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Effects of indomethacin, piroxicam and selected prostanoids on gastric acid secretion by the rat isolated gastric mucosa

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1 The effects of the cyclo-oxygenase inhibitors indomethacin and piroxicam have been investigated on histamine- and dibutyryl cyclic AMP-induced acid secretion in the rat isolated gastric mucosa. The relative potencies of a number of prostanoids as inhibitors of histamine-induced acid secretion were determined in an attempt to classify the prostaglandin receptor mediating this response.

2 Indomethacin (8×10^{-9}–2.7×10^{-6} M) and piroxicam (3×10^{-6} M) potentiated the secretory responses elicited by histamine. This effect might be due to inhibition of the biosynthesis of antisecretory prostanoids. Indomethacin (2.7×10^{-6} M) and piroxicam (3×10^{-6} M) also potentiated the secretory response to dibutyryl cyclic AMP, but since prostaglandin E_2 (PGE_2, 10^{-5} M) did not inhibit this secretory response, the mechanism of the potentiation may differ from that of histamine.

3 The potency of the thromboxane mimetic U-46619 as an inhibitor of histamine-induced acid secretion was markedly reduced in the presence of indomethacin, suggesting that U-46619 may release endogenous antisecretory prostanoids.

4 In the presence of indomethacin (2.7×10^{-6} M) all the prostanoids tested produced concentration-related inhibitions of histamine-induced gastric acid secretion. PGE-analogues were the most potent compounds, the rank order of potency being 16,16 dimethyl $PGE_2 > PGE_2 \geq PGF_{2\alpha} > U\text{-}46619 \geq PGD_2 > PGI_2$. This order of potency is very similar to that obtained in smooth muscle preparations containing 'EP' receptors, suggesting that this receptor type also mediates inhibition of histamine-induced acid secretion in the rat.

Introduction

It is well established that prostaglandins, particularly of the E series, are potent inhibitors of gastric acid secretion in many species including rat (Frame & Main, 1980; Boughton-Smith & Whittle, 1981), dog (Robert *et al.*, 1967) and man (Karim *et al.*, 1973; Konturek *et al.*, 1976). Although the inhibition of acid secretion in the rat is well documented no attempt has been made to classify the prostaglandin receptor mediating this response. A system for the characterization of prostanoid receptors has been proposed by Kennedy *et al.* (1982), who compared the rank order of agonist potency of the naturally occurring prostaglandins, prostaglandin D_2 (PGD_2), PGE_2, $PGF_{2\alpha}$ and PGI_2 as well as the thromboxane A_2 mimetic, U-46619, on a variety of isolated smooth muscle preparations and suggested that distinct receptor types (DP, EP, FP, IP and TP respectively) existed for each of these compounds.

The rat stomach is capable of producing prostaglandins, notably PGE_2 and PGI_2 (Coceani *et al.*, 1967; Singh 1980; Boughton-Smith & Whittle, 1983), but the possible role of these endogenous substances remains unclear. Indomethacin, an inhibitor of prostaglandin synthesis has been found to potentiate acid secretion induced by pentagastrin in the anaesthetized rat and this might indicate an inhibitory role of prostaglandins in the rat stomach. However, in other studies indomethacin had no effect on basal, pentagastrin or histamine-stimulated acid secretion but resulted in a potentiation of dibutyryl adenosine 3′:5′-cyclic monophosphate (db cyclic AMP)-induced secretion *in vitro* (Main & Pearce, 1978; Frame & Main, 1980; Donaldson & Main, 1981). This was surprising since prostaglandins lower intracellular cyclic AMP levels in parietal cells (Major & Scholes, 1978; Soll, 1980) but do not inhibit gastric acid secretion induced by exogenous db cyclic AMP (Main & Pearce, 1978).

The aims of the present study in the rat isolated

gastric mucosal preparation were two fold: firstly to determine the effects of two cyclo-oxygenase inhibitors, indomethacin and piroxicam, on histamine- and db cyclic AMP-induced acid secretion; secondly to determine the antisecretory potencies of a number of prostanoids. A comparison could then be made between the rank order of potency obtained and that reported for these prostanoids on other preparations (Kennedy *et al.*, 1982) in an attempt to identify the prostanoid receptor mediating the inhibition of gastric acid secretion.

A preliminary account of this work has been presented to the British Pharmacological Society (Bunce *et al.*, 1983).

