee of the Royal Colleges of Surgeons and Physicians were approached, whilst at the same time Wellcome and his staff rallied influential support. The debate, of allowing a commercial manufacturer privileges previously accorded only to professional medical men, continued for more than eighteen months. Wellcome's ultimate success permitted him to employ physiologists and pharmacologists to perform a wide range of experimental research and of his early staff, eight became Fellows of the Royal Society, including one of his most distinguished employees, Henry Dale. This strong research commitment has continued to the present day, although the laboratories are now firmly within the company structure of the Wellcome Foundation (Vane 1980).

The registration of Wellcome's laboratories also created an important precedent within the Home Office and future applications by pharmaceutical companies were routinely assessed along with other such requests and were not subject to the special enquiries that had accompanied Wellcome's application.

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INTRACEREBROVENTRICULAR [D-PEN^{2,5}]-ENKEPHALIN, A DELTA OPIOID AGONIST, CAUSES HYPERTENSION AND TACHYCARDIA IN CONSCIOUS RABBITS

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Intracerebroventricular (icv) administration of the mu opioid agonist [D-Ala^2-, MePhe^4-,Gly^5-ol]-enkephalin (DAGO) to conscious rabbits causes hypertension, bradycardia, hyperglycaemia, respiratory depression and sedation; these effects are blocked by intravenous (iv) naloxone (30nmol/kg) (Mathias, May & Whitehead, 1987). Smaller changes were seen following intracisternal (ic) administration. In the present study we have examined the effects of the delta opioid agonist [D-Pen^{2,5}]-enkephalin (DPDPE) given in 25ul saline via the same routes to two groups of conscious rabbits previously implanted with either icv or ic cannulae.

After icv DPDPE (1.0nmol/kg) mean arterial pressure (MAP) increased from 95±2 (mean±SEM, n=6) to 129±4mmHg after 15 min ($p<0.05$). Heart rate (HR) increased from 193±8 to 253±16 beats/min ($P<0.05$). There were no changes in respiration rate, arterial pCO_2 or arterial pO_2. Plasma glucose increased from 6.7±0.2 to 7.8±0.3mM after 60 min ($p<0.05$). Following this dose the animals were more alert and started grooming. Smaller changes occured following 0.1nmol/kg icv DPDPE with no effects after 0.01nmol/kg. Naloxone (300nmol/kg iv) given 10 min after DPDPE (1.0nmol/kg icv) did not alter the hypertension or tachycardia. After ic DPDPE (1.0nmol/kg) MAP increased from 93±6 to 116±9mmHg after 30 min ($p<0.05$). HR increased from 202±17 to 260±15 beats/min after 60 min ($p<0.05$). There were no changes in respiration rate, arterial gases or behavior after ic DPDPE. The lower doses of DPDPE (0.1 & 0.01nmol/kg) were without effect.

In conclusion, icv DPDPE in conscious rabbits causes hypertension and tachycardia with no effects on respiration. The effects of DPDPE probably result from stimulation of delta opioid receptors and are different from the changes produced by stimulation of central mu opioid receptors by DAGO. These findings demonstrate that specific opioid agonists cause distinct effects suggesting that the various opioid receptor subtypes may serve different functions in central cardiovascular and respiratory control.

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EVIDENCE FOR A VASOPRESSIN-MEDIATED PRESSOR RESPONSE TO PARA-VENTRICULAR NUCLEUS INJECTIONS OF NORADRENALINE IN CONSCIOUS RATS

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The hypothalamic paraventricular nucleus (PVN) is an important integrative centre for ascending and descending influences on cardiovascular regulation (Sawchenko & Swanson, 1981). There is a dense ascending noradrenergic projection from the brainstem to vasopressin (AVP) containing cells of the PVN (McNeil & Sladek, 1980 Swanson et al, 1981) suggesting that noradrenaline (NA) released in the PVN may influence AVP release (Benetos et al, 1986) and blood pressure (BP). In the present study we have investigated the cardiovascular effects of NA injected directly into the PVN of conscious Long Evans (control) and AVP-deficient Brattleboro rats.

Male Long Evans (312±5 g, n=15, mean±s.e.m.) and Brattleboro (304±4 g, n=7) rats were prepared, under sodium methohexitone anaesthesia (60 mg/kg i.p.) with unilateral 23 gauge stainless steel guide cannulae targeted 2 mm dorsal to the PVN. Seven days later, the animals were re-anaesthetised with sodium methohexitone and a catheter placed in the abdominal aorta via the caudal artery. Continuous recordings of systolic and diastolic BP and heart rate (HR) were begun following overnight recovery (17-18 hr). Basal levels of BP and HR were 142±2/80±2 mmHg (systolic/diastolic and 357±11 beats/min for Long Evans rats and 148±3/86±3 mmHg and 376±15 beats/min for Brattleboro rats (n=7 in each case). The maximum changes in BP and HR produced by PVN microinjections of NA (10 and 30 nmol in 0.3 µl) are summarised below.

Table 1 Maximum changes in BP (mmHg) and HR (beats/min) following PVN injection of NA

	dose of NA (nmol)	
	10	30
Long Evans (n=7)		
Δ systolic BP	+18±5	+32±8
Δ diastolic BP	+18±5	+27±6
Δ HR	-35±5	-71±19
Brattleboro (n=7)		
Δ systolic BP	-2±4	+9±4
Δ diastolic BP	-6±3	+9±4
Δ HR	-9±6	-45±8

In a separate group of Long Evans rats (n=8) intravenous pretreatment with $d(CH_2)_5$Tyr[Et]DAVP (10 µg/kg bolus followed by 10 µg/kg/hr infusion) abolished the pressor effect of a 10 nmol microinjection of NA, but the bradycardia was not significantly attenuated.

These results are in agreement with the findings of Benetos et al (1986) and suggest that the pressor response produced by microinjection of NA into the PVN of conscious rats is dependent on AVP release.

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60P

CORRELATION OF POSITIVE INOTROPIC RESPONSES IN HUMAN ISOLATED MYOCARDIUM WITH PREOPERATIVE CARDIAC FUNCTION

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Heart failure is the inability of the heart to supply enough blood to adequately perfuse peripheral tissues. Therapy with presently available positive inotropic agents, the aim being to reverse this decline in contractility, often becomes less successful as heart failure progresses. The development of a refractory state makes it important to differentiate between global or specific decreases in contractility. Such a specific receptor abnormality may allow the introduction of new compounds which can bypass this abnormality and utilize the contractile reserve of the failing myocardium.
Positive inotropic responses of dobutamine (β-adrenoceptor agonist), isobutylmethylxanthine (phosphodiesterase inhibitor, IBMX), histamine, Bay K 8644 (calcium channel activator), dihydroouabain and digoxin (cardiac glycosides) and calcium were compared in isolated papillary muscle strips. Preoperative ventricular function (ejection fraction, LVEDP) was measured by routine cardiac catheterization shortly before surgery.
In muscle strips from mitral valve replacement patients, the maximal inotropic response to Bay K 8644 of 2.0 ± 0.4 mN (n=7) was significantly reduced compared to dihydroouabain (5.6 ± 1.1 mN; n=7) and calcium (6.2 ± 0.6 mN; n=8). We have shown similar reductions for compounds acting through increases in cAMP (Brown et al 1986). In contrast, all compounds gave similar maximal inotropic responses in guinea pig isolated papillary muscles (Brown & Erdmann 1985). Thus, these reductions could be either species differences or disease-induced changes.
In muscle strips from patients with terminal cardiac insufficiency undergoing cardiac transplantation, the maximal positive inotropic response to compounds acting through cAMP or calcium channel activation was further reduced: dobutamine, 0.9 ± 0.4 mN (n=13); histamine, 1.8 ± 0.4 mN (n=8); IBMX, 0.5 ± 0.2 mN (n=9); Bay K 8644, 0.8 ± 0.2 mN (n=9). In contrast, the positive inotropic responses to digoxin (6.0 ± 0.2 mN, n=8) and calcium (6.4 ± 0.7 mN, n=12) were not altered. There was a significant negative correlation between the maximal inotropic effect of dobutamine, IBMX, or Bay K 8644 (as a fraction of the calcium response in each muscle strip) and the degree of heart failure.

