NEUROMUSCULAR BLOCKING ACTIVITY OF H_2 RECEPTOR ANTAGONISTS

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Many drugs other than those used in anaesthesia interfere with neuromuscular transmission. The importance of this action is the possibility of respiratory depression or prolonged apnoea induced by interaction with curare or other relaxants.

We have recently reported that cimetidine produces neuromuscular blockade in the isolated phrenic nerve-diaphragm preparation of the rat at a final bath concentration of 0,75 - 2mg/ml. The preparation recovered completely after washing. Using a tyrode with half calcium the neuromuscular blockade of cimetidine was potentiated. The neuromuscular blockade produced by cimetidine was reversed by $Ca\ Cl_2$ and not by neostigmine.

In the presence of lower concentrations of cimetidine there was an increase of neuromuscular blockade induced by non-depolarising relaxants and aminoglucoside antibiotics (Bossa et al., 1980, Bossa et al., 1981, Bossa et al., 1982).

On the basis of these observations we suggest that neuromuscular block produced by cimetidine could be ascribed to an action at a presynaptic level by competing with calcium ions unrelated to its specific effect on H_2 histamine receptor.

In fact neuromuscular blockade produced by cimetidine was reversed by 4-aminopyridine and not by dimaprit (a selective H_2 agonist).

We have also considered the activity of ranitidine, a new H_2 blocking compound.

Ranitidine produced neuromuscular blockade (1-2mg/ml) only in the presence of tyrode with half calcium and the block was reversed by calcium, 4-aminopyridine and not by neostigmine or dimaprit.

Ranitidine like cimetidine increased the neuromuscular blocking activity of curares and aminoglucoside antibiotics.

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ANTISECRETORY AND ANTIULCER EFFECT OF A POTENT H_2-ANTAGONIST: FCE 23067

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FCE 23067 (2-guanidino-5-(N-isopropylcarbamoyl)-4,5,6,7-tetrahydrothiazole [5,4-c] pyridine) has potent inhibiting activity (about 10 times that of ranitidine (R) and cimetidine (C)) on the positive chronotropic action of histamine on the guinea pig isolated atria, but does not fulfil the criteria for competitive antagonism.

Rat basal gastric acid secretion (1) was strongly reduced by FCE 23067 with an ED_{50} of 1.48 mg/kg i.d. on acid output and 6 mg/kg i.d. on volume. The ED_{50} for R and famotidine (F) were respectively 13.2 and 1.36 mg/kg i.d. (output) and 37.8 and 6 mg/kg i.d. (volume). Gastric acid secretion stimulated by histamine (2 mg/kg i.v.) was inhibited by FCE 23067 and R with ED_{50} values of 0.03 and 0.1 mg/kg i.v. and by pentagastrin (20 mcg/kg i.v.) with ED_{50} 0.3 and 1 mg/kg i.v.

On ASA-induced gastric ulcers, FCE 23067 was more potent than R (ED_{50} = 0.021 and 0.29 mg/kg p.o.) and F (ED_{50} = 0.041 mg/kg p.o.). FCE 23067 prevented stress-induced gastric ulcers (2) with an ED_{50} = 7.1 mg/kg p.o.; R and C were less potent (ED_{50} = 11.6 and 27.7 mg/kg p.o.) and F equipotent (ED_{50} = 7.8 mg/kg p.o.). In cysteamine-induced duodenal ulcers, FCE 23067 showed 14 times the activity of R (ED_{50} = 0.74 and 10.5 mg/kg p.o.) and about 2 times that of F (ED_{50} = 1.34 mg/kg p.o.).

In the rat FCE 23067 did not modify gastric emptying (at 10 mg/kg p.o.), basal choleresis, or mean systolic blood pressure in the conscious spontaneously hypertensive animal (at 45 mg/kg p.o.). FCE 23067 had no antiandrogenic or anticholinergic activity and did not raise prolactin levels.

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α_2 AGONISTS AND ANTAGONISTS AND GASTRIC ACID SECRETION

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The effects of alpha-2 agonists and antagonists on gastric acid secretion are still unclear. Opposite excitatory and inhibitory effects on gastric acid secretion have been reported for the alpha-2 agonist clonidine (Del Tacca et al., 1982 ; Soldani et al., 1984) as well as for the alpha-2 antagonist yohimbine (Goldberg and Robertson, 1983). In the case of the alpha-2 blocker tolazoline only excitatory effects have been observed (Waltz and Van Zwieten, 1970). The present study investigates the effects of the alpha-2 agonists clonidine, xylazine and guanabenz, as well as those of the alpha-2 antagonists tolazoline and yohimbine in Shay rats, Schild rats and isolated guinea-pig gastric fundus by following the methods previously described (Del Tacca et al., 1982).

In Shay rats clonidine, xylazine and guanabenz displayed marked antisecretory effects, whereas tolazoline and yohimbine had no effect. Under the same conditions, yohimbine fully prevented the effects of clonidine, xylazine and guanabenz, while tolazoline showed partial antagonism. In Schild rats both clonidine and xylazine displayed a stimulant secretory action, but guanabenz was without effect; cimetidine fully prevented the excitatory effects of clonidine and xylazine. Both tolazoline and yohimbine increased gastric acid secretion in Schild rats : the effect of tolazoline was inhibited by cimetidine, while the effect of yohimbine was prevented by pirenzepine or vagotomy. On isolated guinea-pig gastric fundus, clonidine xylazine and tolazoline exerted a stimulatory activity, whereas guanabenz and yohimbine had no effect: these excitatory responses were fully prevented by cimetidine.

The overall results indicate that alpha-2 agonists and antagonists affect gastric acid secretion, and their action seems to be only partially dependent on their main pharmacological activity. Clonidine and xylazine have opposite inhibitory and excitatory effects mediated by the vagal or histaminergic pathways respectively. The stimulant secretory effect of tolazoline appears to be due to its imidazoline-like structure allowing its interaction with histamine H2 receptors; this effect is thus lacking for guanabenz which is structurally unrelated to imidazoline. The excitatory effect of yohimbine appears to be exerted at central cholinergic sites, as suggested by its disappearance in vagotomized rats.

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MORPHINE OR LOPERAMIDE-INDUCED CONSTIPATION IN MICE: COMPARATIVE ANTAGONISM BY SR 58002 C AND NALOXONE

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In a search for peripherally selective narcotic antagonists we found that SR 58002 (N-methyl levallorphan), administered s.c. to mice, was better than other narcotic antagonists in antagonizing morphine constipation, without impairing morphine analgesia (Dragonetti et al.). N-methyl levallorphan as the methane sulfonate salt (SR 58002 C) should be effective orally for selectively relieving opioid constipation. We studied its oral and s.c. peripheral selectivity and its potency against morphine (M) and loperamide (L) constipation, as compared to those of naloxone (N).

CD albino mice (C. River, Italy), 21±3 g, were used. Antagonism of M (3 and 12 mg/kg s.c.) or L (4 mg/kg p.o.) constipation was assessed by charcoal meal test (Bianchetti et al.), giving M immediately and L 10 min before charcoal and killing mice 30 min after charcoal. Antagonists were given 5 min before charcoal. Antagonism of M analgesia was assessed by the hot plate test (Bianchetti et al.), giving M (24 mg/kg. s.c.) 30 min before the test and antagonists 5 min before morphine.

SR 58002 C, s.c. completely prevented the constipation induced by L and 3 mg/kg M (Table 1). However, unlike N, it only partially inhibited (max. inhib. 60%) the constipating effects of 12 mg/kg M, which is mediated, at least in part, centrally. SR 58002 C, in spite of its quaternary nature, was anticonstipating, p.o., (about 1/10 as potent but longer lasting than N). Even at the highest s.c. and oral doses, SR 58002 C did not impair M analgesia, which was more susceptible than constipation to naloxone antagonism.

These data support the efficacy and the peripheral selectivity, even after oral administration, of SR 58002 C, currently the most promising compound being evaluated clinically for prevention of constipation induced by exogenous and, possibly endogenous (Kreek M.J. et al.) opioids.

Table 1 - Antagonism (ID_{50} mg/kg) of opioid-induced constipation and analgesia.

		Constipation by:			Morphine analgesia
		Morphine		Loperamide	
		12 mg/kg	3 mg/kg		
SR 58002 C	s.c.	8(4-13)●	0.8(0.5-1.3)	1.0(0.6-1.4)	> 60
	p.o.	~ 200●	20(13-34)	12(8-19)	> 400
Naloxone	s.c.	0.8(0.5-1.2)	0.13(0.1-0.2)	0.3(0.2-0.7)	0.06(0.02-0.15)
	p.o.	10(7-16)	1.8(1.0-3.1)	1.0(0.4-2.3)	0.6(0.3-1.3)

In parentheses : 95% conf. limits. ●Maximal inhib.: ~ 60%

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HEXAPRAZOL ACTIVITY ON GASTRIC ELECTRICAL POTENTIAL FALL BY ACETYLSALICYLIC ACID IN RAT

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Hexaprazol, [(N-cyclohexylcarbamoyl)methyl-1] piperazine hydrochloride is a new antiulcer drug with antisecretory activity (SCURI et al., 1984).
Many experimental tests proved its pharmacological action: gastric ulcers induced in rats by phenylbutazone + histamine [ED50 and 95% confidence limits in mg kg^{-1} = 64 (44-93)os] , by fasting [177 (104-301)os] , by Shay method [180 (106-306)os] , by immobilization [210 (116-296)os] ,by reserpine [240 (188-304) os] , by indomethacin [312 (240-406)os] , by HCl 0.6N [233 (138-393) endo-duodenal] , by absolute ethanol [113 (80-161) e.d.] , by NaOH 0.2N [239 (176-324)e.d.] and duodenal ulcers induced by cysteamine [135 (93-195)os].
Its antisecretory activity was proved by reduction of volume and pH increase of gastric juice in pylorus ligated rats (ED50 respectively 145 and ~100 mg kg^{-1} e.d.).

As previously reported by Lücker (1983), hexaprazol demonstrated protective activity on gastric mucosa in healthy volunteers acting as an antagonist of electrical potential (PD) fall by acetylsalicylic acid (ASA). This clinical trial induced the authors to set up a similar method to ascertain hexaprazol protective action in the rat also. Moreover, in order to measure drug potency, other well known antiulcers have been tested in the same experimental conditions: carbenoxolone: (50 to 200 mg kg^{-1} e.d.), pirenzepine (200 to 400 mg kg^{-1} e.d.) and cimetidine (100 to 400 mg kg^{-1} e.d.).

The method consists in placing a polyethylene tube filled with KCl saturated solution in agarose into the rat stomach and connecting it to a millivoltmeter by a calomel electrode. A similar bridge was used to connect the blood stream with the millivoltmeter. No ligation was made on the gastrointestinal tract to block spontaneous flow. Antiulcers were administered e.d. one hour before ASA and PD was registered at fixed times during 60 min after ASA. As indicated by Lücker P.W. (1983), Area Under Baseline (AUB) of the PD vs. time graph and Reiz Index (RI, i.e. irritancy index = AUB * max PD fall) were calculated.

It was proved that hexaprazol has an anti-barrier breaker activity of marked intensity and duration; this efficacy was always dose-dependent.
The relative potency of hexaprazol compared with other drugs followed this order: carbenoxolone> hexaprazol> pirenzepine> cimetidine, by inhibiting both AUB and R.I. Advantages of the method were also discussed.

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INTRAGASTRIC pH-METRY IN THE ANAESTHETISED FERRET: EFFECT OF CIMETIDINE, TIMOPROZOLE AND OMEPRAZOLE

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It has previously been reported that the H_2 receptor blocker cimetidine, and the proton pump inhibitor timoprazole, both inhibited pentagastrin-induced gastric acid secretion in anaesthetised ferrets (Ghelani & Radziwonik, 1985). In these experiments gastric secretion was measured by aspiration and titration of the gastric juice at 15 min intervals, but usually it was only practical to measure one dose of drug in each animal. We now report a method for determining cumulative dose-response effects of drugs in anaesthetised ferrets using intragastric pH-metry.

Male ferrets weighing 1.26-1.73kg, starved for 20-24h but allowed water ad libitum, were anaesthetised and maintained under anaesthesia on pentobarbitone, and the pylorus ligated, as described previously. A pre-calibrated combined pH/reference electrode (GK2801C, Radiometer) was introduced into the stomach via the oesophagus, and positioned in the corpus next to the oxyntic mucosa. The pH was recorded continuously, and converted to hydrogen ion concentration using an Apple IIe computer, and displayed graphically on a VDU. Gastric juice was allowed to empty spontaneously from the stomach by means of a XRO dual flow gastric tube (Vygon). Cimetidine was dissolved in 0.9% saline, while timoprazole and omeprazole were dissolved in 40% polyethylene glycol 400 in phosphate buffered saline.