Methods

All experiments were carried out using a rat isolated gastric mucosal preparation similar to that of Main & Pearce (1978).

Female Wistar rats weighing between 70 and 110 g were anaesthetized with sodium pentobarbitone, 60 mg kg^{-1} i.p., the stomach exposed and the muscle separated from the mucosa by injecting saline between the two layers. After removing the muscle layers the mucosa was tied over a small perfusion chamber (Figure 1) which was then immersed in a 20 ml organ bath containing Krebs-Henseleit solution at 37°C (serosal solution) gassed with 95% O_2/5% CO_2. The mucosal surface of the tissue was continuously perfused at 0.5 ml min^{-1} with an unbuffered Krebs solution (mucosal solution) which had been gassed with 100% O_2. The ionic composition (mM) of the solutions were: serosal, NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.7, $MgSO_4$ 0.6, KH_2PO_4 1.2, $CaCl_2$ 1.3 and glucose 11.1; mucosal, NaCl 144.7, KCl 4.7, $MgSO_4$ 0.6, $CaCl_2$ 1.3 and glucose 11.1. The pH of the effluent perfusate was continuously monitored and acid output measured as nmol H^+ min^{-1} cm^{-2}.

The effect of indomethacin or piroxicam on the acid secretory responses to histamine or db cyclic AMP

Sequential secretory concentration-response curves were constructed to histamine on mucosae bathed in serosal solution containing indomethacin (0 to 2.7 μM) or piroxicam (3.0 μM). Because the secretory responses to histamine were slow in onset and offset only 5 concentrations of histamine were tested on each preparation. In the presence of the highest concentration of indomethacin (2.7 μM), histamine was tested at concentrations from 5×10^{-7} to 3×10^{-5} M, but since the maximum response to histamine was not clearly attained a second series of experiments was carried out on histamine concentrations from 10^{-6} to 10^{-4} M, and results from the two series of experiments combined. Secretory curves were also obtained for db cyclic AMP in control solutions, or solutions containing 2.7 μM indomethacin or 3 μM piroxicam. Since the secretory responses to db cyclic AMP were even slower in onset and offset than those to histamine a full concentration-response curve could not be obtained in a single experiment and results have been combined from two series of experiments. In the first series db cyclic AMP was tested at concentrations from 10^{-5} to 3×10^{-4} M,

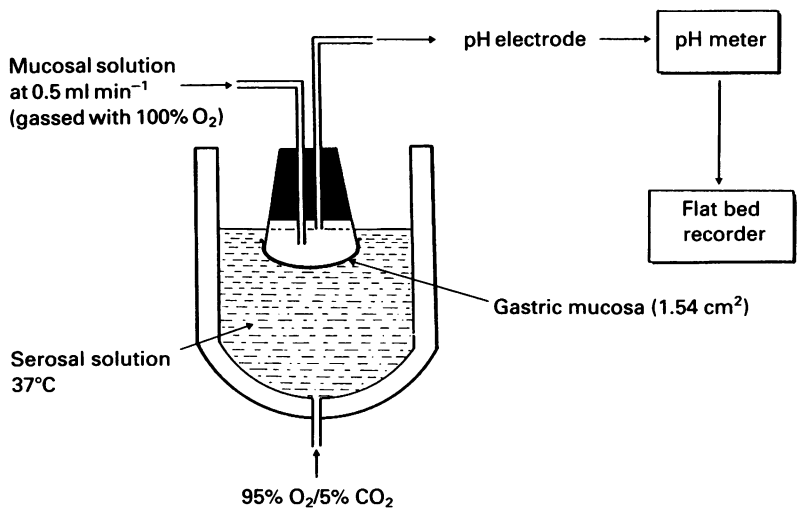

Figure 1 Experimental apparatus for the rat isolated gastric mucosa preparation.

and in the second series at concentrations of 10^{-4} M and 10^{-3} M only. Secretory responses to db cyclic AMP at 10^{-4} M were of similar magnitude in both series of experiments. Each treatment was tested on at least 6 separate preparations.