Thus, these results provide evidence that the reduced inotropic responses are specific, disease-induced changes in access to the contractile reserve rather than global decreases in contractility or species differences; the contractile reserve is maintained even in severe heart failure. We suggest that presently available inotropic therapy either cannot utilize this reserve (compounds acting through cAMP, calcium channel activators) or causes toxicity before maximal inotropic responses are achieved (digitalis).

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PROTECTION OF THE GLOBALLY ISCHAEMIC ISOLATED HEART (RABBIT) WITH SODIUM NITROPRUSSIDE

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Cardioplegic solutions should protect the heart during the ischaemia associated with the suspension of coronary perfusion. Their success in doing so is known to depend largely upon their ability to maintain cytosolic calcium concentration at normal diastolic levels. Slight elevations will stimulate calcium-dependent metabolism, deplete energy reserves and reduce myocardial tolerance of ischaemia. The design of present cardioplegic solutions influences cytosolic calcium concentration only by preventing influx of this ion from the extracellular space, but it ignores the important intracellular calcium stores and their regulation. In the case of smooth muscle, sodium nitroprusside (SNP) causes relaxation, possibly by acting intracellularly to reduce the cytosolic calcium concentration (Cheung & MacKay 1985). Although any effects on cardiac performance seen during the infusion of SNP have generally been regarded as reflex in origin due to a drop in blood pressure, more recent evidence suggests that SNP may act directly on cardiac muscle (Mirro et al 1979). If this is the case SNP may represent a novel approach to the control of cytosolic calcium concentration in the ischaemic myocardium.

36 isolated hearts (NZW rabbits) were perfused with McEwen's solution by the Langendorff method, at 65 cm H_2O pressure and 37°C. After a 30 minute equilibration period a control group of 6 hearts was perfused with McEwen's solution, and 5 groups of 6 with McEwen's solution containing SNP (10^{-3} to 10^{-7}M), for a period of 2 minutes. The coronary perfusion was then suspended for 30 minutes before being restored for a 30 minute recovery period. This sequence was followed 5 times for each heart, each time with the same pre-ischaemia solution.

Large increases in coronary flow and slight increases in heart rate were seen following 2 minutes of perfusion with solutions containing SNP. The recovery of coronary flow and force of contraction following ischaemia was greatest in those hearts which had received SNP. These apparent cardioprotective effects became more noticeable as the number of ischaemic periods increased. The number of observations of V.F. seen during recovery was greatly reduced when high concentrations (10^{-3}, 10^{-4} M) of SNP were present before the preceding ischaemia.

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EFFECTS OF U46619 ON 'MILDLY ISCHAEMIC' MYOCARDIAL TISSUE IN VITRO

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Thromboxane A_2 (TXA_2), has been implicated in early arrhythmias associated with acute myocardial ischaemia (Coker 1982). One possible mechanism is that TXA_2 has direct electrophysiological proarrhythmogenic activity on normal and/or ischaemic cardiac tissue. This study investigated the electrophysiological effects of the stable TXA_2 mimetic, U46619, on isolated mammalian Purkinje fibres in vitro. U46619 was also studied under conditions mimicking mild ischaemia.

Sheep hearts were obtained from an abbatoir. Purkinje fibres were dissected from the left ventricular endocardium and superfused with Krebs solution (6mls.min^{-1}) at 37°C. The composition of the Krebs was as follows (mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 5.0, Na pyruvate 2.0, 0.5 mMoles of Na EDTA prechelated equimolar $CaCl_2$, and 2.5 mM $CaCl_2$. Krebs solution was gassed with 95% O_2/5% CO_2 to give a pO_2 of 648±5 mmHg and a pH of 7.4. For experiments mimicking mild ischaemia, Purkinje fibres were superfused with mild ischaemia mimicking solution (M.I.M.S.). The composition of M.I.M.S. is identical to Krebs except (mM): NaCl 133, KCl 6.9, $NaHCO_3$ 10 and $MgSO_4$ 1.8 M.I.M.S. was gassed with 95% N_2/5% CO_2 to give a pO_2 of 64±1 mmHg and a pH of 6.95. Fibres were electrically field stimulated at 1 Hz. Action potential recordings were made using standard microelectrode techniques. Single cell impalements were maintained throughout the experiments. U46619 was added cumulatively with an equilibration time of 30 minutes.

In Krebs, 10^{-9}M, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} U46619 produced no significant changes in action potential amplitude (APA), maximum rate of depolarisation (V.max), action potential duration to 50% (APD_{50}) and 90% repolarisation (APD_{90}). Control mean values ± S.E.M.: APA 118±2.2, V.max 628±37, APD_{50} 233±19, APD_{90} 313±23, (n=4). In experiments mimicking mild ischaemia, the transition from krebs to superfusion of the fibres with M.I.M.S., resulted in a significant attenuation of all the action potential parameters recorded ($p<0.5$). Mean values ± S.E.M. in Krebs verses M.I.M.S.: APA 126±2.2, 109±2.2; V.max 642±17, 498±33; APD_{50} 205±17, 142±11; APD 90 298±23, 221±14. In M.I.M.S., the same concentration range of U46619 had no significant effects on action potential configuration (n=5).

The results suggest that TXA_2 has no electrophysiological actions on mammalian Purkinje fibres, in either normal or ischaemic-mimicking conditions. Thus, it is likely that the observed proarrhythmogenic activity of TXA_2 is not due to direct electrophysiological activity, but an alternative mechanism. e.g. coronary vasoconstriction and/or platelet aggregation.

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THE ANTIFIBRILLATORY ACTION OF SK&F 94836

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SK&F 94836 2-Cyano-1-methyl-3-[4-(4-methyl-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl)phenyl]-guanidine is a potent and specific inhibitor of phosphodiesterase type III with inotropic and vasodilator activity (Gristwood et al 1987). The purpose of the present study was to determine what effect SK&F 94836 might have on ischaemia and reperfusion induced arrhythmias in the anaesthetised rabbit (Coker 1987).

Male New Zealand white rabbits were anaesthetised with Saffan 12 mg i.v. and α-chloralose 100 mg kg^{-1}. The carotid artery and jugular vein were cannulated for phasic blood pressure measurement and drug administration. Limb lead II E.C.G. was monitored. The chest was opened at the 4th intercostal space. Ligatures (Ethicon Merskil 6/0 reverse cutting needles) were placed under the circumflex artery close to its origin and the left anterior descending artery (LAD) at a point close to the margin of the atrial appendage. Animals in which this procedure: reduced blood pressure below 60 mmHg; resulted in arrhythmia; or S-T segment changes consistent with ischaemia, were rejected. The animals were then allowed to stabilise for 15 minutes. In the drug treated group (selected randomly) SK&F 94836, 31.6 $\mu g.kg^{-1}$ i.v., resulted in a significant increase in heart rate (268±7 to 285±9 bpm $P<0.001$) but had no effect on mean blood pressure (75±7 to 76±5 mmHg). After 15 minutes the circumflex artery was occluded resulting in a marked E.C.G. S-T segment changes indicative of ischaemia. Animals surviving 20 minutes (4 control, 7 drug) were subjected to LAD occlusion resulting in further E.C.G. changes. Rabbits surviving 20 minutes (3 control, 6 drug) then had the circumflex artery reperfused following by LAD reperfusion 5 minutes later (1 death control, 0 drug).

The results of these experiments are shown in the table below. As can be seen SK&F 94836 had no significant effect on the mean number of ventricular ectopic beats (VEB) or on the duration of ventricular tachycardia (VT). However, SK&F 94836 significantly reduced the incidence of ventricular fibrillation and consequently markedly reduced mortality.

The effects of SK&F 94836 on cardiac dysrhythmias as result of coronary artery ligation and reperfusion in the anaesthetised rabbit. Figures represent means ± SEM, those in brackets show number of animals exhibiting this arrhythmia.

Group	N	VEB	VT(s)	Ventricular Fibrillation %	Mortality %
Control	10	158±55(10)	29,8(2)	80	80
SK&F 94836 - 31.6μg/kg^{-1}	9	137±59(9)	159±144(3)	44*	33*

Thus, these data indicate that the potent inotropic and vasodilator agent SK&F 94836 is cardio-
protective against ischaemia and reperfusion induced fibrillation and reduces mortality in this model. Therefore, SK&F 94836 is likely to be particularly useful in the treatment of congestive heart failure a disease state often associated with myocardial ischaemia. * $P<0.05$ Fisher's exact test two sided.

