EFFECT OF THE LEUKOTRIENE ANTAGONIST FPL57231 ON THE ACUTE PHASE OF ENDOTOXIN SHOCK IN CATS.

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Recent studies have provided evidence that leukotrienes may be mediators of endotoxin shock. Lipoxygenase products are released during endotoxin shock in sheep (Ogletree et al, 1982) and the leukotriene antagonist FPL55712 improves survival in a mouse endotoxin model (Hagmann & Keppler, 1982). The purpose of this study was to assess the possible role of leukotrienes in the acute phase of endotoxin shock in cats using the Fison leukotriene antagonist FPL57231 (Sheard et al, 1984).

Cats were prepared as described previously (Ball et al, 1983), with measurements including pulmonary arterial pressure (PAP), dynamic lung compliance (C_{dyn}) and airways resistance (R_{aw}). LTC_4 and LTD_4 were administered intravenously (0.1 - 3.0 µg kg^{-1}) before and after an infusion of FPL57231 (2mg kg^{-1} min^{-1}). At 0.3, 1.0 and 3.0 µg kg^{-1} there were significant rises in arterial blood pressure (ABP) with both LTD_4 and LTC_4. These responses were attenuated in the presence of FPL57231 although the antagonism was more marked with LTD_4. In the doses administered LTC_4 and LTD_4 had little or no activity on PAP, C_{dyn} or R_{aw}. During an infusion of FPL57231, E. coli endotoxin (2mg kg^{-1}) was administered and measurements were made for a further 30 min. Results are presented in Table 1. FPL57231 prevented the increase in PAP and R_{aw} and the decrease in ABP and C_{dyn}, effects which were all observed in the control cats given endotoxin.

Table 1 The effects of FPL57231 on the acute phase of endotoxin shock. Values are means ± s.e.m. of n observations.

	Control (n=6)		FPL57231 (n=7)	
Time (min)	-5	+2	-5	+2
ABP (mmHg)	123 ± 9	69 ± 14*	101 ± 7	95 ± 6
PAP (mmHg)	14 ± 1	31 ± 5*	18 ± 2	19 ± 2
C_{dyn} (ml cm H_2O^{-1})	4.5 ± 0.2	2.9 ± 0.3*	5.0 ± 0.4	5.0 ± 0.4
R_{aw} (cm H_2O $l^{-1}s^{-1}$)	10.2 ± 0.6	20.9 ± 5.2*	8.5 ± 0.7	8.5 ± 0.7

* $P < 0.05$ (Wilcoxon Rank Test).

Radio-immunoassay of blood samples taken from the cats given FPL57231 showed that levels of 6-ketoPGF$_{1\alpha}$ and TXB_2 were not significantly increased by endotoxin, as would normally be expected (Coker et al, 1983). This suggests that FPL57231 acts as a cyclooxygenase inhibitor at this dose and may thus be attenuating the acute endotoxin response by this known mechanism (Parratt, 1983). It is unlikely that leukotrienes are involved in the acute phase of the feline endotoxin response because of their minimal actions on the pulmonary vasculature and airways.

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THE EFFECT OF CYCLO-OXYGENASE AND LIPOXYGENASE INHIBITION ON THE RELEASE OF PAF-ACETHER IN VITRO

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Platelet activating factor (PAF-acether) is formed by a two stage process in which the 1-0-alkyl-2-acyl-sn-glycerol-3-phosphorylcholine ether phosphatide precursor is catalytically degraded to lyso-PAF by the enzyme phospholipase A_2 and then acetylated by a specific Co-A dependent acetyl transferase (Albert & Snyder, 1983). It has been recently demonstrated that in human neutrophils lipoxygenase metabolites can modulate the biosynthesis of PAF-acether by enhancing the expression of PLA_2 (Billah et al, 1985). We have shown that PAF-acether is released after challenge with antigen by sensitized guinea-pig lungs perfused through the airways (Fitzgerald et al., 1985). As alveolar macrophages are the most likely cellular source of this mediator in the lungs, we have investigated : (i) the capability of these cells to synthesize PAF-acether in response to inflammatory stimuli; (ii) the effect of inhibitors of both cyclo-oxygenase and lipoxygenase on the formation of PAF-acether.

Cells were harvested by the lavage of the lungs of male Dunkin-Hartley guinea-pigs (250-350g.) with heparinized sterile saline. Alveolar macrophages were purified by adherence to tissue culture plates in Dulbecco's Modified Eagle's Medium containing 10% foetal calf serum. After 4 hours, the non-adherent cells were removed and the adherent cells were shown to be >99% macrophages by positive esterase staining. The macrophages were incubated for 30 minutes with either the vehicle or the drugs (BW755C or indomethacin) and for a further 30 minutes with the inflammatory stimuli (Ca^{2+} ionophore A23187 $2x10^{-6}$M or formyl methionyl-leucyl-phenyl-alanine (FMLP) 10^{-6}M). At the end of the incubation PAF-acether was extracted from the supernatant, identified and bioassayed as previously described (Parente & Flower, 1985). The levels of thromboxane B_2 (TXB_2) and leukotriene B_4 (LTB_4) in the supernatant were measured by radioimmunoassay.

Alveolar macrophages incubated for 30 min with A23187 released PAF-acether (1.49±0.08ng;m±s.e.m), TXB_2 (43.4±4.5ng), and LTB_4 (0.6±0,05ng) per 10^6 cells/(n=4). BW755C (1-20μM) caused a dose dependent inhibition of TXB_2 and LTB_4 formation. Indomethacin (1μM) completely inhibited TXB_2 formation with no effect on LTB_4. Neither drug had any effect on PAF-acether release. Alveolar macrophages incubated for 30 min with FMLP released PAF-acether (0.78±0.06 ng), TXB_2 (94.9±5.1 ng), and LTB_4 (1.35±0.04 ng) per 10^6 cells (n=4). BW755C (1-50μM) inhibited the formation of TXB_2 in a dose dependent fashion. The inhibition of LTB_4 was statistically significant but less pronounced (21,4% at 50μM; $P<0.001$). The release of PAF-acether was unaffected by the drug.

These results demonstrate that guinea-pig alveolar macrophages are capable of synthesizing PAF-acether in response to the Ca^{2+} ionophore A23187 and FMLP. In these cells the inhibition of cyclo-oxygenase and lipoxygenase does not interfere with the formation of PAF-acether.

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THE METABOLISM OF LEUKOTRIENES BY PORCINE PULMONARY ARTERY

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Porcine pulmonary arteries have the capacity to generate leukotrienes (LTs) upon stimulation with the calcium ionophore A23187 (Piper and Galton, 1984). The release is time-dependent; after 60 min the main LTs generated are LTB_4 and LTE_4 whereas, at 30 min, LTC_4 and LTD_4 are also produced. In these experiments we have investigated the metabolism of exogenous LTs by these arteries.

Porcine pulmonary arteries, obtained from an abattoir, were weighed, chopped, suspended in Tyrode's solution (6ml/g) and agitated at 37°C. LTB_4, LTC_4 or LTD_4 was added (final concentration 1nM) with tracer amounts of the respective [^{3}H]-LT. 2ml aliquots were removed at time intervals from 0 to 180 min and partially purified using Waters C18 Sep-Paks. The material was further purified by RP-HPLC (Spherisorb 5ODS column; solvent MeOH, H_2O, HAc 75:25:0.02, pH5.4; flow rate 1.0 ml/min). Synthetic LTs were co-injected with each sample. Fractions were collected and counted using a liquid scintillation counter. The effect of the inhibitors L-cysteine and serine borate on LT metabolism were also studied. In large scale experiments, synthetic LTs were not added during HPLC and the fractions were assayed for LTs on superfused strips of guinea-pig ileum smooth muscle (GPISM) in the presence and absence of FPL55712 (2.3×10^{-6}M).

The results show that LTC_4 was rapidly converted to LTD_4 with a t1/2 of approximately 3 min. The level of LTD_4 reached a maximum at 10 min, after which it slowly declined with a concomitant rise in the level of LTE_4. After 120 min, 75% of the radioactivity co-eluted with synthetic LTE_4. There was no further metabolism beyond LTE_4, all the radioactivity being in either the LTC_4, LTD_4 or LTE_4 fractions. Incubation with LTD_4 resulted in the formation of LTE_4, the t1/2 being approximately 20 min. After 180 min, 90% of the radioactivity was associated with LTE_4 and, as with LTC_4, there was no further metabolism. Experiments with boiled tissue showed no conversion of LTC_4 or LTD_4.

The addition of L-cysteine (10mM) to the incubation of LTC_4 had no effect on the conversion of LTC_4 to LTD_4 but markedly reduced the conversion of LTD_4 to LTE_4. Serine-borate (45mM) inhibited the conversion of LTC_4 to LTD_4.

Assay of the HPLC fractions on GPISM confirmed the identity of the radioactive fractions as the corresponding leukotriene.

Similar experiments performed with LTB_4 revealed that no metabolism was occurring, in that no radioactivity co-eluted with either 20-OH or 20-COOH LTB_4.

These results show that pulmonary arteries metabolise LTC_4 to LTD_4 and then to LTE_4 but that no further metabolism occurs. There appeared to be no metabolism of LTB_4. In view of these findings, it would appear that these arteries may have a role in reducing the biological activity of the peptido-LTs and in eliminating them from the circulation by promoting the early stages of their metabolism.

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THE EFFECT OF AGE ON THE INVOLVEMENT OF THROMBOXANE A_2 IN ANAPHYLAXIS IN THE ANAESTHETIZED GUINEA PIG

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Thromboxane A_2 (TxA_2) is involved in anaphylaxis induced by exposure of conscious, sensitized guinea-pigs to aerosolized ovalbumin (Barnes and Goadby, 1985). Pretreatment of anaesthetized, sensitized guinea-pigs with dazoxiben, a thromboxane synthetase inhibitor, was found to offer no protection against anaphylaxis induced by intravenous ovalbumin in initial experiments. Thus the conscious and anaesthetized models of anaphylaxis appear to differ. This present study aimed to investigate anaphylaxis in anaesthetized animals using two thromboxane receptor antagonists, EP092 (Jones et al, 1984) and AH23848 (Humphrey and Lumley, 1984).

Male sensitized guinea-pigs (450 - 1390g) were anaesthetized with urethane (1.5g/kg, i.p.) and the trachea cannulated. Sodium pentobarbitone (6mg/ml in saline) was administered, via a cannulated jugular vein, until spontaneous respiration ceased. Guinea-pigs were then attached to a respiratory pump and increases in respiratory overflow volume were detected using the method of Gardiner (1971). Drugs were administered via the jugular vein cannula, 10 min prior to ovalbumin.

Guinea-pigs were divided into two groups; young (5 weeks old; 525±12g;n=33) and old (>15 weeks old; 986±24g; n=26). Table 1 shows the effect of phenidone, dazoxiben, EP092 and AH23848 on ovalbumin-induced increases in respiratory overflow volume.

Table 1. Responses to ovalbumin (0.5mg/kg) of anaesthetized, artificially respired guinea-pigs.

Treatment	Dose (μmol/kg)	% maximum respiratory overflow volume (mean±s.e.mean (n)) young		old	
None		99.4 ± 0.6	(7)	94.1 ± 2.4	(9)
Phenidone	3.7	21.7 ± 5.5	(4)	100.0 ± 0	(4)
Dazoxiben	3.7	40.0 ± 9.0	(6)	88.0 ± 5.4	(5)
EP092	2.4	45.7 ± 17.4	(6)	100.0 ± 0	(4)
AH23848	0.2	64.4 ± 13.1	(8)	100.0 ± 0	(4)

The control animals of both groups responded similarly to ovalbumin challenge with regards to the severity of response. However the mechanisms involved in the response must differ with age as interference with the action of TxA_2 offered protection to young but not to old guinea-pigs. It remains to be determined if this is due to a difference in sensitivity to or release of TxA_2 within the lung.

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PREVENTION OF PULMONARY OEDEMA AND ALTERED PULMONARY PHARMACOKINETICS INDUCED BY α-NAPHTHYLTHIOUREA (ANTU) IN RAT LUNG

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Pulmonary oedema is induced in rats by intra-peritoneal(i.p.) injection of ANTU. This type of lung injury is accompanied by defects in the pulmonary pharmacokinetics of prostaglandin E_2(PGE_2), thromboxane B_2 and adenosine (Bakhle & Grantham,1985 a and b). Here we describe the effects of three different agents -dimethyl sulphoxide(DMSO), N-acetyl cysteine(NAC) and a synthetic steroid, budesonide(BUD) on the pulmonary oedema induced by ANTU. In addition we have studied the effects of one of these agents(NAC) on the pulmonary pharmacokinetics of adenosine and PGE_2 in the same model.

Male rats(200-270g) were injected subcutaneously(s.c.) with NAC(500mg/kg) or BUD(1.2 mg/kg) or with an i.p. injection of DMSO(7.8g/kg), 1h before an i.p. injection of ANTU(10 mg/kg) in olive oil. A further group of rats received the same dose of DMSO s.c. Lungs were removed from anaesthetised rats at fixed times after ANTU injection and the lung dry weight: wet weight ratio determined. This ratio was below that for lungs from untreated rats(21.0±0.7%, n=6), i.e., oedema was present, between 2h and 28h after ANTU injection with the maximum effect, a dry:wet ratio of 16.0±0.1% (n=19), present at 4h after ANTU. Pretreatment with DMSO(s.c.) decreased the oedema at 4h after ANTU, giving a dry:wet ratio of 18.4±1% (n=4). This agent given i.p. totally prevented the oedema at 2h and 4h, giving dry:wet ratios of 20.8±0.2% and 20.1±0.3% respectively (n=3-4), and decreased that present at 6h. Pretreatment with NAC or BUD reduced oedema at 4h after ANTU only, with dry:wet ratios of 18.9±0.6% and 16.8±0.3% respectively(n=4).

Pulmonary pharmacokinetics were assessed in the isolated perfused lung by measuring the $T_{1/2}$ value, the time taken for 50% of injected radioactivity to emerge from the lung after an injection of labelled substrate(Bakhle & Grantham, 1985a). The substrates(^{3}H-adenosine, 10nmol, 100nCi and ^{14}C-PGE_2, 1nmol, 20nCi) were injected together as a 100ul bolus into the perfusate entering the lung. Lung effluent was collected in 40 fractions of 4 drops(each equal to about 3s). The radioactivity in each fraction was measured and the $T_{1/2}$ value calculated. Treatment with ANTU produced $T_{1/2}$ values for adenosine of about 20s at 2,4, and 6h after injection, compared with $T_{1/2}$ of more than 120s in sham- treated or untreated lungs. Pretreatment with NAC lengthened the $T_{1/2}$ for adenosine, i.e., towards the normal value, only at 4h to 25.3±0.8s (ANTU alone, 20.3±1.5s, n=4). After ANTU, the $T_{1/2}$ for PGE_2 increased from 44.2±2.0s at 2h to 50.4±0.7s at 6h(n=4), compared with a value of 39.8±1.5s (n=24) in untreated lungs. Pretreatment with NAC did not prevent the ANTU-induced changes in the $T_{1/2}$ for PGE_2 at any time up to 6h.