Antisecretory activity of prostanoids

Effect of PGE_2 and U-46619 on histamine-induced acid secretion in untreated mucosae Four consecutive secretory responses to a submaximal concentration of histamine (1.4×10^{-5} M) were obtained in each mucosa. After each secretory response the serosal solution bathing the tissue was changed several times and secretion allowed to recover to basal levels. Each secretory response was expressed as the increase in gastric acid output (in nmol H^+ min^{-1} cm^{-2}) over the basal level measured immediately before addition of the histamine dose. Saline (0.05 ml), PGE_2 (10^{-8}–10^{-7} M) or U-46619 (10^{-8}–10^{-6} M) was added serosally 15 min before the third addition of histamine and percentage change in secretion calculated between the second and third histamine responses. Each treatment was tested on at least 3 preparations. From these data the concentration of each prostanoid required to inhibit gastric secretion by 50% (IC_{50}) was calculated. The fourth secretory response to histamine was used to measure the degree of recovery of the tissue after washing out the prostanoid. Since full recovery was achieved after all the prostanoids tested, data on these fourth secretory responses are not included in this paper.

Effect of prostanoids on histamine-induced acid secretion in mucosae treated with indomethacin Throughout these experiments indomethacin (2.7×10^{-6} M) was present in the serosal bathing solution. The protocol for these experiments was the same as above except that the histamine concentration was 10^{-5} M. The antisecretory effects of 16,16 dimethyl PGE_2 (0.003–0.03 μM), PGE_2 (0.03–0.3 μM), PGI_2 (10–30 μM), $PGF_{2\alpha}$ (0.3–3 μM) PGD_2 (3–30 μM) and U-46619 (3–30 μM) were investigated. Six preparations were tested at each concentration of the prostanoid. Inhibitory IC_{50} values were calculated for each prostanoid and equipotent concentration-ratios calculated relative to PGE_2 (i.e. $PGE_2 = 1$).

Effect of PGE_2 on db cyclic AMP-induced acid secretion The effect of PGE_2 (10^{-5} M) on submaximal responses to db cyclic AMP (5×10^{-5} M) was investigated in 6 indomethacin-treated mucosae using the same protocol.

Statistical analysis

Results are expressed as mean ± s.e.mean. IC_{50} values, with 95% confidence limits, were calculated by the method of least squares.

Drugs and solutions

The solutions of the drugs used were prepared as follows: histamine acid phosphate (BDH), and dibutyryl cyclic AMP (dibutyryl adenosine 3′:5′-cyclic monophosphate sodium salt; Sigma) were dissolved in 0.9% w/v NaCl solution (saline). Indomethacin and piroxicam (Sigma) were dissolved in 10% sodium bicarbonate solution. U-46619 (11α, 9α-epoxymethano-prostaglandin H_2) 16,16 dimethyl PGE_2, PGD_2, PGI_2 (synthesized by Glaxo Group Research Ltd) and PGE_2 (Glaxo Group Research Ltd or East Anglia Chemicals) were dissolved in 3% ethanol/0.01% Tween 80 solution. $PGF_{2\alpha}$ was obtained in solution as dinoprost tromethamine (Upjohn). All dilutions were made in saline.

Results

Effects of indomethacin and piroxicam

The rat gastric mucosa spontaneously secreted acid reaching a steady basal secretory rate of approximately 60 nmol H^+ min^{-1} cm^{-2} within 2 h. This secretory rate did not alter in the presence of indomethacin or piroxicam.

In untreated preparations, concentration-related increases in acid secretion were produced by histamine

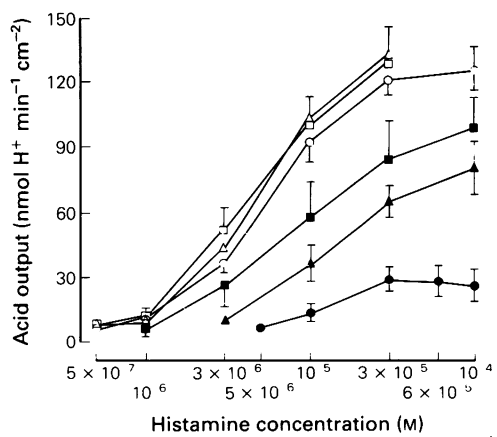

Figure 2 The effects of indomethacin 0.008 (▲), 0.027 (■), 0.27 (□), and 2.7 μM (○) and piroxicam 3.0 μM (△) on histamine-induced gastric acid secretion in the rat isolated gastric mucosa. (●) Control secretion in absence of indomethacin or piroxicam.