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CARDIAC ELECTROPHYSIOLOGICAL EFFECTS OF ANTIRRHYTHMIC DRUGS ON NORMAL AND MILDLY DEPRESSED SHEEP PURKINJE FIBRES

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The aim of this study was to determine whether the electrophysiological effects of the antiarrhythmic drugs, lignocaine, disopyramide, propranolol, sotalol and verapamil were modified by conditions that mimic myocardial ischaemia. Action potential characteristics were recorded in sheep Purkinje fibres superfused in vitro with a normal or an altered physiological salt solution (P.S.S.). The P.S.S. was altered to mimic some of the conditions occurring during ischaemia by raising the KCl to 8 mM, reducing the pH to 6.8 and gassing with 95% N_2 5% Co_2 rather than 95% O_2 5% Co_2. The Po_2 in the organ bath was reduced from >600 to <40 mmHg. The preparations were stimulated at a frequency of 1.5 Hz. In the experiments using normal P.S.S., each drug was added cumulatively and action potentials recorded before and 30 min after each drug addition. In order to examine drug effects under 'ischaemic' conditions readings were taken before and 30 min following superfusion with the altered P.S.S. and subsequently following the cumulative addition of 3 concentrations of each drug dissolved in altered P.S.S., each exposure period being 30 min. Control experiments had shown that the altered P.S.S. caused a reduction in resting membrane potential and in the upstroke and duration of the action potential but that these changes were maximal by 30 min and did not change further between 30 and 120 min of exposure.

Table 1. The % change in maximum rate of depolarisation (MRD), in action potential duration at 50 (APD_{50}) and 90% (APD_{90}) of repolarisation induced by lignocaine, disopyramide, propranolol, sotalol and verapamil in sheep Purkinje fibres superfused with a normal or altered P.S.S.

		Normal			Altered		
Drug	(μM)	MRD	APD_{50}	APD_{90}	MRD	APD_{50}	APD_{90}
Lignocaine	14	-9±7	-43±4	-31±4	-40±6*	-21±7*	-17±5*
Disopyramide	23.6	-42±6	-37±5	-24±5	-46±4	+4±5*	+12±3*
Propranolol	1.4	-14±4	-29±4	-23±3	-47±9*	-18±3	-12±1*
Sotalol	30	-7±7	+6±4	+17±3	-14±6	-8±5*	+3±2*
Verapamil	2.0	-2±3	-33±3	+1±2	-25±5*	-37±6	-18±6*

*P<0.05 significantly different from change in normal P.S.S. n = 4-8.
Typical values for MRD, APD_{50} and APD_{90} in normal P.S.S. were 650±66 Vs^{-1}, 158±6 and 248±8 ms respectively.

Lignocaine, propranolol and verapamil but not disopyramide and sotalol depressed the upstroke of the action potential (MRD) to a greater extent under ischaemic than normal conditions. The shortening of the action potential caused by lignocaine and propranolol was attenuated and disopyramide lengthened rather than shortened the action potential in the presence of the altered compared with normal P.S.S. Under ischaemic conditions, sotalol did not prolong the action potential and verapamil shortened both APD_{50} and APD_{90}. In normal fibres, verapamil shortened APD_{50} only.

We conclude that the electrophysiological profile of these selected antiarrhythmic agents is modified by conditions that mimic myocardial ischaemia.

D.J. Pacini is an MRC scholar.

DIFFERENTIAL ANTI-ISCHAEMIC EFFECTS OF NIFEDIPINE AND R 58735 IN THE GUINEA-PIG HEART-LUNG PREPARATION

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R 58735 (4-[(2-benzothiazolyl)methylamino]-[(4-fluorophenoxy)methyl]-α-piperidine ethanol) has been demonstrated to possess anti-ischaemic activity in isolated guinea-pig hearts, which differs from that of calcium entry blockers (Boddeke et al., 1987). In order to further investigate the anti-ischaemic activity of R 58735, the protective effects of nifedipine and R 58735 upon 35 and 60 min of ischaemia and reperfusion in the guinea-pig heart-lung preparation were compared. Guinea pigs were anaesthetized with urethane (1.5 g/kg) and respirated at positive pressure with carbogen. The vena cava inferior was connected to a reservoir containing donor blood and the aorta was connected to a Starling resistance conducting the blood back to the reservoir. LVP and dp/dt were measured via a needle inserted in the left ventricle. Cardiac preload and afterload were 10 and 53 cm H_2O, respectively, and hearts were paced at a frequency of 5 Hz. Global ischaemia (35 or 60 min) was induced by clamping the venous and aortic cannulae. During ischaemia the temperature was maintained between 36.9 and 37.3°C. Hearts were reperfused retrogradely via the aorta for 10 min and were then switched to the working mode. After 30 min of reperfusion functional recovery (LVP, dp/dt and cardiac output) was calculated as a percentage of the pre-ischaemic values. Tissue CrP, ATP and adenylate charge were determined. Both in 35 and 60 min ischaemia experiments maximal protective concentrations of nifedipine and R 58735 were used. Upon 35 min of ischaemia nifedipine and R 58735 at concentrations that did not affect contractility (10^{-9} and 10^{-6} M, respectively) improved the post-ischaemic recovery of functional and biochemical parameters to a similar extent. After ischaemia for 60 min for R 58735 (10^{-6} M) a pronounced recovery of functional parameters was observed. For nifedipine a smaller recovery of functional parameters compared with R 58735 was found. Moreover, this effect was observed at a higher concentration of 10^{-8} M, which induced a cardiodepressant effect of approximately 14%. After 60 min of ischaemia no differences in recovery of biochemical parameters between nifedipine and R 58735 were found. Although nifedipine and R 58735 were equiprotective after 35 min of ischaemia, a more pronounced effect of R 58735 was observed after 60 min of ischaemia. This may indicate a more pronounced effect of R 58735 preventing irreversible injury.

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THE ANTI-ISCHAEMIC ACTION OF NIFEDIPINE, DILTIAZAM, LIDOFLAZINE AND MIOFLAZINE IN THE ISOLATED WORKING HEART OF THE GUINEA-PIG

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Nifedipine (N), diltiazem (D), lidoflazine (L) and mioflazine (M) induce negative inotropic effects in the isolated guinea-pig working heart. The cardioprotective effects of concentrations of the drugs, which reduce contractile force by 10% were compared. Since L and M are well-known nucleoside transport inhibitors (van Belle, 1985), we investigated whether reduction of loss of adenine pool precursors contributes to the anti-ischaemic action of these drugs. Guinea-pig isolated working hearts (paced at 5 Hz) were perfused with a Tyrode solution containing 1.8 mM calcium. The hearts were pretreated with the drug for 15 min, after which ischaemia (37°C) was maintained for 45 min by clamping the atrial filling line. After a 10 min reperfusion period according to 'Langendorff' the hearts were switched to the working mode for 15 min. During the first 5 min of reperfusion the coronary perfusate was collected in order to assay the efflux of adenosine, inosine, hypoxanthine, xanthine and uric acid. These metabolites were determined by means of HPLC. Mean values ± S.E.M. (n=6) of the sum of purines and oxypurines in the perfusate are expressed as nmoles/5 min/mg dry weight and are presented in Table I. Statistical significance ($p < 0.05$) was evaluated using student's tailed t-test.

Table I

	concentration (M)	purines and oxypurines
normoxia		0.356 ± 0.312
ischaemia		10.21 ± 1.061
N	8×10^{-9}	8.58 ± 1.120
D	2×10^{-7}	7.26 ± 0.871
L	3×10^{-7}	4.66 ± 0.871
M	3×10^{-7}	2.79 ± 0.373

Table II shows the effect of N, D, L and M on the recovery of the aortic dP/dt and cardiac output (CaO) in % of the initial values as well as ATP and ∿P (calculated as creatine phosphate + 2ATP + ADP) as nmoles/mg dry weight.