In summary, ANTU-induced pulmonary oedema was decreased in the early stages by all three pretreatments and most effectively by the anti-oxidants,DMSO and NAC. With NAC, decrease of oedema was accompanied by an improvement in pulmonary pharmacokinetics of adenosine, but not that of PGE_2. Thus adenosine may prove to be the better substrate to use as a biochemical index of pulmonary oedema in this model.

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9α,11β-PROSTAGLANDIN F_2, A METABOLITE OF PGD_2, IS A POTENT CONTRACTILE AGONIST OF HUMAN AIRWAYS IN VITRO AND IN VIVO

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Prostaglandin D_2 (PGD_2) is generated in large amounts with allergen challenge of human isolated lung mast cells in vitro, and is a potent bronchoconstrictor in asthmatic subjects, being 3.5 times more potent than $PGF_{2\alpha}$ (Hardy et al 1984). PGD_2 is metabolised by the initial formation of the 11 -hydroxyl epimer of $PGF_{2\alpha}$, 9α,11β -PGF_2 , which has been found in substantial quantities in the plasma during mast cell activation in vivo in humans, and is biologically active in a number of test systems (Liston & Roberts, 1985). We now report the airway effects of 9α,11β - PGF_2 in isolated human airways and in patients with asthma.

Human airways of 4 mm diameter were dissected from lung tissue obtained from patients undergoing surgery for bronchial carcinoma. The airways were cut spirally and suspended in organ baths containing Krebs' solution at 37°C, aerated with 95% O_2 and 5% CO_2 and contractions measured isometrically. Both prostanoids caused concentration-related smooth muscle contraction in the range of 0.32 - 32 x 10^{-6} M. 9α,11β -PGF_2 was 4.27 times more potent than PGD_2 (n = 6, 3 lungs), this difference being statistically significant (P<0.001).

To extend these observations, a comparative study was carried out in 8 male subjects with mild allergic asthma (mean age 29 years, mean FEV_1 70% predicted, mean provocation concentration causing a 20% fall in FEV_1 ($PC_{20}FEV_1$) histamine 2.4 x 10^{-3} M.) On three separate occasions subjects inhaled doubling concentrations of nebulised histamine, 9α,11β -PGF_2 and PGD_2, and airway calibre followed as changes in FEV_1 and specific airways conductance (sGaw). Concentration response curves were constructed and the $PC_{20}FEV_1$ and provocation concentrations causing a 35% fall in sGaw (PC_{35}sGaw) were derived for each agonist.. Histamine, 9α,11β -PGF_2 and PGD_2 caused concentration-related bronchoconstriction with geometric mean $PC_{20}FEV_1$ values of 2.4, 0.17, and 0.15 x 10^{-3} M respectively; and PC_{35} sGaw values of 2.3, 0.14,and 0.11 x 10^{-3} M respectively . There was no significant difference between the bronchoconstrictor effects of 9α,11β -PGF_2 and PGD_2, however both were significantly more potent (P<0.005) than histamine. The effect of a single inhalation (0.71 x 10^{-3} M) of 9α,11β -PGF_2 and PGD_2 was investigated in 6 asthmatic subjects. Airway calibre was measured with sGaw, and the change expressed as percentage fall from the baseline values. Both 9α,11β -PGF_2 and PGD_2 caused maximum bronchoconstriction 3 to 7 minutes post inhalation. The mean maximum fall for 9α,11β -PGF_2 was 48% and this was significantly greater than the fall caused by PGD_2 (39% P<0.01). Despite the greater magnitude of bronchoconstriction observed with 9α,11β -PGF_2 , the rates of recovery of airway calibre were not significantly different between the two prostaglandins.

Our studies suggest that 9α,11β -PGF_2 is at least equipotent with PGD_2 as a bronchoconstrictor agonist, and as a metabolite of PGD_2, may contribute to the bronchoconstrictor effect of this mast cell-derived mediator in asthma.

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RELEASE OF LEUKOTRIENES BY RAT AND HUMAN GASTRIC MUCOSA AND ITS PHARMACOLOGICAL MODIFICATION

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Vasoconstriction and stasis of blood flow (Guth et al., 1984) are prominent features of ethanol (EtOH)-induced gastric damage in rats. Similar effects are evoked by exogenous leukotriene (LT) C4 (Whittle et al., 1985). We have determined gastric mucosal damage and LTC4 formation induced by EtOH and the effects of pretreatment with the anti-ulcer drugs carbenoxolone (CX) and prostaglandin (PG) E2 and the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA). Furthermore, we have studied the effect of CX on LT release by human gastric mucosa in vitro.

Rats were treated orally with 2 ml of water or 20%, 50% or 100% EtOH. After 5 min the animals were killed and the ulcer index was determined. Fragments of gastric corpus mucosa were incubated in Tyrode solution at 37°C for 20 min. Eicosanoids were determined radioimmunologically. HPLC revealed formation of LTC4 but not of LTD4 and LTE4. Effects of CX on LT release from human gastric mucosa were studied using normal tissue obtained from surgical specimens. Chopped tissues were preincubated in the absence or presence of various concentrations of CX at 37°C for 20 min. Then the medium was replaced by fresh buffer containing the same concentrations of CX plus 0.005 mg/ml ionophore A23187.

Gastric mucosa of control rats released 58 + 7 ng/g LTC4 (mean + SEM, n=18). Instillation of various concentrations of EtOH enhanced mucosal LTC4 formation ex vivo in a dose-dependent manner. Thus, treatment with 100% EtOH resulted in an about fivefold increase in LTC4 release (267 + 25 ng/g, n=43, $p<0.001$). Mucosal LTC4 formation correlated with gastric damage induced by various concentrations of EtOH ($r=0.7$, $n=32$, $p<0.001$). Oral pretreatment of rats with CX (33-300 mg/kg) or NDGA (10-100 mg/kg) 30 min prior to instillation of 100% EtOH resulted in dose-dependent gastric protection and inhibition of mucosal LTC4 release. Both drugs did not affect formation of cyclooxygenase-derived products of arachidonate metabolism. Pretreatment with oral PGE2 (0.2 mg/kg) completely protected the mucosa against EtOH-induced damage, but did not reduce mucosal LTC4 release.

Human gastric mucosa released less LT than rat mucosa. Thus, ionophore-stimulated release (ng/g/20 min, n=19) of LTB4 was 3.9 + 0.7 and of sulfidopeptide-LT (expressed as immunoreactive (i.r.) LTC4) was 3.6 + 0.5. HPLC analysis demonstrated that human gastric mucosa released a mixture of LTC4, LTD4 and LTE4. CX (0.025-0.4 mM) dose-dependently reduced mucosal release of i.r. LTC4 (IC50 0.07 mM) and LTB4 (IC50 0.2 mM).

The results indicate that 1.) Increased formation of LTC4 and possibly other 5-lipoxygenase-derived products of arachidonate metabolism could contribute to EtOH-induced mucosal injury. 2.) In this type of acute gastric damage inhibition of LT formation may be an important mechanism of action of protective drugs. Protection by exogenous PGE2, however, is not due to inhibition of mucosal LT release, but may be mediated by functional antagonism of LTC4 effects. 3.) Similar to the rat, the 5-lipoxygenase pathway of arachidonate metabolism may be a target for anti-ulcer drugs in man.

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INHIBITION OF PAF-INDUCED GASTRIC MUCOSAL DAMAGE BY CORTICO-STEROIDS.

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We have recently reported that platelet-activating factor (PAF) is a potent ulcerogenic agent (Rosam et al., 1986) and augments the gastric damage induced by topical irritants (Wallace & Whittle, 1985). It is possible that eicosanoids such as thromboxane A_2 or leukotriene C_4 (Whittle et al., 1985) mediate some of the gastric damaging effects of PAF. Hence, in the present study, we have examined the effects on the gastric mucosal damage and systemic hypotension induced by intravenous PAF of the cyclooxygenase inhibitor, indomethacin, and the dual cyclooxygenase-lipoxygenase inhibitor, BW755C. Further, we have investigated the actions of the corticosteroids, dexamethasone and prednisolone, which inhibit phospholipase A_2 activity through lipocortin biosynthesis (Flower & Blackwell, 1979).

Male rats (4 to 12 per group) were anaesthetized with sodium pentobarbitone. Systemic arterial blood pressure (BP) was measured throughout the 60-min experiment. A 10-min infusion of PAF, at a dose (100 ng/kg/min) previously shown to produce gastric mucosal erosions (Rosam et al., 1986), was started at minute 20. Rats were pretreated with dexamethasone (0.2 or 2 mg/kg) administered s.c. 120 min or i.v. 15 min prior to PAF administration, while prednisolone (20 mg/kg s.c.), BW755C (50 mg/kg p.o.) and indomethacin (5 mg/kg s.c.) were given, respectively, 120, 60 and 45 min prior to PAF. The stomach was subsequently opened, photographed and mucosal damage was scored (0 to 3 scale) in a randomized, blind manner.

In the control group, i.v. infusion of PAF for 10 min caused a rapid fall in BP (Δ 75 ± 3 mm Hg; mean ± SEM; n=10). The BP remained depressed throughout the infusion period and, during the ensuing 30 min, slowly recovered to 38 ± 8% of pre-PAF levels. The infusion of PAF also resulted in severe hyperaemia and haemorrhage in the stomach, with a mean gastric damage score of 2.9 ± 0.1. While none of the drugs tested significantly affected the fall in BP induced by PAF, all produced a significant acceleration of the recovery of BP after PAF. Thus, in the indomethacin, BW755C, dexamethasone (2 mg/kg s.c.) or prednisolone groups, the BP recovered to (as % of pre-PAF levels): 69 ± 5% ($p<0.05$), 77 ± 8% ($p<0.05$), 82 ± 3% ($p<0.01$) and 87 ± 4% ($p<0.005$), respectively. However, only the corticosteroids, when administered 120 min prior to PAF, and BW755C significantly reduced the gastric damage score. Prednisolone reduced the gastric damage score to 1.0 ± 0.5 ($p<0.05$) while dexamethasone (0.2 and 2 mg/kg) and BW755C reduced the gastric damage score to 1.6 ± 0.6 ($p<0.05$), 1.2 ± 0.3 ($p<0.01$) and 1.2 ± 0.4 ($p<0.01$) respectively.

The reversal of the hypotensive effects of PAF can be significantly accelerated by pretreatment with indomethacin and by the dual inhibitor, BW755C, as well as by dexamethasone and prednisolone. These results suggest a role for the generation of cyclo-oxygenase products in the prolonged hypotension induced by PAF. However, only the corticosteroids, when given 2 hours before PAF, and BW755C significantly reduced gastric mucosal damage. Although it is thus unlikely that cyclo-oxygenase products play an important role in the ulcerogenic actions of PAF in the gastric mucosa, the role of lipoxygenase products in such damage cannot be excluded.

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EVIDENCE OF A ROLE FOR PLATELET-ACTIVATING FACTOR IN ANTIGEN-INDUCED CORONARY VASOCONSTRICTION IN GUINEA-PIG PERFUSED HEARTS.

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The consequences of cardiac anaphylaxis, which include impaired myocardial contractility, dysrythmias and coronary vasoconstriction, have been attributed to antigen-induced release of mediators such as histamine, thromboxane A_2 and cysteinyl-containing leukotrienes (LTs). However, administration of platelet-activating factor (PAF) to guinea-pig or rat isolated, perfused hearts results in disturbances of function resembling those occurring during cardiac anaphylaxis (Benveniste et al, 1983; Levi et al, 1984; Piper & Stewart, 1985). Furthermore, antigen-challenge of sensitized guinea-pig hearts is accompanied by the release of PAF (Levi et al, 1984). The present study investigates the role of PAF in cardiac anaphylaxis using the selective PAF receptor anatagonists, BN52021 and L652,731.

Male Dunkin-Hartley guinea pigs (150-200g) were sensitized to ovalbumin (40µg OA + 10mg $Al(OH)_3$/kg, i.p.) and experiments were performed 14 to 21 days later (Andersson, 1980). Hearts were perfused via the aorta using Kreb's solution (37°C, gassed with 95% O_2, 5% CO_2) at a constant flow rate (8 ml/min) for 30 min before receiving an infusion (0.1 ml/min) of vehicle (30% dimethylsulphoxide), BN52021 (3, 10 or 30 µM) or L652,731 (10 µM). Recordings of coronary perfusion pressure (CPP) were made 10 min after the beginning of the infusion and for a further 10 min following the administration of 100 µg OA. Antigen or vaso-constrictors were administered as bolus doses (10 to 100 µl).

BN52021 (30 µM) significantly attenuated ($P < 0.05$, unpaired Student's t-test) the increase in CPP induced by PAF (100 pmol) whereas responses to LTC_4 (30 pmol, LTD_4 100 pmol) and the thromboxane A_2-mimetic, U44069 (100 pmol) were unaffected. Similarly, L652,731 selectively inhibited the PAF-induced increase in CPP. Neither BN52021 nor L652,731 significantly altered resting CPP. Administration of OA evoked increases in CPP which reached a maximum at 20-40 s (74±10%, n=13), declined after 2 min (33±8%) and were maintained up to 10 min after OA (29±6%). BN52021 (30 uM) attenuated the maximum increase in CPP (39±12%, n=9), whereas both BN52021 (30 µM) and L652,731 (10 µM) markedly inhibited the increases in CPP at 5 (8±6%, 6±4% n=7, respectively) and 10 (9±8%, 3±4%, respectively) min after OA.

The inhibitory effects of selective concentrations of the PAF-receptor antagonists BN52021 and L652,731 on antigen-induced coronary vasoconstriction suggest a role for PAF in cardiac anaphylaxis.

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INTRACRANIAL CIRCULATORY EFFECTS OF PLATELET-ACTIVATING FACTOR AND INDOMETHACIN IN THE ANAESTHETISED PIG

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Platelet-activating factor (PAF, 1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphoryl-choline) is synthesised by white cells, platelets and other cell types and has been shown to possess both vasodilator and vasoconstrictor properties (Kenzora et al, 1984). In view of a possible role in pathophysiological processes, the effects of PAF on special circulations are of interest.

We have examined the effects of bolus injections of PAF and a thromboxane mimetic, U-44069, on intracranial blood flow (ICBF) in the pig. The influence of indomethacin was studied in this system.

Eleven female pigs (32.5-55 kg) were sedated with azaperone (Suicalm, Janssen) i.m. and anaesthesia was induced and maintained with metomidate (Hypnodil, Janssen) i.v. The animals were placed on a heating pad, intubated through a tracheotomy incision and ventilated with O_2-enriched air. Blood pressure (BP), ECG, heart rate and core temperature were recorded. Blood gases were closely monitored and maintained at PCO_2 5.28±0.50 kPa, pH 7.41±0.04 (mean ± s.d.), PO_2 > 10.5 kPa. The intracranial circulation was isolated as previously described (Piper & Stanton, 1985) and intracranial blood flow recorded using an electromagnetic flow cuff (Gould). Drugs were administered via a polyethylene cannula (Portex) in the lingual artery.

