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PAF AND A THROMBOXANE MIMETIC STIMULATE RAT GASTRIC CONTRACTILITY AFTER LOCAL ARTERIAL INFUSION

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Local intra-arterial infusion of PAF-acether or the thromboxane mimetic, U-46619, induces extensive damage in the rat gastric mucosa (Esplugues & Whittle, 1987). In the present study, we have investigated the effects of these mediators following local administration on gastric contractility in vivo.

Male rats (230-250g) were anaesthetised with pentobarbitone and the stomach exposed by a mid-line incision. The left gastric artery was freed from connective tissue under a stereomicroscope and cannulated with a modified short 23g teflon cannula. After ligating the oesophagus, 3 ml of saline were instilled into the gastric lumen and changes in resting intragastric pressure recorded from a catheter, introduced into the stomach via the duodenum through the pyloric sphincter and ligated in place. Close arterial infusion (12.5 µl/min) was started once the basal intragastric pressure (3.3 ± 0.1 mm Hg, mean ± s.e. mean, n=58) had remained constant for at least 15 min.

Local intra-arterial infusion of U-46619 (100 and 1000 ng/kg/min) induced immediate and dose-dependent contractions of the stomach, increasing intragastric pressure ($p<0.05$) by 2.3 ± 0.5 mm Hg and 10.8 ± 0.8 mm Hg (n=4) respectively above basal, which remained stable throughout the 10 min infusion period. Thereafter, intra-gastric pressure slowly decreased, reaching basal levels after 20 min. Pretreatment with the thromboxane receptor antagonist (Patscheke & Stegmeier, 1984) BM 13.177 (5 mg/kg i.v.) significantly inhibited ($p<0.01$) the increase in intragastric pressure induced by U-46619 (500 ng/kg/min) by 68±3% (n=3).

Local intra-arterial infusion of PAF (25 and 50 ng/kg/min) caused a rapid and dose-dependent increase in tonic contractions of the stomach, which resulted in increases ($p<0.01$) of intra-gastric pressure of 5.4 ± 0.5 mmHg (n=5) and 7.7 ± 0.9 mmHg (n=7) above basal, respectively. Following termination of the 10 min infusion of PAF, a further rise in intragastric pressure was observed, which peaked approximately 15 min later. This post-infusion increase was also dependent on the dose of PAF, reaching values of 7.9 ± 1.2 mmHg and 9.4 ± 0.7 mmHg above basal following infusion of 25 and 50 ng/kg/min of PAF respectively. Pretreatment with atropine (1.5 mg/kg, i.v.), dexamethasone (2mg/kg s.c.), the thromboxane synthetase inhibitor benzyl-imidazole (50 mg/kg s.c.), indomethacin (5mg/kg s.c.) or the dual lipoxygenase-cyclo-oxygenase inhibitor BW 755C (50 mg/kg, p.o.) failed to modify the gastric pressure responses during or after PAF (50 ng/kg/min). However, the PAF antagonist (Wu et al, 1986) L-652,731 (2.5 mg/kg i.v.,) significantly ($p<0.05$) reduced both the immediate and post-infusion changes in intragastric pressure induced by PAF (by 53±8% and 55±9% respectively, n=3).

The present study shows that low doses of the thromboxane mimetic and of PAF can stimulate gastric contractility in vivo, although the mechanisms underlying these actions have not yet been elucidated. These findings raise the possibility of a pathophysiological role of either mediator in the stimulation of gastric motility.

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HISTAMINE DOES NOT MEDIATE THE *IN VITRO* COLONIC SECRETORY RESPONSE TO COMPOUND 48/80 IN RAT

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Anaphylactic reactions in epithelial mucosae may lead to secretory responses but the identity of the mediators is still not clear. For example, challenge of the muscle-stripped mucosa of rat colon (Baird et al., 1985) and treatment of dog tracheal epithelium with mast cell-derived mediators released from anaphylactically-challenged lung parenchyma (Lazarus et al., 1986) both lead to a chloride secretory response. Bearing in mind that muscle-stripped sheets of rat colonic mucosa still contain the lamina propria and associated cells of the immune system, we have investigated whether mast cell activation might lead to changes in colonic epithelial function. We have challenged the colonic mucosal sheets with compound 48/80, a well-recognised mast cell activating agent, and have compared the effects on short circuit current, I_{sc}, with those caused by addition of exogenous histamine, prostaglandin D_2 (PGD_2) and platelet activating factor (PAF-acether).

Stripped mucosal sheets from rat descending colon were mounted in Ussing-type chambers as described (Hoult & Phillips, 1986). Transepithelial p.d. and I_{sc} were measured continuously using a computer-controlled voltage clamp, and the mucosal and serosal bathing solutions were removed 15 min after drug addition for subsequent assay for histamine (by spectrophotofluorimetry) and PGE_2 (by RIA).

Compound 48/80 produced a dose-dependent increase in I_{sc}, reaching a value of 190 ± 20 $\mu A/cm^2$ at the highest dose tested (10 µg/ml). Mepyramine at 10^{-4}M did not reduce the I_{sc} response to 1 µg/ml 48/80, but reduced the effect of 10^{-6}M histamine by 75 ± 5% ($P < 0.01$, 6 experiments). However, 10^{-5}M indomethacin completely abolished the responses to 48/80, but also prevented those to histamine (reduced by 90 ± 9%, $P < 0.01$, 6 tests), suggesting a non-specific effect of this cyclo-oxygenase inhibitor. Applied PGD_2 and PGE_2 both caused comparable changes in I_{sc} over the range 10^{-8}-10^{-6}M, and these responses were reduced ca. 75% by 10^{-4}M diphenylamine-2-carboxylic acid (DPC), a known chloride channel blocker. Applied PAF-acether (10^{-7}M) caused a small increase in I_{sc} (20 ± 5 $\mu A/cm^2$), which was reduced to 3 ± 3 $\mu A/cm^2$ by DPC.

Treatment of colonic mucosa with 48/80 caused small releases of histamine (56 ± 8 ng/cm^2/min up to 102 ± 12 ng/cm^2/min, $P < 0.05$, 10 tests) and of PGE_2 (0.85 ± 0.2 ng/cm^2/min up to 1.4 ± 0.35 ng/cm^2/min, n.s., 10 tests). PGD_2 and PAF-acether release were not measured.

Our results show that compound 48/80 elicits a secretory response in rat colon, but it is not clear whether this is due to mast cell activation. However, it is unlikely that the response is due to release of either PGE_2 or histamine, but the contribution of other mediators known to be released by mast cells (PAF-acether, PGD_2) needs to be evaluated. The lack of importance of histamine may reflect the fact that mucosal mast cells contain much smaller amounts of histamine than those present in connective tissue (Enerback & Wingren, 1980).

We thank the SERC for support.

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PYROGENIC AND THERMOGENIC EFFECTS OF RECOMBINANT HUMAN INTERLEUKIN-1β IN THE RABBIT AND THE RAT

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Several cytokines produced by recombinant DNA technology have been assayed for intrinsic pyrogenic activity with the aim of identifying the endogenous pyrogen mediating fever. To date, recombinant human interleukin-1 (IL-1), tumour necrosis factor and interferon-α have been shown to be pyrogenic after i.v. injection in rabbits (Dinarello et al, 1984, 1986). In view of the need for large (μg) quantities of cytokines to produce responses in the rabbit assay, we have compared the effects of recombinant human IL-1β on body temperature and thermogenesis in rabbits and rats, and have investigated whether these are associated with increases in brown adipose tissue (BAT) activity in the rat.

Injections were given intravenously to male rats (Sprague-Dawley, 100-150 g; Wistar 110-210 g) and female rabbits (half lop, 2-3.5 kg), and into the third cerebral ventricle (i.c.v.) through implanted cannulae in male Wistar rats (170-270 g). Colonic temperature was measured in hand-held rats and rabbits restrained in stocks at a room temperature of 25 ± 1 or 21 ± 1°C respectively. Whole body oxygen consumption (VO_2) was measured at 24°C using a closed circuit indirect calorimeter in response to i.c.v. (unanaesthetized) or i.v. (anaesthetised, urethane 0.15g/100g i.p.) IL-1β in rats. Thermogenic activity of BAT was assessed from the binding of guanosine diphosphate (GDP) to mitochondria isolated from the interscapular depot. Purified recombinant human IL-1β (Cistron, specific activity 1-2 x 10^6 units/mg protein) was injected in phosphate-buffered saline containing 20 mM dithiothreitol (protein concentration 750 μg/ml) diluted with 0.9% saline. Doses of IL-1β are expressed as the amount of recombinant protein/animal.

IL-1β (1 μg i.v.) produced a monophasic rise in temperature in rabbits, with a maximum of +0.8 ± 0.1°C (mean ± s.e.m.) at 43 ± 1 min ($n = 5$, $P < 0.001$), but a lower dose (100 ng i.v.) had no effect. Both Sprague-Dawley and Wistar rats responded to IL-1β (100 ng and 1 μg i.v.) with significant increases in body temperature (maximum rise at 20 min after i.v. injection 0.5 - 0.7°C, $P < 0.05$). This was similar in magnitude for both doses, but the duration of the response to 1 μg was longer (4-5h) than that to 100 ng (< 90 min). Rats responded with hyperthermia to i.c.v. injections of IL-1β in doses of 1, 10 and 100 ng ($n = 5$-7), the latter dose producing a rise in temperature up to 1.5 ± 0.3°C above control values which lasted for more than 5h. Dithiothreitol in phosphate-buffered saline had no effect on temperature in rats. VO_2 was increased by 6 ± 1% ($P < 0.05$) 50-60 min after i.v. injection of IL-1β (1 μg, $n = 7$), and by up to 49 ± 6% ($P < 0.001$) 80-100 min after i.c.v. injection (dose range 20-80 ng, $n = 4$-5). BAT activity (GDP binding measured 1h after injection) was increased from 63 ± 3 to 88 ± 7 pmol/mg protein by 1 μg IL-1β i.v. ($P < 0.05$) and from 84 ± 5 to 156 ± 14 pmol/mg protein by 80 ng IL-1β i.c.v. ($P < 0.01$).

These data show that ng quantities of human IL-1β injected i.c.v. cause pyrexia in the rabbit and the rat. The increases in VO_2 and BAT activity observed in the rat may contribute to this pyrexia. We thank Dr R.C. Newton and DuPont for the gift of IL-1β.

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INFLUENCE OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS ON SERUM THROMBOXANE AND PLATELET FUNCTION IN THE HORSE

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Non-steroidal anti-inflammatory drugs (NSAIDs) are used in equine medicine for their anti-inflammatory and analgesic properties (Lees and Higgins, 1985). It has recently been proposed that they may also be of value in the treatment of conditions, such as navicular disease, verminous arteritis, endotoxaemia, laminitis and colic, which may involve arteriolar thrombosis or vasoconstriction. This study of equine platelet function was therefore undertaken to determine the time course of inhibition of serum TXB_2 synthesis by clinical dose rates of two commonly used NSAIDs, phenylbutazone (4.4 mg/kg i.v.) and flunixin (1.1 mg/kg i.v.). Aspirin as lysine acetylsalicylate (19 mg/kg i.v.) was also studied as an irreversible cyclo-oxygenase inhibitor.

Five or six Welsh Mountain ponies each received phenylbutazone (phase 1), flunixin (phase 2) and aspirin (phase 3), at intervals of 5 weeks. Following each drug, serial blood samples were collected for determination of plasma drug and serum TXB_2 concentrations obtained from blood allowed to clot at 37°C for 90 min. Mean (± s.e.mean) elimination half-life and total body clearance values, respectively, were 6.11 ± 0.34 h and 16.3 ± 1.0 ml/kg/h (phenylbutazone, n=6), 1.94 ± 0.1 h and 57.3 ± 2.9 ml/kg/h (flunixin, n=6) and 0.11 ± 0.01 h and 530.0 ± 32.7 ml/kg/h (aspirin, n=5). Mean values of percentage inhibition of serum TXB_2 synthesis are presented in Table 1. Phenylbutazone and flunixin inhibition was reversible, whereas aspirin produced prolonged and presumably irreversible inhibition. This may have been due to inhibition of cyclo-oxygenase in megakaryocytes as well as circulating platelets.

Table 1. Mean percentage inhibition of serum TXB_2 by NSAIDs

Time (h or day)	Phenylbutazone	Flunixin	Aspirin
0.5 h	100	100	100
4 h	88	98	97
12 h	76	77	99
24 h	50	63	99
2 days	7	-4	100
7 days	-25	-22	100
21 days	-	-	65

In a second experiment New Forest and Welsh Mountain ponies were divided into three groups of six animals and treated as follows : no treatment (group 1), low dose aspirin (4 mg/kg i.v., group 2) and high dose aspirin (12 mg/kg i.v., group 3). In groups 2 and 3 template bleeding time was increased within 15 min of dosing and the effect persisted for 48 h. Aspirin treatment did not affect ADP-induced platelet aggregation, whereas aggregation in response to collagen (1 µg) was completely suppressed by aspirin for 48 h and partially inhibited for 72 h. A higher dose of collagen (5 µg) was only slightly affected by aspirin treatment. These preliminary findings suggest that relatively small doses of aspirin, administered every second or third day, may be of value in the treatment of cyclo-oxygenase dependent thromboembolic diseases in the horse.

Supported by the Horserace Betting Levy Board (Project 334).

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INFLUENCE OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS ON EQUINE LEUCOCYTE LOCOMOTION *IN VITRO*

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The principal mode of action of non-steroidal anti-inflammatory drugs (NSAID) is believed to be blockade of the cyclo-oxygenase enzyme system. However, these drugs have other actions which may contribute to their therapeutic effects. It has been shown, for example, that NSAID inhibit aggregation, locomotion, superoxide generation and lysosomal enzyme release by neutrophils.

In this study we investigated the direct effects of NSAID on equine polymorphonuclear (PMN) and mononuclear (MN) leucocyte locomotion using Boyden chamber (Sedgwick *et al.*,1982) and agarose microdroplet (Smith and Walker,1980) assay methods. PMN and MN leucocytes were separated from heparinised equine blood samples on Percoll gradients and re-suspended in Hanks Balanced Salt Solution. In both assay systems 100 µM N-formyl-methionyl-leucyl-phenylalanine (FMLP) was used as a standard chemoattractant for MN cells and zymosan activated plasma (ZAP) was used as the standard for PMN leucocytes.

Inhibition of the accelerated cell movement induced by ZAP or FMLP was determined for a range of six concentrations of three NSAID, indomethacin (50 µM to 10 mM), phenylbutazone (10 µM to 1 mM) and flunixin (0.1 µM to 50 µM). For the last two drugs concentrations were selected to include those previously shown to occur in equine plasma and acute inflammatory exudate following the administration of clinical dose rates of phenylbutazone and flunixin (Lees and Higgins,1985). All three NSAID inhibited cell movement and the responses were usually dose related. Concentrations producing significant inhibition ($P<0.05$) are given in Table 1.