Table II

	Aortic dP/dt	CaO	ATP	∿P
normoxia	100	100	21.08 ± 2.24	63.28 ± 4.87
ischaemia	31.5 ± 10.4	30.1 ± 10.5	8.50 ± 1.44	35.81 ± 3.63
N	46.7 ± 13.8	54.7 ± 11.0	11.44 ± 1.88	45.46 ± 3.60
D	60.1 ± 9.4	57.7 ± 13.2	10.83 ± 1.12	44.13 ± 4.05
L	59.0 ± 13.2	62.2 ± 11.8	10.68 ± 0.82	44.39 ± 3.86
M	56.8 ± 8.4	65.5 ± 9.9	11.82 ± 0.38	49.31 ± 3.20

Pretreatment with L or M very strongly affects the ischaemia-induced loss of adenine nucleotide breakdown products, while N and D only slightly reduce the loss of purines and oxypurines. However, at these concentrations the drugs similarly improve the recovery of the mechanical function of the heart and do not differentially increase the ATP resynthesis. The results strongly suggest that nucleoside transport inhibition does not contribute to the protective activity of L and M, at least in this model of severe ischaemia.

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EFFECTS OF ISCHAEMIA ON THE RESPONSE OF THE IN SITU, BLOOD PERFUSED RAT MESENTERY

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Following experimental ischaemia, there is a reduction in ACh-induced vasodilation, which may be a consequence of endothelial cell damage (Altura et al 1985). The present study investigates further the effect of ischaemia on the vasodilator response to acetylcholine (Ach), isoprenaline (Iso), an endothelium-independent vasodilating agent (Furchgott and Zawadzki 1980), and, on the vasoconstrictor actions of angiotensin II (AII), 5-hydroxytryptamine (5-HT), and phenylephrine (PhE).

The in situ, blood perfused mesentery was prepared according to the method of Jackson and Campbell (1980). Compounds were injected into an extra-corporeal circuit (10µl bolus), proximal to the superior mesenteric artery, and changes in mesenteric and carotid blood pressure were monitored. Ischaemia was induced by occlusion of the mesenteric artery (30 min.) prior to cannulation and perfusion. Dose-response (d/r) curves were constructed for AII, 5-HT, PhE, ACh and Iso. For ACh and Iso a sub-maximal increase in tone (77±6mmHg, n=4) was first induced with an infusion of PhE (10^{-3}M, 75µl min^{-1}).

Following ischaemia, the ACh-induced vasodilation was significantly decreased (see table). Ischaemia also attenuated responses to Iso, but the changes in E.E.D. and max. were not significant. 5-HT and PhE produced a dose-dependent vasoconstriction of the mesenteric bed, ischaemia resulting in a significantly increased E.E.D. The responses to AII were small, and formed a bell-shaped d/r curve. Preliminary studies indicate that this may reflect tachyphylaxis. The responses to AII were not significantly reduced by ischaemia.

	Ach		Iso		AII		5-HT		PhE	
	log EED (M)	max (%)	log EED (M)	max (%)	log EED (M)	max (mmHg)	log EED (M)	max (mmHg)	log EED (M)	max (mmHg)
Cont	−5.25* ±0.36	93±4*	−4.65 ±0.26	104±5	N.A.	23±3	−3.81* ±0.45	51±12	−3.37* ±0.13	148±5
Isch	−4.16 ±0.28	78±2	−3.90 ±0.23	96±5	N.A.	17±4	−2.44 ±0.47	31±6	−2.81 ±0.07	130±11

preconstricted tissues-vasodilation expressed as %PhE tone reversal
(all other responses expressed as mmHg change in mesenteric pressure)
E.E.D., equi-effective dose ie that dose producing 50% max. control response
N.A., not applicable-ED_{50}'s not calculated as true max. not achieved.
* $p<0.05$, unpaired 't' test, 2 tailed. n = 4-5.

In conclusion, the ischaemia-induced reduction in responses to ACh may not be exclusively a result of impaired endothelial cell-mediated relaxation, since responses to the spasmogens PhE and 5-HT were also reduced. The reductions in responsiveness observed may reflect ischaemic damage to the vascular smooth muscle. Furthermore, the apparent differential effects of ischaemia on the individual agents requires investigation.

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[TYR]-HCGRP IS A PARTIAL AGONIST AT HCGRP RECEPTORS ON BOVINE AORTIC ENDOTHELIAL CELLS

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Human calcitonin gene-related peptide (hCGRP) is a potent vasodilator whose effects are mediated (at least in part) by activation of adenylate cyclase (Sigrist et al, 1986). Whether the vasodilation in vitro is dependent on the presence of an intact endothelium remains controversial. We have reported previously to this society that hCGRP activates adenylate cyclase in bovine and human endothelial cell membranes (Grossman et al, 1986; Crossman et al, 1987). [tyr°]-hCGRP is an analogue of hCGRP, designed for ease of iodination at the tyrosine residue and use as a radioligand. We have now examined the effect of the non-iodinated [tyr°]-hCGRP on adenylate cyclase activity of bovine endothelial cell membranes.

Bovine aortic endothelial cells, AG4762 (NIA, Aging Cell Repository, USA) were used at passage 16-19. Adenylate cyclase activity was measured in membrane preparations by the method of Salomon et al (1974). The maximum increase in adenylate cyclase activity produced by [tyr°]-hCGRP was less than that obtained in the presence of hCGRP. hCGRP produced a dose-dependent increase in adenylate cyclase activity from a basal level of 62 ± 28 pmol cyclic AMP/min/mg protein to a maximum of 307 ± 59 pmol cyclic AMP/min/mg protein while [tyr°]-hCGRP produced a dose-dependent increase to 171 ± 7 pmol cyclic AMP/min/mg protein (n = 3, mean ± SEM). The concentration for half-maximal activation of adenylate cyclase (Kact) was 770 ± 283 nM for hCGRP and 2.21 + 1.45 µM for [tyr°]-hCGRP.

The effect of [tyr°]-hCGRP on adenylate cyclase activity was determined at selected concentrations between 40 and 10,000 nM in the presence of 4 µM hCGRP. [tyr°]-hCGRP partially inhibited the hCGRP-dependent activation of adenylate cyclase (Ki = 2 µM).

No specific antagonists of hCGRP are available, and little is known of the pharmacology of the hCGRP receptor or the structure-activity relationships of hCGRP. The present study shows that addition of tyrosine to the N-terminal end of hCGRP confers partial agonist characteristics. Further modification in this region may result in the synthesis of an antagonist, which would be a useful tool for the pharmacological investigation of hCGRP and for the assessment of the role of endogenous hCGRP in physiological vasodilatation.

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TWO VASCULAR MECHANISMS OF ACTION FOR THE HUMAN α-CALCITONIN GENE-RELATED PEPTIDE (hαCGRP)

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Human α-calcitonin gene-related peptide (hαCGRP) is a potent vasodilator and acts in an endothelial dependent manner in rat aorta (Brain et al, 1985) but is un-altered by the removal of the endothelium from rings of cat middle cerebral artery (Hanko et al, 1985). The present investigation compares the action of hαCGRP in rings of rat mesenteric artery and rat isolated perfused mesenteric vasculature.

Rings of rat mesenteric artery in Krebs solution were contracted with noradrenaline (10^{-7} M, tension 167±42 mg, mean ± s.e.mean). The rat isolated perfused mesenteric vasculature was perfused at constant flow (5.8 $ml.min^{-1}$) with Krebs solution. The perfusion pressure was raised with noradrenaline (10^{-5} M, pressure 98±9 mmHg). HαCGRP (10^{-9} - $3x10^{-7}$ M in rings, $3x10^{-11}$ - $3x10^{-9}$ mol in perfused mesentery) was compared with the endothelium dependent vasodilator acetylcholine (10^{-9} - 10^{-6} M in rings, 10^{-11} - $3x10^{-8}$ mol. in perfused mesentery) and the endothelium independent vasodilator sodium nitroprusside (10^{-9} - 10^{-6} M in rings; 10^{-10} - $3x10^{-8}$ mol. in perfused mesentery).