Platelet-activating factor (C16, Bachem) $3x10^{-15}$ - $3x10^{-10}$ mol produced a dose-dependent decrease in ICBF. A transient increase in flow of <8.0% sometimes preceded the decrease but this was not dose-related. U-44069 ($2.9x10^{-9}$ mol) reduced ICBF by 51.1±4.9% (n=4). Indomethacin (5 $mgkg^{-1}$ + 0.5 $mgkg^{-1}h^{-1}$, i.v.) caused a rise in BP and a fall in ICBF of 64.0±4.9% (n=4). This was followed by gradual recovery. In indomethacin-treated animals PAF elicited a biphasic response: a dose-related increase in flow, threshold dose $3x10^{-12}$ mol, was followed by a more sustained decrease in flow, threshold dose $3x10^{-11}$ mol. The dose-response curve for the second phase was steeper than in untreated animals. U-44069 again reduced ICBF.

The results show that PAF and U-44069 can decrease ICBF, which probably reflects active cerebral vasoconstriction. In the presence of indomethacin, the altered PAF response characteristics may indicate that a different part of the intracranial vasculature is reacting. A further possibility is that the PAF-induced decrease in ICBF observed at low doses was mediated by a cyclo-oxygenase product.

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240P

THE PRINCIPAL SITE OF KININ-STIMULATED PGE_2 RELEASE IN RABBIT COLON IS FROM SUBEPITHELIAL CELLS WHICH DO NOT DISPLAY SIDEDNESS

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Bradykinin-induced secretion in mammalian colon involves prostaglandin generation, notably PGE_2, and displays "sidedness" in that the responses are elicited after serosal kinin application and prostanoids are released predominantly into the serosal bathing medium. However, in recent experiments using colonic sheets stripped of epithelial cells (Hoult & Phillips, 1986), it was shown that the kinin-induced PGE_2 in rat colon derives principally from the lamina propria and not from the epithelial cells as previously supposed, and that when the epithelial cell layer is removed the sidedness of the responses is no longer evident. In the present work we have extended the validity of this concept to a "low conductance epithelium" by comparing PGE_2 release induced by lysyl-bradykinin (LBK) in "epithelial-intact" and "epithelial-removed" preparations of rabbit colon.

Sections of muscle-stripped epithelial-intact and epithelial-removed rabbit colon were prepared and mounted in Ussing chambers as described previously (Hoult & Phillips, 1986). In epithelial-intact colon, LBK at 10^{-6} M caused significant PGE_2 release when applied serosally (0.25± 0.1 to 1.61 ± 0.05 ng cm^{-2} min^{-1} $P < 0.05$) but was ineffective when applied mucosally. Data shows serosal PGE_2 concentrations; those in the mucosal bathing medium were lower and not significantly increased after LBK application (eg 0.1 ± 0.1 to 0.2 ± 0.1 ng cm^{-2} min^{-1}, respectively). In colon with epithelial cells removed (verified in sections using PAS and haematoxylin stains and whose intactness was checked by adding radiolabelled PG to one compartment), LBK significantly enhanced PGE_2 release when added to either serosal or mucosal bathing solution (eg on mucosal side from 0.1 ± 0.05 to 1.79 ± 0.77 ng cm^2 min^{-1} $P < 0.05$).

The calcium dependency of the PG generation was investigated by incubating tissues in Ca^{2+}-free Krebs containing 1 mM EGTA for 30 min before LBK application. Removal of calcium from the serosal but not mucosal side abolished the increased potential difference evoked by LBK in epithelial-intact tissue, and reduced the generation of PGE_2. Removal of Ca^{2+} from both sides had a greater effect on PGE_2 release: 3.0 ± 0.66 serosally after LBK reduced to 1.0 ± 0.1 ng cm^{-2} min^{-1}. In contrast, in epithelial-removed tissues, kinin-induced PGE_2 release was reduced after Ca^{2+} removal from either bathing solution.

Our results show that kinin-induced PGE_2 release in rabbit colon is from subepithelial cells and that the apparent sidedness of the effects in terms of route of LBK application, release of PGE_2 and sensitivity to calcium removal occur because of the barrier property of the epithelial cell layer.

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PROSTAGLANDINS AFFECT GAP JUNCTION RECRUITMENT IN OVINE URETERAL SMOOTH MUSCLE

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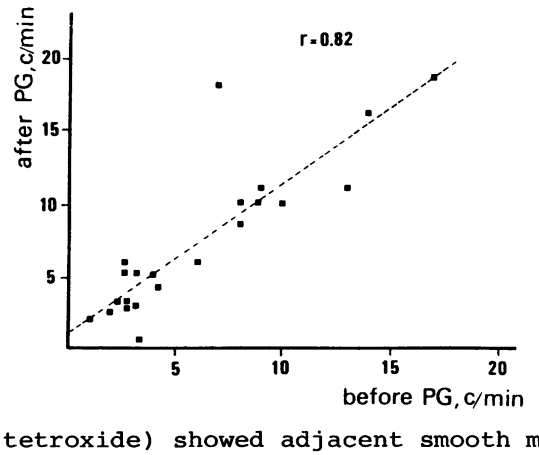
Indomethacin blocks motility in isolated sheep ureteral preparations (Thulesius and Angelo-Khattar, 1985). The present investigation deals with the role of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on establishment of ureteral contractions and transmission of contractile waves.

Ureteral ring preparations (length 4 mm) were obtained from the local abbatoire from freshly slaughtered Merino sheep. Contractions were recorded in an organ bath. After spontaneous rhythmic contractions had been established for 1 hour indomethacin (10^{-5}M) was added. After stop of contractions $PGF_{2\alpha}$ was added and motility invariably restarted with a frequency (c/min) and amplitude similar to the preceding spontaneous rhythm characteristic for each preparation. This applies to three concentrations of $PGF_{2\alpha}$, 10^{-6}, $5 \cdot 10^{-6}$ and 10^{-5}M. Thus there was no concentration dependent increase in frequency or amplitude.

The diagram below shows a good correlation ($P < 0.001$) of frequency of ureteral contraction before and after $PGF_{2\alpha}$ ($5 \cdot 10^{-6}$M). Correlation coefficients (r) for 10^{-6} and 10^{-5}M $PGF_{2\alpha}$ were 0.83 and 0.87.



In a 40 mm long tandem preparation contractions were recorded from both ends with separate transducers but in the same bath. This enabled measurement of transmitted contractions and determination of speed of transmission of contractile waves which was determined at 33.1 ± 1.4 mm/s (n=10). In case of uncoordinated contractions (each end beating at its own rhythm) $PGF_{2\alpha}$ re-established coordination in a large number of experiments but did not alter pacemaker rate.

Electron microscopy from ultra thin sections (fixed in 3 % gluteraldehyde in cacodylate buffer postfixed in 1% osmium tetroxide) showed adjacent smooth muscle cells to possess membrane structures characteristic of gap junctions.

The present findings can be interpreted on the basis of a $PGF_{2\alpha}$ induced recruitment and coordination of a large number of contractile units governed by pacemaker cells and is consistent with an effect on gap-junctions rather than pacemaker cells.

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CYCLOSPORIN A INHIBITS RAT NEUTROPHIL PHOSPHOLIPASE A_2 AND POSSESSES ANTI-INFLAMMATORY ACTIVITY

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The immunosuppressive action of cyclosporin A (CSA) is mainly attributed to a specific action on T-lymphocytes; in particular an inhibition of Interleukin-2 synthesis and receptor expression (Thomson et al. 1984). However, at higher concentrations this compound may also interfere with macrophage Interleukin-1 release (Thomson et al. 1983) and phospholipase action (Fan and Lewis. 1984). These latter effects may predict an anti-inflammatory action, although it has been previously reported that CSA does not inhibit acute inflammation (Borel et al. 1976). We now report that CSA can inhibit rat peritoneal neutrophil phospholipase A_2 and, although it is devoid of anti-inflammatory action against the rat paw oedema induced by carrageenan, CSA does inhibit the primary rat paw oedema induced by Freund's complete adjuvant as assessed at 24 hours.

The effect of CSA on the release of free (^{14}C) arachidonic acid (AA) from A23187 stimulated (^{14}C) AA prelabelled rat peritoneal neutrophils was measured using a modification of a previously reported method (Annfelt-Ronne et al. 1982). CSA inhibited the release of (^{14}C) AA with an IC_{50} of 0.8 micromolar. The 5-lipoxygenase products released by these cells were also reduced with an IC_{50} of 2.3 micromolar. Two separate and differentially inhibited lipolytic reactions are catalysed by freeze/thaw disrupted (^{14}C) AA prelabelled rat neutrophils in the presence of calcium. (^{14}C) 2-arachidonyl phospholipid is hydrolysed by a phospholipase A_2 and AA esterified in the neutral lipid fraction is also hydrolysed to yield free AA. The former, but not the latter, enzyme activity was inhibited by CSA with an IC_{50} of 20 micromolar. Hence, in both intact and disrupted rat peritoneal neutrophils CSA acts in a manner consistent with phospholipase A_2 inhibition.

The effects of CSA and dexamethasone on the rat paw oedema induced by sub-plantar injection of Freund's complete adjuvant (FCA) were examined at 6 and 24 hour time points. Groups of rats (n=8 or 10) were orally dosed with either vehicle, 30mg/kg CSA or 0.1mg/kg dexamethasone 30 minutes before FCA injection. The results (table) show CSA to possess significant activity at the 24 hour, but not the 6 hour time point. Dexamethasone, however, demonstrated greater activity at the earlier time.

Table

% INHIBITION OF CFA OEDEMA at	6	24hours
CSA 30mg/kg	-8	48*
Dexamethasone 0.1mg/kg	70*	35*

* $p < 0.002$

CSA did not inhibit 3 hour carrageenan oedema at 20 or 100mg/kg when dosed either 30 minutes or 24 hours before irritant injection.

The mechanism of anti-inflammatory action of CSA as detected by inhibition of primary FCA induced rat paw oedema is unknown. However, the ability of CSA to inhibit the phospholipases of inflammatory cells and Interleukin-1 synthesis (Thomson et al. 1983) suggests these factors should be investigated in this model.

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EFFECTS OF INTERLEUKIN 1 ON LEUKOCYTE MIGRATION AND CARTILAGE DESTRUCTION IN VIVO

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Interleukin 1 (IL-1) is a potent chemotactic agent in vitro (Sauder, et al., 1984) and induces proteoglycan loss from cultures of articular cartilage (Krakauer et al, 1985). These observations, coupled to the detection of IL-1 in rheumatoid synovial fluid (Nouri, et al., 1984), support the hypothesis that IL-1 is an important mediator of the erosive processes in chronic arthritis. We have now investigated the effects of IL-1 on leukocyte accumulation and cartilage destruction following intra-articular injection in rabbits.

Highly purified human IL-1 (Genzyme)was injected (0.2-20 units) into the knee joints of groups of New Zealand White rabbits (2.5-3.5 kg) and the contralateral joint of each animal received vehicle alone. The animals were killed 4h to 3 days after injection, the joints were washed with 1 ml sterile saline and resultant joint fluids were taken for total and differential leukocyte counts. Articular cartilage (20-60 mg) was dissected from the ends of both femurs of each animal and digested by incubation with papain. The concentration of sulphated proteoglycan in the digest was then measured by the 1, 9 dimethylmethylene blue binding assay (Farndale, et al., 1979) and expressed as µg proteoglycan/mg wet weight of cartilage.

Interleukin 1 caused a dose - dependent increase in the number of leukocytes in joint washes collected 24h after intra-articular injection. Total numbers increased to $0.47 \pm 0.2 \times 10^6$ cells/ml (mean ± s.e. mean; n= 7) with 5 units IL-1 and to $2.1 \pm 0.5 \times 10^6$ cells/ml with 20 units IL-1. At 24h the cells were predominantly mononuclear (60 - 80%). Joint washes from animals killed 4h after injection of 10 units IL-1 contained $8.1 \pm 4.5 \times 10^6$ cells/ml (n=5) and at this time the majority of cells (>90%) were polymorphonuclear. Three days after injection of IL-1, the numbers of leukocytes in joint washes had decreased to less than 10^4 cells/ml. Injection of vehicle into the contralateral joints induced the accumulation of leukocytes but total numbers did not exceed 0.2×10^6 cells/ml and were significantly ($P<0.05$) lower than the numbers of cells in joints receiving 5-20 units IL-1.

Intra-articular injection of IL-1 (5-20 units) caused a significant ($P<0.01$) loss of proteoglycan from cartilage which was maximal 24h after injection but had recovered by 3 days. There was no loss of proteoglycan from cartilage of joints injected with saline, vehicle or 0.2-1 unit IL-1. The percentage loss of proteoglycan 24h after the injection of 5 units IL-1 was 25.9 ± 6.2% (n=7). There was not any greater loss of proteoglycan after injection of 10 or 20 units IL-1.

These experiments demonstrate that highly purified IL-1 can induce responses which are characteristic of chronic erosive arthritis. The pattern of mononuclear leukocyte infiltration and cartilage breakdown 24h after intra-articular injection of 5 units IL-1 is similar to that seen in animals with antigen-induced arthritis of one week's duration. These findings are further support for the proposal that IL-1 is an important mediator of tissue damage in chronic arthritis.

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IDENTIFICATION OF AN INTERLEUKIN-1-LIKE PEPTIDE IN PSORIASIS

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The lesions of the inflammatory and proliferative skin disease psoriasis are characterised by neutrophil infiltrates and have been shown to contain lipid neutrophil chemoattractants, including leukotriene B_4 and 12-hydroxyeicosatetraenoic acid (Camp et al, 1985). In preliminary experiments we have demonstrated the presence of peptide chemokinetic activity which was distinct from C5a des arg on gel filtration high performance liquid chromatography (HPLC) (Cunningham et al, 1985). Further characterisation of this material has now been carried out.

An agarose microdroplet chemokinesis method (Smith & Walker, 1980) was used to compare neutrophil chemokinetic activity in 10,000g supernatants of aqueous homogenates (15 mg tissue per 0.8 ml assay buffer) of psoriatic and normal scale, obtained by abrading lesions or large areas of the skin of healthy subjects. Psoriatic scale supernatants (10,000g) were next ultrafiltered on Amicon YM30 membranes, the filtrates refiltered on YM10 membranes and the retentates and filtrates assayed for chemokinetic activity. Chemokinetic activity in the YM10 retentates was also compared with that in lipid extracts of the same psoriatic scale samples. High speed supernatants were then prepared from samples of psoriatic scale and YM30 ultrafiltrates purified on a 12.5 cm Nucleosil 5 C_{18} column eluted with a linear gradient of 0.1% trifluoroacetic acid/acetonitrile (80:20 to 20:80, by vol, over 32 min). Fractions (1 min) were evaporated and assayed for neutrophil chemokinetic activity and murine thymocyte stimulating activity (Gery et al, 1972).