Table 1. Inhibition of leucocyte movement by NSAID

Drug	Drug concentration range (µM) producing inhibition			
	Boyden chamber		Agarose microdroplet	
	PMN	MN	PMN	MN
Indomethacin	100-10,000	1,000-10,000	100-10,000	1,000-10,000
Phenylbutazone	10-1,000	10-1,000	10-1,000	none
Flunixin	0.1-50	0.1-50	0.1-50	0.1-50

Phenylbutazone and flunixin are used extensively in equine medicine. Since concentrations inhibiting equine PMN and MN cell movement in two *in vitro* assay systems were similar to those achieved in body fluids with clinical dose rates this action could contribute to the anti-inflammatory properties of these drugs in the horse. However, acute and chronic inflammation in the whole animal are complex processes and many interactions, which are absent from *in vitro* systems, may occur.

Supported by the Horserace Betting Levy Board (Project 334).

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INHIBITION OF ACUTE CELLULAR ACCUMULATION BY CATALASE

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The possible involvement of free radicals in general and hydroxyl radicals in particular in inflammatory reactions has been the subject of considerable discussion (Fantone and Ward, 1982). Catalase has been employed as an inhibitor of hydroxyl radical production through enhanced breakdown of hydrogen peroxide and has been shown to inhibit complement and polymorphonuclear leukocyte (PMN) - dependent vascular injury in rabbit skin (Rampart and Williams, 1985) and rat lung (Till and Ward, 1986). To further elucidate the role of radicals we have investigated the effect of catalase on the acute cellular accumulation observed in several inflammatory models.

Acute inflammation and cellular accumulation were induced in male CFHB rats (Interfauna, 230-250g) either by the ventral s.c. implantation of two saline-soaked polyester sponges (Doyle et al, 1983), or by the injection of carrageenan (10mg in 1ml of normal saline), carboxymethylcellulose (20mg in 1ml of normal saline) or zymosan (10mg in 1ml of normal saline) into preformed 6 day dorsal air pouches (Edwards et al, 1981). After 6 hours the exudates were harvested into EDTA-saline and the cells counted using a Coulter counter. Animals were treated before sponge implantation or irritant injection with either cobra venom factor (CVF 2 x 50 units/kg i.p. on the day before) or catalase (4.10^5 units/kg i.v. 30 minutes before).

The cell responses in all models fell within the range $30\text{-}80.10^6$ total cells and were PMN dominated (>90%). Sponge induced cellular accumulation was partially complement dependent with CVF giving 69% inhibition of accumulation. The models of cellular accumulation in air pouches appeared less complement dependent as complement depletion gave only 38, 51 and 37% inhibition of inflammation induced by carrageenan, carboxymethylcellulose and zymosan respectively. Parallel experiments showed CVF treatment to reduce circulating complement levels to <5% of control values at the time of initiation of inflammation. Catalase treatment reduced 6 hour cell accumulation in sponges by 71% and in air pouches by 65, 74 and 51% respectively after carrageenan, carboxymethylcellulose and zymosan.

The results suggest an important role for hydrogen peroxide and probably also hydroxyl radicals in the acute cell response, possibly related to the generation of chemotactic complement fragments (Shingu and Nobunaga, 1984).

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THE EFFECTS OF SOME ANALOGUES OF BRADYKININ ON BRADYKININ-INDUCED RAT PAW SWELLING

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In this study, the effects of four analogues of BK, B4148 (N-Lys, Lys, 2-Hyp, 5,8,Thi, 7-DPhe BK), B4146 (3-Hyp, 5,8,Thi, 7-DPhe BK), B4144 (5,8,Thi, 7-DPhe BK), and B4162 (N-DArg, 3-Hyp, 5,8,Thi, 7-DPhe BK) which were synthesised as potential antagonists, (Vavrek and Stewart,1985) were examined on BK-induced swelling of the rat paw.

Groups of at least five male Sprague-Dawley rats (200-250g) were used. Oedematous paw swelling was measured as dorso-ventral thickness using a simple constant load lever system. Swelling was expressed as percentage increase in thickness from pre-injection levels. Test agents (0.1ml) were injected s.c. into the right hind paw. Left hind paws were used as controls and injected with saline. Paw thickness was measured at 5 min intervals for 45 min and at 15 min intervals for a further 45 min. Peak responses, and overall degree of swelling, measured as area under the time-course curve (AUC),were compared using the Mann-Whitney U test.

Bradykinin, at a concentration of 10^{-9}M, produced swelling of the rat paw which showed an initial rapid peak at 5 to 10 minutes, thereafter subsiding towards control levels over the time course. At 10^{-7}M, all four analogues showed BK-like agonist activity on their own. However, at 10^{-8}M, all four analogues produced a significant reduction of the AUC ($P<0.05$). At this concentration, B4148 did not significantly reduce peak swelling, although the remaining three did ($P<0.05$). At 10^{-9}M, all four analogues significantly depressed both peak swelling and AUC ($P<0.05$). At 10^{-10}M, all four analogues significantly reduced peak swelling ($P<0.05$) and all but B4148 significantly reduced the AUC ($P<0.05$).

	AUC	P	PEAK	P
NO DRUG	1983.7±120.3		59.9±5.9	
B4148	842.9±161.5	★ ★	40.2±3.0	★
B4146	1320.1±135.9	★ ★	43.3±3.3	★
B4144	1395.0±158.1	★ ★	39.7±4.3	★
B4162	1074±178.9	★ ★	41.4±4.9	★

Table 1. The effect of the analogues at 10^{-9}M on BK-induced paw swelling.

These analogues therefore, in this model, appear to have a twofold action. At concentrations from 10^{-10}M to 10^{-8}M, they reduce BK-induced swelling. Above this concentration, they themselves stimulate paw swelling.

AG was supported by an SERC studentship.
We are grateful to Professor J. Stewart and Dr. R. Vavrek for the gift of the BK analogues.

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THE EFFECT OF DIMETHYLSULPHOXIDE ON SUPEROXIDE GENERATION AND INOSITOL PHOSPHATE PRODUCTION IN HUMAN NEUTROPHILS AND HL60 CELLS

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Dimethylsuphoxide (DMSO) has been used clinically as a potential anti-inflammatory agent (Jimenez and Wilkens, 1982). While its mode of action is unclear, it has been shown to inhibit the bactericidal properties and chemotaxis of human neutrophils in vitro (Repine et al, 1981; Antony et al, 1983). We have further investigated the anti-inflammatory effects of DMSO on human neutrophils by measuring inhibition of formylmethionylleucyl-phenylalanine (FMLP) - stimulated superoxide anion (O_2^-) generation. As this response is coupled to receptor activation via phosphoinositide metabolism, the effect of DMSO on FMLP-stimulated inositol phosphate production has been assessed in differentiated promyelocytic leukemia (HL60) cells, a convenient model of the neutrophil (Carey et al, 1987).

DMSO inhibited O_2^- production induced by 10^{-7}M FMLP in human neutrophils with an approx. IC_{50} of 0.5% (v/v). The inhibition was not overcome by increasing the FMLP concentration to 10^{-5}M nor enhanced by decreasing the FMLP concentration indicating that it was not due to competitive antagonism of FMLP. DMSO up to 1% (v/v) had no effect on O_2^- generation induced by 10^{-7}M phorbol myristate acetate (PMA) which is thought to act by direct activation of protein kinase C.

The kinetics of FMLP-stimulated inositol phosphate production was measured in the presence and absence of 2.5% DMSO in differentiated HL60 cells. This concentration of DMSO caused significant reduction in the levels of inositol mono-, bis- and tris+tetrakisphosphate (IP1, IP2 and "IP3") produced up to 3 minutes, levels of IP1 and IP2 being maximal after 30 seconds and "IP3" after 15 seconds. When the effect of increasing concentration of DMSO on FMLP-stimulated inositol phosphate production after 30 seconds was investigated, it was found that DMSO inhibited the production of IP1, IP2, and "IP3" with IC_{50}s of 2.5%, 3.05% and 4.1% (v/v) respectively.

These results indicate that DMSO may inhibit FMLP-stimulated O_2^- generation due to interference with the events leading to the hydrolysis of membrane phosphatidylinositol 4,5-bisphoshate (PIP2) by phospholipase C which releases the intracellular messengers IP3 and diacylglycerol. It has been suggested that DMSO may reduce the steady state concentration of PIP2 in rat hepatocytes (Creba et al, 1983). Whether this occurs in neutrophils and HL60 cells and could account for inhibition of O_2^- generation and inositol phosphate production requires further investigation.

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THE EFFECTS OF DEXAMETHASONE ON OEDEMA FORMATION IN RABBIT SKIN

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Glucocorticosteroids have multiple anti-inflammatory effects, including inhibition of phospholipase A_2 and hence of prostaglandin generation. Since vasodilator prostaglandins are important in the development of acute inflammatory oedema (Williams & Peck, 1977), we have compared the effects of dexamethasone with those of indomethacin upon the responses of rabbit skin to the direct-acting mediators, histamine, bradykinin and PAF.

Californian rabbits of either sex weighing approximately 3kg were anaesthetised with Sagatal. Vascular permeability was measured by the extravasation of [^{125}I]-human serum albumin injected i.v. in Evans' Blue solution (2% w/v) prior to intradermal injections (0.1 ml) of mediators. 30 min after the last injection, blood samples were taken, the animals killed, skin injection sites punched out using an 18 mm punch, and radioactivity estimated in a gamma counter. Dexamethasone (1mg/kg) and indomethacin (3mg/kg) were given i.v. 5 h and 30 min respectively prior to intradermal injections.

The responses to intradermal histamine (H, 3×10^{-9} moles/site), bradykinin (BK, 3×10^{-8} moles/site) and PAF (5×10^{-9} moles/site) with or without PGE_2 (3×10^{-10} moles/site) are shown in the table. PGE_2 alone caused less than 10 µl exudation.

Table 1: The anti-inflammatory effect of indomethacin and dexamethasone

mediator	µl plasma exudate/site m±s.e.m. control (n=16)	indomethacin (n=5)	dexamethasone (n=5)
H	7.8 ± 2.1	8.0 ± 1.4	0.3 ± 0.3*
H + PGE_2	38.1 ± 5.4	33.1 ± 5.7	4.1 ± 2.6*
BK	24.1 ± 3.3	20.8 ± 4.2	6.2 ± 1.7*
BK + PGE_2	85.0 ± 10.0	92.6 ± 19.9	31.6 ± 9.8*
PAF	14.5 ± 2.0	10.4 ± 2.8	3.7 ± 2.4*
PAF + PGE_2	27.7 ± 4.6	34.2 ± 8.5	5.8 ± 3.1*

*$P < 0.05$

Although indomethacin blocked the potentiating action of arachidonic acid with the mediators (data not shown) and was evidently blocking cyclooxygenase in the dose employed here, it had no effect on the actions of the mediators alone or the synergistic action of PGE_2. In constrast, dexamethasone blocked the direct action of the mediators and this could not be fully restored by PGE_2.

It has been postulated that oedema is caused by a combination of vasodilation and increased vascular permeability (Williams and Peck, 1977). Our data, and that of Svensjo and Grega (1986) suggest that dexamethasone exerts its effect by preventing the increase in vascular permeability caused by these mediators.

We thank Mrs M Norris and Mrs L Moore for their technical assistance, Mr RD Taylor for preparing iodinated albumin and Dr TJ Williams for help and advice.

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INVOLVEMENT OF CORTICOSTERONE IN THE ANTI-INFLAMMATORY ACTIVITY OF THE OPIOID κ-AGONISTS TIFLUADOM AND U50488

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The kappa agonists tifluadom and U50488 have previously been reported to exhibit anti-inflammatory activity in the carrageenan paw oedema model. (Russell et al, 1985). These agents have also been reported to elevate circulating plasma corticosterone levels with a central locus of action (Iyengar et al, 1986). As the anti-inflammatory effect of corticosteroids is well documented we have investigated the role of elevated plasma corticosterone levels in the anti-inflammatory activity of the kappa-agonists. Anti-inflammatory activity of tifluadom and U50488 was investigated in sham operated and adrenalectomised rats.

Male Alderley Park rats (approx. 100g) were housed at 3 per cage for 4 days prior to surgery and for at least 7 days following bilateral adrenalectomy or sham operation. Adrenalectomised rats were maintained on 0.9% (w/v) saline pre and post-operatively. Carrageenan paw oedema was induced by subplantar injection of 1% (w/v) carrageenan (Viscarin, Marine Colloids) 30 minutes after s.c. dosing with U50488, tifluadom or vehicle. Paw volume was measured 3 hours post carrageenan.

ED_{50} values for inhibition of carrageenan oedema in intact animals by tifluadom and U50488 were found to be 3.4mg/kg and 4.6mg/kg s.c. respectively. Approximate ED_{50} doses for tifluadom and U50488 of 3mg/kg and 5mg/kg s.c. respectively produced the expected reduction in paw volume in sham operated animals when compared to vehicle controls ($p < 0.001$, $n = 9$). This effect was totally abolished in adrenalectomised animals. Inhibition of carrageenan oedema by the glucocorticoid dexamethasone (0.3mg/kg s.c.) was unaffected by adrenalectomy. Plasma levels of corticosterone in adrenalectomised animals were below the level of detection of the corticosterone radioimmunoassay (<50ng/ml plasma).

At the same dose levels U50488 and tifluadom produced approximately 7 fold ($p<0.005$, $n = 9$) increases in plasma corticosterone levels one hour following s.c. dosing in intact animals housed 3 per cage with daily handling for 4 days prior to use.

Taken together these observations indicate that the previously observed anti-inflammatory activity of U50488 and tifluadom is mediated by a rise in plasma corticosterone levels.

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PLATELET ACTIVATING FACTOR IN THE BRONCHOALVEOLAR LAVAGE FLUID OF OXYGEN TREATED RATS

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Asthma has been described as an inflammatory condition (Page et al, 1983). We have previously reported the presence of Platelet-Activating Factor (PAF-acether) in the bronchoalveolar lavage (BAL) fluid from some asthmatic subjects (Court et al, 1987). Since the exposure of rats to oxygen can cause inflammation of the pulmonary endothelium (Kistler et al, 1967), we have examined the BAL fluid of rats previously exposed to oxygen. This was to determine whether or not inflammation could also induce the release of PAF-acether and lyso PAF from the lungs of these animals.

Male Wistar rats (250-300g) were placed in a sealed chamber, allowed free access to food and water, and exposed to either air or oxygen at a rate of 190ml/min/rat for periods of 12, 24 and 48h respectively. The rats were then anaesthetised with urethane (1.6mg/g) and a tracheal cannula was inserted. Sterile saline (0.9% w/v) containing bovine serum albumin (0.25% w/v) [0.04ml/g body weight] was infused into the lungs over a period of 1 min, and 1 min later it was withdrawn (over a period of 1.5 min). The procedure was then repeated and the two aspirates from each rat were pooled. The BAL fluid was centrifuged at 110xg for 10 min prior to extraction, and then half the sample was acetylated to enable lyso PAF to be measured (Court et al, 1987). The acetylated and the unacetylated samples were then assayed for PAF-acether activity using guinea-pig platelet-rich plasma (Court & Kingston, 1987).