Secretion was measured until hydrogen ion concentration was $\leq 20 \mu mol\ ml^{-1}$ and was then stimulated by a continuous intravenous infusion of pentagastrin at a dose of $4 \mu g\ kg^{-1} h^{-1}$. Once the response had plateaued drugs were administered intravenously as cumulative bolus doses, and the effects calculated as a percentage of the plateau value.

All the compounds produced dose-related reductions of hydrogen ion concentration (Table 1). The ED_{50}s for cimetidine, timoprazole and omeprazole were 11.0, 6.3

Table 1 % Reduction of hydrogen ion concentration produced by cimetidine, timoprazole and omeprazole (mean ± s.e. mean, n = 4)

Compound	Dose mol kg^{-1}				
	3×10^{-7}	10^{-6}	3×10^{-6}	10^{-5}	3×10^{-5}
Cimetidine			24±6	48±7	72±5
Timoprazole			15±7	72±9	91±5
Omeprazole	8±4	37±6	80±8	94±4 (n=3)	

and 1.4 $\mu mol\ kg^{-1}$, respectively. These values agree well with the previously reported effects of cimetidine and timoprazole in aspiration experiments in anaesthetised ferrets. In the present studies the dose-response lines for timoprazole and omeprazole appeared to be parallel, whereas the effect of cimetidine did not parallel that of the pump inhibitors, thus indicating that the test may distinguish between anti-secretory agents with different modes of action.

The ferret, therefore, can be used to detect H_2 antagonists and H^+/K^+ - ATPase inhibitors. pH-metry provides a continuous measure of gastric acid secretion which does not involve perfusion of the stomach, and which enables cumulative dose-response curves to be established in a single ferret more readily than aspiration techniques allow.

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EFFECT OF ANTERIOR HYPOTHALAMIC LESIONS ON ANAPHYLACTIC CONTRACTION AND HISTAMINE SENSITIVITY OF GUINEA-PIG TRACHEA

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Anterior hypothalamic (AHA) lesions have been shown to decrease anaphylactic reactions in vivo, as well as several other immune reactions (Stein et al., 1976). Recently, we have shown that lesions in the AHA prevent the decrease in the number of beta-adrenoceptor binding sites in peripheral guinea pig lung tissue after immune stimulation by bacterial endotoxin (Van Oosterhout & Nijkamp, 1984). In the present study we investigated if the protective effect of AHA lesions against anaphylaxis can also be demonstrated in vitro and furthermore if contraction of isolated airways to the anaphylactic mediator histamine and relaxation to a beta-adrenoceptor agonist was changed.
Bilateral electrolytic lesions in the AHA (< 1.0 mm diameter) were made in male guinea pigs, weighing 300-350 g. Sham operated animals served as controls. Ten days after operation the animals were immunized (i.p.) with ovalbumin (2 mg) in aluminiumhydroxide adjuvans. Three weeks after the immunization trachea's were isolated, spirally cut and divided in two parts of seven rings and accordingly mounted in an organ bath. On one part a histamine dose-response relationship was established, followed after flushing by an antigen-induced contraction (10 ug/ml). The other part was half maximally contracted by carbachol and subsequently relaxed by the beta-adrenoceptor agonist isoprenaline.
The anaphylactic contraction induced in the tracheal spirals from AHA lesioned animals was significantly decreased (35%, $p<0.01$, Student's t-test) as compared to the reaction in control spirals. The dose-response curve for the histamine-induced contraction was significantly decreased ($p<0.05$, ANOVA) in tracheal spirals from AHA lesioned guinea pigs. At 10^{-4} M histamine, the difference in contraction between lesioned and control animals was 38%. No difference in beta-adrenoceptor function was measured.
It can be concluded that AHA lesions induce decreased antigen-induced contraction of isolated tracheal spirals as compared to sham operated animals. This reduced antigen-induced contraction coincides with a decreased sensitivity to histamine. A possible difference in reagenic antibody titers seems not to be important, since the pooled serum of AHA lesioned guinea pigs was as potent as the pooled control serum in passively immunizing guinea pig tracheal spirals, in vitro (results not shown). Studies to investigate the mechanism by which these lesions affect histamine sensitivity, and possibly also the sensitivity for other bronchoconstrictor agents, in the airways of the guinea pig are now in progress.

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ROLE OF NEUTROPHILS AND ARACHIDONIC ACID METABOLITES IN EXPERIMENTAL MODELS OF EAR INFLAMMATION IN MICE

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The roles of certain products of arachidonic acid (AA) metabolism and of neutrophils (PMNs) were investigated in two models of cutaneous inflammation in mouse ears: AA-induced (Young et al 1984) and cantharadin-induced (Tarayre et al 1984) swelling. Inhibitors of cyclooxygenase (CO) (indomethacin), lipoxygenase (LO) (phenidone) and both CO and LO enzymes (BW 755C) were also studied, topically, for their effects on AA metabolism and on the oedematous responses of both models.

Female, VAM 1 mice (25-32g) were used for all studies in groups of not less than 5. Oedema was measured by the wet weight of excised ears and PGE_2 and LTB_4 formation was determined by radioimmunoassay of methanolic homogenates of ears. The presence of mono and diHETEs was investigated by hplc following extraction from the methanolic homogenates using C-18 Sep Pak cartridges (Waters) according to the method of Powell (1980).

Parallel, dose-related increases in swelling and PGE_2 formation were observed 1h after application of AA (0.0625 - 4mg per ear) in acetone to the ears. Large amounts of mono HETEs found in the freshly prepared AA prevented the accurate estimation of mono and diHETEs in vivo. Inhibition of AA (4mg) - induced PGE_2 formation, in the ears by indomethacin (IC 50 7.0ug), BW 755C (IC 50 82.2ug) and phenidone (IC 50 394 ug) did not correlate with inhibition of swelling (indomethacin IC 50 245 ug; BW 755C IC 50 312 ug, phenidone IC 50 84.1 ug). These observations agree with those reported recently by Opas et al (1985). Pre-treatment with nitrogen mustard (NM) (2mg kg^{-1}; i.v.) 3d prior to topical application of 4mg AA reduced the number of circulating PMNs by 74% but did not reduce swelling or PGE_2 formation by the ears. Studies with colchicine (2mg kg^{-1}; s.c.), a compound known to impair the motility of PMNs (Fordham et al 1981) also failed to reduce swelling and PGE_2 formation when administered 1h prior to application of 4mg AA. These results suggest a role for LO products but not for PGE_2 or PMNs in the oedematous response.

Topical application of cantharadin (25ug) in acetone produced marked swellings of the ears by 6h and 10-20 fold increase in PGE_2 formation compared with control ears. Mono and diHETEs (including LTB_4) were not raised significantly at 6h. Inhibition of PGE_2 formation by indomethacin (IC 50 0.34ug) BW 755C (IC 50 27.4ug) and phenidone (IC 50 66.4ug) did not affect swelling significantly. Pretreatment with NM (as above) reduced circulating PMNs by 82%, swelling by 29% and PGE_2 formation by 30%. Colchicine, pretreatment (as above) reduce swelling by 76% and PGE_2 formation by 84%. Histologically, PMN adhesion to the vascular endothelium was observed in this model, but few PMNs were seen extravascularly. These results suggest that PMNs may be involved in the oedematous response in the 6h cantharadin treated ears, but it is unlikely that CO or LO products play a significant role.

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AN ASSESSMENT OF THE NEUROGENIC COMPONENT IN MODELS OF INFLAMMATION

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Neurogenic inflammation arising from the release of neuropeptides from small diameter sensory nerves is now a widely accepted phenomenon (Lembeck 1983). However, in the most commonly employed laboratory models of inflammation (yeast, carageenan, formalin-induced paw oedema and adjuvant arthritis) it remains to be firmly established whether a neurogenic component contributes to the initiation or maintenance of these lesions. Capsaicin (8-methyl-N-vanillyl-6-nonenamide) administered systemically either to adult or neonatal animals or applied topically to a peripheral nerve can be used as a selective sensory neurotoxin to cause loss of function of a population of non-myelinated sensory afferents which are responsible for neurogenic inflammation (Jancso, Jancso-Gabor and Szolcsanyi, 1967).

In the present study, pretreatment of adult rats with capsaicin (2 x 50mg/kg s.c. 7 days prior) caused a small (15-20%) inhibition of paw swelling due to yeast or carrageenan at 3 hours, a substantial (65%) inhibition of formalin oedema at all time points, and no significant inhibition of the inflammatory response in adjuvant arthritis over 14 days. In contrast, neonatal pretreatment with capsaicin (50mg/kg s.c., 3 months recovery) did not inhibit paw swelling in any of the four models. Despite this range of effects on inflammation, all animals pretreated either as neonates or adults with systemic capsaicin consistently showed a maximal inhibition of mustard oil-induced plasma extravasation in the contralateral paw and maximal inhibition of the wiping response to a single topical application of 0.01% capsaicin to the cornea which is in accordance with the widely reported effects of capsaicin on sensory function.

In a further series of experiments, sensory denervation of the adult rat hind paw was achieved by section of the saphenous nerve and topical application of capsaicin (1% for 15 min, 7 days prior) to the sciatic nerve. Under these conditions the inhibition of paw swelling in yeast, carrageenan and adjuvant models increased to around 30% while the inhibition of formalin oedema remained at 65%. In these animals no other effects of capsaicin could be observed. This may therefore give a truer indication of the presence and extent of the neurogenic component in this range of inflammation models and raises the possibility that compensatory mechanisms can be established which contribute to the inflammatory response after systemic capsaicin treatment (Gamillsheg, Holzer, Donnerer and Lembeck 1984).

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PERIPHERAL OPIOID EFFECTS UPON NEUROGENIC PLASMA EXTRAVASATION AND INFLAMMATION

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Antidromic stimulation of non-myelinated sensory nerves or local application to the skin of chemical irritants results in the development of a neurogenic inflammatory response mediated largely by release of neuropeptides from sensory terminals. This release can be modulated by opioids (Bartho and Szolcsanyi 1981; Lembeck, Donnerer and Bartho, 1982) acting at a peripheral site (Smith and Buchan, 1984). The present experiments were designed to confirm this effect with other opioid agonists and to determine whether similar mechanisms might apply in animal models of acute inflammation.

Plasma extravasation in the rat hind paw induced by either antidromic saphenous nerve stimulation at strengths sufficient to recruit non-myelinated afferents or topical application of mustard oil to the skin was inhibited by tifluadom in a dose-related manner (5% inhibition at 0.05mg/kg up to 32% at 1mg/kg s.c.). The action of tifluadom was stereospecific and naloxone reversible. (+)Tifluadom inhibited extravasation by 37% at 0.5mg/kg whereas (-)tifluadom was inactive at this dose and naloxone at 0.5mg/kg reversed the effect of tifluadom. Ethyl-ketocyclazocine was also effective and both k-agonists were more potent than morphine. Extravasation induced by an intraplantar injection of Substance P (10pmol) could not be inhibited by tifluadom (0.5mg/kg s.c.).

In carrageenan-induced paw oedema in the rat tifluadom, U50,488 and morphine produced a dose-related inhibition of the increase in paw volume with calculated ED_{30} values of 1.1, 1.4 and 5.0mg/kg s.c. respectively, which broadly correspond to the doses required to inhibit plasma extravasation by this extent. The kappa agonists had an immediate and naloxone reversible effect when dosed prior to the carrageenan but were without effect when dosed two hours after the oedema was established. Taken together, these data demonstrate the ability of opioids to inhibit oedema formation in acute inflammatory conditions probably through an action at peripheral receptor sites which modulate neuropeptide release from sensory nerve terminals.

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ULTRASONIC VOCALIZATION IN RAT PUPS: EFFECTS OF EARLY POSTNATAL EXPOSURE TO HALOPERIDOL

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Ultrasonic calls represent one of the few response patterns emitted by rat pups which may be quantitatively evaluated and which may be produced by different environmental variables, such as thermal, olfactory and cutaneous stimuli (Allin & Banks, 1971; Okon, 1971; Sewell, 1968). Recently, the diagnostic usefulness of the measurement of ultrasonic vocalization in studies of developmental toxicity has been suggested (Zbinden, 1981). Therefore, since it has been shown that the prolonged exposure to neuroleptics during the neonatal period produces behavioural and neurochemical changes in the adult rat (Cuomo et al., 1981, 1983), it was of interest to evaluate in the present study whether ultrasonic vocalization elicited by the removal of rat pups from their nest could be altered by repeated administration of haloperidol (H) and whether it could represent an early sensitive indicator of subtle behavioural abnormalities caused by prolonged postnatal exposure to this neuroleptic.