Dilution-related chemokinetic activity was present in psoriatic scale supernatants, the maximum distance moved being three-fold greater than that seen with supernatants of normal scale (1.11 $\pm$ 0.07, n=9 vs 0.36 $\pm$ 0.03 mm, n=4; mean $\pm$ s.e.mean; $p<0.01$, Mann-Whitney U-test). This dilution-related chemokinetic activity was found in YM10 retentates, with little activity in the YM10 filtrates or YM30 retentates (n=4). The maximum chemokinetic movement caused by the YM10 retentates (1.06 $\pm$ 0.06 mm) was greater than that generated by lipid extracts obtained from equivalent amounts of scale (0.62 $\pm$ 0.09 mm, mean $\pm$ s.e. mean, n=3), although this activity was seen at the same dilution with both samples. Two reproducible peaks of chemokinetic activity were seen in HPLC purified psoriatic scale extracts (fractions 10-11 and 13-15, n=8). Marked thymocyte co-stimulating activity was consistently found in the second but not the first peak (n=3).

These results indicate that aqueous extracts of psoriatic scale contain greater peptide chemokinetic activity than those of normal scale, and that the molecular weight of the active material is between 10-30kd. Although corrections for recovery have not been made, peptide (>10kd) chemokinetic activity in psoriatic scale appears to be as important as that due to acidic lipids. Two chemokinetic peptides were resolved by HPLC, one of which had the thymocyte stimulating properties of interleukin-1. The identity of these compounds and their specificity to psoriasis remain to be established.

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EFFECT OF PRO-INFLAMMATROY STIMULI ON EICOSANOID BIOSYNTHESIS IN HUMAN BLOOD.

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Direct measurement of eicosanoids in human plasma may be used in the determination of the bioavailabiltiy of novel agents that inhibit eicosanoid biosynthesis. When applied ex vivo this approach takes into account the existence of biologically active metabolites, the degree of plasma protein binding, cell penetration of a drug and its ability to inhibit enzymes of intracellular origin. We previously described the measurement and characterisation of immunoreactive LTB_4 in plasma obtained from human blood incubated in the presence of A23187 (Carey and Forder, 1986) and now describe the effects of several pro-inflammatory agents on LTB_4 and TXB_2 biosynthesis. Blood was collected from drug-free donors in the presence of 100 U ml^{-1} heparin and incubated in the presence of different stimuli for 30 minutes at 37^0C. Plasma was obtained by centrifugation and measurement of LTB_4 and TXB_2 was carried out by direct RIA. A23187 (10 $\mu g ml^{-1}$) caused a time and dose-related stimulation of both TXB_2 and LTB_4 production and plasma levels of TXB_2 were 300±42 and 342±56 ng ml^{-1} and LTB_4 267±31 and 251±36 ng ml^{-1} for a group (n=8) of female and male volunteers respectively. Selective stimulation of LTB_4 was obtained by incubation with zymosan and the chemotactic peptide FMLP. Zymosan caused a statistically significant rise in basal LTB_4 levels ($p < 0.01$ Students t test) which was dose related and maximal at 300 $\mu g\ ml^{-1}$ when levels of LTB_4 were 56±4.2 ng ml^{-1}. FMLP over similar concentrations caused a dose-related increase in LTB_4 levels which were maximal at 6.7± 1.3 ng ml^{-1}. Other inflammatory stimuli eg. bradykinin, carrageenin, lipopolysaccharide and compound 48/80 had no effect on TXB_2 and LTB_4 levels in the whole blood assay. Benoxaprofen, indomethacin, aspirin and flurbiprofen caused no significant inhibition of LTB_4 but did inhibit formation of cyclo-oxygenase products. BW755c and NDGA produced dose-related inhibition of A23187 stimulated LTB_4 and TXB_2. BW755c had an IC_{50} of 1 and 0.5 $\mu g\ ml^{-1}$ and NDGA an IC_{50} of 0.5 and 20 $\mu g\ ml^{-1}$ against LTB_4 and TXB_2 respectively.

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THROMBIN INDUCES RAPID AND TRANSIENT CHANGES IN THE PERMEABILITY OF THE INTACT PLATELET PLASMA MEMBRANE TO CALCIUM.

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Resting platelets, in common with other cells, exist in an environment which produces a 10,000-fold concentration gradient of calcium from the extracellular milieu to the platelet cytosol. The aim of this study was (a) to determine the relationship of the extracellular calcium concentration ($[Ca^{++}]_e$) to agonist-induced increases in the free intracellular calcium concentration ($[Ca^{++}]_i$) and dense granule release, and (b) to quantitate the changes in the permeability of the plasma membrane to calcium following agonist stimulation. Prostacyclin washed, quin-2 (a fluorescent indicator of $[Ca^{++}]_i$)-and ^{14}C-5HT (a marker of dense granule release)-loaded human platelets were used (Poll and Westwick 1985).

Platelets suspended in hepes buffered tyrode containing 10nM; 10, 30, 100, 300μM; 3 and 10mM $[Ca^{++}]_e$ were stimulated with either sub-maximal (0.77nM) or maximal (7.7nM) human α-thrombin (HαT); the resulting peak increases in $[Ca^{++}]_i$ and ^{14}C-5HT release were determined in the same aliquot of platelets. Sub-maximal HαT-induced elevation of platelet $[Ca^{++}]_i$ and 5HT release was very dependent upon the platelet $[Ca^{++}]_e$, such that an overlapping bell-shaped $[Ca^{++}]_e$ response curve for HαT-induced $\Delta[Ca^{++}]_i$ and 5HT release was produced. That is, threshold, peak and post-peak threshold responses of increases in both $[Ca^{++}]_i$ and 5HT release occurred at $[Ca^{++}]_e$ of 10μM, 1 and 10mM respectively. However, maximal thrombin-induced elevation of platelet $[Ca^{++}]_i$, but not the dense granule release, was dependent upon the platelet $[Ca^{++}]_e$. For example, 5HT release and $\Delta[Ca^{++}]_i$ induced by 7.7nM HαT in the presence of 10nM or 1mM $[Ca^{++}]_e$ was 54±2 or 80±3% 5HT, and 80±2 or 3000nM $[Ca^{++}]_i$ respectively.

To analyse the time course of the changes in plasma membrane permeability to Ca^{++} following agonist stimulation, platelets were suspended in hepes buffered tyrode containing nominal $[Ca^{++}]_e$ ($\simeq$10μM) and were stimulated with thrombin followed by the rapid addition of 1mM Ca^{++} at 0, 5, 15, 30, 60, 120 and 240 s post HαT addition. The fluorimeter output was computer-transformed, so that a linear trace of the elevation of $[Ca^{++}]_i$ versus time was produced. Rapid and transient increases in the permeability of the plasma membrane to calcium were induced by thrombin or PAF. For example, the mean ± se of the plasma membrane calcium flux at 0, 5, 30, 60, 120 s post 7.7 nM HαT was 1±0, 600±40, 131±32, 60±15, 20±4, 5±1 and 3±2 $\Delta[Ca^{++}]_i$ nM per s respectively.

Mn^{++} has the ability to bind to quin 2 and quench the fluorescence and is thought to be transported via Ca^{++} translocating pores in other cells (Hesketh et al 1983, Hallam and Rink 1985, Almers and Palade 1981). Addition of 1mM Mn^{++} at similar time intervals post thrombin produced a quenching of quin-2-Ca^{++} complex indicating qualitatively similar time-dependent changes in the membrane permeability to Mn^{++}. In conclusion, dynamic changes in the permeability of the platelet plasma membrane to Ca^{++} are pivotal in platelet activation.

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ATP AND BRADYKININ STIMULATE INCREASED CYTOPLASMIC CALCIUM CONCENTRATION IN CULTURED PIGLET AORTIC ENDOTHELIAL CELLS.

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Several agents, including ATP, bradykinin and Ca^{2+} ionophores, are known to cause the release of PGI_2 and EDRF from cultured endothelial cells (Pearson et al; 1983, Cocks et al., 1985). To determine whether ATP and bradykinin elevate cytoplasmic free calcium, $[Ca^{2+}]_i$, endothelial cells from aortas of 6-week old piglets, were grown in culture until confluent, and then subcultured onto glass coverslips. After 2-3 days confluent monolayers of cells were loaded with the fluorescent Ca^{2+} indicator, fura2 (Tsien et al, 1985). The coverslips were then arranged vertically across the diagonal of a 1 ml cuvette in a dual-wavelength excitation fluorescence spectrofluorimeter. In the presence of 1 mM external Ca^{2+}, 10 μM ATP, a maximally effective dose, caused a rise in $[Ca^{2+}]_i$ from the resting level of near 0.1 μM to a peak reached within 5-10 seconds of between 0.7 μM and 1 μM. $[Ca^{2+}]_i$ then rapidly decreased with a half-life of 15 seconds and steadied after 2 minutes at about 0.2 μM. Addition of a second dose of ATP produced no further rise in $[Ca^{2+}]_i$. 0.3 μM ATP was the minimally effective dose to cause a detectable change in $[Ca^{2+}]_i$.

In the absence of external Ca^{2+}, with 1 mM EGTA, similar changes in $[Ca^{2+}]_i$ could be evoked but the peak levels stimulated with maximally effective concentrations of ATP were markedly smaller at between 500 nM and 700 nM and then declined to the resting level. Addition of a subsequent dose of ionomycin produced a further elevation in $[Ca^{2+}]_i$. Ionomycin, added alone in the presence of 1 mM EGTA, caused an elevation in $[Ca^{2+}]_i$ to above 1 μM which rapidly decreased again to the resting level. ATP now had no effect on $[Ca^{2+}]_i$. These results suggest that ATP can cause the partial release of Ca^{2+} from intracellular stores. The difference in peak stimulated $[Ca^{2+}]_i$ in 1 mM Ca^{2+} and Ca^{2+}-free medium suggests that under normal conditions ATP may promote some influx across the plasma membrane.

Bradykinin, 2 μM, produced similarly rapid elevations in $[Ca^{2+}]_i$ to between 700 nM and 1 μM in the presence of external Ca^{2+}, and to 500-700 nM in the absence of external Ca^{2+}. However, $[Ca^{2+}]_i$ declined slowly from the peak with a half-time of 70 seconds. Possible explanations why the $[Ca^{2+}]_i$ response to ATP is short-lived are that its half-life at the cell suface is very short due to ectonucleotidase activity, or that the P_2-receptor is rapidly desensitised. As a second addition of ATP is without effect yet bradykinin can cause a further rise in $[Ca^{2+}]_i$, some form of desensitisation appears likely.

50-100 mM K^+ had no effect on the resting level of $[Ca^{2+}]_i$ and had no effect on the $[Ca^{2+}]_i$ responses stimulated by ATP or bradykinin. These results suggest that ATP and bradykinin can cause an increase in $[Ca^{2+}]_i$ mainly by discharge of Ca^{2+} from an intracellular membrane-bounded pool with some influx across the plasma membrane, though probably not through voltage-operated channels. The results are consistent with the premise that ATP and bradykinin act to cause the release and/or the formation of PGI_2 and EDRF by elevating $[Ca^{2+}]_i$.

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NICOTINE-STIMULATED CATECHOLAMINE RELEASE FROM BOVINE ADRENAL CHROMAFFIN CELLS IS NOT STEREOSPECIFICALLY BLOCKED BY NICARDIPINE.

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The mechanism by which acetylcholine (or nicotine) causes calcium influx and stimulus-secretion coupling in adrenal chromaffin cells is not clear. Release of catecholamines in response to nicotine is not inhibited by tetrodotoxin (Kilpatrick et al. 1981) indicating that calcium channels are not opened by sodium channel propagated depolarisation. The requirement for external sodium is controversial. Little information is available on the role of dihydropyridine-sensitive voltage-sensitive calcium channels. An earlier report (Cena et al. 1983) describes inhibition of nicotine-stimulated release by the dihydropyridine calcium channel blocker nitrendipine, but with an IC_{50} considerably higher than when release was stimulated by high potassium.

In the present study, we have investigated the inhibition of potassium and nicotine-stimulated release of endogenous catecholamines by stereoisomers of nicardipine.

We used primary cultures of bovine adrenal chromaffin cells maintained as monolayers in tissue culture multiwell dishes. Cells were used after 3-7 days in culture. Release studies were at 37^{o}, with nicardipine present as appropriate during an 8 min preincubation period as well as during the 3 min stimulation of release period in the presence of either 3×10^{-5} M nicotine or 50 mM potassium.

We constructed dose-response curves to (+)-nicardipine and (-)-nicardipine and monitored release of endogenous noradrenaline and adrenaline by HPLC and electrochemical detection. For both these amines, nicardipine potently and stereospecifically inhibited the response to 50 mM potassium. Release by 50 mM potassium in the presence of 10^{-7} M (+)-nicardipine was 23 ± 3% of control for noradrenaline and 30 ± 2% for adrenaline (mean ± s.e.m., n = 4), with IC_{50} values of approx. 4×10^{-8} M and 2×10^{-8} M. However, when release was stimulated by nicotine, no high potency inhibition by (+)-nicardipine was observed, with little difference between the stereoisomers. Release by nicotine in the presence of 10^{-7} M (+)-nicardipine was 100 ± 2% (noradrenaline) and 106 ± 4% (adrenaline).

At 3×10^{-5} M nicotine, release of catecholamines is maximal. To investigate dihydropyridine sensitivity further, dose-response curves to nicotine were constructed in the presence and absence of 10^{-7} M (+)-nicardipine. The presence of the calcium channel antagonist produced a significant reduction in catecholamine release at submaximal concentration of nicotine, resulting in a small shift in the curve (EC_{50} of $4.1 \pm 0.3 \times 10^{-6}$ M nicotine in the absence of nicardipine compared to $6.1 \pm 0.6 \times 10^{-6}$ M in the presence of nicardipine).

In conclusion, functional dihydropyridine-sensitive calcium channels are not required for maximal stimulus secretion coupling by bovine chromaffin cells in response to nicotine. We suggest therefore, that calcium either enters via voltage-sensitive calcium channels which are not sensitive to dihydropyridine calcium channel antagonists, or that calcium enters the cell directly through the acetylcholine receptor channel.

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ISCHAEMIA-INDUCED FALLS IN BRAIN DOPAMINE AND ITS PROTECTION BY NICARDIPINE, A CALCIUM ANTAGONIST.

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Ligation of one common carotid artery of the Mongolian gerbil, Meriones unguiculatus, has pronounced effects upon cerebral oxidative metabolism and energy balance resulting in unilateral infarction (Levine and Payan, 1966). We reported previously that, based upon neuroanatomical observation of the circulus arteriosus, a given population of gerbils could be classified as "stroke prone" and "stroke resistant" and that only "stroke prone" animals experience an ipsilateral decrease in corpus striatal dopamine following cerebral ischaemia (Alps et al, 1984). This study examined the effect of pretreatment with nicardipine and nimodipine (type 1) and flunarizine (type 3) calcium antagonists (Spedding & Middlemiss, 1985) on the ischaemia-induced fall in dopamine in "stroke prone" and "stroke resistant" animals.