(5×10^{-6} to 3×10^{-5} M), with a maximum increase in output over basal of 29.7 ± 5.3 nmol H^+ min^{-1} cm^{-2}. Indomethacin (8.0×10^{-9} to 2.7×10^{-7} M) produced concentration-related increases in both threshold sensitivity and maximum response to histamine (Figure 2). Increasing the indomethacin concentration to 2.7×10^{-6} M produced no further potentiation of the histamine response. The maximum increase in gastric acid output elicited by histamine in the presence of indomethacin 2.7×10^{-6} M was 126.7 ± 10.1 nmol H^+ min^{-1} cm^{-2}, a four fold greater response than that produced in the absence of indomethacin. Piroxicam 3.0×10^{-6} M had a similar potentiating effect on histamine-induced acid secretion.

Dibutyryl cyclic AMP (10^{-5} to 10^{-3} M) produced large concentration-related increases in gastric acid secretion, even in the absence of indomethacin (Figure 3). For example the secretory response to 10^{-3}M db cyclic AMP of 154.5 ± 8.8 nmol H^+ min^{-1} cm^{-2} was even greater than the maximum response to histamine in the presence of indomethacin 2.7×10^{-6} M. This concentration of indomethacin neither increased the maximum response nor the threshold sensitivity to db cyclic AMP, but responses at intermediate concentrations (3×10^{-5}–3×10^{-4} M) were potentiated. Thus the potentiation of the effect of db cyclic AMP by indomethacin was different in profile from, and much less marked than that of histamine. Piroxicam (3×10^{-6} M) had a similar potentiating effect to indomethacin on the response to db cyclic AMP, for example in this series of experiments the response to db cyclic AMP 10^{-4} M was increased from 32.8 ± 6.0 to 102.8 ± 16.2 nmol H^+ min^{-1} cm^{-2}.

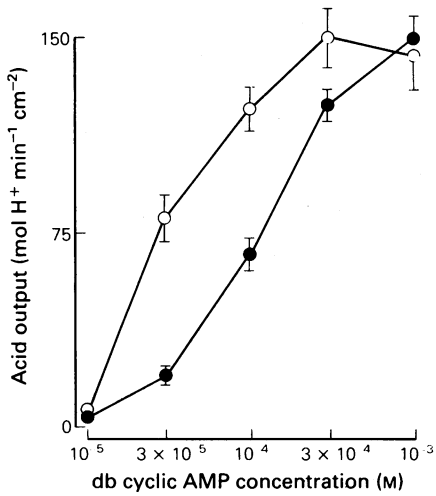

Figure 3 The effect of indomethacin 2.7 μM (○) on dibutyryl cyclic AMP-induced gastric acid secretion in the rat isolated gastric mucosa. (●) Control secretion in absence of indomethacin.

Antisecretory effects of prostanoids

Effect of PGE_2 and U-46619 in untreated mucosae Repeated exposure to histamine (1.4×10^{-5} M) produced consistent acid secretory responses, the mean change between the second and third response to histamine being $+ 6.0 \pm 7.5\%$. In these experiments histamine produced a mean secretory output of 24.1 ± 2.6 nmol H^+ min^{-1} cm^{-2} above basal levels. Both PGE_2 and U-46619 inhibited this secretory response in a concentration-related manner and were of similar potency in this respect (Table 1). PGE_2 had no effect on basal acid secretion whereas U-46619 inhibited basal acid secretion by $50.3 \pm 3.4\%$ at 10^{-6} M.

Effect of prostanoids in indomethacin-treated mucosae In these experiments, performed on mucosae treated with indomethacin (2.7×10^{-6} M), histamine (10^{-5} M)

Table 1 The effect of prostaglandin E_2 (PGE_2) and U-46619 on histamine-induced acid secretion in untreated mucosae

	Molar conc.	*Mean (± s.e.) % change in the secretory response to histamine*	*IC_{50} (μM) (95% confidence limits)*
Saline Control	—	+ 6.0 ± 7.5 (7)	—
PGE_2	10^{-8}	− 7.5 ± 4.6 (4)	0.044
	3×10^{-8}	− 40.2 ± 10.8 (4)	(0.031–0.071)
	10^{-7}	− 72.0 ± 7.6 (4)	
U-46619	10^{-8}	− 29.8 ± 10.8 (6)	0.035
	10^{-7}	− 64.3 ± 12.6 (6)	(0.008–0.088)
	10^{-6}	− 100 ± 0 (3)	

The number of experiments (n) is shown in parentheses.