Mechanical rubbing of the intimal surface of the mesenteric artery ring resulted in a loss of relaxation both to acetylcholine (from a maximum percentage relaxation of 70 ± 13% to a maximum of 9 ± 6%) and to hαCGRP, while the relaxant effect of sodium nitroprusside was increased. Chemical removal of the endothelium with sodium deoxycholate (Byfield et al, 1986) in the perfused mesentery abolished acetylcholine vasodilatation even after the acetylcholine dose was increased 30 fold (10^{-6} mol). Sodium deoxycholate did not alter the effect of hαCGRP and increased the response to some doses of sodium nitroprusside. Therefore the vasodilator effect of hαCGRP, unlike acetylcholine and sodium nitroprusside, appeared to differ in dependence on the endothelium in the two preparations. In the mesenteric rings haemoglobin (10^{-6} M) shifted the concentration-relaxation curve of acetylcholine about 30 fold to the right and reduced the maximum (from 62 ± 7 to 38 ± 3%). Haemoglobin also shifted the curve for hαCGRP to the right but not that of sodium nitroprusside. In the perfused mesenteric vasculature haemoglobin (10^{-6} M) shifted to the right only the acetylcholine dose-response curve and reduced the maximum percentage fall in pressure (from 55 ± 4 to 41 ± 1%). In the mesenteric artery rings methylene blue (10^{-5} M) shifted the acetylcholine and hαCGRP concentration-relaxation curves to the right but not that of sodium nitroprusside. In the perfused mesenteric vasculature hαCGRP, like sodium nitroprusside, was even more effective in the presence of methylene blue (10^{-5} M) than in its absence, while the acetylcholine vasodilatation curve was shifted to the right with a reduced maximum effect (from 55 ± 4 to 39 ± 5%).

HαCGRP has been described previously as endothelium dependent and endothelium independent in its action as a vasodilator. This is unusual and may be reflected in the two mechanisms of action reported here, for the first time, in a single vascular bed. Haemoglobin and methylene blue which reduce the effect of many vasodilators acting via the endothelium (Furchgott, 1984), provide further support for two vascular mechanisms of action for hαCGRP.

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INFLUENCE OF CHRONIC TREATMENT BY THE CALCIUM ENTRY BLOCKER NISOLDIPINE ON ARTERIES OF SPONTANEOUSLY HYPERTENSIVE RATS

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In a previous study, Godfraind et al. (1985) reported that when aortas isolated from spontaneously hypertensive rats (SHR) had been contracted by depolarization and were thereafter rapidly immersed in physiological solution, the time-course of their relaxation to resting level was much slower than the time-course observed with aortas from normotensive rats (WKY). Concentrations of nisoldipine as low as 10 pM that produced only a slight reduction of contraction of aortas from both groups of rats, tended to normalize the relaxation of SHR aortas. The present experiments had two purposes. First, to examine if this delayed relaxation could be related to the age-dependent evolution of hypertension and if it could be observed in arteries other than aortas. In addition, we have examined how a treatment of rats by a dose of nisoldipine already reported to attenuate the development of hypertension (Kazda et al., 1982 ; Godfraind et al., 1986) could modify the ex-vivo responsiveness of aortas and mesenteric arteries to depolarization and to repolarization.

At the age of 8 weeks, rats (48 SHR and 48 WKY) were divided at random into two groups, one (control) receiving ordinary food and the other food containing 1000 ppm nisoldipine, which produced a blood pressure reduction of 70 mm Hg ($P < 0.01$). After dissection and fixation in organ chambers, rings of arteries were maintained during 90 minutes at rest. The physiological solution employed in these experiments contained (mM) : NaCl, 112; KCl, 5; $NaHCO_3$, 25; KH_2PO_4, 1; $MgSO_4$, 1.2; $CaCl_2$, 1.25 and glucose, 11.5. The depolarizing solution used to evoke arterial contraction comprised (mM) : NaCl, 17; KCl, 100; $CaCl_2$, 1.25; $NaHCO_3$, 25; KH_2PO_4, 1 and glucose 11.5. These solutions were maintained at 37°C and aerated with a gas mixture (95% O_2 - 5% CO_2). The physiological solution was replaced by K-depolarizing solution and the subsequent isometric contraction was recorded during 35 min. After, the K-solution was changed for physiological solution (a procedure achieved in 8 sec) and the smooth muscle relaxation to resting level was recorded as long as necessary. Four rings of each artery were recorded simultaneously and one artery from control and one from treated rat were processed during the same experiment.

The force of contraction in response to depolarization was not different between WKY and SHR aortas, but the rate of relaxation after repolarization was slower in SHR than in WKY and it was age-dependent as shown by relaxation $t_{1/2}$ values of aortas [12 weeks old : WKY, 141 ± 23 sec (12) ; SHR, 233 ± 8 sec (12) ; 36 weeks old : WKY, 131 ± 11 sec (12) ; SHR, 1804 ± 222 sec (12)]. Treatment of rats with nisoldipine which reduced hypertension, reduced the contractile response of WHY and SHR aortas to the same extent and attenuated the difference in relaxation time between SHR and WKY isolated arteries. Similar observations were made with mesenteric arteries.

These observations show that arteries obtained from SHR behave differently from arteries of WKY when the voltage-operated calcium channels are activated by K-depolarization to produce smooth muscle contraction. The difference is not related to contractile behaviour but to relaxation following repolarization. Indeed, the relaxation of SHR arteries is slower than the relaxation of WKY arteries and this difference increases markedly with aging. Treatment with nisoldipine attenuated this difference between normotensive and hypertensive animals.

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INHIBITION OF CALCIUM OVERLOAD CONTRACTURES BY NICARDIPINE BUT NOT BY NIFEDIPINE OR NIMODIPINE IN EMBRYONIC CHICK MYOCYTES

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Diastolic tone exhibited by embryonic heart cell aggregates appears to be a sensitive reflection of intracellular calcium levels and is increased by positive inotropic agents and reduced when automaticity is inhibited or when cells are superfused with calcium antagonists or low calcium solutions. Tonic contractures can be induced in these cells by application of veratrine (Patmore,1986). We have compared the effects of the dihydropyridine calcium antagonists nicardipine, nifedipine and nimodipine on these contractures and have investigated the effects of veratrine on whole-cell sodium currents.

Embryonic chick myocytes were prepared by trypsin digestion and cultured for 3-4 days. Whole-cell voltage-clamp recordings were made using conventional patch-clamp techniques on single myocytes cultured on 35mm dishes. For measurements of contractions cells were grown on silicon elastomer treated plates which enhanced the formation of aggregates. Contractions were detected by measuring edge movement as described by Patmore & Whiting (1985). Contractile responses are described as % of the twitch contraction for each aggregate. All data shown are mean values ± s.e.mean. Inward sodium currents were observed on clamping the membrane potential at -85 mV and stepping to -30 mV. These currents were blocked by TTX and inactivated rapidly (within 20 ms, t(0.5)= 1.0 ± 0.1 ms). Veratrine (100 µg/ml) caused a reduction of the peak inward current and markedly slowed the time course of inactivation such that it was not complete 300 ms after the start of the pulse. This resulted in a 25 (± 3, n=3) fold increase in the total inward sodium current. In aggregates veratrine caused cessation of automaticity and a tonic contracture to around 320% of the twitch, which could be explained by attenuation of calcium extrusion by sodium-calcium exchange as a result of increased levels of intracellular sodium. These contractures were insensitive to the calcium channel blocker diltiazem at a concentration of 10 µM which completely inhibits twitch contractures (Patmore, 1986). Thus the pathway for calcium entry appears to be independent of the normal form of the voltage-sensitive calcium channel. The effects of nicardipine, nifedipine and nimodipine are shown in table 1.

Table 1. **Effects of calcium antagonists on veratrine-induced contractures**

		Control	(n)	+ Drug	(n)	% Control
Nicardipine	(5µM)	318 ± 23	(17)	22 ± 15	(5)	6.8
Nifedipine	(5µM)	323 ± 36	(16)	322 ± 54	(11)	99.6
Nimodipine	(5µM)	331 ± 51	(7)	319 ± 47	(4)	96.4

All three antagonists were equipotent inhibitors of the twitch contracture but only nicardipine protected against veratrine-induced calcium overload. The effects were concentration-dependent with an IC_{50} of 3 µM. These data show that nicardipine has additional properties to nifedipine and nimodipine in that it can prevent Na^+/Ca^{2+} overload.