Starting on day 2 after birth and every two days through to day 16, Wistar rat pups were given single subcutaneous injections of H (1 mg/kg) or H-vehicle (V). Ultrasonic calls were recorded for 15 sec 30 min after H or V injection.

The results show that the number of ultrasonic calls was significantly reduced by H-exposure from the 8th until the 14th day after birth. Conversely, this neuroleptic significantly increased the duration of ultrasounds from the 4th up to the 16th day of age. Moreover, notable changes in the sound pressure levels as well as in the frequency of calls were produced by early postnatal treatment with H.

The alterations in ultrasonic vocalization caused by neonatal H exposure seem to be due to an impaired functional maturation of the dopaminergic system. Furthermore, our results suggest that ultrasonic vocalization may be considered as an early sensitive indicator of subtle changes elicited by the postnatal treatment with a dopamine receptor blocking agent at dose levels below those associated with overt signs of neurotoxicity.

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790P

EFFECTS OF CIMETIDINE, RANITIDINE AND THE NEW H_2 RECEPTOR ANTAGONIST CM 57755 ON DRUG METABOLISM IN RATS

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CM 57755 is a new histamine H_2 antagonist as potent as but longer acting than cimetidine (C) (Lavezzo et al., 1984). C is known to impair drug metabolism by inhibiting hepatic mixed-function oxidases. In previous experiments, CM 57755, unlike C, did not interact with cytochrome P-450, suggesting it should not interfere with metabolism of co-administered drugs (Picard-Fraire et al., 1984). We studied the effect of CM 57755 on the pharmacodynamics and/or pharmacokinetics of pentobarbital (P), zoxazolamine (Z) and aminopyrine (A), in the rat.

CD male rats (C. River, Italy), 200±15 g, were used. In P studies, CM 57755 (30 and 120 mg/kg), C (30, 60, 120 mg/kg), ranitidine (R) (120 mg/kg) or saline were injected i.p. 30 min before P (35 mg/kg i.p.) and sleeping times measured. Brain P levels at awakening were checked sometimes. In a separate experiment brain P levels were measured in rats given 120 mg/kg CM 57755, 120 mg/kg C or saline 30, 60 and 90 min after P. Paralysis times were measured in rats given Z (75 mg/kg, i.p.) 30 min after 120 mg/kg CM 57755, C, R or saline, i.p. To study the metabolism of A (Mavier et al., 1983) CM 57755 and C were given i.p., 30 min before injection of 40 mg/kg A into a cannulated jugular vein of urethane-anesthetized rats. Serum levels of both A and its metabolite, 4-amino-antipyrine were determined by HPLC 15, 30, 60 and 90 min after A.

P sleeping time (min±s.e.) after CM 57755 (30 mg/kg: 55±5, 120 mg/kg: 62±5) and R (60±7) was comparable to controls (47±5), but increased dose-dependently after C (30 mg/kg: 78±7, 60 mg/kg: 93±15, 120 mg/kg: 149±16). Waking brain P levels (µg/g) after 120 mg/kg C (21±2) were the same as in controls (20±2) and 120 mg/kg CM 57755 (20±1). Disappearence of P from brain was comparable in controls and CM 57755, but slower in C (controls: 13±2, CM 57755: 14±3, C: 26±2 µg/g at 90 min). Z paralysis times (min±s.e.) were significantly longer than controls (232±12), only in C (337±24), but not after CM 57755 (245±36) and R (238±8). Disappearance of A from serum was comparable in CM 57755 and controls (half-lives: 60 and 56 min), but seriously impaired in C (serum A levels, µg/ml: 46±2 at 15 min and 43±1 at 90 min). In C the appearance of 4-amino-antipyrine at 90 min (µg/ml±s.e.) was also impaired (4±0.4) compared to controls (18±1) and CM 57755 (16±1), which confirms the metabolic effects of C.

The results indicate that CM 57755, unlike C, does not interfere with the in vivo metabolism of co-administered drugs and that it is safe for treatment of ulcer and gastric hypersecretion in patients given other medications.

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THE EFFECT OF STREPTOZOTOCIN-INDUCED DIABETES ON THE BLOOD FLOW TO THE REPRODUCTIVE ORGANS

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Pregnancy in diabetics is associated with many complications, including spontaneous abortion, premature birth, perinatal mortality and congenital malformations (Golob et al, 1970; Pedersen, 1967). It is generally thought that reduction in the rate of blood flow to the uterus and its consequent effect on uterine and foetal growth may be involved in some of the complcations of diabetic pregnancy. We have thus studied the rate of blood flow to the reproductive organs of control and streptozotocin - diabetic rats during the pro-oestrous and oestrous phases of the cycle and on day 21 of pregnancy.

Diabetes was induced by a single intravenous administration of streptozotocin. The pregnant rats were treated on day 12 or 13 of pregnancy at a dose of 40 mg kg^{-1} (mild diabetes) and 50 mg kg^{-1} (severe diabetes). All the non-pregnant rats received 40 mg kg^{-1} 3-4 days prior to test. Blood glucose levels were determined using the Reflotest glucose analyser. Blood flow was measured in the anaesthetised rat by the radioactive microsphere (15 μm) technique (Phaily & Senior, 1978). Organ blood flows were expressed using tissue wet weights.

The uterine (170±48 ml min^{-1} $100g^{-1}$) and ovarian (296±49 ml min^{-1} $100g^{-1}$) hyperaemia observed during pro-oestrous in control rats (n=7) was significantly reduced ($P<0.01$, $P<0.05$) in diabetic rats (n=6) also in pro-oestrous (uterine 23±4, ovarian 103±20 ml min^{-1} $100g^{-1}$). No significant difference was observed in the blood flows to the uterus, ovaries and placentae between control (n=16) and diabetic (n=13) rats on day 21 of pregnancy. The blood flow and % cardiac output to the kidneys (441±28 control, 279±34 diabetic, ml min^{-1} $100g^{-1}$) and the spleen (216±22 control, 147±26 diabetic, ml min^{-1} $100g^{-1}$) were, however, highly reduced ($P<0.05$, $P<0.01$) in diabetic pregnant rats.

These results are as yet insufficient to formulate the mechanisms by which diabetes depresses uterine and ovarian hyperaemia during pro-oestrus. It would appear that the reduction in uterine and ovarian blood flow in diabetics at pro-oestrus, at a time when oestrogen secretion is known to be at the highest (Harvey & Owen 1976), may be due to an alteration in the sensitivity of the uterus to oestrogen (since in diabetics no alteration in the concentration of this hormone has been observed, (Kirchick et al 1978)). Alteration in oestrogen receptor dynamics and/or a reduction in the production and/or metabolism of the known oestrogen action mediators such as prostaglandin and histamine may be involved. The results in the pregnant rats indicate that a homeostatic mechanism aimed at maintaining blood flow to the pregnant uterus at the expense of other organs, namely the kidneys and the spleen, may play a role in the diabetic state.

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EFFECT OF THYROXINE ON VENTRICULAR ADENOSINE TRIPHOSPHATASE ACTIVITY IN GUINEA-PIGS

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Male, Dunkel-Hartley albino guinea-pigs were treated daily with L-thyroxine for 1 to 11 days (100 µg/kg body weight i.p.). Control animals received similar volumes of saline over the same time period.

The hearts were removed and rinsed in a solution (0°C) containing 5 mM EDTA, 0.25 M sucrose, and 5 mM histidine-HCl (pH 7.4). The ventricles were isolated and minced with a scapel and then homogenised at 0°C by 12 strokes with a rotating blade homogeniser in 12 ml of a solution containing 0.15% deoxycholate, 5 mM EDTA, 0.25 M sucrose, and 5 mM histidine adjusted with Tris Buffer to pH 6.8. The homogenate was centrifuged at 1,100 g for 10 min to remove the nuclear fraction and at 12,300 g for 20 min to remove the mitochondrial fraction. The supernatant was then centrifuged at 35,600 g for 1.5 h to obtain the microsomal fraction. The microsomal fraction was rehomogenised in 5 ml of a cold solution containing 2.5 mM EDTA, 1 M NaI, and 2.5 mM histidine (pH 6.8), and then stirred for 1 h between 1 to 4°C. The microsomal fraction was recovered by cetrifugation (35,600 g for 1.5 h) and resuspended in 10 mM imidazole-HCl (pH 7.4) to be stored overnight at 4-7°C.

The assay medium for total ATPase activity contained in 2 ml (in mM): 120 NaCl, 10 KCl, 40 Tris-HCl (pH 7.5 at 37°C), 2 ATP, 2.5 $MgCl_2$, 0.5 EGTA and 33± 2 µg of microsomal protein. The reaction mixture was incubated for 1 h in a water-bath at 37°C, and the reaction terminated by 0.1 ml of 50% trichloroacetic acid. Phosphate was assayed by forming the phosphomolybdate complex which was then measured spectrophotometrically. Na^+, K^+-ATPase activity (µmoles phosphate released/mg protein/min) was measured as the ouabain-inhibitable fraction of total ATPase activity. In the presence of 1 mM ouabain, Ca^{2+}-ATPase activity was measured as the difference in specific activity between the measured ATPase activities in the presence and absence of 2 mM $CaCl_2$ in the assay medium.

The ventricular microsomal Na^+, K^+-ATPase did not vary with the thyroid status of the animal. Even after 8-11 days of thyroxine-treatment, Na^+, K^+-ATPase activity was similar to controls. However, the ATPase activity of the non-ouabain-inhibitable fraction was 7-10% greater than control after 1 day, and approximately 30% greater than control after 8-11 days of thyroxine administration. The myocardial Ca^{2+}-ATPase activity was found to be significantly increased even after only 1 day of thyroxine-treatment. Hence, thyroid hormone modulates the Ca^{2+}-ATPase activity in the ventricles. Other workers have come to various conclusions. For instance, Curfman et al (1977) reported that thyroid hormone significantly enhanced the ventricular Na^+, K^+-ATPase in the guinea-pig; whereas others have shown that Ca^{2+}-ATPase activity of cardiac myosin is markedly increased in thyrotoxic animals (Thyrum et al (1970); and Banerjee and Morkin (1977)).

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ADRENAL STIMULATION OR CORTICOSTEROID REPLACEMENT TREATMENTS COUNTERACT A UNIQUE HYDROXYUREA TOXICITY IN PITUITARY-ABLATED RATS

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Lethality at 100 and 85% was observed following 800 mg/kg/day anticancer drug HYD over 5 days in hypophysectomized or adrenalectomized animals respectively against 0% in intact controls (Vacca *et al.*,1984). The above dose strongly stimulates the hypophyseal adrenal axis in intact rats (Vacca & Preziosi, 1984). Reagent grade corticosterone (B) in olive oil alone or, in a more evident manner, in association (2:1 ratio) with deoxycortone acetate (Cortiron 10 mg/ml, DOCA) at twice-daily doses of 2-4 and 1-2 mg/kg respectively given i.m. to hypophysectomized or adrenalectomized animals delay lethality and partially protect against the increased HYD (dose as above) toxicity. A preparation of tetracosactrin ($ACTH_{1-24}$) for prolonged action, 1 mg as hexa-acetate/ml (Synacthen depot, LATH), given i.m. at 0.5 mg/kg every second day from the 4th day before HYD treatment up to the 5th treatment day proved most effective in delaying HYD lethality in pituitary-ablated rats.

Table 1. Lethality in hypophysectomized rats treated with hydroxyurea and receiving long-acting tetracosactrin hexa-acetate or corticosteroids.

An. N.	HYD	Other treatments	% lethality at the following days 1	2	3	4	5	Plasma B $\mu g\ dl^{-1} \pm s.e.$*
80	NO	-	0	0	2.5	2.5	2.5	4.3±0.9
110	YES	Controls	0.76	49.6	87.1	94.1	100	4.4±0.9
30	YES	LATH 0.5	0	0	6.6	33.3	90	44.7±2.7**
40	YES	B 2	0	36.6	45	85	97.5	21.6±2
60	YES	B 4	0	18.3	46.1	73.3	91.6	35.9±2.1
40	YES	DOCA 1	2.5	25	60	80	85	-
50	YES	DOCA 2	0	47.5	72.6	90	92.5	-
40	YES	B 2 + DOCA 1	0	7.5	22.5	47.5	70	12.9±1.6
30	YES	B 4 + DOCA 2	0	10	30	60	83.3	36.6±2.5

For the abbreviations and treatment schedules see text. Controls received olive oil 1 ml/kg/day i.m. over 5 days and saline (2 ml/kg/day) *per os*. Animals drunk glucose 5% in saline. *Values on the third day at 4 p.m., six hours after morning treatment. ** In these rats previously given two doses of LATH, plasma B values were 30.3±0.5 on the morning before the first HYD administration (in 60 intact untreated rats given saline 6.2±0.8).