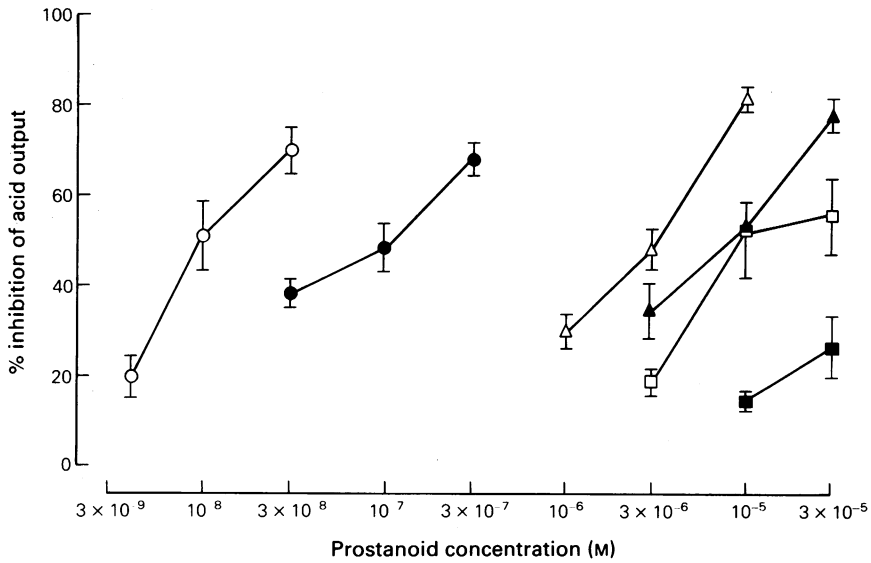

Figure 4 The inhibitory effect of 16,16 dimethyl prostaglandin E_2 (○), prostaglandin E_2 (PGE_2) (●), $PGF_{2\alpha}$ (△), PGD_2 (□), PGI_2 (■) and U-46619 (▲) on histamine-induced gastric acid secretion in the rat isolated gastric mucosa.

produced consistent secretory responses of 85.7 ± 7.1 nmol H^+ min^{-1} cm^{-2}.

All the prostanoids tested produced concentration-related inhibitions of the acid secretory response to histamine. The results are summarized in Figure 4 and Table 2. Indomethacin had no significant effect on the antisecretory potency of PGE_2, but produced a 200 fold reduction in the potency of U-46619. In the presence of indomethacin, 16,16 dimethyl PGE_2 was the most potent compound tested, being approximately 8 times more potent than PGE_2. The PGE_2 analogues were markedly more potent than $PGF_{2\alpha}$ which in turn was more potent than PGD_2 and U-46619. PGI_2 was virtually inactive at concentrations up to 3×10^{-5} M. Table 2 also shows the equipotent concentration-ratios (relative to PGE_2) for these prostanoids for their ability to contract strips of guinea-pig fundus muscle (from Kennedy *et al.*, 1982). The equipotent concentration-ratios for 16,16 dimethyl PGE_2 and $PGF_{2\alpha}$ on the guinea-pig fundic strip are similar to those on the rat gastric mucosa. U-46619 and PGD_2 are of similar potency on the same tissue, but are relatively more potent on the mucosa than the fundic strip, and PGI_2 is markedly more potent on the fundic strip. However, with the exception of PGI_2, the rank order of potency of these prostanoids is very similar in both preparations.

None of the prostanoids, with the exception of PGI_2

Table 2 A comparison of the antisecretory potencies of selected prostanoids in rat gastric mucosa

Prostanoid	*Rat isolated gastric mucosa*		*Guinea-pig fundic strip**
	IC_{50} (μM) (95% confidence limits)	*Equipotent conc. ratio (PGE_2 = 1)*	*Equipotent conc. ratio (PGE_2 = 1)*
PGE_2	0.088(0.054–0.14)	1.0	1.0
16,16,DMPGE$_2$	0.011(0.008–0.017)	0.13	0.1**
$PGF_{2\alpha}$	2.7(2.1–3.4)	30.7	33
U-46619	7.2(4.5–10.0)	81.8	660
PGD_2	8.9(5.8–35.0)	101.1	650
PGI_2	> 30	> 340	20

* From Kennedy *et al.* (1982).
** Unpublished data.