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EFFECTS OF HYPERTENSION AND AGE ON RESPONSES TO CALCIUM IN RAT CAUDAL ARTERIES

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As it has been suggested that (1) hypertension may be associated with modifications of voltage- (VOC) and receptor-operated (ROC) calcium channels (Lederballe Pedersen, 1979), and (2) hypertension may represent an accelerated form of vascular aging (Soltis *et al.*, 1986), we have compared the vasoconstrictor response to calcium of caudal arteries from young hypertensive (13 weeks old) and old, normotensive (36-52 weeks old) rats. Hypertension was induced in male Wistar rats (6 weeks old) by placing a solid silver clip (gap 0.2 mm, Atkinson *et al.*, 1987) on the left renal artery for 7 weeks. Following measurement of systolic arterial pressure (SAP, mmHg) in preheated rats, reserpine (2.5 mg/kg i.p.) was administered. Eighteen hours later arteries were removed and perfused/superfused firstly with modified Krebs bicarbonate: no calcium and 0.2 mM Na_2EDTA with either 120 mM KCl (and no sodium) or 10 µM phenylephrine (PHE) at 2 ml/min for 30 min, then with the same solutions without Na_2EDTA but with 0.3, 1 or 3 mM $CaCl_2$ for 2 min, with 5 min between each.

Group	n	Body weight (g)	SAP (mmHg)	Increase in perfusion pressure at 3 mM calcium	
				KCl	PHE
Young					
Sham-clipped	16	250±16	112±5	15±2	85±8
Clipped	6	224±35	174±8[a]	38±15[a]	71±10
Old	7	487±3[a,b]	109±4[b]	8±3[b]	95±24

Results are given as means ± SEM. t-test comparison ($P < 0.05$): 'a' with young, sham-clipped rats, 'b' with young, clipped rats.

Our results show that (1) responses to calcium in arteries perfused with PHE are not modified by either hypertension or age, (2) there is a greater response to calcium in potassium-depolarized arteries from renovascular hypertensive rats, and (3) responses to calcium in arteries from old normotensive rats are not different from those of young normotensive rats. We suggest that renovascular hypertension, but not age, may modify VOC.

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EFFECT OF THE SOMATOSTATIN ANALOG, BIM 23014 AND THE PAF ANTAGONIST, BN 52021 ON PAF-INDUCED BRONCHOCONSTRICTION IN GUINEA-PIGS

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The effects of the somatostatin analog, BIM 23014 (D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2) and low doses of the platelet-activating factor (PAF) antagonist, BN 52021 (Braquet et al., 1987), either alone or in combination, on PAF-induced bronchoconstriction were investigated in the guinea-pig. Bronchopulmonary alterations were recorded using the Konzett and Roosler's method. BIM 23014 (1, 5, 50 and 500 µg/kg) and BN 52021 (125, 250 or 500 µg/kg) were injected intravenously 5 minutes before PAF (60 or 100 ng/kg) challenge. Given alone, neither BIM 23014 nor BN 52021, even at the highest dose studied, exhibited a significant inhibitory effect on the bronchoconstriction induced by PAF. In contrast, when given in association with BN 52021 at 125 µg/kg, an inhibitory effect is observed with BIM 23014 at 50 and 500 µg/kg. When BIM 23014 is associated with BN 52021 at 250 µg/kg or 500 µg/kg, significant inhibition is observed starting at 1µg/kg of BIM 23014 (table 1).

Table 1 Inhibiton of PAF-induced bronchoconstriction in guinea-pig by the association BIM 23014 + BN 52021

Treatment	dose µg/kg	n	percent bronchoconstriction (% inhibition)	statistical significativity
Control		13	78.8 ± 5.5	
BN 52021 BIM 23014	125 50	6	53.9 ± 12.1 (-31.6)	$p < 0.05$
BN 52021 BIM 23014	125 500	7	38.6 ± 3.4 (-51.0)	$p < 0.01$
BN 52021 BIM 23014	250 1	6	48.3 ± 10.0 (-38.7)	$p < 0.01$
BN 52021 BIM 23014	250 50	12	34.7 ± 8.8 (-56.0)	$p < 0.001$

The present results indicate that the association of a somatostatin analogue with BN 52021, markedly reduced the bronchopulmonary effects of the phospholipid mediator. Since the PAF-induced bronchoconstriction is dependent upon platelet activation, the effect of the association of both drugs on these blood elements was investigated. Addition of BIM 32014 to a inactive concentration of BN 52021 (10 nM) inhibited PAF-induced platelet aggregation in a dose-dependent manner. These latter data suggested that the combination BN 52021 and BIM 23014 may be used in various physiopathological models where platelet activation is involved.

Braquet et al. (1987) Parmacol Rev.39, 97-145.

TRANSITION METALS AND PERTUSSIS TOXIN MODULATE PAF-INDUCED RISE IN CYTOSOLIC FREE CALCIUM ION CONCENTRATION OF U937 CELLS

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We have previously demonstrated that U937 cells loaded with the fluorescent calcium indicator Fura-2 possess platelet activating factor (PAF) and leukotriene B_4 receptors which are closely coupled to calcium mobilisation (Ward and Westwick 1987; Ward, Pleass and Westwick 1987). To determine the relative contribution of extracellular Ca^{++} ($[Ca^{++}]_e$) to PAF-induced elevation of cytosolic free calcium concentration ($[Ca^{++}]i$), we have examined the effectiveness of the Ca^{++} chelator EGTA and the transition metal ions Ni^{++}, Co^{++} and Mn^{++}. These cations in a number of cell types either block or permeate calcium translocating pores (see Hille 1984) and in contrast to Ca^{++} quench Fura-2 fluorescence (Grynkiewicz et al, 1985). We have also determined the relative contribution of pertussis toxin sensitive components (Ui 1984) in the PAF-induced elevation of $[Ca^{++}]i$.

Replacement of 1mM $[Ca^{++}]_e$ with 10µM-1mM Mn^{++} or Co^{++} produced a gradual quench of the Fura-2 fluorescence which was not accelerated upon addition of PAF (0.3-100nM). Replacement of 1 mM $[Ca^{++}]_e$ with either 1 mM Ni^{++} or 5mM EGTA produced an immediate small quench then a steady signal, accompanied by a significant prolongation of the response time and a marked decrease in the peak elevation of $[Ca^{++}]i$ upon addition of PAF (Table 1).

	ONSET OF RESPONSE (secs).					
TREATMENT	0.3	1	3	10	30	100 nM (PAF)
1 mM Ca^{++}	18±2	14±4	5±1	4±1	2±0.5	2 ±1
1 mM Ni^{++}	>120	>120	>120	18±5	16±0.5	10 ±2
5 mM EGTA	>120	29±6	12±1	8±1.5	4±1	4.5±1

In addition, Ni^{++} produced a 100, 100, 98.4, 86, 89.5 and 86% inhibition respectively of 0.3, 1, 3, 10, 30 and 100nM PAF-induced elevation of $[Ca^{++}]_i$ compared to values in the presence of 1mM Ca^{++}. Pretreatment of $5x10^7$ U937 cells/ml with 5 µg/ml pertussis toxin for 4 h produced a 39±9 ($p < 0.05$), 31.9±9 ($p > 0.05$), 35±6.4 ($p < 0.05$), 40±7.5 ($p < 0.05$) and 51±5% ($p < 0.05$) inhibition respectively of 1, 3, 10, 30 and 100nM PAF-induced elevation of $[Ca^{++}]_i$.

Thus, resting U937 cells are relatively permeable to Co^{++} and Mn^{++} in contrast to platelets (Hallam and Rink 1985; Poll and Westwick 1986), but their permeability is not enhanced upon PAF stimulation, suggesting that entry of these cations into U937 cells is not via PAF operated Ca^{++} pores. The results with Ni^{++} and EGTA suggest that $[Ca^{++}]_e$ is a major contributor to PAF-induced elevation of $[Ca^{++}]_i$ and probably an influx of Ca^{++} occurs prior to intracellular Ca^{++} mobilisation. In addition, a pertussis toxin-sensitive component regulates, in part, the elevation of $[Ca^{++}]_i$.