One hundred and sixty four male gerbils (60 - 80 g) were pretreated i.p. with 50 $\mu g.kg^{-1}$ nicardipine or nimodipine, 100 $\mu g.kg^{-1}$ flunarizine or saline, 30 min before being anaesthetised with 6 mg pentobarbital. The right common carotid artery was exposed in the paratracheal region. The artery was dissected free and ligated. In sham operated controls the carotid artery was exposed and ligatures put in place but not tied. After 3 h the animals were decapitated, the corpus striata separated into left and right hemispheres and stored under liquid nitrogen until analysed for dopamine content by high performance liquid chromatography coupled to electrochemical detection (Alps et al, 1985).

Table 1 Effect of calcium antagonists on ischaemia-induced ipsilateral decrease in dopamine

		Dopamine ($\mu g.g^{-1}$)				
		"stroke prone"			"stroke resistant"	
Treatment	n	non-occluded striatum	occluded striatum	n	non-occluded striatum	occluded striatum
Control	11	1.31 ± 0.14	1.27 ± 0.14	21	1.30 ± 0.11	1.33 ± 0.11
Ligated	12	1.52 ± 0.18	0.57 ± 0.14	23	1.41 ± 0.09	1.43 ± 0.07
Nicardipine	13	1.47 ± 0.17	0.91 ± 0.10*	20	1.33 ± 0.14	1.30 ± 0.14
Flunarizine	12	1.46 ± 0.15	0.90 ± 0.07*	18	1.33 ± 0.10	1.25 ± 0.12
Nimodipine	14	2.79 ± 0.44**	1.02 ± 0.10**	20	1.15 ± 0.07*	1.16 ± 0.07**

Statistical significance relative to ligated group: *$p < 0.05$ **$p < 0.01$

Pretreatment with nicardipine, flunarizine and nimodipine reduced significantly the ipsilateral decrease in corpus striatal dopamine that occurs as a consequence of cerebral ischaemia in "stroke prone" animals. Nimodipine, in contrast to nicardipine and flunarizine, enhanced significantly the level of dopamine in the non-occluded hemisphere of "stroke prone" animals. The significant protection of dopamine by nicardipine in this model is of functional importance in relation to the marked neuro-cytoprotection observed in vivo in rats (Alps & Hass, 1985) and gerbils (Alps et al, 1985) subjected to forebrain ischaemia. It must be considered that nicardipine may be of benefit in the treatment of diseases occurring as a result of cerebral ischaemia.

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THE EFFECT OF NICARDIPINE ON "DELAYED NEURONAL DEATH" IN THE ISCHAEMIC GERBIL HIPPOCAMPUS.

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The use of the Mongolian gerbil (Meriones unguiculatus) in studies on cerebral ischaemia and infarction is due to its lack of communicating arteries between the carotid and the vertebro-basilar circulation (Kahn, 1972). The exceptional susceptibility of the hippocampal CA1 neurones following 5 min transient bilateral occlusion of the common carotid circulation is well-documented (Kirino, 1982).

The aim of this study was to investigate the cytoprotective activity of the calcium entry blocker, nicardipine, on delayed death of the CA1 neurones.

Mongolian gerbils weighing 50 - 70 g were anaesthetised with 5% halothane in a 70% nitrous oxide/30% oxygen gas mixture. After induction the halothane was decreased to about 1.5% for the remainder of the experiment. The left and right common carotid arteries were exposed through a paratracheal incision and simultaneously occluded with a microvascular clamp. Both clamps were removed after 5 min and the wound dusted with antibiotic powder and surgically closed. Nicardipine was injected i.p. at 500 $\mu g.kg^{-1}$ 15 min prior to ischaemia and repeated twice daily during a 72 h survival period. At this point the gerbils were reanaesthetised and the brains perfuse-fixed in situ, by intra cardiac injection of 10% buffered formalin. The heads were removed and stored overnight in fresh formalin at 4°C. The brains were then removed to fresh formalin and fixed for one week prior to wax embedding, sectioning, and staining with cresyl fast violet.

Mean percent neuronal death in the hippocampal CA1 subfield was determined from a count made in five fields per hemisphere, extending from the paramedian area to the junction with the CA2 neurones (n = 10 areas per animal).

Highly selective vulnerability of the CA1 neurones was confirmed by light microscopy in untreated control animals. For 8 controls (80 observations) the percent neuronal death was 78.0 ± 4.2%, whereas for 8 nicardipine treated animals the value was 43.8 ± 4.7% ($p < 0.001$ vs control by Student's t-test).

The neuronal cytoprotective effect of nicardipine against cerebral ischaemia in a new model of rat 4 vessel occlusion has been previously reported (Alps & Hass, 1985). The present results lend further support to the theory that rapid intracellular influx of calcium may be the most critical event leading ultimately to cell death (Hass, 1981). This hypothesis strengthens the concept of treating cerebral ischaemia with calcium entry blocking agents such as nicardipine.

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DISSIMILARITIES BETWEEN THE EFFECTS OF VERAPAMIL AND HYPOXIA ON RAT ARTERIES IN VITRO.

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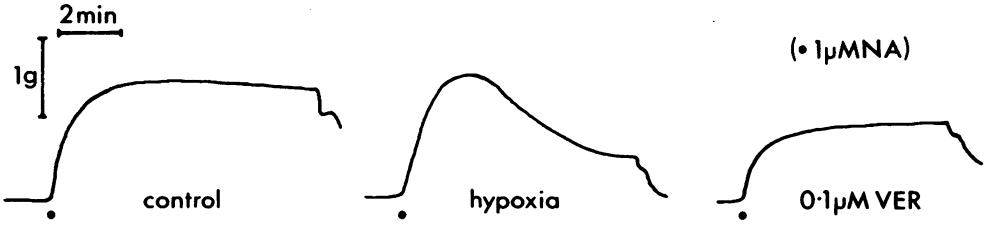
Ebeigbe (1982) has suggested that a decrease in calcium entry may be a mechanism for hypoxic relaxation of rabbit aortic muscle. We have investigated this hypothesis in the rat by comparing the effects of the calcium entry blocker verapamil (VER) with the effects of hypoxia in rat aorta (AO), superior mesenteric artery (MA) and rat femoral artery (FA). All preparations were obtained from male Wistar rats (180-250g) killed by cervical dislocation. 5-7mm circular preparations were mounted in organ baths containing Krebs' solution ($CaCl_2$ 2.5mM) gassed initially with 5% CO_2 in O_2 maintained at 37°C. The resting tension applied to vessels was AO 3g;MA + FA 1.5g. Following a 1hr equilibration period reproducible responses to NA (1μM) were obtained (normoxic response : pO_2 = 395 mmHg). After washout and recovery tissues were gassed with 5% CO_2 in N_2 for 30 min and again contracted with NA (1μM) (hypoxic response : pO_2 = 63 mmHg). In a separate series of experiments the effect of increasing concentrations of VER (10^{-9}-10^{-5}M) on the response of each type of vessel to NA was studied. Results are expressed as percent inhibition of the response to NA by hypoxia or VER.

In all three types of vessel VER caused a dose dependent inhibition of the NA induced contraction. FA was most sensitive to VER with a maximum inhibition by VER (10^{-5}M) of 95 ± 0.9% n=12. RA was least sensitive to VER with a maximum inhibition by 10^{-5}M VER of 51.1 ± 8.4% (n=12). The sensitivity of MA was found to be intermediate between AO and FA with a maximum inhibition of 64.5 + 3.4% (n=6). However, when comparing the effect of hypoxia it was found that AO was most sensitive with a 39.7 + 6.5% (n=10) reduction in hypoxia. Hypoxic responses of FA to NA were reduced by 30.0 + 7.4% (n=19) and of MA by 5.6 + 4.7% (n=15). So whilst VER sensitivity lies in the order FA>MA>AO sensitivity to hypoxia lies in the order AO>FA>MA. Furthermore the responses of FA to NA (1μM) in hypoxia were not maintained and this change in time course of the response of FA to hypoxia was not mimicked by VER (see Figure 1).

Fig 1. Representative traces showing effects of 30min hypoxia and VER (0.1μM) on NA (1μM) induced contactions of femoral artery.



These results suggest that there is no correlation between sensitivity of individual arteries to VER and their susceptibility to hypoxia. Furthermore the difference in the time course of the effects of VER and hypoxia on FA suggests that the effects of hypoxia on isolated rat arteries cannot be wholly attributed to a decrease in verapamil sensitive calcium entry.

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ACTION POTENTIAL CONDUCTION AND TRANSMITTER RELEASE FROM NON-ADRENERGIC, NON-CHOLINERGIC NERVES: EFFECTS OF Ca^{2+}-ANTAGONISTS

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Organic Ca^{2+}-entry blockers e.g. verapamil, diltiazem and amlodipine, inhibit action potential (AP) conduction in sympathetic nerves. They do this at high concentrations (approximately 10^{-4}M), by a local anaesthetic effect and so prevent transmitter release from these nerves (Beattie et al., 1985). Inorganic Ca^{2+}-entry blockers e.g. cobalt and manganese, however, prevent transmitter release in concentrations which leave AP conduction unaffected, by preventing Ca^{2+}-entry at nerve terminals.

The present communication extends these observations to non-adrenergic, non-cholinergic (NANC) nerves, the transmitter from which, in the tissues studied, hyperpolarizes the membrane and relaxes the muscle. Two tissues were selected for study using verapamil, nifedipine and cobalt. First the effects of Ca^{2+}-entry blockers on AP conduction were measured (Beattie et al., 1984) using the rat anococcygeus extrinsic nerve preparation (McKirdy & Muir, 1976). These nerves contain separate adrenergic and NANC fibres (Gibson & Gillespie, 1973). To destroy adrenergic nerves, rats were pretreated (daily, i.p.) with guanethidine (30 mg/kg for 4 weeks) and reserpine (1 mg/kg for 2 days). The destruction of adrenergic nerves was confirmed by Falck histochemistry (Gillespie & Muir, 1970). Secondly, the effect of Ca^{2+}-entry blockers was assessed on transmitter release, by measuring inhibitory junction potentials (ijps) in the guinea-pig internal anal sphincter (gpias) in the presence of phentolamine, guanethidine and atropine (each 1 x 10^{-6}M). Verapamil (3-5 x 10^{-5}M) abolished AP conduction, while nifedipine (1 x 10^{-4}M) and cobalt (3 x 10^{-3}M) reduced the amplitude and number of APs in the anococcygeus nerves. Verapamil (1 x 10^{-4}M) and nifedipine (5 x 10^{-4}M) each inhibited ijps in the gpias, indicating that transmitter release was inhibited. Lower concentrations (1-5 x 10^{-6}M) of each inhibited the spontaneous spike discharge of the sphincter and abolished tone. Cobalt (1 x 10^{-3}M) inhibited ijps, spontaneous spikes and tone in the ias.

Ca^{2+}-entry blockers clearly inhibit AP conduction and transmitter release from NANC nerves. As in the guinea-pig vas deferens, higher concentrations are required to inhibit presynaptic than postsynaptic events i.e. spike discharge and tone. Compared with their effects on the sympathetic nerves, verapamil and cobalt were more potent inhibitors of AP conduction in the anococcygeus nerves. Nifedipine, ineffective in the vas deferens (Beattie et al., 1984; 1985), inhibited both APs in the anococcygeus nerves and NANC transmitter release in the gpias. The presynaptic activity of organic Ca^{2+}-entry blockers is unlikely to be important therapeutically since the doses required are high compared with those required to block postsynaptic events in muscle. Cobalt inhibits both presynaptically (transmitter release) and postsynaptically (spontaneous spike discharge and tone) at equal concentrations by blocking Ca^{2+} channels. The ability to block Ca^{2+} channels presynaptically offers the possibility of developing new Ca^{2+}-entry blockers which preferentially affect nerves.

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THE EFFECT OF PERTUSSIS TOXIN ON AGONIST-INDUCED PHOSPHOINOSITIDE HYDROLYSIS AND CHANGES IN CYTOSOLIC FREE Ca^{2+} IN HUMAN NEUTROPHILS

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Neutrophil responsiveness may be regulated by the actions and interactions of at least two second messenger molecules; namely cytosolic free calcium ($[Ca^{2+}]i$) and 1,2 Diacylglycerol (DAG) (Di Virgilio et al, 1984) that are produced as a consequence of receptor mediated phosphoinositide hydrolysis (Nishizuka, 1984). However, recent studies have indicated that the arachidonic acid metabolite, leukotriene B_4 (LTB_4), unlike the chemotactic tripeptide, formylmethionylleucylphenylalanine (FMLP) and the ether lipid, platelet activating factor (PAF), may induce the activation of rabbit (Volpi et al, 1984) and human (MacIntyre & Rossi, 1985) neutrophils independently of phosphoinositide hydrolysis. Although the role of guanine nucleotide binding proteins (G proteins) in the adenylate cyclase system is well established, there is conflicting evidence concerning their involvement in the regulation of agonist-induced changes in $[Ca^{2+}]i$ and phosphoinositide hydrolysis (Joseph, 1985). Pertussis toxin inactivates the inhibitory guanine nucleotide binding protein, Ni, which is linked to receptor mediated inhibition of adenylate cyclase. In the present study we examined the effects of pertussis toxin on phosphoinositide metabolism and changes in $[Ca^{2+}]i$ in human neutrophils elicited by FMLP, LTB_4 and PAF.

Human neutrophils were prepared by gelatin sedimentation followed by hypotonic lysis of contaminating erythrocytes. $[Ca^{2+}]i$ was determined, directly, by using the fluorescent indicator dye, quin 2 (White et al, 1983) and phosphoinositide hydrolysis was monitored, indirectly, as changes in $[^{32}P]$-phosphatidate (PtdA) in cells prelabelled with $[^{32}P]$-orthophosphate (Dougherty et al, 1984).

FMLP (0.05 - 50 nM), PAF (0.1 - 100 nM) and LTB_4 (0.025 - 2.5 mM) induced a rapid concentration dependent elevation of $[Ca^{2+}]i$ from the basal value of 133 ± 6 nM (mean ± S.E., n = 20) to around 250 - 500 nM in different experiments using neutrophils from different donors. FMLP (1 - 1000 nM) and PAF (1.8 - 1800 nM), but not LTB_4 (< 2.5 μM) induced concentration dependent $[^{32}P]$-PtdA formation. Pretreatment of neutrophils (10^8 cells/ml) with pertussis toxin (15 μg/ml, 2 hours, 37°C) inhibited FMLP, PAF and LTB_4 induced elevation of $[Ca^{2+}]i$ and also inhibited FMLP and PAF induced $[^{32}P]$-PtdA formation.

These results indicate that a pertussis toxin sensitive process, presumably a guanine nucleotide regulatory binding protein is involved, either directly or indirectly, in the regulation of agonist induced phosphoinositide hydrolysis and changes in $[Ca^{2+}]i$.