The best protection was observed when adrenal stimulatory or replacement treatments allowed plasma B concentrations at least corresponding to or even higher than those obtained six hrs after the third morning HYD administration in intact rats ($\mu g\ dl^{-1}$ 26.1±2.3). This could indicate that the HYD-induced adrenocortical activation may represent a unique defence mechanism against the drug toxicity which is lacking in pituitary- or adrenal-ablated animals.

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EFFECT OF POLYETHYLENE GLYCOL 400 ON ADRIAMYCIN INDUCED HISTAMINE RELEASE

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The clinical use of the anthracycline antibiotic adriamycin (A) is limited by acute and subacute cardiovascular effects and by a dose dependent cardiomyopathy. Recently, Bristow et al. (1983) suggested the hypothesis that these side effects are related and are mediated by the release of vasoactive substances such as histamine, catecholamines and prostaglandines.

In previous studies (Bartoli Klugmann et al., 1984) we have shown that pretreatment of mice with the widely used solvent polyethylene glycol (PEG) 400 could prevent the cardiac toxicity induced by a chronic or acute treatment with A. Among the several pharmacological and biological actions of PEGs, some Authors (Magnusson et al., 1982) have pointed out a reduction in spontaneous and polymyxin B induced release of histamine.

The aim of this study was the evaluation of the effects of a pretreatment with PEG 400 on the release of histamine induced by A. Peritoneal rat mast cells were collected and incubated in triplicate for 30 min at 37 °C with concentrations of A (100 and 50 µg/ml) which produced an important histamine release without killing the cells (evaluation by trypan blue dye exclusion). Additional samples were preincubated in triplicate with 0.05, 0.5, 5 and 10% PEG 400, for 30, 60 and 90 min. The amount of histamine released was assayed by a fluorimetric method. The two tested A concentrations induced an important histamine release which was significantly inhibited by the pretreatment with the two highest concentrations of PEG 400, at every considered experimental time.

For in vivo experiment, we have evaluated by means of an histological study under the light and fluorescence microscope, the effect of a pretreatment with 3.45 g/kg of PEG 400 on the degranulation of peritoneal and pericardial mast cells induced by an i.p. injection of 15 mg/kg of A in mice. Also in vivo A caused an important mast cell degranulation, indicating release of histamine and other amines, which was almost completely prevented by the pretreatment with PEG 400.

This effect of PEG 400 on A induced histamine release could explain the important protective action exhibited in vivo on A treated animals, confirming therefore that A cardiotoxicity can be related to the release of histamine and other vasoactive substances.

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CHANGES IN PLATELET FUNCTION IN DIABETES MELLITUS WITH IMPROVEMENT OF GLYCAEMIC CONTROL

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Numerous studies have demonstrated abnormal platelet function in diabetic patients using a variety of techniques including platelet aggregation (Sagel et al, 1975), measurement of platelet-specific protein β-thromboglobulin (Preston et al, 1978), platelet thromboxane synthesis (Buktus et al, 1980) and refractoriness to inhibitory prostaglandins (Betteridge et al, 1982). Their presence early in the diabetic state, before the occurrence of overt vascular complications, suggests that they at least play a part in the pathogenesis of microvascular complications.

Two moderately-well controlled groups of young insulin-dependent diabetics matched for sex, age and duration of diabetes underwent function tests at time 0 (T_0) and 6 months later (T_1). The active group improved their glycaemic control (HbA_1, T_0 11.9 to T_1 9.0%; normal range 6 - 8%) while the control group maintained the same glycaemic control (HbA_1, 11.4 to 11.9%). The patients were free of drugs which influence platelet function and had no evidence of nephropathy (Albustix negative) or macrovascular disease. There was no significant difference between the platelet counts in each group and none of the patients had suffered hypoglycaemic reactions within 24 hours of the venesections.

Platelet aggregation was measured by the photometric method of Born. Dose response curves were constructed for aggregation induced by ADP alone and also in the presence of Iloprost (a prostacyclin analogue) (Casals-Stenzel et al, 1983). Thromboxane (TXB_2) produced in response to exogenous collagen was measured in duplicate by radio-immunoassay using a double antibody separation technique. β-Thromboglobulin was also assayed by RIA (Boulton et al, 1976). With improvement of glycaemic control, there was a statistically significant increase in the responsiveness of the platelets to Iloprost, increased platelet thromboxane production and a reduction in β-thromboglobulin production. There appeared to be an increase in platelet sensitivity to ADP-induced aggregation but this did not reach statistical significance (Table 1).

These results suggest that with improved diabetic control, platelets become more sensitive to inhibiting prostaglandins and that there are fewer activated platelets in the peripheral circulation.

Table 1 Results of platelet function tests with improved glycaemic control

	EC_{50} (± s.e.m.) for ADP (μM)		ADP dose ratio due to Iloprost (1.5×10^{-9} M)		TXB_2 T_1/T_0 Ratio	BTG Ratio
	T_0	T_1	T_0	T_1		
Actives (n = 5)	8 ± 2.3	6 ± 2.2	1.25	*3.3	*2.35	*0.66
Controls (n = 4)	6 ± 2.5	6.5 ± 2.1	2.5	1.9	0.83	1.0

Statistical analysis using Wilcoxon rank sumsigned test (*$P < 0.05$).

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PHARMACOKINETICS OF D-PENICILLAMINE AND ITS DISULPHIDE IN RATS

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D-Penicillamine (PSH) is a second line anti-inflammatory agent used in the treatment of rheumatoid arthritis. Its mode of action is unclear but is believed to be due in part to the reactivity of its sulphydryl (SH) group. In both man and rat the major route of metabolism is the formation of disulphides, both penicillamine-penicillamine (P-P) and penicillamine-cysteine (P-C). There has been much controversy as to the pharmacologically active form(s) of d-penicillamine, but P-C has been implicated. However, the reactivity of the oxidised forms of d-penicillamine are known to be low since the reduction of the disulphide bond is enzyme dependant *in vivo* and difficult *in vitro*. In order to assess the activity of the disulphide, the pharmacokinetics of oral and i.v. doses of PSH and P-P were followed in rats.

Female Sprague Dawley rats weighing between 120-150g were used. They were dosed either orally or i.v. with PSH (200mg/kg) or P-P (202mg/kg) following a twelve hour fast. Water was allowed *ad libirtum*. The animals were housed in metabolism cages that allowed the separate collection of urine and faeces. At intervals (0.25-18 hours) over the subsequent 78 hours, the rats (1-2 per time point) were bled by cardiac puncture and the plasma assayed for total d-penicillamine, i.e. PSH, P-P, P-C, and protein bound drug, using a modification of the HPLC method of Abounassif and Jefferies (1983).
Urine, collected in aliquots for 48 hours, was similarly analysed.

The results, summarised in Table 1, show that there were significant differences between oral and i.v. administration in both the peak plasma concentration and area under the time/concentration curve (AUC) for P-P indicating that the disulphide is poorly absorbed from the g.i. tract. Peak plasma concentration but not AUC was decreased for PSH between oral and i.v. dosing. Following i.v. administration of these compounds, the AUC was significantly lower after P-P than after PSH indicating the low reactivity of the oxidised metabolite and that the presence of a free SH group is necessary to react with endogenous components (e.g. plasma proteins) to remain in the system. This is substantiated by the urinary excretion data showing that after i.v. dosing, excretion is greater after P-P than PSH.

In summary, both the absorption and ultimate plasma concentration of PSH may depend on the reactivity of its SH group rather than on an oxidised metabolite.

Table 1: A comparison of certain pharmacokinetic parameters following i.v. and oral administration of PSH (200mg/kg) and P-P (202mg/kg) to rats.

	PSH		P-P	
	oral	i.v.	oral	i.v.
Peak plasma concentration (mg/L)	75.5	274.1	4.67	231.1
Terminal plasma half-life (h)	47.0	46.3		72.5
AUC_{0-78} (mg.h/L)	1087	1078	5.00	384.6
Urinary excretion (mg/48h)		2.86		5.34

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THE EFFECT OF ADDED GLUCOSE ON THE IN VITRO ACETYLATION OF SULPHAMETHAZINE BY HUMAN WHOLE BLOOD IN HEALTHY AND DIABETIC SUBJECTS

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Increased in vivo acetylation of sulphamethazine (SMZ) has been reported in diabetic subjects. (Shenfield et al 1982) and the reported decrease in the half life of isoniazid in normal volunteers following a glucose load (Thom et al 1981) suggests that elevated blood glucose may increase production of acetyl CoA. Recently we reported an HPLC assay for the in vitro measurement of SMZ acetylation using human whole blood (Lindsay & Baty 1985) and we report here the use of this method to compare the acetylating ability of healthy and diabetic subjects and the effect of adding glucose to blood prior to an in vitro acetylation of SMZ.

Whole blood was incubated for 24 hours with SMZ (180 µmoles litre^{-1}) as previously reported. In a further set of experiments the substrate was augmented with glucose to increase the blood glucose by approximately 5 mmoles litre^{-1}.

Table 1 shows the mean amounts of acetylsulphamethazine (AcSMZ) produced by healthy and diabetic subjects together with their glucose concentrations. Table 2 shows the percentage increase in the amount of acetylated product following the addition of glucose to the blood.

Table 1

Mean Blood Glucose + mmoles/litre (range)	Mean AcSMZ, nmoles (range) ++	No. of subjects
◘ 4.8 (3.4 - 6.8)	3.1 (2.2 - 4.7)	9
* 9.8 (5.8 - 19.1)	4.1 (1.8 - 8.0)	26

Table 2

Mean Blood Glucose + mmoles/litre (range)	% Increase in AcSMZ, nmoles (range) NS	No. of subjects
◘ 9.9 (8.4 - 11.8)	106 (86 - 127)	8
* 13.9 (11.0 - 19.2)	109 (40 - 146)	12

◘ Non age matched healthy subjects
* Diabetic subjects attending routine out-patient monitoring
The value in the two groups was significantly different using the Mann-Whitney U test + $p < 0.001$ ++ $p > 0.05$ NS Not significant

In normal subjects addition of 10 mmoles litre^{-1} of glucose to give concentrations of between 13.4 and 16.8 mmoles litre^{-1} did not change the % increase in acetylation. Table 2 indicates that such levels in diabetic subjects do cause an increase in the acetylation capacity. This might indicate that additional factors are responsible for the increased acetylation capacity of the diabetic subjects.

Using blood from healthy volunteers (n = 4) it was shown that elevation of their initial glucose concentration by the addition of 5, 10, 20 and 50 mmoles litre^{-1} of glucose did not increase the initial rate of acetylation, but extended the linearity of the reaction. Experiments with blood samples from one subject to which acetyl CoA was added (AcCoA/SMZ ratios of 1.9 4.7 and 9.3) showed an increase in the initial rate of acetylation of 9%, 23% and 33% respectively. These results would appear to support the hypothesis that elevated levels of glucose increase the extent of drug acetylation in vitro.

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M_1 MUSCARINIC ANTAGONISTS SELECTIVELY INHIBIT VAGALLY-INDUCED ACID SECRETION

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Muscarinic receptor subtypes, M_1 and M_2, mediate the cholinergically-induced acid secretion. M_1 subtypes, predominantly localized in the enteric neurones (Buckley & Burnstock, 1984) may modulate the secretion evoked through the vagus, whereas M_2 subtypes, distributed on parietal cells, may activate the final secretory process. That the proposed mechanisms may be operant both in "in vitro" and "in vivo" conditions has been shown by using the M_1 selective antagonist pirenzepine which potently inhibits vagal induced secretion but weakly antagonizes direct parietal cell stimulation (Pagani et al., 1984). In the present study we investigated the antisecretory activity of dicyclomine, a drug reported to possess M_1 selectivity in binding to isolated membranes (Luber-Narod & Potter,1983). The aim was to determine wether M_1 receptor selectivity would be reflected on a functional parameter. We wished also to compare the potency of pirenzepine, an hydrophilic compound, with that of dicyclomine which is considerably more lipophilic. Recently, lipophilicity has been shown to influence estimation of antisecretory drug potency (Black et al., 1985).