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EFFECT OF A SOMATOSTATIN ANALOGUE, BIM 23014 AND A SPECIFIC PAF ANTAGONIST, BN 52021, ON CEREBRAL ISCHEMIA

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The effects of a somatostatin analog, BIM 23014 (D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2) and a platelet-activating factor (PAF) antagonist, BN 52021 (Braquet et al., 1987) either alone or in combination were tested on cerebral ischemia in gerbils. Cerebral ischemia was obtained by bilateral ligature of the common carotides for 10 min in male Mongolian adult gerbils. Each animal was examined 1 h after declamping and every hour until 6 h to evaluate the morbidity according to the stroke index chart defined by Mc Graw. After the last observation, animals were killed by decapitation to allow the isolation of brain mitochondria. Respiratory rates, respiratory control ratio (R.C.R ; state 3 / state 4) was determined according to Chance and Williams. Given one hour after declamping, BN 52021 (10 mg/kg i.p.) induced a significant protection of cerebral ischemia (Spinnewyn et al., 1987) BIM 23014 caused a regular decrease in the stroke index and a significant increase in R.C.R. value. When the gerbils were treated with an association of an inactive low dose of BN 52021 and increasing doses of BIM 23014, the protection against cerebral ischemia was greater than that observed with BIM 23014 treatment alone. Circulating leucocytes count was decreased (- 20 % at 2 h, - 36 % at 4 h) after ischemia and reperfusion and remained identical at 0.2 and 4 h when gerbils were treated with BN 52021 (0.5 mg/kg i.p.) and BIM 23014 (50 µg/kg i.p.).

TREATMENT	STROKE INDEX AT 6 H	R.C.R
SHAM OPERATED	0.2 ± 0.2	6.08 ± 0.11
LIGATURED CONTROL	14.8 ± 0.8	3.10 ± 0.42
BN 52021 0.5 MG/KG	12.0 ± 3.3	3.51 ± 0.85
BIM 23014 5 µG/KG I.P.	8.6 ± 1.8 **	4.74 ± 0.47 *
BIM 23014 25 µG/KG I.P.	2.4 ± 2.1 **	4.84 ± 0.14 *
BIM 23014 50 µG/KG I.P.	2.0 ± 1.0 ***	5.05 ± 0.33 *
BN 52021 0.5 MG/KG I.P. + BIM 23014 5 µG/KG	7.4 ± 2.2 **	5.36 ± 0.33 *
BN 52021 0.5 MG/KG I.P. + BIM 23014 25 µG/KG	8.6 ± 1.8 **	5.29 ± 0.17 **
BN 52021 0.5 MG/KG I.P. + BIM 23014 50 µG/KG	2.7 ± 1.8 **	5.61 ± 0.17 **

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

These results suggest that in the post ischemic phase, the association of BN 52021 and BIM 23014 could play an important role by preventing the infiltration and degranulation of blood inflammatory cells.

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CHARACTERIZATION OF PAF-INDUCED HYPERRESPONSIVENESS OF THE GUINEA-PIG TRACHEA TO POTASSIUM

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Platelet activating factor (PAF) induces bronchoconstriction and enhances the bronchoconstrictor effects of certain agonists in the anaesthetized guinea-pig (Mazzoni *et al.*, 1985). The aim of this study was to assess whether PAF could modify K^+-induced contractile responses under *in vitro* conditions on guinea-pig isolated trachea.

Tracheal strips from male Dunkin-Hartley guinea-pigs (420-480 g) were rubbed to remove epithelium, suspended in Krebs solution (composition mM : NaCl 120; $NaHCO_3$ 25; $MgSO_4.7H_2O$ 0.05; KH_2PO_4 1.2; KCl 4.7; $CaCl_2$ 1.25; glucose 1.1; containing 0.25% w/v bovine serum albumin) and prepared for the recording of developed tension under isometric conditions. A basal resting tension of 2 g was applied. One cumulative concentration-response curve to K^+ (10-100 mM) was obtained from each preparation after a 20 min incubation with ethanol (0.04% ; control), PAF (0.1-1.0 μM) or PAF (1.0 μM) plus either WEB 2086, SRI63441 or benzylimidazole. The maximal contractile response to K^+ (E_{max}) was calculated and expressed as mean ± s.e.mean. In a separate series of experiments, contractile responses of the tracheal strip to U-46619 were determined in the absence and presence of WEB 2086.

The concentration-response curve to K^+ was steepened by PAF with an enhancement of the maximum response (E_{max} : ethanol 1.8 ± 0.1 g (n=18); 0.1 μM PAF 2.6 ± 0.3 g (n=8); 1.0 μM PAF 2.5 ± 0.1 g (n=13). The PAF-induced potentiation of the K^+-responses (1.0 μM PAF; E_{max} 2.4 ± 0.1 g, n=14) was abolished by the PAF receptor antagonists (Casals-Stenzel, 1987 ; Handley *et al.*, 1987), WEB 2086 (10 nM; E_{max} 1.8 ± 0.1 g, n=4) and SRI 63441 (10 nM; E_{max} 1.8 ± 0.2 g, n=6) and the thromboxane synthetase inhibitor, benzylimidazole (10 μM ; E_{max} 1.7 ± 0.1 g, n=4). U-46619 (100 nM), a thromboxane receptor agonist (Barnes and Goadby, 1986), evoked a contractile response of the tracheal strip that was unchanged by WEB 2086 (0.1 μM). Finally, in the Konzett and Rössler anaesthetized guinea-pig preparation (method as Barnes and Goadby, 1986), WEB 2086 (2.2 $\mu mol.kg$) failed to modify the bronchoconstriction due to U-46619 (1.4 $nmol.kg^{-1}$). This response to U-46619 was inhibited by the thromboxane receptor antagonist AH 23848 (Barnes and Goadby, 1986).

In conclusion, PAF enhanced the responsiveness of guinea-pig isolated tracheal smooth muscle to K^+ via liberation of thromboxane A2 (TxA2) as indicated by results obtained by benzylimidazole. WEB 2086 failed to exhibit antagonist activity at the TxA2 receptor ; in addition WEB 2086 does not interfere with the synthesis of TxA2 (P. Sedivy, personnal communication). Therefore the enhancement of K^+ responses by PAF appears to result from activation of PAF receptors, which can be blocked by WEB 2086

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SPECIFIC BINDING SITES OF PAF IN IRIS AND CILIARY BODY IN PIGMENTED RABBIT EYES

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Platelet activating factor (PAF), a phospholipid mediator exhibiting a wide range of biological activities, appears to play a major role in inflammation. In some experimental induced eye diseases, BN 52021 a specific PAF receptor antagonist shows important protective effect. In the cornea, BN 52021 inhibits leucocyte infiltration and corneal edema in immune complex induced keratitis and in the iris it prevents the inflammatory responses (increase of intraocular pressure and leakage of serum proteins into the aqueous humor) induced by argon laser coagulation of the rabbit iris. These results strongly suggest the participation of PAF in these inflammatory states of the eye through a potential receptor mediation. Since PAF binding sites have been recently described in the rabbit retina, we examined whether PAF recognition sites are also present in iris and ciliary body of the pigmented rabbit eyes.

After a careful dissection, the iris and the ciliary body were washed in a tris-HCl 50 mM (pH = 7.4), $MgCl_2$ 10 mM, EDTA 2 mM, PMSF 10^{-4} M, trasylol 50 UI per ml, homogenized using a Dounce homogenizer, filtrated through two layers of gauze and centrifugated two times at 40.000 g for 15 min. The pellets were resuspended in the homogenizing buffer. Membrane preparations of these tissus were used for binding assays with ^{3}H-PAF (NEN. Les Ulis France). 40 to 70 µg of tissue were added to a 1 ml final volume containing ^{3}H-PAF without or with 1000 fold more unlabelled PAF for the nonspecific. ^{3}H-PAF specifically bound was separated from the unbound by filtration through presoaked Whatman GF/B filters. In both iris and ciliary body preparations, the binding of ^{3}H-PAF was saturable, specific, time dependent and reversible. Increasing concentrations of ^{3}H-PAF ranged from 8.10^{-10} M to 10^{-8} M showed an apparent two steps saturation curves suggesting the existence of two binding sites : one of high affinity with a K_d of 4 $\pm$ 0.8 nM and a Bmax = 5.1 $\pm$ 0.4 pmol/mg protein and one of lower affinity with a K_d = 20 $\pm$ 1 nM and a Bmax = 15 $\pm$ 2 pmol/mg protein (n = 4). Moreover, the binding of ^{3}H-PAF corresponding to the two sites was displaceable by unlabeled PAF whereas only the binding of high affinity appeared to be inhibited by BN 52021.