PAF-acether was detected in the BAL fluid from animals exposed to oxygen for 12h but not in the other groups. Lyso PAF, however, was detected in the samples from both oxygen and air exposed rats, though the pattern of release from the oxygenated lungs differed from that of the control, air exposed, rats (Table 1).

Table 1. The concentrations of PAF-acether and lyso PAF within the BAL fluid. At each exposure (n=4), the mean concentration (nM) and range, in parenthesis, are indicated.

	12h		24h		48h	
	AIR	OXYGEN	AIR	OXYGEN	AIR	OXYGEN
PAF-acether	0	0.45 (0.06-0.98)	0	0	0	0
Lyso-PAF	0.29 (0.28-0.30)	5.50 (3.01-16.60)	6.04 (0.54-15.50)	9.72 (3.91-15.4)	11.16 (4.29-26.36)	0.52 (0.20-1.16)

PAF-acether has, therefore, been detected in the BAL fluid of two inflammatory conditions; human asthmatics, and artificially inflammed rat lungs.

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INHIBITION OF HISTAMINE-INDUCED BRONCHOCONSTRICTION BY SK&F 94836, SALBUTAMOL AND THEOPHYLLINE IN ANAESTHETISED GUINEA-PIGS

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SK&F 94836 is a potent selective phosphodiesterase III (PDE III) inhibitor (Reeves et al. 1987) with inotrope and vasodilator activity in vivo (Gristwood et al. 1987). PDE III inhibition elevates the intracellular cAMP content and this is a possible mechanism for the relaxation of bronchial smooth muscle. In this study we have examined the effect of SK&F 94836 on resting bronchial tone, inhibition of histamine-induced bronchoconstriction (HIB), and heart rate in guinea-pigs. SK&F 94836 was compared with salbutamol and theophylline.
Guinea-pigs were artificially respired at 40 breathes/min. Overflow pressure on inflation, blood pressure and heart rate were monitored as described by Owen and Pipkin (1985). The animals were repeatedly challenged with bolus i.v. injections of histamine. Reproducible responses to histamine (≃150% increase in overflow pressure) were obtained. At least 1 minute prior to each histamine challenge either vehicle or drug was administered (i.v. bolus). Responses to HIB pre- and post- drug were compared.
SK&F 94836 caused dose related inhibition of HIB, 26±13% and 62±14% inhibition at 10^{-7} and 10^{-6} mol/kg respectively. Salbutamol caused dose related inhibition of HIB, 22±8%, 48±8% and 68±4% at 4 x 10^{-10}, 2 x 10^{-9} and 4 x 10^{-9} mol/kg respectively. Theophylline also caused dose related inhibition of HIB, 32±8% and 84±8% at 3.16 x 10^{-5} and 10^{-4} mol/kg respectively. At the above concentrations none of the drugs decreased resting bronchial tone, but all increased heart rate in a dose-dependent manner.
In conclusion, we have shown that SK&F 94836 inhibits HIB and thus may possess bronchodilator activity that could be of use therapeutically. Comparison of SK&F 94836 with salbutamol and theophylline in their ability to inhibit HIB yields a rank order of potency salbutamol > SK&F 94836 > theophylline.

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SIMULTANEOUS MEASUREMENT OF AGONIST-INDUCED ^{45}Ca EFFLUX AND CONTRACTION IN ISOLATED AIRWAY SMOOTH MUSCLE

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Agonist-induced contraction of airway smooth muscle is largely dependent upon mobilisation of Ca^{2+} from intracellular stores (Giembycz & Rodger, 1987). This being the case then it should be possible to demonstrate agonist-induced Ca^{2+} efflux from this tissue, just as in vascular smooth muscle (see Leijten & van Breemen, 1984). In the present study we have measured simultaneously the tension changes and pattern of ^{45}Ca efflux from guinea pig isolated trachealis induced by histamine, methacholine, KCl and caffeine.

Single rings of trachea were loaded with ^{45}Ca ($2\mu Ci\ ml^{-1}$) for 2h and then, suspended under 2g resting tension in water-jacketed tissue baths at 37°C, superfused with non-radioactive Tris Krebs-Henseleit solution at a rate of $2.2ml\ min^{-1}$. The superfusate was collected every 1 or 2 min and the radioactivity measured by liquid scintillation counting. Tissues were blotted and left overnight in 3ml EDTA ($5mmol\ l^{-1}$) to extract the remaining ^{45}Ca. Tension changes were measured by conventional methods.

^{45}Ca efflux from unstimulated trachealis was bi-exponential, comprising a fast ($t_{1/2}$ = 5±0.2 min) and a slow ($t_{1/2}$ = 22±1 min) component. The effects of the contractile agonists were examined at two points (60 & 90 min) during the slow efflux period. The results are shown in Table 1.

Table 1. Effects of histamine (Hist), methacholine (Mch), KCl and caffeine on contraction and ^{45}Ca efflux from guinea-pig isolated trachealis. Results are mean + S.E. mean, n = 4 tissues.

AGONIST	TENSION GENERATED (g)		INCREASE IN ^{45}Ca EFFLUX RATE (% min^{-1})	
	60 min	90 min	60 min	90 min
Hist (10μM)	0.98±0.1	1.33±0.09	0.14±0.01	0.15±0.01
Mch (10μM)	0.69±0.09	0.98±0.15	No change	0.13±0.02
KCl (90mM)	0.82±0.1	1.25±0.12	0.12±0.02	0.05±0.003
Caffeine (100mM)	Nil	0.03+0.02	0.41+0.02	0.34+0.02

Histamine, methacholine and KCl all elicited sizeable contractions of the trachealis that were associated with accelerated Ca efflux from the tissue (except for Mch at 60 min). In contrast, caffeine only induced small contractions of the trachealis. These contractions, however, were accompanied by the greatest observed increases in Ca efflux.

The results demonstrate that it is possible to measure agonist-induced ^{45}Ca efflux from isolated airway smooth muscle simultaneously with changes in developed tension. The pattern of stimulated Ca efflux from the tissue is, however, complex and the underlying mechanisms require further investigation.

SEB is supported by a scholarship from Organon Laboratories

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EFFECT OF METHACHOLINE AND LEUKOTRIENE D_4 ON CYCLIC AMP CONTENT AND CYCLIC AMP-DEPENDENT PROTEIN KINASE ACTIVITY IN AIRWAY SMOOTH MUSCLE

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There is a wide disparity between the effects of different contractile agonists on the cyclic AMP (cAMP) content of airway smooth muscle (see Torphy et al, 1986). Measurement of the cAMP-dependent protein kinase (A-kinase) activity ratio, however, is regarded as a more sensitive indicator of intracellular cAMP content than measurement of cAMP itself. In this study, therefore, we have examined the effects of methacholine and leukotriene D_4 (LTD_4) on cAMP levels and cytosolic A-kinase activity of guinea-pig isolated trachealis.

Guinea-pig tracheal rings were suspended in Krebs-Henseleit solution, containing flurbiprofen (1μM) and tension changes recorded by conventional methods. Tissues were rapidly frozen in liquid nitrogen at pre-determined periods, between 0 and 15 min, after addition of the agonist. The cAMP content of the tissue was measured by protein-binding assay (Amersham). A-kinase activity was measured using a modification of the method described by Torphy et al, (1986), and is expressed as an activity ratio.

Methacholine (100μM) produced significant reductions ($p < 0.05$, paired T-test) in cAMP content of the tissue after 4 and 15min, of 0.069±0.008 and 0.087±0.018 pmol/mg tissue respectively (n=5). LTD_4 (100nM) only produced a significant reduction in cAMP content after 8min (of 0.37±0.08 pmol/mg) which was maintained through until 15min.

Methacholine produced a significant ($p < 0.05$, Mann-Whitney) decrease in the A-kinase activity ratio over the first minute of agonist stimulation (Fig 1). This effect was blocked by pretreatment with atropine (5μM). In contrast, LTD_4 was without effect on A-kinase activity.

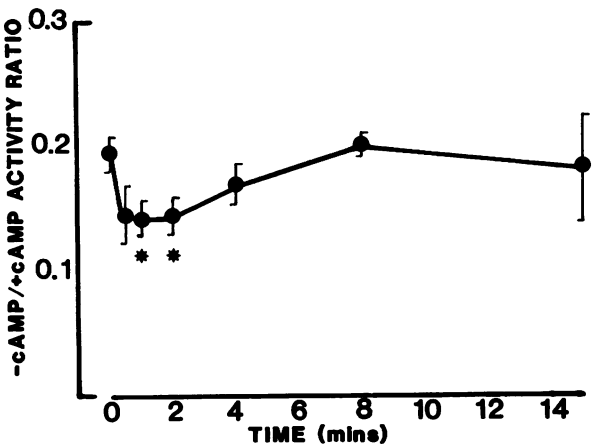

Figure 1.

Time-course of the effect of methacholine (100μM) on the A-kinase activity ratio in guinea-pig isolated trachealis. Values are the mean ± S.E. mean of 6 experiments.
* indicates values significantly different from control (see text).

These results suggest that a reduction in the activity of cyclic AMP-dependent protein kinase may constitute part of the mechanism underlying cholinomimetic-induced contraction of guinea-pig airway smooth muscle. This is unlikely to be the case for LTD_4.

We are grateful to the Asthma Research Council and SHERT for financial support.

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RESPONSES OF THE RABBIT PULMONARY MICROVASCULATURE TO PLATELET ACTIVATING FACTOR

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Intradermal injection of platelet activating factor (PAF) in the rabbit induces an increase in microvascular permeability as assessed by the local accumulation of intravenously injected ^{125}I-albumin, and marked synergism with vasodilator prostaglandins (eg. PGE_2) to produce oedema has been demonstrated (1,2). In the present study we have investigated the effects of PAF on the pulmonary microvasculature of the rabbit by measuring the extravascular accumulation of ^{125}I-albumin and changes in the pulmonary vascular resistance.

Rabbit isolated lungs were perfused via the pulmonary artery at 20ml/min with Tyrode's solution containing 4.5% Ficoll 70 and 0.1% BSA, and perfusion pressure was continuously monitored. Perfusion fluid was pumped for alternating periods of 5 min from two reservoirs, one containing ^{125}I-albumin and one containing unlabelled albumin. Microvascular leakage of albumin was monitored by measuring the extravascular accumulation of ^{125}I-albumin using an external, collimated gamma-scintillation probe. Each experiment comprised six 10 min cycles; each cycle consisted of a 5 min 'wash-in' with labelled perfusate and a 5 min 'wash-out' with unlabelled perfusate. Radioactive counts in the collimated region of the lung were recorded every 30 sec and the residual activity at the end of the 'wash-out' used as a measure of extravasated albumin.

Bolus injections of PAF (1nmole) induced increases in perfusion pressure (peak Δ = 10.5 ± 1.2mmHg, mean ± SEM, n=11) which were reduced by ≈70% in the presence of either indomethacin (10μM) or dazmegrel (10μM) suggesting that PAF induced pulmonary vasoconstriction is mediated in part by a cyclooxygenase metabolite, probably thromboxane. Infusion of PAF (3nmole/min) over 10 minutes (during the fourth cycle) also induced an increase in perfusion pressure (peak Δ = 25 ± 4mmHg, n=10) and resulted in an increased accumulation of extravascular ^{125}I-albumin. At the end of the experiment (ie. at 60 min), 250 ± 33μg albumin had accumulated in control lungs (n=6), 279 ± 27μg in lyso-PAF infused lungs (n=4) and 850 ± 170μg in PAF infused lungs (n=10). PAF-induced increases in pulmonary infusion pressure and protein accumulation were effectively reduced in lungs that were perfused with 10μM L-652731, a selective PAF antagonist (2); albumin accumulation at 60 min was 391 ± 55μg (n=4). Infusion of PAF during retrograde perfusion of the lungs resulted in enhanced albumin accumulation when compared with forward perfusion (2441 ± 338μg at 60 min, n=6). This suggests that PAF constricts arterioles to cause increased perfusion pressure and thus, during retrograde perfusion, hydrostatic pressure within capillaries is higher than during forward perfusion which favours increased protein accumulation. Infusion of a stable thromboxane mimetic U46619 (0.6nmoles/min for 10 min) induced comparable increases in perfusion pressure to those observed with PAF (peak Δ = 31 ± 6mmHg, n=6). In the same lungs, albumin accumulation at 60 min was not substantially different from controls lungs (339 ± 54μg, n=6). Thus, PAF-induced increases in protein accumulation appear to be due to direct action on pulmonary capillaries and are not solely a consequence of increased hydrostatic pressure.

These results suggest that PAF may be an important mediator of oedema formation in the lung.

We thank Pfizer Central Research, Sandwich, Kent, for financial support.

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CONTRASTING ACTIVITIES OF 12(R)- AND 12(S)-HYDROXY-5,8,10,14-EICOSATETRAENOIC ACIDS AS LYMPHOCYTE CHEMOATTRACTANTS

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The elevated amounts of leukotriene B_4 [LTB_4] (Brain et al., 1984) and 12(R)-hydroxy-5,8,10,14-eicosatetraenoic acid [12(R)-HETE] (Woollard, 1986) in the lesional scale of patients with the skin disease psoriasis, may be partly responsible for the neutrophil and lymphocyte infiltrates at lesional sites. 12(R)-HETE has been found to be more potent than the platelet product, 12(S)-HETE, in activating neutrophils in vitro, although considerably less potent than LTB_4 (Cunningham et al., 1987; Evans et al., 1987). Since 12(R)-HETE has recently been shown to be a lymphocyte chemoattractant in vitro (Bacon et al., 1987), the chemotactic activity of 12(R)-HETE for human lymphocytes has now been compared with that of 12(S)-HETE and LTB_4.

The preparation of mixed peripheral blood lymphocytes and measurement of chemotaxis was carried out as previously described (Bacon et al., 1987). Results have been expressed as mean $\pm$ s.e. mean migration index [area of lower surface of filter occupied by cells in response to chemoattractant (mm^2) / area of filter occupied by randomly migrating cells (mm^2)]. LTB_4 was a gift from Dr J. Rokach (Merck Frosst, Canada); 12(R)- and 12(S)-HETE were resolved by chiral column h.p.l.c. from 12(R,S)-HETE, prepared by photooxidation of arachidonic acid, and were supplied by Dr P. Woollard. Serial dilutions of the hydroxy fatty acids were prepared in serum free Eagle's Minimal Essential Medium buffered with N-2-hydroxyethylpiperizine-N-2-ethanesulphonic acid (HEPES) buffer (pH 7.4).

Dose-related chemotaxis was obtained in response to 12(R)-HETE, whilst in contrast, 12(S)-HETE exhibited only weak lymphocyte chemotactic activity.