Rank order of potency for, rat gastric mucosa: 16,16 dimethyl $PGE_2 > PGE_2 > PGF_{2\alpha} > U\text{-}46619 > PGD_2 > PGI_2$; guinea-pig fundic strip: 16,16 dimethyl $PGE_2 > PGE_2 > PGI_2 > PGF_{2\alpha} > PGD_2 > U\text{-}46619$.

and U-46619, affected basal acid output. PGI_2 and U-46619, at a concentration of 3×10^{-5} M, produced inhibitions of $11.1 \pm 2.5\%$ and $36.4 \pm 3.4\%$, respectively.

Effect of PGE_2 on db cyclic AMP-induced acid secretion In these experiments, with indomethacin (2.7×10^{-6} M) present, db cyclic AMP (5×10^{-5} M) elicited reproducible acid secretory responses of 109.1 ± 11.8 nmol H^+ min^{-1} cm^{-2}. PGE_2 at the relatively high concentration of 10^{-5} M, which inhibited histamine-induced acid secretion by $87.2 \pm 2.6\%$, had no effect ($+3.3 \pm 13.0\%$) on the secretory response to db cyclic AMP.

Discussion

In the present study both histamine and db cyclic AMP stimulated acid secretion in a concentration-related manner in the rat isolated gastric mucosa preparation. In the absence of indomethacin, secretory responses to db cyclic AMP were much greater than those to histamine, the response to db cyclic AMP 10^{-3} M being 5 times the maximum response to histamine. The maximum response obtained to histamine in this study was approximately 65% of that reported by Main & Pearce (1978), and secretory responses to db cyclic AMP were similar to those found by Donaldson & Main (1981). It has been previously shown that indomethacin has no effect on secretory responses to histamine (Donaldson & Main, 1981) in the rat gastric mucosa. However, in our study indomethacin, although not affecting basal acid secretion produced a concentration-related increase in both the threshold sensitivity and maximum response to histamine, the maximum being increased four fold by indomethacin 2.7×10^{-6}M. The non-steroidal anti-inflammatory drug, piroxicam, which is of similar potency to indomethacin as an anti-inflammatory agent (Wiseman *et al.*, 1976) and as an inhibitor of PGE_2 synthesis (Egg, 1983) had similar effects to indomethacin on histamine-induced acid secretion. These results suggest that the rat gastric mucosa is producing endogenous substances via the cyclo-oxygenase pathway (e.g. prostaglandins) that have an inhibitory effect on acid secretory responses. This is consistent with the finding that the antisecretory effect, in this preparation, of arachidonic acid, a precursor for prostaglandin biosynthesis, is abolished by indomethacin (Frame & Main, 1980).

The secretory responses to db cyclic AMP were also potentiated by indomethacin or piroxicam but the profile of this effect was different from that of histamine. Neither the threshold sensitivity of the mucosae nor the maximum response to db cyclic AMP was increased, but responses to intermediate concentrations were potentiated. This effect cannot be totally explained by the inhibition of endogenous prostaglandin formation, at least not that of PGE_2, because exogenous PGE_2 at a concentration that almost abolished the secretory response to histamine had no effect on the secretory response to db cyclic AMP. This lack of activity of PGE_2 against db cyclic AMP-induced secretion has been observed previously (Main & Whittle 1974) and it is thought that prostaglandins mediate their effects by inhibiting the histamine stimulated increase in cyclic AMP within the parietal cells (Major & Scholes, 1978; Soll 1980) and thus can only inhibit at a stage prior to cyclic AMP production. It is possible that the potentiation of db cyclic AMP by indomethacin may be due to the inhibition of phosphodiesterase, since other studies suggest that indomethacin can inhibit this enzyme (Beatty *et al.*, 1976; Silvola *et al.*, 1982). However, no information on the effect of piroxicam on phosphodiesterase could be found.