Acid secretion in the mouse stomach (Angus & Black, 1979) was induced by bethanechol (10^{-5} M) or by electrical field stimulation (10 Hz, 0.5 msec 10 V for 10 min). IC_{50} were determined after exposure (30 min) to at least 3 drug concentrations and calculated on the inhibition (%) of the peak output.

	Mouse stomach IC_{50} (μM $\pm$ 95% conf. Lim.)	
	Bethanechol	Field stimulation
Atropine	0.03 (0.02-0.04)	0.07 (0.05-0.09)
Pirenzepine	0.84 (0.57-1.22)	0.33 (0.23-0.49)
Dicyclomine	11.80 (8.10-17.20)	2.56 (1.93-3.38)

Both pirenzepine and dicyclomine preferentially antagonized secretion evoked through the intrinsic neurones by field stimulation. Conversely, atropine was manifestly more potent in inhibiting bethanechol activation of parietal cells. The lipophilicity of dicyclomine does not affect its ability to discriminate between neural evoked and parietal cell stimulation of acid secretion, although it may lead to an underestimation of its antisecretory activity. These results support the hypothesis that M_1 muscarinic subtypes, sensitive to selective antagonists, are involved in neurally evoked acid secretion.

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THE ROLE OF GLUTATHIONE IN THE DISPOSITION OF 2,4-DINOTRO-FLUOROBENZENE IN THE RAT

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2,4-Dinitrofluorobenzene (DNFB) is widely used as a hapten in immunological studies since it reacts readily with proteins under mild conditions. DNFB may thus form immunogenic protein conjugates *in vivo*, and this can result in a hypersensitivity reaction; topical application of dinitrohalobenzenes produces contact sensitivity in a number of species. This type of reaction may be attenuated by prior administration of reduced glutathione (GSH) (Ogura, 1972). We have recently shown that DNFB administered i.v. to rats is excreted predominantly as dinitrophenyl(DNP)-mercapturate and DNP-GSH conjugate (Grabowski et al., 1985). Thus conjugation with GSH may represent an important detoxication mechanism for DNFB. In order to characterize further the role of GSH, we have investigated the depletion of hepatic GSH by DNFB, and the effect of depletion by diethylmaleate (DEM) on DNFB disposition.

DNFB (25mg/kg) given i.v. to urethane anaesthetized male Wistar rats caused depletion of hepatic GSH in a time-dependent manner. Thus, a 20% reduction was seen after 30 min, whereas after 5h the depletion was 57% of the control value.

DEM (480mg/kg) in corn oil (2.4ml/kg) or vehicle alone was given i.p. to anaesthetized rats 30 min before i.v. administration of [^{3}H]-DNFB (91 µCi/kg, 25mg/kg). Tissue distribution, covalent binding to plasma proteins and biliary excretion of radioactivity was determined, and DNFB metabolites in bile were separated using h.p.l.c. (Kitteringham et al., 1985). DEM caused 88% depletion of hepatic GSH after 30 min. No differences between tissue distribution of radioactivity in control and DEM pretreated animals were found. Biliary excretion of ^{3}H was extensive over a 5h experiment (22 ± 4% in control rats) and was not changed by DEM. No differences were seen in the biliary metabolite profile; DNP-mercapturate and DNP-GSH accounting for over 80% of the radioactivity in both control and DEM-treated rats. The majority of radioactivity in plasma after 5h was covalently bound: 76.9 ± 4.5% and 80.5 ± 6.2% in control and DEM-treated rats respectively (not statistically different). The urinary metabolites of DEM-pretreated and control free-range rats were analysed for 3 days after [^{3}H]-DNFB administration. In 24-48h urine from pretreated rats there was a greater proportion ($p < 0.05$, pretreated v. controls) of a minor component which was chromatographically similar to N^2-acetyl-N^6-DNP-lysine (acetyl-DNP-lys). This metabolite has been isolated from urine and characterized by FAB and CI mass spectrometry. Previous studies have shown that acetyl-DNP-lys is a metabolite of synthetic DNP-protein conjugates (Kitteringham et al., 1985).

Thus depletion of GSH by 88% had no observable effect on DNFB disposition over 5h. However, the increase in acetyl-DNP-lys in 24-48h urine indicates that altered protein-DNP conjugate formation may result from GSH depletion. Such changes, although small, may be of toxicological importance.

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800P

EXOGENOUS L-CARNITINE IS ABSORBED BY THE RAT INTESTINE AT A DOSE-DEPENDENT, WIDE DOSE RANGE

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L-Carnitine (CAR) is present in all mammalian tissues and plays an essential role in the metabolism (mitochondrial ß-oxidation) of long-chain fatty acids. To that respect, the required amount of CAR is ensured to the organism by the endogenous synthesis process, mainly at the hepatic level, and by the intestinal absorption of the CAR present in the food. In pathologic carnitine-deficient conditions, it becomes of outstanding importance the possibility for the amount of intestinal absorbed CAR to be raised by administration of adequate oral doses.

This experiment was performed to find out what amounts of the product orally administered to the rat can be absorbed, dose-dependently.

To that purpose, the following parameters were determined: 1) serum carnitine maximal concentration (ΔCmax, 6th h) and area under curve (AUC 0-24 h); 2) the amount of urinary excreted carnitine as total carnitine (TCAR) over the first 24 hrs after treatment.

Results are reported in the table herebelow (mean values $\pm$ S.E.):

Oral dose	SERUM CARNITINE		URINARY EXCRETION AS TCAR
mg/kg	ΔCmax (6th h) μM	AUC (0-24 h) μmoles/l/h	administered dose %
100	-	-	16.3±0.6 (3)
250	54.9±4.7 (6)	406	-
500	98.1±6.0 (9)	-	17.2±1.2 (3)
1000	122±8.6 (10)	1710	17.0±2.8 (3)
2000	186±10 (10)	-	13.0±3.7 (3)

In parentheses, the number of animals.

The above results indicate the following: 1) an increase in serum carnitine ΔCmax, statistically dose-dependent (R= 0.980; P= 1%); 2) an increase in carnitinemia AUC, dose-dependent at 250 and 1000 mg/kg; 3) urinary excretion as TCAR (%) of the administered CAR, dose-dependent in animals treated with 100, 500, and 1000 mg/kg, 16% - 17% - 17%, respectively; the value of animals treated with 2000 mg/kg (13%) L-CAR appears to be less dose-related.

The above results indicate that the increase in the exogenous CAR intestinal absorption in the rat is dose-dependent up to 1000 mg/kg, ceasing to be such at the 2000 mg/kg dose. Therefore, it is reasonable to assume that the capacity of the rat intestine to absorb exogenous CAR starts to be saturated at the 2000 mg/kg dose.

INHIBITION OF HEPATIC DRUG METABOLISM BY QUINOLINE DERIVATIVES IN VITRO

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There is increasing evidence from both in vitro and in vivo studies of the potential of some antimalarial aminoquinoline drugs to inhibit hepatic microsomal drug oxidation (Back et al., 1983; Back et al., 1984a; Murray, 1984; Thabrew & Ioannides, 1984; Riviere & Back, 1985). Using aminopyrine as substrate in vitro, Murray, (1984) found that primaquine (PQ) amodiaquine (AQ) and quinine (Q) were relatively potent inhibitors of the N-demethylase activity but quinidine (QD) and chloroquine (CQ) were essentially non-inhibitory. The present study was designed to investigate the effects of the five drugs tested above plus mefloquine (MQ) on the in vitro metabolism of tolbutamide (TOL) and the steroid ethinyloestradiol (EE_2) by rat liver microsomes.

The 2-hydroxylation of EE_2 (0.01 mM; 1μCi) was studied in the presence of the antimalarial (0.1, 0.2 and 0.5 mM). At the end of the incubation period ^3H-labelled metabolites were analysed by h.p.l.c. (Maggs et al., 1983). The hydroxylation of TOL (0.1 mM) was also studied in the presence of each antimalarial (0.5 mM) and the formation of OHTOL assessed by h.p.l.c. (Back et al., 1984b).

Ether extractable radioactivity from control microsomal incubations with EE_2 was resolved into at least three peaks by reversed-phase h.p.l.c; the major peak was 2-$OHEE_2$. MQ, PQ, AQ and Q significantly inhibited EE_2 metabolism at each of the concentrations studied as evidenced by an increase in the percentage of unmetabolized EE_2. QD significantly inhibited metabolism at 0.2 and 0.5 mM but CQ was without effect. In terms of recovery of 2$OHEE_2$, PQ was the most potent inhibitor. At an inhibitor concentration of 0.5 mM the order of potency was PQ ($1.5 \pm 0.4\%$ 2-$OHEE_2$ recovered; Mean ± S.D.; n = 4) > MQ ($5.1 \pm 3.1\%$) > Q ($18.1 \pm 4.5\%$) > QD ($30.4 \pm 5.2\%$) > AQ ($46.9 \pm 3.4\%$) > CQ ($60.0 \pm 4.0\%$), and compared to control ($60.0 \pm 2.9\%$).

Tolbutamide hydroxylase activity in control microsomes was 82.3 ± 13.5 $pmol.min^{-1}.mg\ protein^{-1}$. The order of potency of the inhibitors was MQ ($19.2 \pm 9.2\%$ of control; n = 6) > PQ ($22.3 \pm 13.3\%$) > Q ($35.5 \pm 7.4\%$) > QD ($55.1 \pm 10.4\%$) > AQ $86.9 \pm 5.1\%$) > CQ ($95.6 \pm 10.1\%$).

The results of the present study are substantially in agreement with those of Murray (1984) pointing to PQ being a potent inhibitor of a number of oxidative enzymes and CQ being essentially non-inhibitory. In addition, MQ is roughly comparable to PQ in its propensity to inhibit the enzymes studied.

J.H.R. is in receipt of an MRC studentship.

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802P

INHIBITION OF ^{111} INDIUM LABELLED PLATELET DEPOSITION BY ASPIRIN, PROSTACYCLIN AND THE THROMBOXANE ANTAGONIST, AH23848

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Aspirin, prostacyclin and the thromboxane antagonist, AH23848 inhibit aggregation to low concentrations of collagen (0.1-3 µg/ml) in human platelet rich plasma (Brittain et al., 1985). We have developed a quantitative in vitro method for investigating the interaction between human platelets in flowing blood and the surface of experimentally damaged blood vessels, and have used this system to study the effects of platelet anti-aggregatory drugs.

Citrated human whole blood containing autologous, functional platelets labelled with ^{111}Indium (Hawker et al., 1980) was circulated over everted, de-endothelialised segments of rabbit aorta at 37°C in a Baumgartner chamber (Baumgartner, 1973). The flow rate was maintained at 160 ml/min for ten min and the rabbit aortic segments were then removed, washed free of blood and counted for radioactivity. The number of platelets adhering to the surface of each vessel segment was calculated from the radioactivity of the circulating blood, the deposited radioactivity and the area of vessel exposed to the blood (Cazenave et al., 1979). The effect of drugs was determined by comparing the numbers of platelets deposited after an initial circulation of blood in the absence of drug, with deposition occuring after a second circulation of the same blood, over different aortic segments, in the presence of drug.

The platelet deposition on rabbit aorta from untreated blood was 312 ± 14 (mean $\pm$ s.e.m.) x 10^3 platelets/mm^2 (n=107). Aspirin (10^{-6}M - 10^{-4}M), prostacyclin (10^{-10}M - 10^{-7}M) and AH23848 (2×10^{-9}M - 2×10^{-5}M) inhibited platelet deposition in a concentration related manner, producing maximum inhibitions of 70-75%. The order of inhibitory activity was prostacyclin > AH23848 >> aspirin, with equipotent concentration ratios of 1, 17, 3000 respectively comparing half-maximal inhibition values of 3×10^{-9}M, 5×10^{-8}M and 9×10^{-6}M.

This method has proved useful for studying the effects of anti-platelet drugs on the interaction of platelets in flowing blood with vascular surfaces, following experimental damage. We have used the technique to demonstrate that the inhibitory effect of AH23848 on platelet deposition upon rabbit vascular sub-endothelium, occurs at similar concentrations to those required to produce significant blockade of platelet thromboxane receptors (Humphrey & Lumley, 1984).

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THE INFLUENCE OF PROTEIN BINDING ON THE POTENCY OF THE PROSTANOID EP_1-RECEPTOR BLOCKING DRUG, AH6809

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AH6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid) is a selective prostanoid EP_1-receptor blocking drug *in vitro* (Coleman et al., 1985). However, in preliminary experiments in anaesthetized guinea-pigs, we have been unable to demonstrate any effect of AH6809 on PGE_2-induced gastric contractility (unpublished observations). One possible explanation for this lack of activity *in vivo* could be that AH6809 is protein bound; we have therefore examined the effect of bovine serum albumin (BSA) on the potency of AH6809 *in vitro*.