Concentration (M)	Migration Index $\pm$ s.e. mean (n=5)	
	12(R)-HETE	12(S)-HETE
5×10^{-7}	1.35 ± 0.12	1.10 ± 0.13
10^{-6}	1.55 ± 0.19	1.12 ± 0.08
5×10^{-6}	2.01 ± 0.21	1.22 ± 0.09
10^{-5}	2.72 ± 0.36	1.37 ± 0.15
5×10^{-5}	1.65 ± 0.29	1.29 ± 0.14

In a further four experiments a comparison of chemotactic responses to 12(R)-HETE and LTB_4 showed that LTB_4 was at least 200 times more potent than 12(R)-HETE on a molar basis, approximate ED_{50} values being 1.9×10^{-8}M and 5.2×10^{-6}M for LTB_4 and 12(R)-HETE, respectively. Maximum migration indices were similar (2.96 ± 0.41 and 2.99 ± 0.36 for LTB_4 and 12(R)-HETE respectively).

12(R)-HETE thus exhibits much greater in vitro activity as a chemoattractant for lymphocytes than 12(S)-HETE. Although LTB_4 is a more potent chemoattractant than 12(R)-HETE, the latter is found in 1000 fold greater amounts in extracts of lesional psoriatic skin, suggesting that both LTB_4 and 12(R)-HETE may be of similar importance in eliciting lymphocyte infiltration in this disease.

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EFFECT OF THE DIACYLGLYCEROL KINASE INHIBITOR R59022 ON AGONIST-INDUCED HUMAN PLATELET ACTIVATION

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R59022 is an inhibitor of the enzyme 1,2-diacylglycerol (DAG) kinase which converts DAG to phosphatidic acid (PA). In human platelets, R59022 has been shown to increase the endogenous levels of DAG in response to agonist stimulation and to increase the activity of the DAG-dependent enzyme protein kinase C (PrKC) [de Chaffoy de Courcelles et al, 1985], the activation of which closely parallels granule secretion [Nishizuka, 1986]. However, this work has only addressed the role of DAG as a potentiatory signal in platelet activation, whereas recent work has suggested that PrKC may also be involved in the inhibition of platelet responses [Krishnamurthi et al, 1986; 1987; Drummond & MacIntyre, 1985]. In the present study, R59022 was used to increase agonist-induced DAG levels in order to assess the relative importance of DAG as a potentiatory versus inhibitory signal in human platelet activation.

All studies were performed using plasma-free suspensions of human platelets suspended in a Hepes-tyrode buffer pH7.4, prelabelled with either [^{14}C]-5HT (a dense-granule marker), [^{3}H]-arachidonate or [^{32}P]-phosphate and concentrations of agonists that were threshold or submaximal with respect to inducing [^{14}C]-5HT secretion. R59022 (30μM) potentiated [^{14}C]-5HT release in response to the thromboxane A_2 mimetic U46619 (100nM), the DAG analogue 1,2-dioctanoyl-glycerol (DiC_8, 60μM), and submaximal thrombin concentrations (0.05-0.2U/ml); the latter two responses correlated with an increase in the incorporation of [^{32}P] label into a 45KDa protein, a marker of PrKC activity [Nishizuka, 1986]. However, a number of observations in the present study point to R59022 acting at a site(s) other than DAG kinase. Platelet aggregation in response to collagen (2μg/ml), platelet activating factor (100ng/ml), the PrKC activators DiC_8 and phorbol 12, myristate-13-acetate (PMA, 6-16nM), U46619 and thrombin were inhibited by R59022 (30-60μM). Furthermore, collagen-induced [^{3}H]-arachidonate release and [^{14}C]-5HT secretion which are aggregation-dependent events, were inhibited by R59022 (30μM). As the inhibitory effects of R59022 on collagen-induced responses are opposite to those obtained with DiC_8 and PMA [Krishnamurthi et al, 1987], and are most likely related to the inhibition of aggregation, the results question the usefulness of R59022 as a tool in elucidating the role of DAG in platelet activation, especially when the latter is aggregation-mediated. Additionally, R59022 (30μM) potentiated [^{14}C]-5HT release in indomethacin (10μM)-treated platelets in response to PMA and to a threshold concentration of the Ca^{2+}-ionophore ionomycin (50nM), and enhanced PMA-induced 45KDa protein phosphorylation. As neither PMA nor ionomycin induces DAG formation under the conditions employed, this potentiation of 5HT secretion by R59022 is unrelated to its previously described effects as a DAG kinase inhibitor, and further underlines the other non-specific effects of the drug on platelets, apart from DAG kinase inhibition. Care should therefore be employed in using R59022 as a tool in investigating platelet stimulus-response coupling based solely on its actions as a DAG kinase inhibitor.

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EVIDENCE FOR A FUNCTIONAL CYTOCHROME P450 - MONOOXYGENASE PATHWAY IN THE ISOLATED LAMB DUCTUS VENOSUS

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The ductus venosus (D.V.) sphincter of mature fetal lambs contracts to indomethacin in vitro (Adeagbo et al 1982) and in vivo (Sideris et al 1982) suggesting that a product of arachidonate cyclooxygenase actively maintains patency of the ductus. PGI_2 rather than PGE_2 has been ascribed this role (Adeagbo et al 1985). The present study examined the effect of cytochrome P450 inhibitors and of 5,6-; 11,12-; and 14,15-expoxyeicosatrienoic acids (ETAs) on the ductus. DV sphincters from near-term fetuses were suspended between platinum hooks in a 10ml bath containing Krebs solution at 37°C bubbled with either 2.5% O_2 and 5% CO_2 in N_2 (PO_2 between 18 and 30 mm Hg; low PO_2) or 5% CO_2 in O_2 (PO_2 between 550 and 670 mm Hg; high PO_2). Tension was recorded isometrically via a transducer (Grass FT .03C) coupled to a Grass polygraph.

Unlike indomethacin, none of the cytochrome P450 inhibitors metyrapone, 4-phenylimidazole in concentrations up to 1mM and 14, 15-isonitrile (50uM) contracted the naive (ie unconstricted) ductus; suggesting that the product of cyto. P450-linked arachidonate monooxygenase if important endogenously may not mediate ductal relaxation. However, these compounds reversed indomethacin-induced tone concentration dependently at low or high PO_2. Sphincters constricted with 20mM K^+ (without indomethacin) were not relaxed by all the compounds indicating that relaxation was not due to non-specific interaction with Ca^{2+} fluxes rather could be due to a specific inhibition of endogenous formation of contractile products via cyto. P450-catalysed pathway.

Of the three ETAs studied, only the 5,6-derivative has remarkable activity under our test conditions. At 10^{-7} - 10^{-5}M, it caused dose-dependent biphasic contractions of the ductus in low and high oxygen media. The mean contractile tension to 10^{-5}M was 322.5 $\pm$ 6.7mg, and contractions are attributable to 5,6-ETA itself since its d-lactone derivative (Kutsky et al, 1983 was completely inactive on the ductus. Phenoxybenzamine (5 x 10-7M) or procaine (10^{-5}M) treatment did not inhibit any of the contractile components implying that the effects were of myogenic origin and could not have been due to indirect release of contractile principles from adrenergic or other neuronal terminals. On the other hand, indomethacin (2.8 x 10^{-6}M) or endothelium removal completely abolished the phasic but not the tonic components suggesting that 5,6-ETA may either cause a release of cyclooxygenase product(s) or is itself transformed by cyclooxygenase in the ductal endothelium to contractile products. The present data thus show that product(s) of cyto P450 - linked reactions may compliment cyclooxygenase products in the regulation of ductal tone.

ASOA was supported jointly by Wellcome (Nig.) Trust Fund and the HSC Foundation.

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EVIDENCE THAT LEUKOTRIENE C_4 AND U46619 INDUCE HYPOXIA IN THE GUINEA-PIG ISOLATED PERFUSED HEART

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We and others have shown that the negative inotropic action of leukotriene C_4 (LTC_4) is a consequence of coronary vasoconstriction (Roth et al, 1985; Trevethick et al, 1987). Furthermore, the thromboxane A_2 mimetic, U46619, a potent coronary vasoconstrictor, also decreased force of contraction in the guinea-pig perfused heart (Kennedy et al, 1983). Vasoconstriction may cause myocardial hypoxia and result in a loss of contractile function. Since inadequate myocardial oxygenation would stimulate anaerobic metabolism, leading to increased lactate formation, we have evaluated the ability of both LTC_4 and U46619 to enhance lactate efflux from the guinea-pig, isolated, perfused heart.

Hearts were perfused with oxygenated (95% O_2 / 5% CO_2) Krebs-Henseleit solution at constant flow (7ml/min) by the Langendorff method (Kennedy et al, 1983). After 30 minutes equilibration, LTC_4 (10^{-7}M), U46619 (10^{-7} M), or verapamil (10^{-7}M) was infused into the heart for 10 and 15 minutes respectively. In a further 5 experiments, hypoxia was induced by perfusion for 20 minutes with Krebs previously equilibrated with 95% N_2 / 5% CO_2. Coronary effluent samples were collected throughout the experimental and recovery periods, and were subsequently assayed for lactate enzymatically (Gawehn et al, 1974).

LTC_4 and U46619 decreased force of contraction, increased perfusion pressure and increased the rate of lactate efflux from the heart. Similarly, perfusion with hypoxic Krebs also decreased force of contraction and increased the rate of lactate efflux, but decreased perfusion pressure. In contrast, verapamil decreased force of contraction, increased perfusion pressure and decreased lactate efflux. This unexpected increase in perfusion pressure may reflect depression of myocardial oxygen demand and loss of metabolic vasodilatation of the coronary circulation. The maximum changes in each of the parameters caused by the different interventions are summarised in Table 1.

Table 1: Comparison of LTC_4, U46619, Verapamil and Hypoxia-induced effects on the guinea-pig isolated perfused heart. (Each value is the mean ± standard error of the mean.)

	n	Force (%)	Pressure (mmHg)	Lactate Efflux (µmol/min)
LTC_4	6	-72 ± 12	+39.5 ± 8.8	+5.91 ± 0.54
U46619	6	-84 ± 11	+45.5 ± 11.5	+3.21 ± 0.41
Verapamil	3	-46 ± 10	+22.0 ± 4.3	-2.55 ± 0.25
Hypoxia	5	-63 ± 2	-13.2 ± 3.3	+6.84 ± 0.61

We conclude that in the guinea-pig, isolated, perfused heart LTC_4 and U46619 cause coronary vasoconstriction, which is sufficiently severe to increase the rate of lactate efflux, suggesting that a significant number of myocytes were rendered hypoxic. Diminished oxygen supply may then be responsible for the decreased force of contraction. Interestingly, this response occurs in a constant flow perfusion model, implying that treatment with these vasoconstrictor agents leads to redistribution of flow through the myocardium.

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ANTAGONISM BY CALCIUM ANTAGONISTS OF LEUKOTRIENE-INDUCED CORONARY CONSTRICTION IN VITRO

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We have previously shown that verapamil, a calcium entry blocker (CEB), antagonises the vasoconstrictor effect of leukotriene (LT) C_4 in the rat isolated working heart (Piper & Stanton, 1984). The possible formation and release of LTs in the circulatory system in vivo (see Lefer, 1986) and the growing use of CEBs for vasospastic disorders in man has led us to examine other drugs for a possible effect on LTC_4-induced coronary constriction. We report the effects of verapamil (VER), nifedipine (NIF) and two purported intra-cellular calcium antagonists, TMB-8 and HA-1004, in this system. TMB-8 has been reported to antagonise LTD_4-induced contractions of monkey respiratory smooth muscle (Weichman et al, 1985) whilst HA-1004 relaxes rabbit aortic strips pre-contracted with A23187 (Ishikawa et al, 1985).

Hearts from Wistar rats (♂, 275-425g) were perfused via their aortae with modified Ringer-Locke solution at 38°C. A constant head of 40 mmHg pressure was maintained and 4g tension applied to the apex. Coronary flow (CF) was recorded by means of a photoelectric drop-counter and perfusion pressure (PP) was monitored continuously. After stabilisation, each heart received three 60-sec infusions of LTC_4 (same concentration) at 15 min intervals. Recovery occurred between infusions. Infusion (Infn) 1 was LTC_4 alone; infn 2 was LTC_4 + antagonist. CEB or TMB-8 infn was commenced 10 sec before and continued for 60 sec with the LTC_4. HA-1004 was infused for up to 5 min before and then with LTC_4. Infn 3 was LTC_4 alone and demonstrated recovery from antagonism of the response. Responses were calculated as ▲coronary vascular resistance (▲CVR) per g wet weight of ventricles, i.e. $PP/CF_{infn} \cdot g^{-1} - PP/CF_{pre\text{-}infn} \cdot g^{-1}$ (units, U). LTC_4 2.5, 5 and 10 x 10^{-9} M induced ▲CVR of $^{+}2.3\pm0.4$, $^{+}4.4\pm0.7$ and $^{+}7.1\pm2.9$ U respectively (n=5, means + s.e.means). VER $5x10^{-7}$ reduced these to $^{+}0.4\pm0.04$, $^{+}0.5\pm0.1$ and $^{+}4.2\pm2.1$ U. NIF $5x10^{-9}$ to $5x10^{-6}$ M, VER $5x10^{-8}$ to $5x10^{-6}$ M, TMB-8 $5x10^{-7}$ to $5x10^{-5}$ M and HA-1004 $5x10^{-7}$ to $5x10^{-5}$ M were examined for their relative potency in this system. LTC_4 $5x10^{-9}$ M was used throughout. NIF, VER and TMB-8 reduced LTC_4-induced increases in CVR in a dose-related manner whereas HA-1004 was without effect. Significant antagonism was achieved at the lowest concentration of each active drug. Maximum antagonism was not significantly different for nifedipine and verapamil (81.9 ± 10.5% and 93.2 ± 2.8%, both $5x10^{-6}$M). Maximum for TMB-8 was only 58.8 ± 4.2%, $5x10^{-5}$M. Higher concentrations resulted in elevation of CVR by themselves. Both NIF and VER $5x10^{-7}$ to $5x10^{-6}$ M produced small changes in CVR when infused without LTC_4. Lower concentrations were without effect on coronary tone. IC_{50} for NIF was $3.3x10^{-8}$M, for VER: $3.5x10^{-7}$M and for TMB-8: $2.9x10^{-5}$M. Experiments in which arginine-vasopressin was substituted for LTC_4 demonstrated that reversal of vasoconstriction by CEBs is not specific for LTs.

Experiments with these and other calcium antagonists may provide insight into the mechanisms of LT-mediated vasoconstriction.

We thank Dr J. Rokach of Merck Frosst Laboratories, Prof F. Hoffmeister of Bayer AG and Prof H. Hidaka for gifts of LTC_4, nifedipine and HA-1004 respectively. This work was supported by BHF grants nos. 84/80 and 85/86.