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ACTIVATORS OF PROTEIN KINASE C INHIBIT α_1-ADRENOCEPTOR RESPONSES IN RAT PINEALOCYTES

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α_1- and β-Adrenoceptors are important in the physiological regulation of the pineal gland (Klein, 1985). α_1-Adrenergic activation amplifies (20- to 100-fold) β-adrenergic stimulation of intracellular cAMP and cGMP (Vanecek et al., 1985); alone α_1-adrenergic agonists have no effect on cyclic nucleotides. α_1-Adrenergic potentiation of the cAMP response, but not that of cGMP, appears to be mediated by protein kinase C(PKC) because activators of the enzyme, phorbol esters and a synthetic diacylglycerol, mimic the effect α_1-agonists have on cAMP and PKC (Sugden et al., 1985). α_1-Adrenoceptor activation also increases phosphatidylinositol(PI) hydrolysis and elevates cytosolic Ca^{++}($[Ca^{++}]i$) by enhancing Ca^{++} influx through a ligand-dependent Ca^{++} channel. PKC activation has been reported to desensitize hepatocyte α_1-adrenergic responses (Lynch et al., 1985); thus it was of interest to investigate whether activators of PKC alter pinealocyte α_1-adrenergic responses.

Dispersed rat pinealocytes were incubated (37°C, 95% air/5% CO_2) for 24 h before testing. Cyclic nucleotides were determined by radioimmunoassay, $[Ca^{++}]i$ by measuring Quin-2 fluoresence, and PI hydrolysis by measuring the production of [^{3}H]inositol monophosphate([^{3}H]IP) by [^{3}H]inositol-labelled cells.

The noradrenaline (NA, 10 μM) stimulation (4- to 5- fold) of [^{3}H]IP formation (Zatz, 1985) was inhibited by 4-β-phorbol 12-myristate 13-acetate (PMA, IC_{50} ~1 nM) and 4-β-phorbol 12,13-dibutyrate (PDBu, 100 nM). Phorbol(1 μM), the parent compound, had no effect. Synthetic diacylglycerols (50 μg/ml), 1-oleoyl, 2-acetyl glycerol and 1,2-dioctanoyl glycerol, also inhibited the [^{3}H]IP response; diolein did not. Similarly, the adrenergic elevation of $[Ca^{++}]i$ was prevented by prior treatment(5 min) with PMA (10 nM), which also rapidly (<2 min) reversed the increase in $[Ca^{++}]i$ produced by NA (1 μM). PMA (IC_{50} ~6 nM) and PDBu (IC_{50} ~35 nM) also inhibited the cGMP response to NA (1 μM), but not to isoproterenol (1 μM). This indicates the α_1-component of NA stimulation of cGMP was inhibited. Under these conditions the cAMP response in the same cells was unaltered, possibly because phorbol esters and the related compounds activate PKC directly and thus potentiate β-adrenergic stimulation of cAMP by a mechanism not involving α_1-adrenoceptors.

These findings suggest that PKC activation desensitizes α_1-adrenergic responses. This, supported by the finding that α_1-adrenergic stimulation activates PKC (Sugden et al., 1985), indicates that PKC may be physiologically involved in homologous desensitization of α_1-adrenergic responses in pinealocytes by acting on an early step in signal transduction.

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EFFECTS OF LITHIUM TREATMENT ON AGONIST-STIMULATED INOSITOL PHOSPHOLIPID HYDROLYSIS IN RAT BRAIN SLICES

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Lithium salts are widely employed as an effective treatment for bipolar affective disorders but their mode of action are unclear. Recently, the ability of Li^+ to inhibit myo-inositol-1-phosphate phosphatase has enabled the development of a sensitive assay for phospholipase C-mediated inositol phospholipid hydrolysis (Berridge et al. 1982), and has prompted the suggestion that alterations in the phosphoinositide cycle may be the basis of Li^+s psychotropic effects. The present experiments describe the acute and chronic effects of Li^+ on agonist-mediated phosphoinositide breakdown in rat brain slices.

For acute experiments, male Sprague-Dawley rats, approx. 250 g, were injected with 6.75 m Equiv/Kg LiCl i.p. and killed 18 h later. In chronic studies rats were fed a diet consisting of 2.3 g Li_2CO_3, 1.5 Kg rat food and 2 L H_2O for 2 weeks. Animals were then killed with or without an 18 h Li^+-free period (Li^+ concentrations in cerebral cortex, measured by flame photometry, were: acute - 1.4 ± 0.2 (m Equiv/Kg wet wt); chronic - 0.4 ± 0.04; chronic (withdrawn) - 0.2 ± 0.02). Cerebral cortex slices were then prepared, preincubated with ^{3}H-inositol in the presence of 5 mM LiCl and water-soluble ^{3}H-inositol phosphates extracted by anion-exchange chromatography (Brown et al. 1984).

After acute treatment, ^{3}H-inositol phosphate accumulating in the presence of 100 μM carbachol was reduced to 63 ± 7% of the untreated level. Stimulation by noradrenaline, KCl, histamine and 5HT was not significantly influenced. When slices were preincubated for 30 min in the presence of 2.5 mM unlabelled myo-inositol, in an effort to reverse the depletion of endogenous inositol induced by Li^+ (Allison & Stewart, 1971), the effects of acute LiCl treatment were enhanced. Stimulation by carbachol (10^{-4} M) now represented 31 ± 4% of the response in untreated tissue; KCl (31 mM), 35 ± 5%; histamine (3 x 10^{-4} M), 67 ± 8%; 5HT (3 x 10^{-4} M), 44 ± 5%. The response to noradrenaline (10^{-4} M) was unaffected.

In the absence of a drug-free period, chronic Li^+ treatment reduced carbachol (10^{-4} M) stimulated ^{3}H-inositol phosphate accumulation to 52 ± 9% and 40 ± 4% respectively in the absence and presence of an unlabelled myo-inositol pre-incubation. A reduction in the noradrenaline (10^{-4} M) response (77 ± 2% of control) could only be seen after preincubation with myo-inositol. Dose-response curves revealed that the reduction in the response to carbachol was due to a fall in E_{max} with no change in EC_{50}. When animals were withdrawn from chronic treatment for 18 h prior to death, the responses to carbachol were enhanced (235 ± 33%) with respect to untreated controls. Neither acute nor chronic Li^+ treatments significantly affected the overall incorporation of ^{3}H-inositol into phospholipids.

It appears therefore, that treatment with lithium reduces receptor-mediated inositol phospholipid hydrolysis in rat cerebral cortex slices and that this effect is maintained on chronic treatment. It remains to be seen if this property of lithium is involved in its therapeutic mode of action.

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POTENTIATION OF H_1-RECEPTOR-MEDIATED INOSITOL PHOSPHOLIPID AND CYCLIC AMP RESPONSES IN GUINEA PIG BRAIN BY 1,4-DITHIOTHREITOL

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We have previously reported that the disulphide reducing agent 1,4-dithiothreitol (DTT) selectively potentiates histamine H_1-receptor-mediated contractile activity in the longitudinal smooth muscle of guinea-pig ileum (Donaldson & Hill, 1985a, 1986). We have now extended these studies to investigate the effect of this agent on two H_1-receptor-mediated biochemical responses in guinea-pig cerebral cortex and cerebellum, namely accumulation of ^{3}H-inositol phosphates (Daum et al., 1984; Donaldson & Hill, 1985b) and the augmentation of adenosine-stimulated cyclic AMP accumulation (Hill et al., 1981).

Accumulation of cyclic AMP (Hill et al., 1981) and ^{3}H-inositol phosphates (Donaldson & Hill, 1985b) was measured essentially as described previously, except that acid extraction (10% perchloric acid w/v) was used to improve the recovery of inositol phosphates (Donaldson & Hill, 1985c). DTT (1 mM) was added 30 min prior to the addition of agonists and the incubations continued for 10 min (cyclic AMP accumulation) or 45 min (inositol phosphate accumulation) in the continued presence of DTT.

Incubation of tissue slices with DTT resulted in a parallel shift of the dose-response curve for histamine stimulated ^{3}H-inositol phosphate accumulation to lower agonist concentrations, in slice preparations of guinea-pig cerebellum (6.0±1.2 fold, 5 experiments; $p<0.005$, analysis of variance according to Delean et al., 1978) and cerebral cortex (6.3±1.2 fold, 3 experiments, $p<0.005$). DTT similarly potentiated histamine stimulated cyclic AMP accumulation in cerebral cortical slices (13.2±3.7 fold sinistral shift, 4 experiments, $p<0.005$) without affecting the basal response to adenosine (0.1 mM). Interestingly the maximal stimulation of ^{3}H-inositol phosphate accumulation elicited by the histamine analogue 2-methylhistamine, which appears to behave as a partial agonist in guinea-pig cerebral cortex, was increased from 33.6±3.7 to 81.9±10.9% ($p<0.005$) of the response to 1 mM histamine (3 experiments) by DTT, without any significant effect on the EC_{50} value.

These results indicate that DTT can potentiate H_1-receptor mediated responses in brain tissues and suggest that this disulphide reducing agent may increase the efficacy of H_1-agonists in addition to any effect on agonist-binding affinity.

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EFFECT OF 1,4-DITHIOTHREITOL ON [^{3}H]-MEPYRAMINE BINDING TO HOMOGENATES OF GUINEA PIG CEREBELLUM AND CEREBRAL CORTEX

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1,4-dithiothreitol (DTT), an agent which reduces disulphide bonds to sulphydryl groups, has been widely used in receptor studies to establish the presence and functional importance of disulphide bonds in receptor structure (Hollenburg, 1985). We have previously reported that DTT potentiates histamine H_1-receptor-mediated responses in guinea-pig ileal smooth muscle (Donaldson & Hill, 1985; 1986a) and brain (Donaldson & Hill, 1986b). This alteration in H_1-receptor activity prompted us to investigate the effect of DTT on the binding characteristics of the H_1-selective ligand, ^{3}H-mepyramine (Hill et al., 1978), in homogenates of guinea-pig cerebral cortex and cerebellum.

^{3}H-mepyramine binding was measured in 50 mM Na-K phosphate buffer, pH 7.4, to membrane fractions of guinea-pig cerebellum and cerebral cortex prepared essentially as described previously (Hadfield et al., 1983). Freshly prepared membranes were incubated for 20 min at 37°C in the presence or absence of DTT (1 mM) before the addition of ^{3}H-mepyramine and, where appropriate, competing ligand. Incubations were then continued for a further 35 min, terminated by the addition of ice-cold buffer containing 1 µM non-radioactive mepyramine (Daum et al., 1982) and filtered through Whatman GF/B filters. The levels of non-specific binding was defined as that insensitive to inhibition by 2 µM promethazine.

Studies of the binding of different concentrations of ^{3}H-mepyramine (0.5-10.0 nM) to brain homogenates showed that DTT had no effect on either the equilibrium dissociation constant (K_D) or specific binding site capacity. The lack of effect on K_D was confirmed in studies of the inhibition of the specific binding of 1 nM ^{3}H-mepyramine by non-radioactive mepyramine. However, in both cerebellar and cerebral cortical membranes DTT shifted the curve for the inhibition of ^{3}H-mepyramine binding (1 nM) by histamine to lower agonist concentrations (cerebellum-IC_{50} = 81±7 & 18±2 µM, $P<0.005$, analysis of variance according to Delean et al., 1978; cortex-IC_{50} = 57±9 & 11±2 µM, $P<0.005$; in the absence and presence of DTT respectively) and lowered the Hill coefficient from 0.96±0.07 to 0.69±0.04 (cerebellum, $P<0.01$) and 0.90±0.11 to 0.72±0.08 (cortex). Analysis of these histamine inhibition curves as double hyperbolae revealed two binding sites in the presence of DTT and only one, low affinity site, in the absence of DTT.

These results show that DTT affects agonist but not antagonist binding to high affinity ^{3}H-mepyramine binding sites in guinea-pig brain and suggest that DTT may stabilise a proportion of the agonist binding sites in a high affinity state.

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RANITIDINE AND FAMOTIDINE DIFFER IN THEIR INTERACTION WITH THE PARIETAL CELL HISTAMINE H_2-RECEPTOR

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After the discovery of burimamide as the first compound to block the histamine-mediated acid response of the parietal cell numerous compound sharing these properties were developed. They are referred to as histamine H_2-receptor antagonists, suggesting a homogeneity in the formal type of interaction of these compounds with the histamine H_2-receptor. Recently it was reported, that one of the newer histamine H_2-receptor antagonists, famotidine, inhibits the dimaprit-mediated chronotropic response of the guinea-pig isolated atrium in an unsurmountable manner (Pendleton, et al., 1983). Therefore a study was designed to find out whether or not ranitidine and famotidine would differ in their interaction with the guinea-pig parietal cell histamine H_2-receptor.

Guinea-pig parietal cells were isolated and enriched up to approximately 70 - 75% by the elutriation technique originally described by Soll (1978). Cell viability was tested with the Trypan blue exclusion technique and found to be >95%. H^+ secretion in response to histamine (0.1 - 1000 µmol/l) in suspensions of 10^6 cells was measured by the ^{14}C-aminopyrine uptake technique developed by Berglindh et al., (1976) and modified in our laboratory (Sewing, et al., 1983) in the absence and presence of ranitidine (1 - 100 µmol/l) or famotidine (0.1 - 10 µmol/l). The transformation of the concentration-response curves according to Arunlakshana and Schild (1959) was used to determine the formal nature of the antagonists' action and their pA_2-values. Furthermore 10^6 cells were exposed for 30 min at room temperature to 30 µmol/l ranitidine or 10 µmol/l famotidine, washed three times in an antagonist-free buffer, and subsequently stimulated to produce H^+ by 0.1 - 1000 µmol/l histamine to find out whether the inhibition was reversible by washing.

Ranitidine and famotidine shifted the histamine concentration-response curve of histamine towards the right in a concentration dependent manner. The Schild-plot revealed a slope of 1.03 ± 0.02 and a pA_2-value of 6.83 ± 0.01 for ranitidine (N = 3) and a slope of 1.29 ± 0.04 and a pA_2-value of 7.58 ± 0.08 for famotidine (N = 4). The slope of the Schild-plot for famotidine was significantly greater than 1, that of the Schild-plot for ranitidine was not. The inhibitory effect of ranitidine was fully reversible by washing, that of famotidine was only partially reversible.

From this study it is concluded that ranitidine - as in all other relevant systems - interacts with the guinea-pig parietal cell histamine H_2-receptor in a competitive fashion. In this system famotidine reacts as a noncompetitive and partially unsurmountable histamine H_2-receptor antagonist and behaves identically as on the dimaprit-mediated chronotropic response of the guinea-pig atrium. The nature of the interaction of famotidine with the histamine H_2-receptor is unknown.