Prostanoid-induced contraction of guinea-pig isolated fundic strip (GPF) has been shown to be mediated by EP_1-receptors (Kennedy et al., 1982), and we have used this preparation to investigate the effect of 4% BSA on the antagonist potency of AH6809 against PGE_2.

Preparations of GPF were mounted in organ baths in modified Kreb's solution containing indomethacin (2.8×10^{-6}M), maintained at 37°C and gassed with 95% O_2 in CO_2 (Kennedy et al., 1982). Cumulative concentration-effect curves to PGE_2 were repeated until constant, and 4% defatted BSA (Chen, 1967) was then added to the bathing solution and a further concentration-effect curve to PGE_2 was obtained. The presence of BSA caused a small rightward shift of PGE_2 concentration-effect curves (concentration ratio = 2.9, 95% C.L. 1.4-5.8, n=4) while there was no shift of concurrent control PGE_2 curves (concentration ratio = 0.9, 95% C.L. 0.8-1.1, n=4). We next examined the effects of various concentrations of AH6809 (10^{-6}, 10^{-5} and 10^{-4}M) on PGE_2 concentration-effect curves in the absence and presence of BSA. The data obtained in the absence of BSA were used to determine the pA_2 for AH6809 by the method of Arunlakshana & Schild (1959), and a value of 6.7 (95% C.L. 6.5-7.2) with regression slope of 0.80 (95% C.L. 0.6-1.0) was obtained. This is similar to the pA_2 value of 6.6 reported previously (Coleman et al., 1985). In the presence of BSA, antagonism of responses to PGE_2 by AH6809 at all concentrations, was markedly reduced, resulting in a -1.6 (95% C.L. - 1.3 to -2.0) log unit parallel shift of the Schild regression and representing a 41-fold loss in potency of AH6809. This is consistent with approximately 98% binding of AH6809 to BSA.

In conclusion, these results suggest that AH6809 is extensively protein bound and may account for its lack of antagonist activity *in vivo*.

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THE USE OF THROMBOXANE RECEPTOR ANTAGONISTS TO DETERMINE THE INVOLVEMENT OF TXA_2 IN GUINEA-PIG ANAPHYLAXIS

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Dazoxiben, a thromboxane synthetase inhibitor, has been found to protect conscious, sensitised guinea-pigs from bronchoconstriction induced by aerosolised antigen (Goadby & Hardman, 1984). It has been reported however that dazoxiben, whilst inhibiting TxA_2 synthesis, redirects arachidonic acid metabolism to increase the production of bronchodilator substances such as prostaglandin E_2 (Defreyn et al, 1982). Thus the use of a thromboxane receptor antagonist, which does not interfere with enzyme activity, is preferable in investigating the role of TxA_2 in anaphylaxis. In this study two such agents, AH23848 and EP092, were compared with results obtained using dazoxiben.

Thromboxane receptor antagonist action was investigated in anaesthetised, unsensitised guinea-pigs using U46619 as the thromboxane receptor agonist. Bronchoconstriction was monitored using a modification of the method of Konzett and Rossler (1940). A reduction in U46619-induced bronchoconstriction (using a single dose of U46619 of 1.4 nmol. kg^{-1} per animal) was seen with EP092 (0.24 - 2.42 $\mu mol.kg^{-1}$) and AH23848 (0.02 - 0.24 $\mu mol.kg^{-1}$) but not with dazoxiben (1.9 - 18.6 $\mu mol.kg^{-1}$) indicating dazoxiben had no thromboxane receptor blocking action. AH23848 appeared approximately 20 times more potent than EP092 and both agents themselves produced a transient bronchoconstriction.

Male guinea-pigs sensitised to ovalbumin were used to determine the involvement of TxA_2 in anaphylaxis.

Guinea-pigs were exposed individually to an aerosol of 1% ovalbumin in a sealed chamber until the onset of dyspnoea and cough. All three agents prolonged the time to onset of symptoms. Dazoxiben (1.9 and 3.7 $\mu mol.kg^{-1}$) caused dose-related protection but the protection observed after 18.6 $\mu mol.kg^{-1}$ was minimal. The dose-response curve to dazoxiben would thus appear to be bell-shaped.

EP092 (2.4 $\mu mol.kg^{-1}$) produced a greater protection than at 12.1 $\mu mol.kg^{-1}$ but there was great individual variation between animals. AH 23848 (2.4 - 12.1 $\mu mol.kg^{-1}$) produced dose-related protection which was similar in its maximum effect to the degree of protection seen with EP092.

The findings of this study support the postulate that TxA_2 is a mediator of anaphylaxis in the guinea-pig but also reveal a possible difference in the effects of aerosol and intravenous antigen.

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5Z-CARBACYCLIN DISCRIMINATES BETWEEN PGI_2-RECEPTORS ON VASCULAR SMOOTH MUSCLE CELLS AND PLATELETS: AGONIST/ANTAGONIST PROPERTIES

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(5E)- and (5Z)-Carbacyclin are chemically stable analogues of prostacyclin (PGI_2), which mimic PGI_2 actions. In particular, they inhibit platelet aggregation and relax vascular smooth muscle, although less efficiently than PGI_2 itself (Whittle et al., 1980). Both the antiaggregating and the vasodilating activities of PGI_2 and other stable analogues are mediated through activation of adenylate cyclase (AC). Therefore, we investigated the characteristics of AC activity modulation by the two isomeric carbacyclins in membranes from human platelets and cultured myocytes from rabbit mesenteric artery.

PGE_1 has been used as a reference compound since we have already demonstrated that this stable prostaglandin acts at platelet and vascular levels via the same mechanism of action (i.e. AC stimulation), and possibly through the same receptors, of PGI_2 (Lombroso et al., 1984; Oliva et al., 1984).

In human platelets, both (5E)- and (5Z)-Carbacyclin stimulated AC activity in a dose dependent fashion to the same extent of PGE_1 (6.5-fold), the order of potency being (5E)-carbacyclin $>$ PGE_1 $>$ (5Z)-carbacyclin ($EC_{50} \simeq 4.5 \times 10^{-7}$, 8×10^{-7}, 2.5×10^{-6} M, respectively).

Both carbacyclins stimulated AC activity in myocytes from rabbit mesenteric artery, as well. However, while (5E)-carbacyclin displayed the same efficacy (3.5-fold stimulation over basal) and approximately the same potency as PGE_1 ($EC_{50} = 8 \times 10^{-6}$ M), the maximal activation elicited by (5Z)-carbacyclin was approximately 50% of that obtained with the other prostaglandins with an EC_{50} of 10^{-4} M. (5Z)-carbacyclin displayed also antagonistic properties, in that it decreased the stimulation of AC elicited by PGE_1.

The stimulating effect on AC correlated well with the functional response of these prostaglandins: in fact, (5Z)-carbacyclin inhibited platelet aggregation 100%, while it did not reach the maximal relaxation of rabbit mesenteric artery attained with PGI_2.

In conclusion, (5Z)-carbacyclin is a full agonist at platelet level, but it is a partial agonist on vascular smooth muscle cells. Therefore, this prostaglandin appears to discriminate between PGI_2 receptors in the two target cells.

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EFFECTS OF OXYTOCIC DRUGS ON PROSTAGLANDIN SYNTHESIS BY HUMAN PREGNANT MYOMETRIUM

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The release of prostacyclin (PGI_2) by human pregnant myometrium (HPM) increases throughout pregnancy reaching a peak at the time of parturition (Seed et al, 1983). However the synthesis is much lower than that seen in rat pregnant myometrium (El Tahir & Williams 1980) indicating PGI_2 may not be the chief arachidonic acid (AA) metabolite of HPM. The present experiments were undertaken to establish the profile of prostaglandin (PG) production by HPM and to assess the influence of a variety of factors on this synthesis.

HPM was obtained at elective caesarian section (37-39 weeks) and suspended in Tris-buffered saline (50% w/v, pH 8.0). Samples were pre-incubated with drugs for 10 mins at 37°C, cooled, chopped and incubated for 30 mins at 20°C (Seed et al, 1983). Incubation media were then subjected to organic solvent extraction, chromatography, derivatisation and quantitative analysis of PGs was performed by stable isotope dilution GC/MS (Black et al, 1982).

Table 1. Effects of arachidonic acid (AA_1), phospholipase A_2 (PLA_2), ergometrine (ERG), and oxytocin (OX) on HPM prostaglandin formation.

Treatment	n	PGE_2	PGD_2	6oxo-$PGF_{1\alpha}$	$PGF_{2\alpha}$
control	4	514+/-66	242+/-35	271+/-80	343+/-64
AA (30uM)	4	1693+/-231 *	1374+/-541 *	1171+/-124 *	2026+/-166 * X
control	4	396+/-83	217+/-52	298+/-69	340+/-66
PLA_2 (1U/ml)	4	1344+/-136 *	1235+/-299 *	1178+/-120 *	1211+/-188 *
control	4	303+/-97	326+/-53	456+/-136	317+/-91
ERG (6.5uM)	4	888+/-218 *	477+/-165	566+/-207	837+/-122 *
control	7	420+/-91	367+/-80	349+/-77	313+/-75
OX (20mU/ml)	7	1306+/-233 *	1059+/-176 *	1004+/-159 *	1837+/-331 * X

*p<0.01 ANOVA (vs control), Xp<0.01 Mann Whitney U test (% total PGs vs control)

Table 1 shows that AA and PLA_2 significantly increased production of all PGs but that only the proportion of $PGF_{2\alpha}$ as % of total is significantly altered by AA. ERG significantly increased production of PGE_2 and $PGF_{2\alpha}$ but did not elevate PGD_2 or 6oxo-$PGF_{1\alpha}$ formation whereas OX increased synthesis of all products, particularly $PGF_{2\alpha}$.

As term approaches it appears myometrial capability to synthesise PGs is limited by precursor availability which is low due to weak PLA_2 activity. Interestingly ERG which produces uterine contracture only stimulates production of PGE_2 and PGF_2 whereas oxytocin which induces phasic contractions also elevates PGI_2 (assayed as 6oxo-$PGF_{1\alpha}$) which relaxes HPM (Omini et al, 1979).

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HIGHLY SPECIFIC ACTION OF THE PROSTACYCLIN MIMETIC ZK 96480 ON SMOOTH MUSCLE PREPARATIONS

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At present the characterization of PGI_2 receptors relies heavily on the ranking of agonist potency. A problem that we have encountered is the PGE-like stimulant activity of certain stable PGI_2 analogues. For example iloprost (Schrör et al, 1981; Casals-Stenzel et al, 1983) contracts the bullock iris sphincter and behaves as a partial agonist at the PGE receptor present in the preparation (Dong & Jones, 1982). We have also found that iloprost contracts the rat stomach strip and guinea-pig trachea. Threshold effects were obtained with doses of iloprost 10-15 times larger than those of 16,16-dimethyl PGE_2. Again a partial agonist action on the PGE-sensitive contractile system was found (Table 1).

We have recently examined two other potent PGI_2 mimetics, 6a-carba-$\Delta^{6,6a}$ PGI_1 (Ogawa & Shibasaki, 1984) and ZK 96480 {[1S,5S,6R,7R]-3-(5-carboxy-3-oxa-1E-pentylideno)-7-hydroxy-6-(3S-hydroxy-4S-methyl-nona-1,6-diynyl)-bicyclo[3.3.0]octane}. The former shows considerable PGE-like activity, whereas the latter does not (Table 1).

Table 1 Equipotent molar ratios on isolated smooth muscle preparations

Prostanoid	Bullock iris	rat stomach strip*	guinea-pig trachea*	rat colon
16,16-dimethyl PGE_2	1.0	1.0	1.0	1.0
PGE_2	2.3	3.7	↑↓	7.1
PGE_1	7.0	8.5	↑↓	↑↓ inhibition
PGI_2	~300	~350	~250	~6.0
6a-carba-$\Delta^{6,6a}$ PGI_1		31	66	18
iloprost	p.a.(33-75%)	p.a.(36-96%)	p.a.(66-83%)	5.7
ZK 96480	>4500	1950	>1000	1.0

Each value is the mean of at least 4 determinations. * Thromboxane antagonist EP 045 (2.5 μM) present. ↑↓ both contractile and relaxant actions were seen.