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CHARACTERISATION OF PROSTANOID RECEPTORS IN THE RAT PULMONARY CIRCULATION

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We have adapted a method (Dyson et al, 1987) for direct measurement of pulmonary artery pressure (PAP) in rats to study depressor as well as pressor responses to prostanoids. Agonists used were PGD_2, PGE_2 and its analogues 16,16-dimethyl PGE_2 (16,16-dmPGE_2) and misoprostol, ICI 81008 (a specific $PGF_{2\alpha}$ receptor agonist), PGI_2 mimetics Cicaprost (ZK 96480) and Iloprost, and the TXA_2 mimetic U-46619. Antagonists used were EP092, a specific thromboxane receptor antagonist (Armstrong et al, 1985), and AH6809 which inhibits the actions of PGD_2 on human platelets (Keery & Lumley, 1985) and the contractile effects of PGE_2 on smooth muscle (Coleman et al, 1985) but not the relaxant effects of PGE_2 or PGI_2.

Male PVG or Hunter rats (180-290g) anaesthetised with i.p. pentobarbitone sodium (70 $mgkg^{-1}$) were prepared as previously described. To study the actions of depressor prostanoids, the ventilation gas was changed from 40% oxygen to air once the pulmonary artery needle was in place. Depressor responses were calculated as % reduction of the hypoxia-induced elevation of PAP.

PGD_2 and ICI 81008 produced pulmonary pressor responses that were slower in onset with lower maxima (<10mmHg) than U-46619 (>20mmHg). The PGI_2 mimetics produced depressor responses only, but PGE_2 and particularly 16,16-dmPGE_2 had biphasic effects, the depressor predominating at low doses and the pressor to a variable degree at high doses. Misoprostol gave pulmonary pressor responses only. Relative activities are shown in Table 1.

Table 1 Comparison of pulmonary pressor and depressor activities

Prostanoid	Dose ($\mu g\ kg^{-1}$ ± s.e.m.) 5 mmHg rise in PAP		Prostanoid	Dose ($\mu g\ kg^{-1}$ ± s.e.m.) 75% reduction in PAP	
U-46619	0.27 ± 0.033	(n = 8)	Cicaprost	0.13 ± 0.038	(n = 5)
PGD_2	5.5 ± 2.2	(n = 7)	Iloprost	0.77 ± 0.17	(n = 6)
ICI 81008	8.7 ± 3.3	(n = 5)	PGE_2	11 ± 2.4	(n = 4)
Misoprostol	15 ± 3.2	(n = 6)			

EP092 (20 $\mu gkg^{-1}min^{-1}$) antagonised the pulmonary pressor effects of U- 46619 (dose ratio 12.3 ± 0.69, n=5), 16,16-dm PGE_2 and misoprostol, but not those of PGD_2 or ICI 81008. The response to ICI 81008 desensitised readily. AH6809 (5 $mgkg^{-1}$) inhibited the effects of PGD_2 (dose ratio 3.5 ± 0.3, n=5). AH6809 slightly inhibited the action of U-46619 (dose ratio 1.24 ± 0.11, n=9); in animals where this was marked, the 16,16dmPGE_2 and misoprostol induced PAP rises were also inhibited slightly. AH6809 did not affect the depressor actions of PGE_2 or Iloprost.

These results suggest that, in addition to a thromboxane receptor system, the rat pulmonary circulation contains specific but less sensitive PGD and PGF pressor systems. The PGI_2 mimetics, Cicaprost and Iloprost, are potent pulmonary vasodilators, and are much more active than PGE_2. Identification of a specific PGE_2 dilator system is complicated by the finding that the PGE_2 analogues have pressor effects which are almost certainly mediated through the thromboxane system.

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EFFECT OF A THROMBOXANE ANTAGONIST ON ORGAN BLOOD FLOW IN SPONTANEOUSLY HYPERTENSIVE RATS OF DIFFERENT GESTATIONAL AGES

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It has previously been reported that thromboxane (TXA_2) plays an increasingly important role in controlling flow to the reproductive organs of a normotensive strain of rat as gestation advances (Kerr & Senior 1987). In this study AH23848 ($[1\alpha(Z),2\beta,5\alpha]$-(±)-7-[5-[[1,1-biphenyl]-4-yl]methoxyl]-2(4-morpholinyl)-3-oxo cyclopentyl]-4-heptanoic acid), a thromboxane receptor antagonist, was employed as a tool to assess the involvement of TXA_2 in control of regional blood flow (BF) in non-pregnant (pro-oestrous (PO) phase of the oestrous cycle) and the pregnant spontaneously hypertensive rat (SHR, Okamoto strain). BF was measured in the anaesthetised (pentobarbitone sodium 60mg kg^{-1} i.p.) non-pregnant and pregnant (days 10, 21 and 23) SHR using the labelled microsphere technique (^{46}Sc, 15μm diameter). BF was expressed using tissue wet weight as ml min^{-1} $100g^{-1}$. AH23848 (2.5mg kg^{-1} i.v.) was administered 3 hours prior to experimentation in a sodium bicarbonate (1% w/v)/sodium chloride (0.9% w/v) vehicle. (Significance levels $P<0.05$*; $P<0.01$**; $P<0.001$***).

As gestational age advanced a number of changes occurred in the SHR. A fall in blood pressure was observed to near normotensive levels as parturition approached (BP mmHg: PO 190±10; day 10 195±12; day 21 150±20; day 23 150±10*). Uterine wet weight (mg) (PO: 348±18; day 10: 664±108*; day 21: 2730±176***; day 23: 2888±210***) and dry weight (PO: 56±8; day 10: 113±9*, day 21: 344±32***, day 23: 350±41***) increased as gestation progressed. BF per uterus was increased 5-fold in the pre-term animal; flow per unit weight was maintained despite large increases in uterine tissue mass. By day 21 blood supply to the ovaries (PO: 365±51, day 21: 1116±242*) and to the stomach (PO: 47±3, day 21: 106±20*) was significantly elevated above non-pregnant levels. AH23848 had no effect on blood flow to the uterus of the pregnant SHR. AH23848 potentiated ovarian flow on day 10 (day 10: 264±64; day 10 + AH23848 690±95**) and again on day 23 (day 23 1102±193; day 23 + AH23848: 1861±192*). Placental blood supply was also observed to be increased by AH23848 treatment in the pre-term SHR (day 23: 102±16; day 23 + AH23848 185±32*).

It would appear that TXA_2 plays some physiological role in controlling the flow to the utero-placental unit in the pregnant SHR. Pre-term placental flow is influenced in part by TXA_2 and in addition ovarian BF in this strain appears to be modified by the action of TXA_2 in mid-gestation and just prior to term. Both stages are characterised by a relatively high ratio of oestrogen:progresterone, which may have an effect on biosynthesis of TXA_2 in vivo.

This effect is similar to the results from the normotensive rat suggesting that there is no greater involvement of TXA_2 in the regulation of ovarian blood flow in the hypertensive rat (Kerr & Senior, 1987).

Parturition occurs in the normotensive rat on day 22 whereas in the SHR it is one day later but the pattern of changes in placental blood flow in the two strains are very similar as are the responses to the TXA_2 antagonist, again supporting the view that the TXA_2 involvement is similar in pregnancy in the two strains.

We thank Glaxo Group Research Ltd. for the supply of AH23848.

Kerr, M.B. & Senior, J. (1987) Proceedings of the British Pharmacological Society, Cambridge Meeting.

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CARDIO-PULMONARY EFFECTS OF INHALED ARACHIDONIC ACID AEROSOLS IN ANAESTHETIZED CATS

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Products of arachidonic acid (AA) metabolism have a presumptive role in the pathogenesis of asthma (Robinson & Holgate, 1985). Consequently there have been many studies of the effect of exogenous AA on bronchial smooth muscle tone, both in vitro and in vivo. In vivo the pharmacological effects of exogenously administered AA differ according to species, and the route of administration (Spannhake et al., 1980). In the present study we have investigated the cardio-pulmonary effects of an inhaled aerosol of AA in either normal or bilaterally-vagotomized anaesthetized cats, and compared these to our previous findings in rats and guinea-pigs (Payne & Lees, 1986).

Male mongrel cats (2.8 - 4.0 kg) were anaesthetized (initially with 5% halothane, thereafter with α-chloralose, 80-100 mg kg^{-1} i.v.), and mechanically ventilated (27 strokes min^{-1} of 15 ml laboratory air kg^{-1} body wt) through a mid cervical tracheostomy. A modified DeVilbiss ultrasonic nebuliser (Lees & Payne, 1986) was incorporated in the afferent limb of the ventilator circuit and used to generate and administer aerosols of AA (modal particle size 3.4 μm, weight mean diameter 9.0 μm) from a 1 mg ml^{-1} solution in 1M TRIS buffer (pH 8.5). Pulmonary inflation pressure (PIP), an index of intrathoracic airway calibre, was measured via a lateral port in the ventilator circuit. Femoral arterial blood pressure (BP) and heart rate (HR) were also recorded.

Sequential administration of aerosols of AA for increasing periods of 10, 20 and 40 sec provoked graded bronchoconstriction measured as a transient increase in PIP of 1.9 ± 0.4, 3.3 ± 0.7 and 5.4 ± 1.2 cm H_2O respectively (mean ± s.e. mean, n=6). This was accompanied by a maximum fall in BP of 26/21 ± 4.6/7.5 mmHg and in HR of 21.4 ± 11.1 b min^{-1}. In bilaterally-vagotomized animals, the bronchoconstrictor effect of AA aerosol (10-40 sec) was maintained (maximum rise in PIP of 5.8 ± 0.8 cmH_2O, mean ± s.e. mean, n=5) whereas the previously seen accompanying cardiovascular effects were abolished. The bronchoconstrictor effect of AA aerosol (10-40 sec) was markedly (>70%) attenuated, ($p<0.05$) following the cyclooxygenase inhibitor indomethacin (10 mg kg^{-1} i.v.).

We can conclude therefore that in anaesthetized cats, administration of exogenous AA as an inhaled aerosol provokes bronchoconstriction through its subsequent cyclooxygenase-catalysed degradation into broncho-active metabolites (probably $PGF_{2\alpha}$ and/or thromboxane A_2). In this respect the cat resembles the guinea-pig more closely than the rat (Payne & Lees, 1986). In contrast, the cardiovascular effects of AA aerosol in the cat are more similar to those in the rat. However, in the cat they are clearly mediated by an indirect vagal-reflex mechanism, possibly through the stimulation of irritant receptors also thought to be responsible for the bradycardia following i.v. administration of AA in this species. (Hintze et al., 1984).

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THE EFFECT OF NITRIC OXIDE ON THE ISOLATED PERFUSED RABBIT HEART

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Prostacyclin and endothelium-derived relaxing factor (EDRF) are important regulators of vascular tone. In addition, both compounds inhibit platelet aggregation, suggesting that they also regulate platelet-vessel wall interactions (for review see Moncada et al. 1987). We have recently demonstrated that nitric oxide (NO) released from vascular endothelial cells accounts for the biological activity of EDRF (Palmer et al., 1987). In view of this we have now studied the effect of authentic NO on the isolated perfused rabbit heart.

Hearts were obtained from rabbits treated with heparin (1000 U/kg, i.v.), anaesthetised with pentobarbitone sodium (30 mg/kg, i.v.) and bled from the carotid artery. The hearts were perfused through the aorta with Tyrode solution according to the Langendorff technique, at a constant flow of 20-26 ml/min. The coronary perfusion pressure (CPP) was raised to between 30 and 40 mm Hg by a continuous infusion of 9,11-dideoxy-9α, 11α methano epoxy-prostaglandin $F_{2\alpha}$ (U46619, 30-130 nM). Nitric oxide was prepared as solutions in Helium- deoxygenated water as described previously (Palmer et al., 1987).

Injections of NO (1.4 - 44.6 pmol, EC_{50} 5.2 ± 1.3, n=5) induced a dose-dependent decrease in CPP, without affecting the contractile force of the heart. Glyceryl trinitrate (GTN; 0.044 - 4.4 nmol, n=4) and sodium nitroprusside (SNP 0.38 - 38 nmol, n=4) also induced a dose-dependent decrease in CPP without affecting the contractile force of the heart.

A continuous infusion of indomethacin (1 μM) did not affect the vasodilatation induced by NO (n=4), however, pyrogallol (30 μM, n=4), an inhibitor of EDRF (Moncada, et al., 1986), inhibited the effect of NO without affecting the action of GTN or SNP.

Infusions of haemoglobin and methaemoglobin (0.1-3.0 μM, n=4) caused dose-dependent vasoconstriction both in control hearts and in hearts whose coronary circulation had been contracted with U46619. Neither compound modified the contractile force of the heart. Only haemoglobin however caused concentration-dependent inhibition of NO-induced vasodilatation (IC_{50} 31 ± 3 nM, n=3). Haemoglobin (1-3 μM), but not methaemoglobin, also caused partial inhibition of SNP-induced vasodilatation without affecting the response to GTN (n=3).

Our data indicate that NO is a powerful vasodilator of the coronary circulation. This action of NO is blocked by haemoglobin, a compound known to bind NO, but not by methaemoglobin. The partial reversal by haemoglobin of SNP-induced vasodilatation is probably due to the spontaneous generation of NO from SNP in the coronary circulation. In addition to being a potent inhibitor of NO, haemoglobin also has a direct vasoconstrictor action which is shared by methaemoglobin. These observations may be relevant to the understanding of vasospasm associated with haemorrhage.

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ENDOTOXIN-INDUCED IMPAIRMENT OF VASCULAR REACTIVITY IN THE PITHED RAT

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Impaired vascular reactivity to various agents was shown in patients with septic shock and in animals after endotoxin administration (Chernow and Roth, 1986) but the mechanism is unknown. We have studied reactivity to noradrenaline (NA)and to angiotensin II (AII) in pithed rats during infusion of E. Coli endotoxin. As prostanoids are known to be released in endotoxin shock in the rat (McKechnie et al 1985) their possible role in modifying vascular reactivity was examined. Male Sprague-Dawley rats (271 ± 12 g) were pithed under ether anaesthesia and artificially ventilated (100% O_2; 53 strokes/min; approximately 1 ml/100g, to keep arterial PCO_2 at 27 ± 2 mm Hg); rectal temperature was maintained at 37 ± 0.5 C. Cannulae were placed in the carotid arteries for blood sampling and for measurement of mean arterial blood pressure (M.A.B.P.) and heart rate (H.R.) and in the femoral vein for drug and endotoxin administration. After 1h equilibration, E.Coli endotoxin (Difco 055: B5, 1 µg or 1 mg/kg) or vehicle (0.9% saline) was infused over 2h. Cumulative dose-response curves to NA or AII were obtained 1h into the infusion. Control pithed rats showed stable M.A.B.P., H.R. and glucose over at least 5h. NA and AII (0.005-500 µg/kg) produced dose-dependent pressor responses, with $E_{max\ 50}$ values of 0.92 ± 0.11 and 0.24 ± 0.05 µg/kg respectively. Endotoxin infusion produced a dose-dependent fall in M.A.B.P. (-11.7 ± 2.2, n = 11 and -22.9 ± 2.7, n = 12 mm Hg with 0.001 and 1.0 mg/kg respectively). $E_{max\ 50}$ values for both NA and AII were significantly, and dose-dependently, increased by endotoxin (e.g. for 1 mg/kg endotoxin E_{max50} values for NA and AII were 21.3 ± 0.6 and 1.5 ± 0.1 µg/kg respectively, $p<0.01$ vs. control values). Pretreatment with flurbiprofen, a cyclooxygenase inhibitor (5 mg/kg 30 min pre-endotoxin) significantly reduced endotoxin-induced hypotension ($p<0.05$) and restored normal vascular reactivity to NA and to AII (two way ANOVA, $p<0.01$ and $p<0.05$ for interaction, NA and AII respectively). Infusion of the stable prostacyclin analogue, iloprost, to produce a hypotension (-22.4 ± 2.9 mm Hg, n = 6) similar to that produced by endotoxin (1 mg/kg) did not modify reactivity to NA. Thus endotoxin-induced hypotension is unlikely to explain the impairment of vascular reactivity to pressor agents. Moreover, although the flurbiprofen results suggest the involvement of a cyclooxygenase product, it seems unlikely that this is prostacyclin.