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NEW CHEMICAL PROBES FOR THE H_2 RECEPTOR

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Black et al. (1972) showed that varying the position of a methyl substituent on histamine imidazole ring was critical to achieve selectivity for H_1 or H_2 receptor subtypes. This finding provided a valuable basis for the development of a series of H_2 antagonists, e.g. metiamide and cimetidine, all containing a 4-methyl substituted imidazole ring, a moiety seemingly crucial for H_2-receptor recognition. Recently, we described a class of H_2 selective antagonists, of which mifentidine is a representative compound, characterized by a semirigid conformation in which an amidino group is linked to an imidazole through a phenyl ring. We postulated that receptor recognition occurs through the amidine moiety, the imidazole ring providing only additional attachment (Donetti et al., 1984). Thus, considering the ionization properties, the imidazoles in the mifentidine and in the cimetidine series would play a different role.

To test this hypothesis we studied the consequences of methyl substitution on mifentidine and cimetidine imidazoles. The results on antagonist affinity for H_2 receptors mediating positive chronotropic effect in guinea pig atria are summarized in Table 1.

Table 1 Effect of methyl substituents on mifentidine and cimetidine imidazoles

methyl position	K_B (nM) mifentidine analogues	cimetidine analogues
-	21 (mifentidine)	1400*
2	52	58000
4	980	365 (cimetidine)

* from Durant, G. J. et al. (1977) J. Med. Chem. 20, 901-6

Optimum affinity in the cimetidine series is observed introducing the methyl group in 4 position, while occupancy of the 2 position results in a dramatic loss of affinity. Conversely, in the mifentidine series, 4 substitution is the least favourable, while absence or presence of the radical in 2 position does not ensue in significant affinity change. The H_2 site appears to recognize primarily the N-C-N group both in its linear (formamidine in mifentidine) or cyclized (imidazole in cimetidine) form.

These results suggest that the imidazole of mifentidine behaves differently from that of cimetidine in respect of the H_2-receptor site.

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STEPWISE INCREASE IN HISTAMINE CONCENTRATION CAUSES A PROTRACTED MICROVASCULAR LEAKAGE.

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Intravital microscopy of the hamster cheek pouch has shown that histamine stimulation for 5 min results in a shortlasting permeability increase and tachyphylaxis to repeated stimulation at intervals (<30 min) (Svensjö and Joyner 1984). Continuous stimulation with histamine also results in a shortlasting increase in leakage of macromolecules from postcapillary venules. Now we have exposed the hamster cheek pouch to increasing conc. of histamine. In some experiments terbutaline was present during the histamine stimulation.

The cheek pouch of pentobarbital anesthetized hamster was prepared for intravital microscopy and fluorescein labelled dextran (FITC-dextran 150 KD) was injected i.v. Svensjö et al. (1978). The FITC-dextran conc. of the cheek pouch superfusate was measured and used to quantitate permeability changes in parallel to intravital observations on venular leaky sites as indicated by fluorescent spots. The cheek pouch was exposed to histamine 2×10^{-6}M resulting in a reversible increase in the number of leaky sites and FITC-dextran conc. of the superfusate. Thirty minutes later the preparation was exposed to stepwise doublings of histamine conc. from $0.5\text{-}64 \times 10^{-6}$M and each lasting for 10 min except the highest conc. which was given for 60 min. Mean results from eleven animals are shown in Table 1. For each histamine conc. there was a reversible increase in number of venular leaks until the conc. reached 8×10^{-6}M. However, the FITC-dextran exudation continued to rise until 64×10^{-6}M. At this stage both the number of leaks and FITC-dextran conc. in the artificial lymph declined despite the fact that the preparation was exposed to the very high histamine conc.

Table 1. Mean maximal number of leaky sites/cm^2 cheek pouch (L) and FITC-dextran efflux ng/10 min (E) following histamine (n=7) and after terbutaline and histamine stimulation (n=4).

	Histamine conc. x 10^{-6}M											
	2	0.5	1.0	2.0	4.0	8	16	32	64	64	64	64
HIST (L)	229	45	107	174	217	212	201	166	146	96	61	51
HIST (E)	296	358	317	448	888	1162	1434	1535	1735	1583	1507	1375
HIST+TERB (L)	219	0	10	7	15	17	21	21	7	0	0	0
MINUTES	0	30	40	50	60	70	80	90	100	110	120	130

The response to histamine 2×10^{-6}M prior to terbutaline treatment was not different from that seen in the histamine group. 30 min local application of terbutaline 10^{-6}M resulted in a 90% reduced response to histamine. Even at the highest histamine conc. the terbutaline inhibition was unchanged.

Conclusion. The short term tachyphylaxis to inflammatory mediators like histamine can apparently be overcome in the hamster by increasing the strength of the stimulus. Terbutaline, 10^{-6}M given prior to and during mediator exposure effectively counteracted even the highest histamine dose. These results might be helpful in understanding the nature of prolonged vascular leakage in chronic inflammation.

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AF-DX 116, AN M_2 ANTAGONIST, DISCRIMINATES AMONG PERIPHERAL MUSCARINE RECEPTORS.

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The muscarine M_2 receptor subtype located in effector organs and recognized by pirenzepine with a uniformly low affinity, may not constitute a homogeneous population. Evidence comes from compounds able to discriminate among muscarinic - mediated responses. Thus, some neuromuscular blockers, tipyfied by gallamine, act more potently at the cardiac than at the ileal receptors (Clark & Mitchelson, 1976), while drugs such as 4-DAMP and hexahydro-siladifenidol show higher affinity for the ileal muscarine receptors (Barlow et al., 1976; Mutschler & Lambrecht, 1984). We report the characterization of AF-DX 116 (11[[2- [(diethylamino)methyl] -1-piperidinyl] acetyl] -5, 11-dihydro-6H pyrido [2,3-b] [1,4] benzodiazepine-6-one), a novel M_2 selective antagonist, endowed with a marked cardioselectivity (Giachetti et al., 1986). We estimated its affinity towards muscarine receptors in smooth muscle and heart.

Cardiac preparations consisted of guinea pig left atria (paced at 3 Hz, 2 msec, supramaximal voltage), and spontaneously beating right atria. Smooth muscle contractility was studied in guinea pig ileum, taenia coli, and trachea, and in rat urinary bladder.

Table 1 AF-DX 116 affinities in antagonizing muscarinic-mediated responses

preparation	pA_2 (± s.e.)	slope (± s.e.)
left atria*	7.32(0.05)	1.036(0.026)
right atria	7.47(0.06)	0.910(0.024)
ileum	6.44(0.08)	0.932(0.074)
taenia coli	6.40(0.16)	1.002(0.124)
bladder*	6.39(0.06)	0.953(0.063)
trachea	6.32(0.06)	0.959(0.045)

agonist : bethanechol, except *carbachol

AF-DX 116 parallely displaced the agonist dose-response curves. Schild's analysis indicated competitive antagonism (slopes not different from 1).

AF-DX 116 possesses a tenfold greater affinity for atrial receptors, in comparison to that estimated for smooth muscle. Greater affinity for the cardiac muscarine receptor is a feature common to the neuromuscular blockers. However, in contrast with the latter drugs, which interact allosterically with the receptor (Clark & Mitchelson, 1976), AF-DX 116 behaved competitively over a wide concentration range (0.3-30 μM). As a corollary to its competitive nature, AF-DX 116 antagonism is not influenced by the agonist used. In conclusion, the discriminative behaviour of AF-DX 116, might be useful in the classification of the M_2 muscarine subtype.

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MODULATION OF PGD_2-INDUCED CYCLIC AMP FORMATION IN BLOOD PLATELETS BY PHORBOL 12-MYRISTATE 13-ACETATE.

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1,2-Diacylglycerol (DG), cytosolic free calcium ions (Caf) and cAMP act as second messengers to control platelet reactivity (Kawahara et al, 1980; Feinstein et al, 1981). DG acts via stimulation of protein kinase C (PKC), an affect that is mimicked by phorbol 12-myristate 13-acetate (PMA). Besides acting independently or synergistically with elevated Caf to mediate platelet activation, DG (via activation of PKC) can inhibit or reverse agonist-induced phosphoinositide hydrolysis and Ca^{2+} flux indicating that it can act as a bidirectional regulator of cellular reactivity (MacIntyre et al, 1985). In other cell types, activation of PKC by PMA may modulate the effects of agonists that influence adenylate cyclase (e.g. Heyworth et al, 1984). We examined the effects of PKC activation on cAMP formation in intact human platelets.

All studies were performed using plasma-free suspensions of human platelets in a modified Tyrodes solution. Platelet cAMP content was measured by radioimmunoassay (Bushfield et al, 1985).

Incubation of platelets (<4 min, 37°C) with PMA(<300 nM) did not alter the basal level of cAMP (8.3 ± 0.4 pmol/10^8 cells; mean ± S.E.; n = 7 experiments). PGD_2 induced a concentration-dependent elevation of cAMP content to 120 ± 10 pmol/10^8 cells with an $EC_{50}$of around 300 nM. PMA (3-300 nM; 2 min; 37°C) but not 4α-phorbol 12,13-didecanoate (4α-PDD; 4-400 nM) inhibited PGD_2 (300 nM)-induced cAMP formation with an I_{50} of around 7.5 nM. These inhibitory effects of PMA were still evident in the presence of the phosphodiesterase inhibitor IBMX (1 mM), the cyclooxygenase inhibitor flurbiprofen (10 μM) and sufficient apyrase (10 units of ADPase activity per ml) to suppress the inhibitory effects of ADP (1-10 μM) on PGD_2 (300 nM)-induced cAMP formation.

These results indicate that PMA but not the non-tumour promoting 4α-PDD, interferes with PGD_2-induced elevation of platelet cAMP content and that this effect is not dependent upon ADP release, thromboxane A_2 formation or active phosphodiesterase. These inhibitory effects of PMA could result from (a) an inhibitory effect at the PGD_2 receptor; (b) inactivation of the stimulatory guanine nucleotide-binding regulatory protein, Ns; (c) activation of Ni or (d) a direct inhibitory effect on the adenylate cyclase. Recently it has been reported that PMA exerts no direct effect on forskolin- or PGI_2-stimulated cAMP formation in intact platelets (Ashby et al, 1985), but attenuates Ni- but not Ns-dependent regulation of adenylate cyclase in isolated platelet membranes (Jakobs et al, 1985). These observations suggest that the inhibitory effects of PMA on PGD_2-induced cAMP formation reported here might best be explained by an effect on the PGD_2 receptor.

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POSSIBLE SUBTYPES OF PROSTACYCLIN RECEPTORS IN PLATELETS FROM DIFFERENT SPECIES.

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In addition to specifically blocking thromboxane receptors in smooth muscle, the prostaglandin endoperoxide analogues EP 035 and EP 157 inhibit the aggregation of human platelets induced by a variety of agents. Ligand binding and cAMP measurements indicate that these compounds activate prostacyclin receptors linked to adenylate cyclase (Armstrong et al, 1985). However we have observed that they do not inhibit the aggregation of rat platelets induced by ADP, whereas prostacyclin and its stable analogues (e.g. iloprost) are highly active. To investigate this difference further we have examined the activity of EP 035, EP 157 and three close analogues of prostacyclin on plasma-free suspensions of platelets from man, horse, rat and rabbit.

Iloprost and ZK 96480 (Mueller et al, 1984) are equi-effective inhibitors on each of the four platelet preparations. They are always the most active agents although absolute sensitivity varies considerably : iloprost IC_{50} values - human 0.19, horse 23, rat 6.0, rabbit 34 nM. Equipotent molar ratios (EPMR) for both inhibition of aggregation induced by PAF (ADP for rat platelets) and for elevation of cAMP are given in Table 1.

Table 1 Species variation in sensitivity to PGI_2 mimetics

prostanoid	Human	Horse	Rat	Rabbit
	EPMR : Inhibition of platelet aggregation			
iloprost	1	1	1	1
carbacyclin	25	14.5	4.2	3.5
EP 157	66	120	>2500	1167
EP 035	666	375	>20,000	>2000
	EPMR : Stimulation of platelet cAMP production			
iloprost	1	1	1	1
carbacyclin	25	12.8	8.2	2.2
EP 157	264	156	>>1000	2198
EP 035	2840	417	>>1000	>2500

It must be appreciated that the human cAMP system is very highly tuned such that although 3μM EP 035 increases cAMP levels 14.8 ± 2.6 fold (n = 4) and 3μM EP 157 25.8 ± 3.2 fold (n = 5), less than a 2 fold increase is required for total inhibition of aggregation. However, on rat and rabbit platelets 30 μM EP 157 can only increase cAMP levels by 1.35 ± 0.17 fold (n = 4) and 1.79 ± 0.20 fold (n = 4) respectively. Furthermore, when these high concentrations of EP 035 and EP 157 are used on rabbit and rat platelets the weak inhibitory effects produced are little affected by the adenylate cyclase inhibitor SQ 22536 (Harris et al, 1979) suggesting that another mechanism is operating.

The marked differences between human/horse and rat/rabbit platelets, especially in relation to the relative activities of carbacyclin and EP 157, suggest the existence of subtypes of the prostacyclin receptor.

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THE INHIBITION OF PLATELET AGGREGATION BY AMBROXOL, A MUCOLYTIC AGENT

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Ambroxol (Mucosolvan-NA872-trans-4-(2-amino-3,5,-dibromo-benzyl amino) - cyclohexanol hydrochloride), a mucolytic agent, inhibits phospholipases A from rabbit lung lysosymes and alveolar macrophage lysates (Heath and Jacobson, 1986). Because phospholipases A_2 and C are believed to be important in platelet activation, we studied the effects of ambroxol upon this cell and compared its action with that of mepacrine, a known inhibitor of phospholipase A_2.

Ambroxol (125 -500 μM) inhibited aggregation of both human and rabbit platelets in response to collagen (0.5 - 10 μg/ml), ADP (0.1 - 10 μM) PAF (0.2 - 10 ng/ml) and low concentrations of thrombin (0.2 - 0.6 U/ml), but not to high concentrations of thrombin (> 0.8 U/ml), or to arachidonic acid (10 - 50 μg/ml). A similar pattern of inhibition was observed with mepacrine (50 μM). The inhibition by ambroxol was concentration-dependent, and appeared to be "competitive" in that increasing doses of agonist could partially overcome the effects of a given concentration of ambroxol e.g. the IC_{50} against aggregation in rabbit platelets induced by 0.5 ng/ml PAF was $\sim$ 200 μM, but $\sim$ 400 μM was required to achieve the same effect when the concentration of PAF was 4 ng/ml. The dose response curve of ambroxol inhibition was very steep e.g. 100 μM ambroxol was ineffective against collagen-induced aggregation of rabbit platelets, whereas 500 μM could completely inhibit the response. The inhibition was selective, since concentrations of 2mM and above did not affect platelet responses to arachidonic acid or high dose thrombin. Ambroxol was 4-5 fold more potent on washed rabbit or human platelets than on PRP, suggesting either extensive binding to plasma components or rapid metabolism. The latter seems unlikely, since preincubation of PRP with ambroxol before the addition of aggregating agents did not affect its inhibitory activity.