In the absence of indomethacin, PGE_2 and the thromboxane mimetic U-46619 were equipotent inhibitors of histamine stimulated acid secretion. However, the activity of U-46619 was dramatically decreased when indomethacin was present (see Tables 1 and 2) suggesting that U-46619 may act, at least partly, through an indirect mechanism involving the formation of endogenous prostaglandins (eg PGE_2). U-46619 also tended to inhibit basal acid secretion, an effect that does not appear to have been previously described for prostanoids tested on the rat isolated gastric mucosa. This may be a direct effect of U-46619, since it occurred both in the absence and presence of indomethacin, and PGE_2 did not inhibit basal secretion. The mechanism involved is not known and merits further investigation.

Studies comparing the antisecretory potencies of selected prostanoids were carried out in the presence of indomethacin to eliminate the possible influence of endogenous prostanoids. PGI_2, which is produced in comparatively large quantities in the gastric mucosa (Whittle *et al.*, 1978b) and is reported to be a potent antisecretory prostaglandin in the rat (Whittle *et al.*, 1978a), had virtually no effect in this study on histamine stimulated acid secretion. This lack of activity could result either from rapid inactivation of PGI_2 in the aqueous serosal solution (Johnson *et al.*, 1976) or possibly the antisecretory effect of PGI_2 seen in other preparations may not involve direct inhibition at the parietal cell. PGI_2 has been found to be approximately 100 times less potent than PGE_2 on canine isolated parietal cells (Skoglund *et al.*, 1982).

In the present study PGE_2 and the synthetic analogue 16,16 dimethyl PGE_2 were the most potent antisecretory prostanoids tested, being at least 30 times more potent than $PGF_{2\alpha}$. The rank order of potency of the prostanoids tested was 16,16 dimethyl

$PGE_2 > PGE_2 > PGF_{2\alpha} > U\text{-}46619 > PGD_2 > PGI_2$. This order of potency is very similar to that found for these prostanoids in another PGE-sensitive preparation, the guinea-pig isolated fundic strip (Table 2), classified by Kennedy *et al.* (1982) as an 'EP' receptor containing preparation. We therefore suggest that the inhibition of histamine-induced acid secretion in the rat mucosa by prostanoids is mediated by 'EP' receptors. 'EP'-receptors may also be involved in the inhibition of acid secretion in other species since a similar order of potency was described in canine isolated parietal cells (Skoglund *et al.*, 1982). Further characterization of this antisecretory response awaits the development of specific antagonists for the effects of E-prostaglandins.

In conclusion, the results of the present study suggest that products of the cyclo-oxygenase pathway are produced by the rat isolated gastric mucosa preparation which could have modulatory effects on acid secretory mechanisms. Prostaglandins of the E series are the most potent inhibitors of acid secretion in this preparation, suggesting that inhibition is mediated via 'EP'-receptors.

We wish to thank members of the Chemical Research Department at Glaxo Group Research Ltd, for synthesizing PGE_2, 16,16 dimethyl PGE_2, PGD_2, PGI_2 and U-46619 used in this study.

References

BEATTY, C.H., BOCEK, R.M., YOUNG, M.K. & NOVY, M.J. (1976). Effect of indomethacin on cyclic AMP phosphodiesterase activity in myometrium from pregnant rhesus monkeys. *Prostaglandins*, **11**, 713–725.

BOUGHTON-SMITH, N.K. & WHITTLE, B.J.R. (1981). The gastric antisecretory actions of prostaglandin E_2 and stable prostacyclin analogues against different secretagogues in perfused whole stomach of rat or mouse *in vitro*. *Br. J. Pharmac.*, **72**, 291–298.

BOUGHTON-SMITH, N.K. & WHITTLE, B.J.R. (1983). Stimulation and inhibition of prostacyclin formation in the rat gastric mucosa and ileum *in-vitro* by anti-inflammatory agents. *Br. J. Pharmac.*, **78**, 173–180.

BUNCE, K.T., REEVES, J.J. & STABLES, R. (1983). Effects of indomethacin, piroxicam and selected prostanoids on gastric acid secretion *in vitro*. *Br. J. Pharmac.* **79**, 565P.

COCEANI, F., PACE-ASCIAK, C., VOLTA, F. & WOLFE, L.S. (1967). Effect of nerve stimulation on prostaglandin formation and release from the rat stomach. *Am. J. Physiol.*, **213**, 1056–1064.

DONALDSON, K.M. & MAIN, I.H.M. (1981). A comparison of the effects of indomethacin and methylxanthines on basal and stimulated gastric secretion *in vitro*. *Br. J. Pharmac.* **74**, 970–971P.