GAG is supported by an M.R.C. studentship.

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EVIDENCE THAT 8-OH-DPAT INDUCED HYPERPHAGIA IS MEDIATED BY 5-HT_{1A} RECEPTORS

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Previous studies have shown that 8-OH-DPAT and other putative 5-HT1A agonists cause hyperphagia in non-food-deprived rats (Dourish et al., 1986). In the case of 8-OH-DPAT hyperphagia depends on presynaptic 5-HT stores (Dourish et al., 1986) and is elicited by infusing the drug into the raphe nuclei (Hutson et al., 1986). We have now investigated the hyperphagic response to 8-OH-DPAT further using various antagonists in order to clarify which subtype of the 5-HT receptor is involved.

Individually housed, male Sprague-Dawley rats (250-300 g) fed ad lib on diet 22F (Labsure, Poole, Dorset) were given either vehicle or antagonist and 30 min later were injected with either 0.9% NaCl or 8-OH-DPAT (1 mg/kg s.c.). Food intake was measured in the home cage over the next 2 h. Vehicle treated rats consumed between 0.12 $\pm$ 0.04 (11) and 0.37 $\pm$ 0.08 g (15) (Mean $\pm$ SEM, no. rats in brackets) of diet in different experiments. Data were analysed by Kruskal-Wallis ANOVA followed by Mann-Whitney U test.

Antagonist (mg/kg)	8-OH-DPAT Induced Food Intake (g) Without Antagonist	With Antagonist	P	Main Receptors Blocked
Metergoline (5)	1.5 $\pm$ 0.36 (6)	0.46 $\pm$ 0.22 (5)	<0.05	5-HT1A,1B,1C,2,DA
(-)Pindolol (4)	1.5 $\pm$ 0.36 (6)	0.32 $\pm$ 0.09 (7)	<0.002	5-HT1A,1B > 1C
Spiperone (0.05)	2.0 $\pm$ 0.51 (6)	0.60 $\pm$ 0.19 (8)	<0.05	5-HT1A > 1B, DA
(+) Pindolol (4)	1.5 $\pm$ 0.36 (6)	1.29 $\pm$ 0.31 (9)	ns	low affinity for 5-HT1A,1B,1C, 2
Haloperidol (0.1)	2.0 $\pm$ 0.51 (6)	1.33 $\pm$ 0.26 (10)	ns	DA, α1
Idazoxan (3)	2.0 $\pm$ 0.51 (6)	2.75 $\pm$ 0.36 (6)	ns	α2
Methysergide (10)	1.83 $\pm$ 0.22 (17)	2.11 $\pm$ 0.32 (7)	ns	5-HT2
Ketanserin (2.5)	2.56 $\pm$ 0.57 (6)	2.67 $\pm$ 0.68 (6)	ns	5-HT2,1C
MDL 72222 (2)	1.83 $\pm$ 0.22 (17)	1.53 $\pm$ 0.37 (10)	ns	5-HT3
ICS 205 930 (1)	1.83 $\pm$ 0.22 (17)	1.68 $\pm$ 0.41 (6)	ns	5-HT3

Apart from (-) pindolol which caused a small though significant increase, none of the above antagonists significantly affected food intake in the absence of 8-OH-DPAT.

The lack of effect with haloperidol, idazoxan, (+) pindolol, ketanserin, methysergide, MDL 72222 and ICS 205 930 suggest that DA, α1/α2, 5-HT2 and 5-HT3 receptors are not involved in the response to 8-OH-DPAT. Its blockade by the non-selective 5-HT antagonist, metergoline and the 5-HT1 selective antagonist (-) pindolol indicates a role for 5-HT1 sites. Finally, as spiperone which has a greater affinity for 5-HT1A than 5-HT1B sites, also blocked the hyperphagia at the dose used by Tricklebank et al. (1984) to indicate that forepaw padding is 5-HT1A dependent, the hyperphagic action of 8-OH-DPAT is suggested to be mediated by 5-HT1A recognition sites.

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HYPERTONIC SALINE INTAKE IN THE RAT AFTER ADMINISTRATION OF 5-HT_{1A} AGONISTS, BENZODIAZEPINE PARTIAL AGONISTS AND ZOLPIDEM

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Fluid intake is depressed in rehydrating rats if hypertonic saline is available to drink instead of water. Anxiolytics, including benzodiazepines and barbiturates, increase the acceptability of strong salt solutions, and this increase in consumption has been taken to reflect anxiolytic activity in animals (Falk & Burnidge, 1970; Schmidt, 1966). To characterize this behavioural test more completely, the present series of experiments investigated effects of benzodiazepine (BZ) partial agonists (which have few or no sedative or ataxic side-effects); of zolpidem, which is a selective hypnotic (Arbilla et al, 1985; Depoorteere et al, 1986); and of several putative 5-HT_{1A} agonists, for which anxiolytic activity has been suggested (Peroutka, 1985).

Adult male rats (hooded General strain, University of Birmingham) were adapted to a daily 22h water-deprivation schedule and were familiarized with drinking either 1.8% or 2.7% NaCl solution in a 30 min test. Consumption was measured to the nearest 0.5 ml. Data were analysed using an ANOVA for independent groups, and Dunnett's t test. Drug effects were considered significant, if a significance level of at least $P<0.05$ was reached.

Hypertonic salt consumption was stimulated by several putative 5-HT_{1A} agonists. The potent and selective agonist, 8-hydroxy-2(di-n-propylamino)tetralin(8-OH-DPAT), significantly increased intake of 2.7% NaCl solution at 0.01 and 0.03 $mg.kg^{-1}$ s.c. Ipsapirone (TVX Q 7821) significantly increased 1.8% NaCl consumption at 1.0 and 3.0 $mg.kg^{-1}$ s.c., and gepirone hydrochloride increased consumption at 0.1 and 3.0 $mg.kg^{-1}$ s.c.

BZ partial agonists, Ro16-6028 and Ro17-1812 (Haefely, 1984) both produced significant dose-related (0.01 - 10.0 $mg.kg^{-1}$ i.p.) increases in 1.8% NaCl consumption, in the absence of sedation or ataxia. In contrast, the selective hypnotic compound zolpidem, which also binds to BZ recognition sites, had no effect (either increase or decrease) at clearly sedating doses (0.3 - 3.0 $mg.kg^{-1}$). We conclude that the hypertonic saline consumption test may have value in pre-clinical assessment of drugs for potential anxiolytic activity. Increased saline intake can be completely dissociated from sedating effects of BZ receptor agonists, and can also be induced by selective 5-HT_{1A} agonists.

We thank Synthélabo for zolpidem, Hoffman-La Roche for Ro16-6028 and Ro17-1812, Bristol-Myers, Evansville, for gepirone HCl, and Troponwerke, Cologne, for ipsapirone. A. Desa is supported by a grant from the Malaysian Government.

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MDL 72832, A NOVEL 5-HT_{1A} RECEPTOR LIGAND, STEREOSELECTIVELY STIMULATES PALATABLE FOOD CONSUMPTION IN RATS

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MDL 72832 (8-[4-(1,4-Benzodioxan-2-ylmethylamino)butyl]-8-azaspiro[4,5]decane-7,9-dione) has been characterized as a potent and selective ligand for central 5-HT_{1A} recognition sites (Fozard et al, 1987). Its S(-) enantiomer was found to be 32 times more active than R(+)MDL 72832 in its affinity for the 5-HT_{1A} site. Furthermore, in animals trained to differentiate 8-hydroxy-2(di-n-propylamino) tetralin (8-OH-DPAT) from saline in a drug discrimination paradigm (Tricklebank et al, 1987), (-)MDL 72832 generalized to the 8-OH-DPAT cue at 0.01 - 0.1 $mg.kg^{-1}$ s.c., whereas (+)MDL 72832 had no effect at doses up to 0.3 $mg.kg^{-1}$ s.c. (Fozard et al, 1987). Hence, comparisons between the behavioural effects of the two enantiomers of MDL 72832 should help to identify potential 5-HT_{1A} receptor mediation.

Putative 5-HT_{1A} agonists, including 8-hydroxy-2-(di-n-propylamino)tetralin(8-OH-DPAT), reliably increase food intake in free-feeding (Dourish et al, 1985; Hutson et al, 1986) and sham-feeding (Cooper & Neill, 1987) rats. Recently, 8-OH-DPAT was shown to stimulate palatable food consumption in partially-satiated rats (Cooper, 1987), and we have used this paradigm to investigate effects of the two enantiomers of MDL 72832 on feeding responses. The 5-HT_{1A} agonists, gepirone and ipsapirone (TVX Q 7821) were also tested. Individually-housed adult male hooded rats (General strain) were trained to eat a sweetened wet mash for 30 min to induce partial satiety, were then injected s.c. with the test compound, and after 30 min, were given a further 30 min access to the palatable diet (Cooper, 1986). The post-injection intake was recorded. Drugs were dissolved in isotonic saline.

(-)MDL 72832(0.1 - 1.0 $mg.kg^{-1}$) had a pronounced effect on food consumption. The minimally effective dose was 0.03 $mg.kg^{-1}$, when intake was increased from a control level of 6.7 $\pm$ 1.5g to 11.4 $\pm$ 1.0g ($P<0.05$; N=8 per group). (+)MDL 72832 also significantly increased food intake, but the minimally effective dose was 1.0 $mg.kg^{-1}$. At this dose, intake was increased from a control level of 7.0 $\pm$ 1.2g to 11.6 $\pm$ 1.1g ($P<0.01$; N=7 per group). In addition, both gepirone (0.01 - 1.0 $mg.kg^{-1}$ s.c.) and ipsapirone (0.03 - 3.0 $mg.kg^{-1}$ s.c.) produced significant increases in palatable food consumption. In conclusion, these data provide strong support for 5-HT_{1A} receptor-mediation of increased feeding responses, since MDL 72832 stereospecifically enhanced palatable food consumption.

We thank Merrell Dow Research Institute, Strasbourg, for the enantiomers of MDL 72832, Bristol-Myers, Evansville, for gepirone hydrochloride and Troponwerke, Cologne for ipsapirone. J. Neill is supported by Dept. Education, Northern Ireland.

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OPIOID RECEPTORS INVOLVED IN EMESIS IN THE DOG

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Emesis is a common, but poorly characterised side-effect of opiate analgesics in the clinic. This study attempts to elucidate the opioid receptor type(s) involved in opiate-induced emesis in the dog.

Experiments were performed in adult male beagle dogs, weight range 9-16 kg, 30 minutes after a test meal (n=3-6). Morphine (5 mg/kg, s.c.) was emetic within a few minutes of administration. The emesis elicited by morphine (5 mg/kg, s.c.) was presumed to be mediated by opioid receptors since it was prevented by pretreatment with the opioid receptor antagonist naloxone (0.5 mg/kg, s.c.; 15 minutes). The peripherally active quaternary opioid antagonist N-methyl naloxone (Russell et al., 1982) at a dose of 1 mg/kg, i.v. (5 minutes) also prevented morphine emesis.

Morphine-induced emesis was prevented by both the mu selective opioid antagonist naltrexone (0.01 mg/kg, s.c.; 15 minutes) and a mu/delta selective dose (1 mg/kg, s.c.; 15 minutes) of the opioid antagonist M8008 (Smith and Carter, 1986).

In contrast to morphine which has relatively poor access to the brain (Kutter et al., 1970), diamorphine (1 mg/kg, s.c.) and the selective mu opioid agonist, sufentanil (James and Goldstein, 1984) at a dose of 0.01 mg/kg, s.c., did not elicit emesis in the dog. Fifteen minutes pretreatment with diamorphine and sufentanil prevented the emesis elicited by morphine (5 mg/kg, s.c.).

The kappa agonist, tifluadom (1 mg/kg, s.c.; Romer et al., 1982) which passes the blood brain barrier (BBB) was itself neither emetic nor able to prevent the emetic effect of morphine (5 mg/kg, s.c.) in this model.

These results support the suggestion of Costello and Borison (1977) that there are two sites at which opiates modulate emesis. According to the present data both sites probably contain opioid receptors of the mu type with stimulation of those receptors outside the BBB (Chemoreceptor Trigger Zone) inducing emesis and those receptors at a second site inside the BBB (Emetic Centre) being anti-emetic.

Blancquaert et al. (1986) suggested that mu and/or kappa opioid receptors were involved in the anti-emetic effects of opioid agonists against apomorphine-induced emesis. In contrast, data from the present study suggest that the anti-emetic effect of opioid agonists against opioid-induced emesis is mediated via mu opioid receptors alone.

The donations of N-methyl naloxone (Dr. H. Merz, Boehringer) and tifluadom (Dr. D. Romer, Sandoz) are gratefully acknowledged.

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ANTAGONISM BY PARA-CHLOROPHENYLALANINE OF CISPLATIN-INDUCED EMESIS

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The ability of 5-HT_3 receptor antagonists to inhibit cisplatin-induced vomiting and retching in the ferret indicates a role for 5-hydroxytryptamine (5-HT) in the mediation of emesis (Miner and Sanger, 1986; Costall et al, 1986). In the present study the possibility that the effects of cisplatin are mediated via the release of 5-HT was investigated using para-chlorophenylalanine (PCPA) to inhibit the synthesis of 5-HT.

Male ferrets (1.5-2.0kg) were housed individually and presented with cat food at 12.0-13.0h. At 13.30-14.30h the ferrets were anaesthetised with fluothane (N_2O/O_2 carrier) and cisplatin (10mg/kg) was injected into the jugular vein. The animals were placed individually in cages for recovery from anaesthetic and observation of emesis over a 4h period. In each animal the time to onset of emesis and the number of vomits and retches were recorded. PCPA (100mg/kg, i.p.) was administered daily for 4 days prior to the injection of cisplatin. Levels of dopamine (DA), homovanillic acid (HVA), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and noradrenaline (NA) were determined in the area postrema (AP) (an area involved in the mediation of emesis) and caudate nucleus (CN) (a 'reference' area) 90 min after cisplatin or vehicle injection using HPLC with electrochemical detection.