The present results demonstrate for the first time the existence of specific PAF binding sites in iris and ciliary body of rabbit eye which could mediate the action of PAF in eye inflammatory processes and explain the protective effect observed with BN 52021.

INHIBITION OF PAF-INDUCED EFFECTS IN THE GUINEA PIG RIGHT ATRIUM BY BN 52021 AND SOMATOSTATIN

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Platelet-activating factor (PAF) has been implicated in conduction arrhythmias, whereas somatostatin was reported to restore sinus arrhythmia (Levi et al., 1984, Day et al., 1985). PAF effects are specifically inhibited by BN 52021 (ginkgolide B). This study investigates the effects of PAF on spontaneously beating guinea-pig right atria and the interaction of BN 52021 (Braquet et al., 1987), somatostatin and a somatostatin analogue, BIM 23014 (D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2), on these modifications.

Spontaneous atrial force and rate were recorded. PAF (10^{-12} M - 10^{-9} M) produced chronotropic and inotropic positive effects on the right atria and induced tachyphylaxis. BN 52021 did not affect the basal rythmn but dose-dependently (IC_{50} - 1.34 x 10^{-5} M) inhibited the maximal effect of PAF (10^{-10} M). Somatostatin (10^{-8}M - 10^{-6} M) induced a dose-dependent negative inotropic effect with slight modifications of the atrial rate whereas the somatostatin analogue, BIM 23014 (10^{-5}M) was without effect. Both products significantly reduced PAF effects and induced a similar additive effect when combined with BN 52021.

		BEAT/MIN	% VARIATION
PAF	10^{-10} M	46.4 ± 1.8	
BN 52021	10^{-5} M	27 ± 3.8	- 41.8 ± 8.2
BIM 23014	10^{-5} M	31 ± 2.0	- 33.2 ± 4.4
SOMATOSTATIN	10^{-8} M	29 ± 4.0	- 37.1 ± 8.7
BIM 23014 + BN 52021	10^{-5} M 10^{-5} M	18 ± 3.3	- 60.5 ± 7.2
SOMATOSTATIN + BN 52021	10^{-8} M 10^{-5} M	13 ± 1.0	- 72.7 ± 1.7

Thus, PAF effects on guinea-pig right atria are dose-dependently inhibited by BN 52021. Interaction with somatostatin and its analogue, BIM 23014, exhibited an additive effect with BN 52021. The mechanisms of these effects deserved further investigations.

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EFFECTS OF AGENTS MODIFYING ENDOCYTOSIS ON INTESTINAL IRON UPTAKE FROM PERMEANT AND NON-PERMEANT IRON COMPLEXES IN THE RAT

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It is known that iron bound to transferrin in the plasma enters target cells via interaction with specific transferrin receptors on the cell surface and internalization of the whole iron-transferrin-receptor complex (Huebers & Finch, 1987). A similar mechanism may be involved in the uptake of inorganic iron from the gut lumen into intestinal mucosal cells (Huebers et al, 1983). To investigate this possibility, ^{59}Fe uptake into isolated fragments of rat small intestine (Barrand et al, 1986) has been studied in the presence or absence of agents known to interfere with endocytotic iron uptake into other cell types. Iron in 16mM Hepes buffer at pH 7.4 was maintained in soluble form either by binding to transferrin or by complexing to nitrilotriacetic acid (NTA, a ligand which cannot itself enter cells and must therefore donate its iron to the endogenous system) or to 3-hydroxy-2-methyl-4-pyrone (maltol, a ligand which is able to enter cells and may therefore by-pass the normal iron uptake system).

No uptake of ^{59}Fe was evident after 15min exposure to 0.025mM iron bound either to human (initially 99% iron-free) transferrin or to rat transferrin prepared from the plasma of iron-deficient animals.

NH_4Cl (1-100mM), chloroquine (0.1mM), monensin (0.001-0.1mM) and spermine (10-50mM), agents which have been shown at these concentrations to raise intravesicular pH and so to prevent iron release from transferrin within target cells (Loh et al, 1985), were without effect on uptake of ^{59}Fe (0.02 and 0.1mM) in the presence of maltol (metal:ligand ratio 1:4) and only spermine was able to reduce iron uptake from iron:NTA (metal:ligand ratio 1:5): iron uptake in pmol $min^{-1}mg^{-1}$ wet tissue from 0.1mM iron:NTA was 2.4±0.6 without and 0.7±0.4 with 30mM spermine (n=8) whilst from 0.1mM iron:maltol it was 4.8±1.0 without and 4.2±2.5 with 30mM spermine (n=8). However, spermine at this concentration modifies the iron:NTA complex. Thus its effect on iron uptake is possibly unrelated to any action on the tissues. Cytochalasin B (0.1mM), an agent which inhibits endocytosis by interfering with cytoskeletal function was without effect on ^{59}Fe uptake with iron-NTA.

Uptake of ^{59}Fe (0.02mM) bound to NTA or to transferrin was also studied *in vivo* from the lumen of tied-off segments of rat duodenum. ^{59}Fe appeared rapidly in the blood when given as iron:NTA but there was a 30 min lag before ^{59}Fe entry from iron:transferrin with about 50% of the ^{59}Fe dose remaining unabsorbed in in the gut lumen after 90 mins. Neither cytochalasin (0.5mM) nor chloroquine (0.5mM) had any effect on *in vivo* uptake of ^{59}Fe from either ligand.

Thus there is no evidence either *in vitro* or *in vivo* to suggest that endocytosis is involved in the uptake of inorganic iron across the luminal surface of the intestinal mucosa.

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STRUCTURE-ACTIVITY RELATIONSHIPS OF DERIVATIVES OF TRIPHENYL-ETHYLENE TO CONTROL REPLICATION OF BREAST CANCER CELLS IN VITRO

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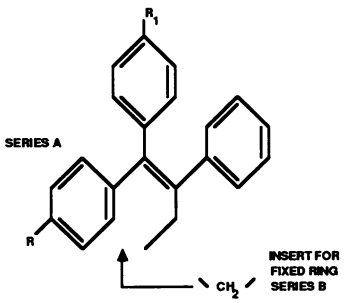
We have developed an oestradiol-responsive subline of T47D human breast cancer cells (Keydar, et al., 1979) to evaluate the structure-activity relationships of derivatives of tamoxifen, a nonsteroidal antioestrogen used in breast cancer therapy (Jordan, 1986). Compounds (Figure 1) were synthesized as previously described (McCague, et al., 1986) and fixed-ring analogues were used to avoid isomerization of hydroxylated derivatives.



Compound	R	R_1
I A, B	H	$OCH_2CH_2N(CH_3)_2$
II A, B	OH	$OCH_2CH_2N(CH_3)_2$
III A, B	$OCH_2CH_2N(CH_3)_2$	H
IV A, B	$OCH_2CH_2N(CH_3)_2$	OH
V A, B	H	OH
VI A, B	OH	H

Figure 1. Compounds used for study.

In dose-response studies, oestradiol (10^{-13}-10^{-9} M) increased cell replication to a maximum of 5 fold over control in a 7-day growth assay. Tamoxifen (1A) derivatives were tested for antioestrogenic activity against oestradiol 10^{-10} M. Both series A and B of Compounds I and II were antioestrogens, with Compounds II (IC_{50} 1 x 10^{-9} M) 100 fold more potent than Compounds I (IC_{50} 1 x 10^{-7} M). Compounds III (A and B) were both oestrogens (EC_{50} 3 x 10^{-8} M), but paradoxically Compounds IV (A and B) were antioestrogens. The unstable A form of Compound IV isomerized to the more potent A form of Compound II. However, the stable fixed-ring B series of Compound IV was antioestrogenic (IC_{50} 1 x 10^{-7} M). Similarly, the unstable A series of Compound V converted to the more potent oestrogenic A form of Compound VI (EC_{50} 2 x 10^{-11} M). The B series of Compound V and VI were oestrogens, with Compound V (B) showing only weak partial agonist activity.

The results demonstrate the importance of a hydroxyl at R to produce high potency, and an alkylaminoethoxy side chain at R_1 for antioestrogenic activity. The paradoxical antioestrogenic activity of Compound IV B suggests an orientation of the drug by the hydroxyl at the ligand binding site of the oestrogen receptor to place the alkylaminoethoxy side chain in an "antioestrogenic" area.

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