EGG, D. (1983). Effects of glycosaminoglycan-polysulfate and two non-steroidal anti-inflammatory drugs on prostaglandin E_2 synthesis in chinese hamster ovary cell cultures. *Pharmac. Res. Commun.*, **15**, 709–717.

FRAME, M.H. & MAIN, I.H.M. (1980). Effects of arachidonic acid on rat gastric acid secretion in response to different secretagogues; inhibition of these effects by indomethacin. *Br. J. Pharmac.*, **69**, 171–178.

JOHNSON, R.A., MORTON, D.R., KINNER, J.H., GORMAN, R.R., McGUIRE, J.C. & SUN, F.F (1976). The chemical structure of prostaglandin X (prostacyclin). *Prostaglandins*, **12**, 915–926.

KARIM, S.M.M., CARTER, D.C., BHANA, D. & GANESEN, P.A. (1973). Effect of orally administered prostaglandin E_2 and its 15-methyl analogues on gastric secretion. *Br. med. J.* **1**, 143–146.

KENNEDY, I., COLEMAN, R.A., HUMPHREY, P.P.A., LEVY, G.P. & LUMLEY, P. (1982). Studies on the characterisation of prostanoid receptors: A proposed classification. *Prostaglandins*, **24**, 667–689.

KONTUREK, S.J., KWIECIEN, N., SWIERCZEK, J., OLESKY, J., SITO, E. & ROBERT, A. (1976). Comparison of methylated prostaglandin E_2 analogues given orally in the inhibition of gastric responses to pentagastric and peptone meal in man. *Gastroenterol*, **70**, 683–687.

MAIN, I.H.M. & PEARCE, J.B. (1978). A rat isolated gastric mucosal preparation for studying the pharmacology of gastric secretion and the synthesis or release of endogenous substances. *J. Pharmac. Methods*, **1**, 27–38.

MAIN, I.H.M. & WHITTLE, B.J.R. (1974). Prostaglandin E_2 and the stimulation of rat gastric acid secretion by dibutyryl cyclic 3-5-AMP. *Eur. J. Pharmac.*, **26**, 204–211.

MAJOR, J.R. & SCHOLES, P. (1978). The localisation of a histamine H_2-receptor adenylate cyclase system in canine parietal cells and its inhibition by prostaglandins. *Agents & Actions*, **8**, 324–331.

ROBERT, A., NEZAMIS, J.E. & PHILLIPS, J.P. (1967). Inhibition of gastric secretion by prostaglandins. *Am. J. Dig. Dis.*, **12**, 1073–1076.

SILVOLA, J., KANGASAHO, M., TOKOLA, O. & VAPAATALO, H. (1982). Effects of nonsteroidal anti-inflammatory drugs on rat gastric mucosal phosphodiesterase activity. *Agents & Actions*, **12**, 516–520.

SINGH, J. (1980). Prostaglandin release from rat stomach following vagal stimulation or administration of acetylcholine. *Eur. J. Pharmac.*, **65**, 39–48.

SKOGLUND, M.L., NIES, A.S. & GERBER, J.G. (1982). Inhibition of acid secretion in isolated canine parietal cells by prostaglandins. *J. Pharmac. exp. Ther.*, **220**, 371–374.

SOLL, A.H. (1980). Specific inhibition by prostaglandins E_2 and I_2 of histamine stimulated [^{14}C] aminopyrine accumulation and cyclic adenosine monophosphate generation by isolated canine parietal cells. *J. clin. Invest.*, **65**, 1222–1229.

WHITTLE, B.J..R, BOUGHTON-SMITH, N.K., MONCADA, S.

& VANE, J.R. (1978a). Actions of prostacyclin (PGI_2) and its product, 6-oxo-$PGF_{1\alpha}$ on the rat gastric mucosa *in vivo* and *in vitro*. *Prostaglandins*, **15**, 955–967.

WHITTLE, B.J.R., MONCADA, S. & VANE, J.R. (1978b). Formation of prostacyclin by the gastric mucosa and its actions on gastric function. *Prostaglandins*, **15**, 704–705.

WISEMAN, E.H., CHANG, Y.H. & LOMBARDINI, J.G. (1976). Piroxicam, a novel anti-inflammatory agent. *Arzneim. Forsch.*, **26**, 1300–1303.

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