Table 1. Changes in neurotransmitter/metabolite levels following cisplatin/PCPA treatment.

Treatment	DA	HVA	5-HT	5-HIAA	NA
			Area Postrema		
Vehicle	10.2±1.2	4.8±0.6	20.5±0.5	4.8±0.4	87.8±6.0
Cisplatin	16.9±1.7	3.1±0.3	24.4±1.7	3.6±0.4	54.6±4.6**
PCPA	7.6±0.5	2.4±0.3*	3.7±0.6***	1.8±0.1**	51.0±3.2**
PCPA + Cisplatin	8.7±1.3	6.1±0.9	4.3±1.3***	0.7±0.1***	54.3±5.8*
			Caudate Nucleus		
Vehicle	193 ± 25	96±23	10.4±1.2	4.2±0.5	6.8±0.8
Cisplatin	320 ± 54***	112±11	7.2±1.0	3.4±0.2	- ***
PCPA	636 ± 45***	125±12	3.1±0.3**	1.3±0.1**	3.0±0.4*
PCPA + Cisplatin	999 ±207*	241±26*	- ***	- ***	5.7±0.5

n = 4, S.E.M.s shown. Significant differences from vehicle responses are indicated as *P<0.05, **P<0.01, ***P<0.001 (Dunnett's t test). All values are given as ng/mg protein.

Emesis was induced in all ferrets receiving cisplatin alone; the onset of action was at 70±5 min with 85±14 retches and 14±6 vomits within the 4h period. Pretreatment with PCPA abolished cisplatin-emesis in all animals and markedly reduced the levels of 5-HT and 5-HIAA in both the AP and CN. PCPA failed to reduce the levels of DA and caused only small reductions in NA. The data support an involvement of 5-HT in the emetic actions of cisplatin in the ferret.

This work was supported by the MRC.

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NEUROLEPTIC INTERACTION WITH THE EFFECTS OF 5-HT ON THE RABBIT VAGAL NERVE

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Metoclopramide and other neuroleptic agents are used clinically in the treatment of chemotherapy-induced nausea and emesis. In a ferret model, the 5-hydroxytryptamine (5-HT) receptor antagonists MDL 72222, ICS 205-930 and GR38032F which act on the 5-HT_3 receptor subtype, have also been shown to exert a potent antagonism of cisplatin-induced emesis (Costall et al. 1986a, b; Miner and Sanger, 1986). It was considered possible that neuroleptic agents, similarly to metoclopramide, may have additional 5-HT_3 receptor antagonist effects (Fozard and Mobarok-Ali, 1978) and in the present study we investigate the potential 5-HT_3 receptor antagonist actions of neuroleptic drugs and reference agents using the rabbit vagal nerve preparation.

The rabbit cervical nerve was removed, desheathed and placed in a two compartment bath, each compartment being individually perfused with Krebs-Henseleit solution. The DC potential between the two compartments was recorded via Ag/AgCl electrodes and displayed on a Grass recorder. Drugs were applied in the perfusate to one compartment only and 5-HT perfused onto the tissue for 90s (at 12 min intervals) to obtain a depolarisation of the resting membrane potential. Antagonists (30 min pretreatment) were used at a minimum of 4 concentrations to obtain dose-response curves for the construction of Schild plots and the determination of pA_2 values.

The application of 5-HT ($4x10^{-7}$-$2x10^{-5}$M) induced immediate and concentration-related falls in resting membrane potential to a maximum of 3 to 4mV. Repeated applications of 5-HT failed to cause tachyphylaxis. ICS 205-930 (10^{-11}-10^{-8}M) and metoclopramide (10^{-8}-10^{-5}M) caused parallel shifts in the dose-response curves to 5-HT without significant reductions in the maximum attainable response; the slopes of the Schild plots did not significantly differ from unity, and the pA_2 values were 10.2 and 7.5 respectively. Methysergide and ritanserin (10^{-6}M) failed to antagonise the effect of 5-HT. The neuroleptic agents (selected for their ability to attenuate the emetic effects of cisplatin in the ferret model) (Costall et al. unpublished data), spiroperidol, haloperidol, sulpiride and tiapride (10^{-6}M), failed to antagonise the depolarisation caused by 5-HT. A higher concentration of tiapride (10^{-5}M) antagonised the effect of 5-HT but with a significant reduction in the maximum attainable response and similar comments would apply to the effects of domperidone. Cisapride (10^{-7}-10^{-5}M) caused parallel shifts in the 5-HT dose-response curves but the slope of the Schild plot was significantly less than unity.

The data indicates that neuroleptic agents such as haloperidol and sulpiride are without potential to antagonise at 5-HT_3 receptors on the rabbit vagal nerve, and therefore the antiemetic action of such compounds probably reflects dopamine receptor antagonism. However, tiapride, domperidone and cisapride did exert some antagonism of the actions of 5-HT on the vagal nerve preparation but the precise nature of the interaction and relevance to an anti-emetic effect remains to be established.

J.B. Owera-Atepo is supported by the British Council.

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A SIMPLE HABITUATION TEST IN THE MOUSE

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A simple test is described which allows the measurement of mouse habituation to a test environment in which it is presented with an aversive white, brightly illuminated (60W white light) environment and a more acceptable dark, dimly-lit (60W red light) environment. These environments are presented in a 2-compartment box (45 x 27 x25cm) with access between the white and black painted areas enabled via an opening in the dividing partition. Mice (BKW, 25-30g) were taken from a dark environment, in a dark container, to the experimental room maintained on 'dim' red illumination.

On each test day mice were placed individually in the centre of the white compartment and their behaviour monitored by remote video recording. On the first day of test mice moved cautiously around the brightly-lit area until finding the opening into the dark environment in which they then spent a major proportion of their time showing rearing and locomotor exploration (measured by crossings of 9cm lined squares on the test box floor). The latency for movement into the black environment was initially 12.6±1.3sec and, including this latency, 52-53% of total time was spent in the black environment (29.6±3.1 rears/5 min, 31.3±3.3 crossings/5 min in the white, 43.6±4.2 rears/5 min, 49.3±4.3 crossings/5 min in the black). Over a 3-7 day period of repeated exposure to the test box mice learned to move rapidly into the black compartment (latency 0-2 sec, $P<0.001$) where most behaviour was exhibited (67-80% of time spent in the black, rears increased to 63.5±6.2/5 min, line crossings to 71.2±7.3/5 min, $P<0.001$, with corresponding reductions in the white section).

Mice challenged daily with scopolamine, 0.25mg/kg i.p. 30 min before test, failed to learn to avoid the aversive environment (latency 15.8±1.6 sec, maintained over 20 days, rears in the white 46.3±4.3/5 min, 20.1±1.9/5 min in the black, $P>0.05$ compared with the first day of test): the same dose of methyl-scopolamine did not alter the habituation profile. However, disruptions in habituation followed lesions of the nucleus basalis of Meynert (ibotenic acid, 2µg/0.25µl, or electrolesion, 1mA/10sec, Ant. 2.3, Vert. -4.5, Lat. ±2.1 with respect to Kopf zero) (e.g. for electrolesions latency maintained at 15.5±1.7sec, rears in the white 80.1±8.0/5 min, and 18.6±1.9/5 min in the black, $P<0.001$ compared to habituation of sham operated or normal animals over 8-21 days). The disruption in habituation caused by scopolamine or lesions of the nucleus basalis (which caused selective loss of ChAT in the frontal cortex) could be reversed by arecoline given by persistent intraperitoneal infusion, 50mg/kg/day (maximum dose which could be administered without autonomic problems). Thus, on day 7 when mice normally move immediately into the dark environment and exhibit most behaviour in the dark, the scopolamine treated or lesioned animals given arecoline behaved in a manner indistinguishable from normal, untreated mice (e.g. latencies for control, scopolamine treated and scopolamine plus arecoline treated mice were 1.2±0.2, 14.9±1.6 and 0sec respectively and rears in the white were 18.6±1.9, 79.6±8.2 and 17.4±1.8/5 min respectively, arecoline effect significant to $P<0.001$).

Thus, a simple model is described in which mice learn to avoid an aversive environment. This behaviour is disrupted by scopolamine and lesions of the nucleus basalis which reduce cholinergic function in the frontal cortex, and this disruption can be corrected by persistent treatment with a cholinomimetic. Such a model may be valuable for assessment of drug action on cognition.

MODIFICATION OF MORPHINE ANTINOCICEPTION BY INTRATHECALLY (I.T.) ADMINISTERED BENZODIAZEPINE RECEPTOR LIGANDS

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A part of the antinociceptive effect of opiates is due to an action within the spinal cord. Benzodiazepine derivatives have also been reported to be capable of modifying pain transmission at the spinal level. In the present study, we investigated the putative interaction of benzodiazepine receptor (BZR) ligands with opiates in mediating analgesia at the spinal cord level.

Lumbar subarachnoid catheters were chronically implanted in 28 male rats. After baseline tail-flick latency (TFL) had been determined, drugs were injected i.t. in a volume of 5 ul. TFL was then measured at 15 min intervals for one hour. At least 4 days separated 2 consecutive experiments in any given animal. Correct placement of catheter tip was histologically confirmed.

Table I: Effects of intrathecal BZR ligands and morphine on tail-flick latency [a]

Drug treatment	Minutes after drug administration 0	15	30	45	60
Saline	4.39 (0.12)	4.64 (0.15)	4.57 (0.24)	4.54 (0.28)	4.55 (0.23)
Morphine 3 ug	4.16 (0.15)	6.79 (0.60)	7.80 (0.47)	7.44 (0.56)	7.38 (0.58)
Midazolam 16.5 ug	4.46 (0.10)	5.00 (0.16)	5.02 (0.24)	5.00 (0.18)	4.65 (0.15)
Morphine 3 ug + Midazolam 16.5 ug	4.05 (0.14)	8.06* (0.63)	9.24* (0.52	9.57* (0.43)	9.22* (0.51)
Morphine 3 ug	4.05 (0.12)	7.36 (0.73)	8.39 (0.57)	8.25 (0.60)	7.91 (0.64)
Ro 19-4603 15 ug	4.39 (0.14)	3.93 (0.14)	4.44 (0.09)	4.29 (0.20)	4.24 (0.18)
Morphine 3 ug + Ro 19-4603 15 ug	3.93 (0.16)	4.92* (0.56)	6.47* (0.60)	6.90* (0.64)	7.10 (0.65)

[a] Values are mean (± s.e.mean) in seconds of at least 8 trials in different animals
*Significantly different from morphine 3 ug alone (paired t-test, $P \leq 0.05$).

Midazolam (a BZR agonist) significantly increased, whereas Ro 19-4603 (a BZR inverse agonist) significantly decreased the antinociceptive effects of morphine. These BZR ligands injected alone did not significantly alter TFL.

Our results demonstrate that BZR ligands injected i.t. are able to modify the effects of opiates in the modulation of pain transmission at the spinal level. These results can be explained either by a direct action of BZR compounds on opiate mechanisms or more likely by the interplay of two different mechanisms both acting independently on the same ascending pathway. In any case, our findings with BZR ligands support the notion of a GABA involvement in the control of nociceptive information.

CHRONIC, BUT NOT ACUTE, CLOMIPRAMINE INCREASES INTRUDER-ORIENTATED AGGRESSIVE BEHAVIOUR IN SHORT-TERM SOCIALLY ISOLATED RATS

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Ethology provides a pharmacological tool by which the effects of centrally acting compounds on overt animal behaviour may be studied. We have used the method of Silverman (1965) to examine the effects of acute and chronic clomipramine (CLP) treatment of the "resident" animal on the overt behaviours exhibited during social interaction (SI).

In all experiments male Wistar rats were housed under reverse-daylight conditions from weaning for at least 5 weeks before testing. Animals used in the SI test were housed in groups of 4 for 3 weeks prior to testing. Resident animals were housed individually for 3 days before each test. The effect of acute CLP (10 - 90 µmol Kg^{-1} sc) treatment of the resident animal was studied by introducing the conspecific 30 min. after drug treatment and recording the resulting activity for 10 min. on video tape (under low-intensity red light) for analysis at a later date. Each member of the resident group was tested on 4 occasions at weekly intervals and came into contact with each member of the corresponding intruder group. In chronic studies the resident animals were tested on the first occasion without any treatment, following which Alzet mini-osmotic pumps containing drug or vehicle were implanted sc under Fentanyl/Midazolam anaesthesia. SI tests were carried out after 7 and 14 days treatment and 7 days following removal of the mini-pumps. The effect of acute CLP (10 - 270 µmol Kg^{-1}) on exploratory locomotor activity (ELA) in a novel environment was examined using activity monitors containing infra-red photocells linked to a BBC microcomputer. Locomotor activity was measured for 10 min. immediately following introduction of the animal into the cage.

Acute Studies.		Clomipramine (µmol Kg^{-1})					
Test	Vehicle	10	30	90	270	N	ID_{50}
SI	22.2(2.2)	16.6(2.2)[a]	10.7(1.3)[b]	5.6(1.6)[b]		8	29.5
ELA	179(36)	170(29)	154(6)	141(10)	135(9)	6	>270
SI Test.	Chronic Clomipramine (Target dose: 10 µmol Kg^{-1} day^{-1} sc)						
Treatment (days)		0	7	14	+7 post	N	
Vehicle		12.6(1.3)	10.7(1.8)	10.2(0.7)	13.0(1.6)	8	
CLP		12.2(1.7)	20.1(3.1)[c]	23.0(1.7)[d]	12.2(1.3)[e]	8	

SI: Mean (sem) percentage aggressive behaviours of total behaviours observed. Mann Whitney U-test: a,$p<0.05$; b,$p<0.001$ versus vehicle; c,$p<0.05$; d,$p<0.01$ versus pretreatment; e,$p<0.001$ versus 14 day treatment.
ELA: Mean(sem) locomotor counts. All values not significantly different from those after vehicle control (Dunnett's test).
ID_{50}; µmol Kg^{-1}

The data indicate that the progression from investigation to affective aggression exhibited by the resident animal towards the conspecific intruder is reduced by acute CLP but increased following chronic treatment. The lack of effect on ELA indicates that the acute effect of CLP on behaviour is not due to motor impairment. The differential effects of acute and chronic CLP treatment on rodent behaviour are similar to those reported for other tricyclic antidepressants (see Vogel et al., 1986, and refs cited therein); such effects on rodent behaviour following chronic antidepressant treatment may prove a useful model of the increased drive - and even aggression (Kaplan et al., 1961) - which appears to be an integral part of the recovery process from depression.

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EVIDENCE FROM IN VIVO DIALYSIS THAT REDUCED HIPPOCAMPAL 5-HT AND GLYCINE RELEASE IS ASSOCIATED WITH ANXIETY IN THE RAT

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Subconvulsant doses of pentylenetetrazole (PTZ) cause anxiety in humans and animals. We therefore used PTZ to investigate some of the neurotransmitters that may be important in modulating anxiety. The purpose of the study was to correlate behavioural measures of anxiety (in the elevated plus-maze, Pellow et al, 1985) with in vivo transmitter release.