ZK 96480 is however the most potent inhibitor of the spontaneous rhythmic activity of the isolated rat colon. The proximal 3 cm of the ascending colon is reproducibly sensitive to ZK 96480; IC_{50} = 1.5 - 2.5 nM. PGI_2, iloprost and 6a-carba-$\Delta^{6,6a}$ PGI_1 also completely suppress activity. EPMR are given in Table 1. PGE_1 (20 - 100 nM) showed variable effects - contraction or slight inhibition. PGE_2 and 16,16-dimethyl PGE_2 always produced contraction. The distal ridged portion of the ascending colon is also sensitive to inhibition by prostacyclin analogues. However it is much more responsive to the contractile action of PGE_2. ZK 96480 always produced inhibition of spontaneous activity (IC_{50} = 1.5 - 5 nM). Iloprost and 6a-carba-$\Delta^{6,6a}$ PGI_1 showed contractile activity on those preparations most sensitive to PGE_2 and relaxation on those least sensitive to PGE_2.

These results demonstrate the highly selective action of ZK 96480.

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ICI 159995; A NOVEL THROMBOXANE A_2 RECEPTOR ANTAGONIST

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Thromboxane A_2 (TxA_2) an unstable metabolite of arachidonic acid causes platelet aggregation and contraction of vascular and pulmonary smooth muscle in vitro (Svensson et al, 1976; Svensson et al, 1977). In the present study we utilised the stable TxA_2 agonist U-46619 in order to determine the nature of the antagonism exhibited by the novel agent ICI 159995 (5(z)-7-[2,2-dimethyl-4-phenyl-1,3-dioxan-cis-5-yl]heptenoic acid).

ICI 159995 inhibited U-46619-induced aggregation of human and rabbit platelets in vitro in a dose dependent manner, yielding K_B values (± s.e. of mean) of $3.2 \pm 0.2 \times 10^{-6}$M and $1.3 \pm 0.1 \times 10^{-5}$M respectively. However the platelet inhibitory activity of prostacyclin was not modified by this agent.

ICI 159995 competitively antagonised the contractile effects of U-46619 on both rat (pA_2 6.84±0.03) and rabbit (pA_2 6.24±0.05) aorta in vitro, but did not modify contractions to noradrenaline in either preparation. U-46619-induced contractions of guinea-pig trachea and parenchyma in vitro were antagonised by ICI 159995 in a dose dependent manner (pA_2 6.58±0.04 and pA_2 5.58±0.26 respectively). In both of the above pulmonary preparations the response to histamine was unaffected by ICI 159995. Contractions of guinea-pig ileum induced by PGE_2, 5-hydroxytryptamine, acetylcholine and histamine were all unaffected by ICI 159995. Similarly ICI 159995 did not antagonise acetylcholine or $PGF_{2\alpha}$-induced contractions of rat colon. These results indicate that ICI 159995 is a specific TxA_2 antagonist which acts at platelet, pulmonary and vascular receptors.

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INHIBITION OF INDOMETHACIN-INDUCED GASTRIC EROSIONS IN THE MOUSE BY INTRACEREBROVENTRICULARLY ADMINISTERED SALMON CALCITONIN

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We have previously demonstrated a potent action of the hormone calcitonin to inhibit the development of indomethacin-induced gastric erosions in the rat and mouse (Bates, Buckley & Strettle, 1979a,b). This action would appear to be mediated by an unknown mechanism which is independent of calcitonin's action to inhibit gastric acid and pepsin secretion (Bates, Buckley & Strettle, 1981). One feasible explanation of this action of calcitonin is an effect via the central nervous system (CNS) to alter either hormonal and/or autonomic nervous system outflow. Calcitonin has a variety of CNS actions including effects on behaviour and pain, and has also been shown to reduce the severity of cold-restraint induced gastric ulceration (Morley, Levine & Silvis, 1981). In this paper the possibility of a CNS component in the action of calcitonin to inhibit indomethacin-induced gastric erosion development was investigated.

Gastric erosions were induced in starved (24 h) CFLP mice (20-26g) of either sex over a 5h period by the administration of indomethacin (40 mg kg^{-1}, i.p.) as previously described by Bates et al (1979a). An erosion was counted as being an area of damage greater than 0.5 mm in any cross-sectional direction when viewed perpendicular to the mucosal surface. Salmon calcitonin was administered by either the intracerebroventricular (i.c.v.), according to the method of Cashin & Heading (1968), or the subcutaneous route in an appropriate vehicle.

Following administration of calcitonin by either route an inhibition of erosion development was observed; doses of 0.1, 1, 10 and 100 MRC U kg^{-1} calcitonin produced 24±7, 24±12, 29±11 and 58±19% inhibitions respectively following i.c.v. administration and 5±6, 20±5, 51±5 and 77±7% inhibitionsrespectively following subcutaneous administration (Mean ±s.e. of mean, n = 10). All of these inhibitions apart from the 0.1 MRC U kg^{-1} s.c. dose of calcitonin, were statistically significantly different ($p<0.05$) from control experiments (Mann-Whitney U test).

A dose related fall in plasma calcium concentrations was observed following s.c. administration of calcitonin with significant effects occurring at doses of 10 and 100 MRC U kg^{-1}; however following i.c.v. administration a significant fall in calcium concentrations was only observed at a dose of 10 MRC U kg^{-1}.

Thus at a low dose of calcitonin (0.1 MRC U kg^{-1}) a significantly greater effect ($p<0.05$) was observed to inhibit gastric erosions following i.c.v. administration when compared to s.c. administration; whereas at high doses of calcitonin (10 and 100 MRC U kg^{-1}) s.c. administration gave significantly greater effects ($p<0.05$) than i.c.v. administration. This evidence suggests that following i.c.v. administration of calcitonin at least part of the anti-gastric erosive action of calcitonin must be mediated via the central nervous system.

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CHANGES IN LYSOPHOSPHOLIPIDS AND PHOSPHOLIPASE A_2 ACTIVITY DURING ISCHAEMIA IN THE ISOLATED RAT HEART

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Lysophospholipids have been implicated in the development of ischaemically induced arrhythmias (Shaikh and Downar, 1981, Corr et al., 1982). However, the time course of the accumulation of these amphiphiles during early ischaemia has not been studied. Langendorff perfused rat hearts were used to study the effect of coronary artery ligation (CAL) on lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE) concentrations within the tissue. LPC and LPE were extracted using the method of Shaikh and Downar (1981), separated by HPLC and quantified by phosphate assay. Samples of ischaemic and non ischaemic tissue were taken for analysis 0-30 min following CAL. Sham ligated controls at 15 and 30 min were also carried out. Arrhythmias typically commenced 12 min after ligation and persisted for 10 min before reverting to normal rhythm.

LPC concentration increased 5 min after CAL. The changes in LPE concentration were not significant (Table 1). Control levels of LPC and LPE at 15 and 30 min were not significantly different from controls at zero time. Phosphatidylcholine (PC) and phosphatidyl ethanolamine (PE) levels did not change. Increasing perfusate Ca^{2+} concentration and/or decreasing K^+ concentration, both of which enhance the severity of ischaemically induced arrhythmias caused an elevation of LPC levels. In control hearts at zero time LPC rose from 1.51±0.14 nmol/mg protein with 1.2mM Ca^{2+} plus 5.9mMK^+ to 3.50±0.28 nmol/mg protein with 2.5mM Ca^{2+} plus 2.5mMK^+.

LPC could increase due to an increase in phospholipase A_2 (PLA_2) activity or to a decrease in LPC catabolism. The pH profile of PLA_2 activity was measured in ischaemic and non-ischaemic tissue after 5 or 20 min of CAL using a method based on that of Franson et al. (1978). Five minutes after CAL PLA_2 activity was unchanged in the ischaemic area whereas after 20 min CAL PLA_2 activity in the ischaemic area was signficantly reduced. This suggests LPC catabolism is depressed in ischaemic tissue.

Table 1

n=12	TIME AFTER CAL (MIN)			MEAN± SE n mol/mg protein		
0	5	10	15	20	25	30
LPC 1.51 ±0.14	2.49[a] ±0.36	2.35 ±0.43	2.77[a] ±0.44	3.01[a] ±0.65	1.69 ±0.31	3.19[a] ±0.56
LPE 2.03 ±0.27	2.03 ±0.18	1.99 ±0.58	2.53 ±0.38	3.21 ±0.53	2.55 ±0.56	2.39 ±0.42

a = $P \leq 0.05$ vs time 0 T-Test.

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FLUFENAMIC ACID INHIBITS PROSTAGLANDIN $F_{2\alpha}$, E_2 AND D_2 BIOSYNTHESIS IN GUINEA-PIG LUNG

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Flufenamic acid, a non-steroidal anti-inflammatory drug of the fenamate class, has recently been shown to be a weak inhibitor of soyabean 15-lipoxygenase activity (Davis et al., 1984) and to inhibit 5-lipoxygenase activity associated with either intact rat neutrophils or broken basophil leukaemia (RBL-1) cells. In homogenates prepared from the RBL-1 cells, flufenamic acid was found to be equipotent with respect to its ability to inhibit both 5-lipoxygenase and cyclo-oxygenase activities. The ability of flufenamic acid to inhibit both oxidative pathways associated with arachidonic acid metabolism enables the fenamate to inhibit antigen-induced bronchoconstriction (Sturton et al., 1984). The present study was designed to investigate the effect of flufenamic acid on cyclo-oxygenase activity.

Lung microsomes were prepared from male Duncan-Hartley guinea-pigs, based on the method of Remmer et al. (1967). Cyclo-oxygenase activity was determined by measuring the formation of prostaglandins (PG) E_2, D_2 and $F_{2\alpha}$ from ^{14}C-arachidonic acid, according to the method of Blackwell et al. (1975). Reaction mixtures contained lung microsomes (3-5mg protein), ^{14}C-arachidonic acid (500nM), 2mM adrenaline, 2.5mM reduced glutathione and 100mM Tris-HCl buffer, pH 8.2. When present, flufenamic acid (1-10μM) was added before the substrate. PGs produced during the 15min incubation at 37°C were measured by liquid scintillation spectrometry using a Beckman LS-7500 scintillation counter, following extraction in ethyl acetate and identification by silica gel thin-layer chromatography.

In the absence of flufenamic acid, microsomal fractions from guinea-pig lung tissue converted ^{14}C-arachidonic acid to prostaglandins $F_{2\alpha}$, E_2 and D_2 (Table 1) in the ratio of 4.6 : 2.1 : 1, and a number of unidentified products.

Table 1 $PGF_{2\alpha}$, PGE_2 and PGD_2 Formation by Microsomal Fractions of Guinea-Pig Lung.

Flufenamic Acid (μM)	Prostaglandin Formation (pmol produced min^{-1} mg of $protein^{-1}$)		
	$F_{2\alpha}$	E_2	D_2
Control	1.88 ± 0.30	0.85 ± 0.10	0.41 ± 0.10
1.0	1.33 ± 0.12	0.65 ± 0.05	0.25 ± 0.04
2.5	0.43 ± 0.05	0.43 ± 0.02	0.03 ± 0.01
5.0	0.06 ± 0.01	0.36 ± 0.01	0.01 ± 0.003
10.0	-	0.06 ± 0.01	-

Results are the means of five different experiments ± s.e.m.

In the presence of flufenamic acid (1-10μM), a concentration-dependent inhibition of all three prostaglandins was observed. In terms of IC_{50} values (concentration required to reduce the maximum response by 50%), flufenamic acid was found to be equipotent in preventing the formation of $PGF_{2\alpha}$ (IC_{50} = 1.4 ± 0.2μM, n=5) and PGD_2 (IC_{50} = 1.3 ± 0.1μM, n=5), while PGE_2 formation was found to be significantly less sensitive to the anti-inflammatory agent (IC_{50} = 2.8 ± 0.5μM, n=5, $p<0.05$). The results presented show flufenamic acid to be a potent inhibitor of prostaglandin biosynthesis in lung tissue.

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THE EFFECT OF HEAVY METALS ON THE BIOSYNTHESIS OF PROSTAGLANDINS D_2, E_2 AND $F_{2\alpha}$ IN RABBIT KIDNEY

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Prostaglandin (PG) biosynthesis by prostaglandin synthetase (E.C. 1.14, 99.1.) is known to occur in many tissues including mammalian kidney, the major products being PGE_2, $PGF_2\alpha$ and PGD_2 (Tai, etal. 1976). The PGs thus formed have been shown to exert a variety of actions associated with renal function, including natriuresis, increased glomerular filtration and inhibition of tubular electrolyte reabsorption. Environmental exposure to cadmium and lead rapidly leads to significant accumulation of these metals in renal tissues (Webb, 1975). Thus, exposure of the prostaglandin synthetase complex these metals may well result in either a disruption of PG biosynthesis, or a relative imbalance of PGs in the kidney, leading to the production of the toxic effects seen following exposure to these ions.