The pattern of inhibitory activity shown by ambroxol suggested that it might act by inhibiting arachidonic acid release, which is thought to be at least partly catalysed by phospholipase A_2. This suggestion was supported by the observation that ambroxol (100 μM) completely inhibited the collagen-induced (5 μg/ml) release of radiolabel from the phospholipids of washed rabbit platelets prelabelled with [1-^{14}C] - arachidonic acid. However, in one experiment in which tracer amounts of [1-^{14}C] - arachidonic acid were incubated with rabbit platelet homogenates, ambroxol inhibited the generation of cyclo-oxygenase (but not lipoxygenase) products, with an IC_{50} of $\geqslant$ 50 μM, a concentration which did not affect platelet aggregation in response to arachidonic acid. In addition, ambroxol was only a weak inhibitor of the hydrolysis of 1-palmitoyl, 2-^{14}C-oleoyl phosphatidylcholine by platelet lysates (< 25% inhibition at 500 μM, a concentration which totally inhibited platelet aggregation), or by pig pancreatic phospholipase A_2.

In conclusion, ambroxol inhibits platelet aggregation and has a spectrum of activity similar to that of mepacrine. However, our data suggests that although an anti-phospholipase action seems plausible there may be additional actions of ambroxol which affect platelets. These are currently under investigation.

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MODULATION OF PROSTANOID SYNTHESIS, ARACHIDONATE LIPOXYGENASE AND PLATELET AGGREGATION BY DIMETHYLSULPHOXIDE

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Clinical Studies with Dimethlsulphoxide (DMSO) have suggested that it is efficacious in the treatment of burns, interstitial cystitis, arthritis, scleroderma, and other conditions associated with inflammatory process. In addition, DMSO exerts pharmacological actions that include vasodilation, decrease in body temperature, analgesia and is anti-inflammatory. The mechanism by which DMSO exerts these action is not understood.

Non-steroidal anti-inflammatory drugs (NSAID) produce actions similar to DMSO, and these can be explained by their direct inhibitory actions on prostaglandin (PG) biosynthesis. Because of the similarity in the actions of NSAID and DMSO we reasoned that DMSO may be exerting its effects via an inhibition of PG biosynthesis. Therefore, we investigated the effects of DMSO on prostanoid synthesis, arachidonate lipoxygenase activity, and platelet aggregation, using methods described previously (Strickland et al, 1983; Saeed et al, 1980).

1% DMSO (v/v) had no effect on the biosynthesis of PGE_2, PGF_2 and thromboxane B_2 (TxB_2). At 9% DMSO (v/v) the production of PGE_2 was significantly increased ($P < 0.01$) by 29%, whereas the productions of PGF_2 or TxB_2 remained unchanged. On the other hand, DMSO at a concentration range of 1-30% (v/v) caused inhibition of soybean lipoxygenase activity. DMSO also inhibited platelet aggregation induced by adrenaline (ADR) adenosine-5'-diphosphate (ADP) and arachidonic acid (AA). The results obtained were as follows:

Drug	Aggregating Agents			
	Adrenaline	ADP	Arachidonic Acid	Collagen
DMSO (% v/v)	1.7 ± 0.07	1.3 ± 0.2	3.8 ± 0.45	1.7 ± 0.2
Aspirin (mM)	1.4 ± 0.6	5 ± 0.8	0.17± 0.02	2.8 ± 1.8

Numbers represent IC50 values ± S.E.M. (IC50 is a concentration of drug required to inhibit platelet aggregation by 50 percent).

These results suggest that DMSO inhibits platelet aggregation and exerts differential effects on AA metabolism via Pg endoperoxide synthetase. Lipoxygenase activity by DMSO may result in the attenuation of the rate of biosynthesis of other products of arachidonic acid cascade. It thus appears that DMSO may represent a prototype for a new class of antiplatelet and anti lipoxygenase drugs.

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ROLE OF THE ENDOTHELIUM IN VASODILATOR EFFECTS OF ACETYLCHOLINE, HISTAMINE, HYDRALLAZINE AND TRYPSIN IN RESISTANCE VESSELS.

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Several vasodilators have been shown to act in vascular strips indirectly via the endothelium (Furchgott and Zawadzki 1980). In this study the vasodilator actions of acetylcholine (Ach), histamine (His), hydrallazine (Hyd), and trypsin (Tr) were investigated using the isolated perfused mesenteric arterial bed (MAB) of the rat in the presence and the absence of the endothelium. Bolton et al. (1984) and Criscione et al. (1984) have recently shown that the endothelium of intact vessels can be removed by perfusing the lumen of the artery with distilled water for 10 min without damaging the medial smooth-muscle cells, as shown by light microscopy, and scanning and transmission electron microscopy.

Normotensive Sprague-Dawley rats weighing 280-350 g were anaesthetized with ether and the MAB's removed as described by McGregor (1965). The preparation was kept at room temperature (20-22°C) and perfused at a rate 5 ml/min with a modified Tyrode's solution gassed with 95% O_2 and 5% CO_2. Perfusion pressure (PP), which under constant flow conditions is proportional to vascular resistance, was measured. To study the effects of Ach, Tr and His, vasoconstriction was induced by continuous infusion of noradrenaline (NA) (2 µg/ml). In further studies bolus injections of NA (2 µg) were administered at intervals of 5 min in the absence and presence of Hyd (0.1-10 µg/ml).

The vasodilatation produced by bolus injections of Ach, Tr, and His in intact MAB's, preconstricted with NA, was found to be concentration-dependent. (ED_{50}: 3 ng, n=12; 29 ng, n=8; and 2000 ng, n=11 respectively). The responses to Ach, Tr and His were selectively antagonized by atropine (0.01 µg/ml), soya bean trypsin inhibitor (1 µg/ml) and mepyramine (0.04 µg/ml) respectively. The ED_{50} values in the presence of antagonist were: Ach (320 ng, n=6), Tr (280 ng, n=8); His (>3 mg, n=6). Removal of the endothelium enhanced the pressor response to NA (53 $\pm$ 4 mmHg vs 89 $\pm$ 6 mmHg, $p < 0.001$), abolished the relaxant effect of Ach and Tr, and markedly reduced (68%) that of His.

Hyd inhibited NA-induced vasoconstriction at concentrations between 0.1 and 10 µg/ml (IC_{50}: 0.15 µg/ml, n=7). After removal of the endothelium, pressure responses to NA were enhanced (53 $\pm$ 9 mmHg vs 78 $\pm$ 8 mmHg $p < 0.05$) and a similar inhibition was seen in the same dose-range (IC_{50}: 0.38 µg/ml, n=7).

These results indicate that in isolated MAB's of rats, Ach, Tr, and His acting at three different sites on the endothelium are able to release a substance(s) (probably the endothelium-derived relaxing factor) which in turn produces vasodilatation. These vasodilator effects therefore appear to be indirect, whereas Hyd produces vasodilatation via a direct effect on the smooth muscle of the resistance vessels.

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HAEMOGLOBIN INHIBITS VASODILATATION INDUCED BY STIMULANTS OF SOLUBLE BUT NOT PARTICULATE GUANYLATE CYCLASE IN RABBIT AORTA

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Haemoglobin (Hb) inhibits the vasodilatation and associated increase in cyclic GMP induced by endothelium-derived relaxing factor (EDRF),nitrovasodilators such as glyceryl trinitrate (GTN) and bovine retractor penis inhibitory factor (IF)(Bowman and Drummond, 1984; Martin et al, 1985a). Inhibition is probably mediated by the binding and inactivation of the active principles (Martin et al 1985a; 1985b), so preventing their interaction with the endogenous ferrous haem-containing receptor site on the soluble form of guanylate cyclase (Craven and De Rubertis, 1978). Atrial natriuretic peptides (ANP's) elicit vasodilatation by activating a different isoenzyme, a particulate form of guanylate cyclase (Winquist et al, 1984). It is not known,however, whether the particulate form of the enzyme has a haem-containing receptor site. We therefore utilized Hb as a tool to explore the possible role of haem-containing receptor sites in the activation of particulate guanylate cyclase by ANP's.

Isometric tension recordings were made from endothelium-denuded rings of rabbit aorta bathed in Krebs at 37°C. Following induction of submaximal tone with phenylephrine ($3x10^{-8}$-10^{-7}M) relaxation to cumulative additions of the atrial natriuretic peptide, atriopeptin II (Sigma), were recorded.

Atriopeptin II ($3x10^{-11}$-10^{-7}M) elicited a dose-dependent relaxation of phenylephrine-induced tone (EC_{50} $7.6x10^{-10}$M, n=8). This relaxation was unaffected by pretreatment with Hb (10^{-5}M, 10 min). Furthermore atriopeptin II (10^{-7}M, 3 min) induced a 6.2- fold increase in the cyclic GMP content of endothelium-denuded aortic rings and this increase was unaffected by pretreatment with Hb (10^{-5}M, 20 min). The selective cyclic GMP phosphodiesterase inhibitor, M&B 22,948 (10^{-4}M), relaxed phenylephrine-induced tone by 20.2± 1.8%, n=8, and when tone was reestablished to the preexisting level with additional phenylephrine, subsequent relaxation to atriopeptin II was potentiated 2.2- fold.

The potentiation of atriopeptin II induced relaxation by M&B 22,948 is indicative that the rise in cyclic GMP and vasodilatation are causally-related. The fact that Hb, in concentrations which inhibit vasodilatation and increases in cyclic GMP induced by EDRF, GTN and IF (Bowman and Drummond, 1984, Martin et al , 1985a), did not inhibit the vasodilatation and increased cyclic GMP induced by atriopeptin II implies that a haem-containing receptor site is not involved in the activation of particulate guanylate cyclase. Hb appears to inhibit vasodilatation and increases in cyclic GMP induced by stimulants of the soluble but not of the particulate form of guanylate cyclase.

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A COMPARISON OF THE INHIBITORY EFFECTS OF CRF AND OPIOID AGONISTS ON NEUROGENIC PLASMA EXTRAVASATION IN THE RAT

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Various agents, including opioid agonists, have been shown to inhibit plasma extravasation into rat skin following antidromic electrical stimulation of the saphenous nerve. Opioid-induced effects in this model may be mediated by opioid receptors located peripherally on sensory nerve endings resulting in a diminished release of substance P (Bartho and Szolcsanyi, 1981; Smith and Buchan, 1983). Kiang and Wei (1985) have reported recently that rat corticotrophin releasing factor (CRF) also inhibits neurogenic plasma extravasation in the rat, possibly by acting through its own receptor system. We have extended the studies of CRF in this model and compared its effects with those of the opiate agonists morphine, FK 33, 824 and dynorphin 1-13.

Male Sprague-Dawley rats (200-280g) were anaesthetised with pentobarbitone sodium (60mg/kg i.p.) and the saphenous nerve in the left leg exposed for electrical stimulation (1Hz, 10V, 6msec duration). Evans Blue dye (50mg/kg, 3 min. before stimulation) and all drugs were administered via a cannula placed in the right jugular vein. For each drug, a minimum of 5 animals/dose were used. Immediately after stimulation, the animals were sacrificed and the skin of the dorsal and inside flank of both paws removed. Evans Blue was extracted from the tissue and measured colourimetrically at 600nm. Blood pressure recordings were carried out in anaesthetised rats by means of a cannula placed in the right carotid artery.

Intravenous administration of CRF inhibited neurogenic plasma extravasation in a dose-related manner, the ED_{50} and 95% confidence limits being 5.8nmoles/kg (4.6-6.9). For comparison, the ED_{50} values for the opioid agonists tested were: morphine, 2700nmoles/kg (2340-3110); FK 33, 824, 0.45nmoles/kg (0.31-0.63) and dynorphin 1-13, 1180nmoles/kg (950-1480). CRF produced a long lasting inhibition of extravasation, the effects of a 6nmol/kg dose lasting for at least 1h, whilst that of morphine and FK 33, 824 were of much shorter duration. Dynorphin 1-13, however, also appeared to induce a prolonged inhibition. Unlike the opioid agonists, the inhibitory effects of CRF were not mediated by opioid receptors since whereas naloxone, 3µmoles/kg, completely antagonised the effects of morphine (3000nmoles/kg), FK 33, 824 (0.8nmoles/kg) and dynorphin 1-13 (625nmoles/kg), a higher dose of naloxone, 12µmoles/kg, did not significantly antagonise CRF (6nmoles/kg). As with opioid agonists (Smith and Buchan, 1983), it appears unlikely that CRF acts as a substance P antagonist since CRF (1µM) failed to antagonise the contractile effects of substance P on the field stimulated guinea-pig myenteric plexus - longitudinal muscle preparation.

CRF-induced hypotensive effects also appear unlikely to be responsible for the inhibition of neurogenic plasma extravasation. In the anaesthetised rat, CRF (6nmoles/kg i.v.) produced 30 min. after administration a mean fall in blood pressure of 26mmHg (n=3). Hydralazine (2µmoles/kg i.v.) induced a similar fall in blood pressure at this time, 25mmHg (n=3), but failed to reduce the extravasation response; the amount of dye leaked/paw after hydralazine (2µmoles/kg) being 29.9µg compared to 26.3µg in control animals.

In summary, therefore, CRF and opioid agonists may inhibit neurogenic plasma extravasation by activation of independent receptor systems. The location and implications these actions of CRF remain to be elucidated.

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A SLOW SPINAL SYNAPTIC RESPONSE MEDIATED AT NMA RECEPTORS

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Excitations mediated at N-methylaspartate (NMA) receptors have been shown to be suppressed markedly by concentrations of Mg^{2+} comparable to those normally present in extracellular fluid (Ault et al 1980). Thus synaptic responses sensitive to NMA antagonists are rarely observed in Mg^{2+} containing medium (Herron et al 1985). In the present study three hemisected spinal cord preparations from 1 - 4 day old rats have been used to record long duration synaptic activity in the presence of 1mM Mg^{2+}. Segmental reflexes (L5) were evoked by supramaximal cathodal shocks applied, at 2 per min, to the dorsal root at approximately 3mm from its entry zone. Ventral root responses were measured with d.c. coupling via non-polarizable wick electrodes. Responses were recorded oscillographically for 50msec and for the whole of the interstimulus interval on an ink writing recorder (f.s.d. in 0.1sec). The oscillogaphic records showed the shortest latency component, time to peak 8msec, to be resistant to the NMA antagonist 2-amino-5-phosphonopentanoate (AP5) (100μM) as previously reported. A second component in the oscillographic record, time to peak 17msec, was partially depressed (less than 20%) by AP5(100μM). The latter component corresponds in latency to that previously reported to be depressed by AP5 in the absence of Mg^{2+} (Evans et al 1980). Examination of the pen recording showed a very slow synaptic response, time to peak 6sec, with a duration of 20sec. This very slow synaptic response was abolished by AP5 (50μM).

All components of the synaptic response were markedly depressed by kynurenic acid (0.5mM).

The present observations suggest that it may be appropriate to employ a very long time course when searching for NMA receptor mediated synaptic responses. At present it is not clear whether this slow excitatory potential reflects either reverberation over interneuronal pathways or a slow synaptic response analogous to that of sympathetic ganglia. Observations on transmission in the amphibian spinal cord (Dale & Roberts 1985) would support the latter possibility.

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