Male hooded Lister rats were anaesthetised with halothane and a dialysis loop was inserted into the hippocampus (A -3.3 mm, L 2.0 mm, D 2.8 mm). The next day they were injected with subconvulsant doses of PTZ (15 & 30 mg/kg i.p.) and placed in a high-sided wooden box. The dialysis loop was perfused (1 ul/min) for 45min with artificial CSF, with the initial 15 min of perfusate being discarded. Amino acids, amines and their metabolites in the next 30 min sample were measured by HPLC. Immediately after the sample period, the pump was disconnected and the rat was placed in the elevated plus-maze for a 5-min trial.

PTZ was without significant effect on the release of aspartate, glutamate, serine, glutamine, glycine and taurine; GABA was generally below the limits of detection. PTZ caused a dose-related decrease in noradrenaline release ($p<.01$); the release of other amines & metabolites was not significantly changed.

Since our main interest was in correlating in vivo release with behavioural changes, we divided the animals into two groups, on the basis of their performance in the plus-maze. Rats with zero entries onto the open arms formed the 'anxious' group (n=20); the remainder formed the 'non-anxious' group (n=22) and had 24% entries, and spent 18% of their time, on the open arms. The 'anxious' group had undetectable levels of glycine (vs 6.3 pmoles/ul in the non-anxious group, $p<.001$) and significantly lower concentrations of 5HT (116 vs 21.6 fmoles/100ul, $p<.01$). There were no significant differences between the groups in noradrenaline release, or in any of the other substances measured.

Our finding that anxiogenic effects are associated with a decreased release of 5HT from the hippocampus is consistent with the effects of the anxiolytic, buspirone, which has post-synaptic actions in the hippocampus similar to those of 5HT (Anwal & Rowan, 1986). This suggests that, in the hippocampus, a decreased 5HT function is associated with increased anxiety. This contrasts with other brain regions, such as the dorsal raphe.

HAB has a SERC CASE award. SEF is a Wellcome Trust Senior Lecturer. We are grateful to I. Kilpatrick for allowing us to use his HPLC for amine estimation.

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AGONIST AND ANTAGONIST EFFECTS OF RU 32514, A NEW NON-BENZODIAZEPINE LIGAND FOR BENZODIAZEPINE RECEPTORS

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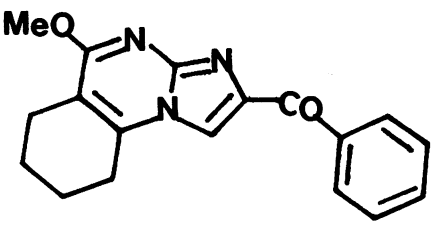
RU 32514, 2-benzoyl-5-methoxy-6,7,8,9-tetrahydro imidazo[1,2-a]quinazoline, has selective affinity for benzodiazepine binding sites (IC_{50} = 61nM for displacing 0.6nM [^{3}H]-flunitrazepam from rat cortical membranes - Squires and Braestrup, 1977). In animal models of anxiety RU 32514 shows benzodiazepine-like potential anxiolytic activity (Table 1). Similarly, RU 32514 (5-20mg/kg p.o.) substitutes for chlordiazepoxide) (CDZP) in rats trained to discriminate 5mg/kg p.o. CDZP from saline.



RU 32514

Table 1

	Conflict		Stress-Induced	Leptazol
	Food[b]	Water[a]	Ultrasounds[c]	Cue
	MED	MED	MED	ED_{50}
RU 32514	5	5	5	20
CDZP	5	2	0.2	3

MED = minimal effective dose. All doses are mg/kg p.o. (1h pre-test) except the ultrasounds model where drugs were given i.p. (30 min pre-test) and food-motivated conflict where drugs were given 25 min prior to test.

However, RU 32514 at doses up to 200mg/kg p.o. showed no anticonvulsant activity against tonic seizures induced in mice by either leptazol, picrotoxin or strychnine. Similarly, the same dose range of RU 32514 was completely inactive in tests for sedative and myorelaxant activity: rotating drum in mice, pull up test in rats (Deacon and Gardner, 1984). In all three seizure types RU 32514 (100mg/kg p.o.) totally antagonised a just sub-maximal effective dose of diazepam.

RU 32514 may be a partial agonist at benzodiazepine receptors with only weak intrinsic activity.

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DIFFERENT EFFECTS ON LEPTAZOL SEIZURES IN RATS OR MICE OF RU 32514, A NEW BENZODIAZEPINE RECEPTOR LIGAND

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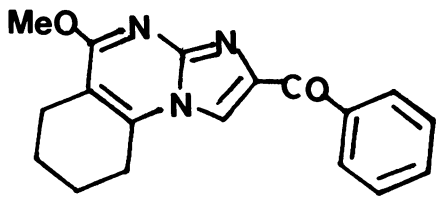
RU 32514 (2-benzoyl-5-methoxy-6,7,8,9-tetrahydro imidazo[1,2-a]quinazoline) is a novel benzodiazepine receptor ligand with some agonist and some antagonist properties. The effects of this compound have been compared on tonic leptazol seizures induced in either male CD-1 mice (25-30g) or male Wistar rats (180-240g). The dose of leptazol (90-125mg/kg s.c.) was adjusted until 9/10 control animals showed seizures within 30 min.



RU 32514

At no dose between 2 and 200mg/kg p.o. (1h pre-test) did RU 32514 affect the seizures induced by leptazol in mice. However, a just sub-maximal anti-leptazol effect of diazepam (0.7mg/kg i.p.) was antagonised by RU 32514 in a dose-related manner with an approximate ED_{50} of 54mg/kg p.o. (1h pre-test) (Table 1).

Table 1

MOUSE			RAT		
	Dose mg/kg p.o.	$^{n}/_{10}$ Seizures		Dose mg/kg p.o.	$^{n}/_{10}$ Seizures
Control		9	Control		9
Diazepam		1,0	RU 32514	1	9
RU 32514	100	9		5	4
Diazepam+RU 32514	25	3		10	0
	50	4		50	2
	100	9		100	6
				200	3/9

In rats, RU 32514 inhibited leptazol seizures maximally at 10mg/kg p.o. (1h pre-test) but was less consistent and less effective at higher doses. Combination of such a higher dose (100mg/kg) with diazepam did not affect the anticonvulsant activity of diazepam.

These data suggest a species difference in benzodiazepine receptor sensitivity in terms of intrinsic activity and are similar to a rat-mouse difference observed in the effects of another agonist/antagonist benzodiazepine receptor ligand, CGS 9896, on leptazol seizures (Keim et al., 1984).

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THE BEHAVIOURAL CONSEQUENCES OF TREATMENT WITH SELECTIVE 5-HT_3 RECEPTOR ANTAGONISTS

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The selective 5-HT_3 receptor antagonist, GR38032F reduces the hyperactivity caused by mesolimbic dopaminergic overactivity and is also active in behavioural tests for anxiolytic activity (Costall et al, 1987; Jones et al, 1987). We have reported previously (Tyers et al, 1987) that the selective 5-HT_3 receptor antagonists, ICS205-930 (Richardson et al, 1985) and MDL72222 (Fozard, 1984) have similar profiles of action to GR38032F in anxiolytic tests. We have now evaluated the potential antipsychotic activity of ICS205-930 and compared the effects of higher doses of GR38032F and ICS205-930 in these tests and in those for anxiolytic activity.

The hyperactivity resulting from the infusion of dopamine (25µg/24h, 0.48µl/h, 13 days) into the rat nucleus accumbens, measured (counts/60min) using photocell cages (256±21 - 212±26; vehicle: 86±7.9), was reduced by ICS205-930, 0.1-1.0µg/kg i.p. b.d. (73±9 - 87±10, $p<0.001$) or by GR38032F, 0.1-100µg/kg i.p. b.d. (79±8.3 - 89±8.6, $p<0.001$). Slightly higher doses of ICS205-930, 10 and 100µg/kg b.d., were less effective or inactive (177±19 and 257±29, $p>0.05$) against the dopamine response. This apparent loss of selectivity was also seen with GR38032F at the high dose of 0.5mg/kg t.d.s. (260±27, $p>0.05$).

In two tests for anxiolytic activity the differences between GR38032F and ICS205-930 at higher doses were more marked. In the light aversion test in the mouse, activities of mice in the dark and brightly illuminated areas of a two-compartment box were measured. Untreated mice spent more time in the dark area which is reflected as increased rearing (32±3.4 rears/5min) compared to the light areas (19±2.1 rears/5min, n=10). Both GR38032F, 0.05-10µg/kg i.p., and ICS205-930, 0.1-10µg/kg i.p., dose-dependently increased rearing in the light by up to 260% with corresponding decreases in the dark area ($p<0.001$). A higher dose of ICS205-930, 100µg/kg i.p., had no effect on rearing in either compartment; in contrast, there was no loss of activity with GR38032F up to 1mg/kg. In the rat social interaction test, unfamiliar animals were placed in a novel brightly lit area and the time spent in social contact (i.e. crawling under or over partner, sniffing or following) was measured. Control animals spent 78±8s/10min in social interaction which was increased dose-dependently by up to 180% by ICS205-930, 0.1-10µg/kg i.p., or GR38032F, 0.05-10µg/kg i.p. This effect was maintained with doses of GR38032F up to 1mg/kg but for ICS205-930 activity was lost at 0.1 and 1.0mg/kg.

Thus, whilst both GR38032F and ICS205-930 were effective in tests which may predict antipsychotic and anxiolytic activity, the action of ICS205-930 appeared to diminish at higher doses. This loss of selectivity of action was not seen for GR38032F in tests for anxiolytic action, but occurred if high doses were given during a mesolimbic dopamine infusion.

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COMPOUNDS SELECTIVE FOR THE 5-HT_{1A} RECEPTORS HAVE ANXIOLYTIC EFFECTS WHEN INJECTED INTO THE DORSAL RAPHE NUCLEUS OF THE RAT

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Both buspirone and ipsapirone may represent a new class of anxiolytic drugs that interact with the 5-HT_{1A} receptor (Peroutka, 1985). At present there are inconsistent data relating to the efficacy of these drugs in animal models of anxiety. Indeed another 5-HT_{1A} selective compound, 8-OH DPAT, has been shown to possess both pro-conflict and anti-conflict activity in rats depending upon whether or not 5-HT synthesis is inhibited (Engel et al., 1984). This may reflect a predominance of post-synaptic or pre-synaptic activity, which has also been proposed to account for the decreased and increased feeding responses observed with 8-OH DPAT (Dourish et al., 1986).

We therefore investigated the effects of discrete microinjections of buspirone, ipsapirone, 8-OH DPAT and a non-selective 5-HT_1 agonist, 5-carboxamidotryptamine (5-CT), into the dorsal raphe nucleus (DRN) of the rat. This region contains a high density of 5-HT_{1A} receptors (Verge et al., 1985) and provides the major source of 5-HT forebrain innervation. Two animal models of anxiety were used : the social interaction test (SI) (File, 1980) and the thirsty rat conflict model (TRC) (Vogel et al., 1971).

Male hooded lister rats (200-250g) were each implanted with a stainless steel guide cannula with its tip 3mm above the DRN. The recovery period was at least 7 days. Five minutes before testing, drugs were injected into the DRN in 0.5µl fresh saline through a 30G injection needle. The SI test was performed under high light/unfamiliar conditions over a 10 minute test period. Only one rat of a pair was treated and assessed for SI and locomotor activity (LMA). Rats were deprived of water for 18h before testing for 3 minutes in the TRC model. All injection sites were subsequently verified histologically.

8-OH DPAT (100ng 21.8±4.2s, vehicle 3.6±1.0s), buspirone (40ng 21.2±4.8s; 200ng 23.3±6.0s; vehicle 4.2±1.3s), ipsapirone (200ng 24.0±5.7s; vehicle 7.3±2.0s) and 5-CT (2ng 30.3±7.2s; vehicle 8.3±2.0s) all significantly increased SI relative to vehicle pretreated controls. LMA was increased at these doses but only the buspirone (40ng) group showed a significant difference from control ($p<0.05$). Similarly, these compounds were effective in the TRC model ($p<0.05$). 8-OH DPAT and ipsapirone were active over a similar concentration range but buspirone was only effective at 200ng and 5-CT at 10-100ng. All drugs failed to modify unpunished drinking at their anticonflict doses.

The 5-HT_{1A} drugs are therefore like the GABA agonist muscimol in that they produce anxiolytic responses upon direct application to the DRN (Higgins et al., 1986). This strongly suggests that the behavioural effects are a consequence of the inhibition of DRN cell firing, thus supporting the theory that a reduction in forebrain 5-HT neuronal activity may be an important factor in relieving anxiety.

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5-HT LIGAND EFFECTS IN THE SOCIAL INTERACTION TEST OF ANXIETY

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Effects of 5-HT receptor ligands in animal models of anxiety vary between tests. The 5-HT_{1A} ligand 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) has been reported anxiogenic (X-maze, Critchley & Handley, 1986), anxiolytic (punished drinking, Engel et al, 1984) and inactive (plus-maze, File et al, 1987); ritanserin, a 5-HT2 receptor antagonist has been shown anxiolytic (X-maze, Critchley & Handley, 1986; light/dark box, Colpaert et al, 1985), anxiogenic (plus-maze, File et al, 1987) and inactive (operant conflict, Colpaert et al, 1985). We now add the effects of these and related compounds in the social interaction test.

Male PVG rats (170-190 g) were housed and tested according to Gardner & Guy (1984). Seconds spent in social interaction (SI) and number of walks (NW) were recorded. There were no significant changes in number of rears except as noted and too little aggression for assessment. Effects of representative doses are shown in Table 1. (Doses in mg/kg ip). 8-OHDPAT (.05 - .1); RU24969 (0.5 - 1); 5-MeODMT (1) and beta-CCE (1) reduced SI while ipsapirone (1), pindolol (.1 - .25) and diazepam (1 - 2) increased it. 5MeODMT increased the number of rears but RU 24969 did not. Ketanserin (0.5) and ritanserin (1.0) had no significant effect on any parameter.

Table 1. Mean differences from saline controls

	dose	SI	NW		dose	SI	NW
beta-CCE	1.0	-16*	+0.3	diazepam	1.0	+19*	+2.7
8-OHDPAT	.05	-17.8*	+17.9	pindolol	0.1	+19.8**	0.0
RU24969	.5	-19.8*	+10.6*	ipsapirone	1.0	+35.4**	-3.0
5-MeODMT	1.0	-58.6**	-4.2	ketanserin	0.5	+2.8	-2.3
total saline ± s.e.m. (N=43)	x	43.3±2.4	9.5±.5	ritanserin	1.0	+11.2	-1.5

doses in mg/kg. N/group 5-9 *p<0.05; **p<0.01

The results for the 5-HT_{1A} ligands are similar to those we have previously obtained for the X-maze but, at the doses used here, ritanserin and ketanserin contrast in being inactive. Further studies on the origins of inter-test differences are necessary.

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