Renal microsomes were prepared from male New Zealand white rabbits based on the method of Remmer et al. (1967). PG synthesis was measured using a reaction mixture that contained 2.5mM reduced glutathione, 2mM adrenaline, rabbit kidney microsomes (3.6 to 4.4 mg of protein) and 100mM Tris-HCl buffer, pH 7.6 to give a find volume of 0.5ml. The reaction was initiated by the addition of 0.6nmol ^{14}C-arachidonic acid. Following a 15 min incubation at 37°C the reaction was stopped by the addition of 25 µl 1M HCl. The PGs formed were extracted into 1.5ml ethyl acetate, evaporated to dryness and the residue dissolved in 25 µl 100% ethanol. The ethanolic solutions were spotted onto silica gel thin layer chromatography plates and developed using the "iso-octane" solvent system of Blackwell et al (1975), Following visualisation by exposure to iodine vapour, the PGs were transferred to glass scintillation vials and the activity determined by liquid scintillation spectrometry using a Beckman LS-7600 Liquid Scintillation Counter.

Rabbit renal microsomes converted ^{14}C-arachidonic acid into a mixture of prostaglandin products, namely, $PGF_2\alpha$, PGE_2 and PGD_2. Lead (0.01 to 1.5 µM), produced a concentration-dependent inhibition of PG synthesis. The production of PGD_2 and PGE_2 was more sensitive to lead. (IC_{50} = 0.05 ± 0,004 µM and 0.08 ± 0.01 µM respectively, n = 4), than that of $PGF_2\alpha$ (IC_{50} = 0.28 ± 0.03 µM, n = 4). Cadmium (0.001 to 1.5 µM) produced a biphasic response on PG synthesis. Low concentrations (< 0.01 µM) produced a significant ($p < 0.05$) stimulation of PGE_2 synthesis from 0.27 ± 0.02 to 0.43 ± 0.03 pmol min^{-1} $mg\ of\ protein^{-1}$ (n = 4). At higher concentrations cadmium (0.01 to 1.5 µM) produced a concentration-dependent inhibition of synthesis of the three PGs tested. As with lead, the synthesis of PGD_2 and PGE_2 was more sensitive (IC_{50} = 0.67 ± 0.05 µM and 0.78 ± 0.06 µM respectively, n = 4) than that of $PGF_2\alpha$ (IC_{50} = 1.13 ± 0.07 µM, n = 4). The results presented here indicate that the production of PGD_2 and PGE_2 in kidney are particularly sensitive to the divalent cations lead and cadmium. This may have serious toxicological consequences in the kidney and other tissues.

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FIBRONOLYTIC EFFECT OF PROSTANOIDS IN THE RAT

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Although the action of prostanoids on platelet aggregation and vascular reactivity has been exhaustively studied in the last few years their effect in other aspects of haemostasis and thrombosis and particularly their role in fibrinolysis has received only scant attention. Recently, prostacyclin (PGI_2) and its enzymatic metabolite 6 oxo PGE_1 have been reported to enhance blood fibrinolytic activity following intravenous injection to rabbits (Korbut et al., 1983). In addition, administration of aspirin to human volunteers inhibits the rise in fibrinolytic activity which follows venous occlusion (Levin et al., 1984) suggesting that endogenous prostanoids may play a part in the control of fibrinolysis in vivo. We describe here experiments to determine the effect of prostanoids on fibrinolysis in the anaesthetised rat.

Male rats (Sprague-Dawley, 250-290g) were anaesthetised with urethane (1g kg^{-1}). Routinely, the carotid artery was cannulated for drug injection and blood collection but in some experiments drugs were injected intravenously via a cannula inserted into the femoral vein. After administration of heparin (100U kg^{-1}) 0.6 ml blood was withdrawn at T=0 and replaced with an equal volume of warm saline. 15 min later prostanoid (or an equal volume of saline) was injected intraarterially and further blood samples (0.6ml) were collected 15, 30 and 60 min thereafter. Fibrinolytic activity was determined using the euglobulin clot lysis time procedure (ECLT) as described by Samama et al., 1975).

The ECLT in control, saline-injected rats was 176.9±13 min (n=6). Intraarterial injection of PGI_2 (ECLT = 107.2±6.4 min, n=6, T=60) and 6 oxo PGE_1 (ECLT= 92±3.4 min, n=6, t=60) enhanced fibrinolysis as shown by a reduction in ECLT. The reduction in ECLT was both dose-dependent (over the range 0.5-5.0 ug kg^{-1}) and time-dependent (significantly reduced 15 min after injection, maximal effect after 60 min). No change in ECLT was observed in animals injected with LtC_4 (163.6 ±3 min, n=6) or in saline-injected controls (166.3±3.2 min, n=6). Neither PGI_2 nor 6 oxo PGE_1 affected the rate of dissolution of pre-formed euglobulin clots prepared from the blood of saline-injected rats.

In order to determine the role of endogenous prostanoids in fibrinolysis, animals were anaesthetised and blood samples collected from the carotid artery before and 30 min after intravenous injection of the cyclooxygenase inhibitor, indomethacin (3 mg kg^{-1}) or the thromboxane synthetase inhibitor, dazoxiben (3mg kg^{-1}). The ECLT was significantly prolonged in indomethacin-treated rats (from 168.7±8.3 min to 198.5±5.4 min, n=6, $P < 0.05$) but significantly reduced in animals injected with dazoxiben (from 162.5±6.8 min to 110.7±4.8min, n=6, $P < 0.001$). Furthermore, the fibrinolytic activity of intravenously administered carbachol (ECLT= 74.2±2.4 min, n=6, T=30 min, c.f. 152.5±4.9 min, n=6 at T=0, $P < 0.001$) was significantly reduced following intravenous indomethacin administration (ECLT= 130±5.8 min, n=6, T=30 min c.f. 155.0±5.6 min, n=6 at T=0).

These results are consistent with the hypothesis that PGI_2 and/or 6 oxo PGE_1 have a role to play in the physiological control of clot dissolution in the rat. The mechanism of this effect is not clear but may be related to increased release of plasminogen activator from vascular endothelial cells.

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PLATELET-ACTIVATING FACTOR INDUCES THE RELEASE OF LEUKOTRIENE-LIKE MATERIAL FROM RAT ISOLATED, PERFUSED HEARTS

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Platelet-activating factor (PAF), a putative mediator of inflammation, exerts both platelet-dependent (Lefer et al, 1984) and platelet-independent actions (Voelkel et al, 1982) in the cardiovascular system. A role for PAF in cardiac anaphylaxis has recently been proposed on the basis of its release from isolated perfused guinea-pig hearts and its ability to mimic cardiac anaphylaxis when exogenously administered (Levi et al, 1984). Although in vivo studies in the dog (Kenzora et al, 1984) and the pig (Feuerstein et al, 1984) provide indirect evidence that leukotrienes (LTs) may be partly responsible for PAF-induced cardiac dysfunction, the mechanism of the platelet-independent cardiac effects of PAF remains to be elucidated. The present study investigates the involvement of LTs in the cardiac effects of PAF in vitro.

Hearts from male Wistar rats (300-350g) were perfused via the aorta using Kreb's solution (37°C, gassed with 5% CO_2 in O_2) at a constant flow rate (8 ml/min) for 45 min before administration of a bolus dose of PAF (0.036 - 4.5 nmol). Recordings of coronary perfusion pressure, cardiac contractility and rate were made immediately before and up to 30 min after administration of PAF. The heart perfusate superfused 3 strips of longitudinal smooth muscle from guinea-pig ileum (GPISM) in series to detect the release of LT-like materials. The last GPISM was continuously superfused with FPL 55712 (1.9 μM) to increase the specificity of the bioassay. Tissues were calibrated with exogenous LTD_4 (0.5 - 20 ng).

PAF (0.036 - 4.5 nmol) induced dose-related increases in coronary perfusion pressure of 20 - 60% and decreases in cardiac contractility of 11 - 27%, whereas heart rate was unaltered. Accompanying the cardiac dysfunction induced by PAF was a dose-related release of LT-like material into the perfusate (1.4 - 15.2 ng/g heart wet weight LTD_4 equivalents). The appearance of LT-like bioactivity in the heart perfusate followed a time-course similar to that of the increase in coronary perfusion pressure reaching a maximum between 2 and 5 min post-PAF which subsided between 20 and 30 min post-PAF. Administration of a second, identical dose of PAF resulted in no further cardiac dysfunction nor was there any release of LT-like bioactivity. The increase in coronary perfusion pressure induced by PAF (0.91 nmol) was inhibited by 86% in hearts pretreated with FPL 55712 (1.9 μM) for 15 min before PAF was injected.

In conclusion, the present findings are consistent with the suggestion that the cardiac effects of PAF are related to the release of lipoxygenase products.

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STIMULATION OF GASTRIC NON-PARIETAL SECRETION BY PROSTAGLANDIN $F_{2\alpha}$ IN THE RAT AND CAT

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The effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on gastric non-parietal secretion has been examined in anaesthetised preparations of the rat and cat, and a comparison made with the activity of 16,16-dimethyl prostaglandin E_2 ($dmPGE_2$).

Rats were anaesthetised with pentobarbitone (50mg/kg i.p.) and treated with atropine (3μmol/kg) to inhibit gastric acid secretion and with indomethacin (14μmol/kg s.c.) to inhibit endogenous prostaglandin formation. The prostaglandins were applied intragastrically and secretion collected over a 1h period. Cats were anaesthetised with chloralose (80mg/kg i.v.) and treated with the H_2-receptor antagonist loxtidine (3μmol/kg i.v.) to inhibit gastric acid secretion and with indomethacin (10μmol/kg i.v.). Prostaglandins were administered either by close-arterial infusion to the stomach or by intragastric instillation. Gastric secretion was collected every 30min and the dose of prostaglandin was increased threefold during each successive 30min period. Since Na^+ is a major constituent of non-parietal juice, a minor constituent of parietal juice and a non-labile ionic species this was used as the index of non-parietal secretion in both animals. Results are expressed as mean ± s.e. mean, n = 4-7 throughout.

In the rat under control conditions, Na^+ output was 27.4 ± 6.1 μEq/h; $PGF_{2\alpha}$ at doses of 0.1, 0.3, 1 and 3μmol/kg increased Na^+ output to 42.0 ± 9.2, 64.4 ± 7.1, 91.2 ± 4.3 and 125.3 ± 24.8μEq/h respectively. For comparison, $dmPGE_2$ at doses of 0.01, 0.03 and 0.1μmol/kg increased Na^+ output to 48.8 ± 8.0, 65.7 ± 5.4 and 130.0 ± 10.2 μEq/h respectively.

The results obtained in the cat are shown in Table 1.

Table 1. Stimulation of gastric non-parietal secretion by $PGF_{2\alpha}$ and $dmPGE_2$ in the anaesthetised cat

CLOSE-ARTERIAL INFUSION			INTRAGASTRIC INSTILLATION		
Dose (nmol/kg/min)	Na^+ output (μEq/30min) $PGF_{2\alpha}$	$dmPGE_2$	Dose (nmol/kg)	Na^+ output (μEq/30min) $PGF_{2\alpha}$	$dmPGE_2$
0	134.2± 9.9	121.5±22.0	0	127.3±20.6	147.3±10.4
0.01	144.0±15.3	176.1±39.3	3	-	160.4±10.6
0.03	155.0±17.4	241.5±50.7	10	-	221.0±27.5
0.1	173.4±16.1	320.4±66.7	30	132.8±16.4	307.4±28.4
0.3	250.6±31.9	383.1±78.0	100	168.7±31.3	371.8±36.7
1	296.3±29.5	456.7±91.4	300	164.9±25.5	-
3	403.5±75.5	-	1000	250.5±23.7	-
10	428.1±66.2	-	3000	267.2±16.3	-

These results confirm the observation that $dmPGE_2$ stimulates gastric non-parietal secretion in the anaesthetised rat (Tao & Wilson, 1984) and also show that $PGF_{2\alpha}$ is active in this test although weaker than $dmPGE_2$. In addition the present study shows that $PGF_{2\alpha}$ and $dmPGE_2$ stimulate non-parietal secretion in the cat by both the parenteral and intragastric routes and that again $PGF_{2\alpha}$ is less active than $dmPGE_2$.

Tao, P. & Wilson, D.E. (1984). Prostaglandins 28, 353-365.