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THE GASTRIC ANTISECRETORY ACTIONS OF PROSTAGLANDIN E_2 AND STABLE PROSTACYCLIN ANALOGUES AGAINST DIFFERENT SECRETAGOGUES IN PERFUSED WHOLE-STOMACHS OF RAT OR MOUSE *in vitro*

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1 The characteristics of the antisecretory actions of prostaglandin E_2 (PGE_2) and two stable prostacyclin analogues during different rates of acid stimulation have been evaluated in the lumen-perfused isolated whole stomach of the rat and mouse.

2 In the rat isolated stomach, histamine induced a dose-dependent increase in acid output. Pre-incubation with PGE_2 caused a dose-related and surmountable inhibition.

3 The stable prostacyclin analogues, 6β-PGI_1 and a 16-phenoxy derivative likewise caused a surmountable inhibition of histamine-stimulated acid output from rat stomach.

4 PGE_2 had inconsistent actions on the acid secretion stimulated by pentagastrin, methacholine or dibutyryl cyclic adenosine 3',5'-monophosphate.

5 In the mouse isolated stomach, acid secretion was stimulated by low concentrations of histamine, pentagastrin or methacholine.

6 PGE_2 failed to inhibit histamine-stimulated acid output from mouse stomach, but high concentrations of the potent 16-phenoxy analogue did show anti-secretory activity.

7 The results indicate the usefulness of the rat isolated stomach for studying the interaction of prostaglandins with the acid secretory process in mammalian gastric mucosa.

Introduction

The recent development of viable, acid-secreting isolated preparations of mammalian stomachs (Wan, Assem & Schild, 1974; Holton & Spencer, 1976; Bunce & Parsons, 1976; Main & Pearce, 1978a; Angus & Black, 1979) has allowed study of the antisecretory actions of drugs, including prostaglandins, without such complicating factors as mucosal blood flow changes. In both the isolated stripped mucosa and lumen-perfused whole stomach of the rat, prostaglandin E_2 (PGE_2) inhibits histamine-stimulated acid output (Main & Pearce, 1978a; Whittle, Boughton-Smith, Moncada & Vane, 1978a). We have now investigated in more detail the characteristics of the antisecretory responses to PGE_2 and two stable antisecretory prostacyclin analogues, 6β-PGI_1 (Whittle, Boughton-Smith, Moncada & Vane, 1978b) and (5α)5,9-epoxy 16-phenoxy PGF_1 (Whittle & Boughton-Smith, 1979) during different rates of acid stimulation by histamine, methacholine, pentagastrin or dibutyryl cyclic adenosine 3',5'-monophosphate (db cyclic AMP) in rat or mouse whole-stomach.

A preliminary account of some of this work was presented to the British Pharmacological Society (Boughton-Smith & Whittle, 1979).

Methods

Isolated lumen-perfused stomach of the rat

The techniques used were similar to those described by Bunce & Parsons (1976). Fed immature male rats (Wistar) weighing between 35 and 45 g were killed by cervical dislocation. The abdomen was opened, the oesophagus ligated close to the stomach and the stomach flushed with warm Krebs-Henseleit solution through an incision in the muscular non-secretory forestomach. A cannula was placed in the gastric lumen through the incision in the forestomach and another through the pyloric sphincter via the duodenum. The stomach was then placed in an organ bath containing 20 ml of a modified Krebs-Henseleit

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solution (serosal solution) at 37°C and perfused (1 ml/min) through the gastric lumen with the modified Krebs-Henseleit solution from which the buffer salts had been omitted (mucosal solution).

Acid secretion from the gastric lumen was measured continuously as pH of the perfusate via a microelectrode system, arranged so that the tip of the pH electrode was mounted 19 cm above the perfused stomach. The pH values were converted to hydrogen-ion concentration. Under these conditions, a spontaneous acid secretion developed which reached steady levels after approximately 20 to 40 min (132 ± 6 nmol/min, $n = 106$). Any preparations that did not reach a 'basal' acid secretion greater than pH 4.5 (32 nmol/min) after 40 min were rejected (<2%) as they were found to be unresponsive to histamine.

The serosal solution was changed 5 to 15 min after the stomachs were set up, to remove any free debris resulting from dissection. After 40 min, when the basal secretion had reached plateau levels, the vehicle (ethanol) or antagonist (prostaglandin) was added to the serosol solution in a maximum volume of 20 μl. After an incubation period of 12 min the agonist (histamine acid phosphate, methacholine, pentagastrin or db cyclic AMP) was added to the bath and the acid secretion measured until peak stimulated secretion was recorded (24 to 40 min). Each preparation was used for a single response only, with treatment being allocated randomly between preparations. Responses to histamine were measured as peak stimulation of acid secretion above the rate of basal acid secretion immediately prior to the addition of histamine (Δ acid secretion).

Isolated perfused stomach of the mouse

Whole stomachs from fed male mature mice (Keebles) weighing between 25 and 30 g were prepared by the method described above for the rat, except that the Krebs-Henseleit solution contained a higher glucose concentration (31 mM) in both serosal and mucosal solution (Angus & Black, 1979).

Bathing solutions

The serosal solution was composed of (mM): NaCl 119, $MgSO_4$ 1.2, KCl 4.7, glucose 5.6, $NaHCO_3$ 30, $K_2H_2PO_3$ 0.5 and CaCl 1 and gassed vigorously with 95% O_2 and 5% CO_2. The mucosal solution was of similar composition but with the buffer salts omitted and gassed with 100% O_2. In the mouse isolated stomach experiments the solutions were similar except the glucose concentration was adjusted to 31 mM which allowed a more sustained secretory response.

Drugs

Histamine acid phosphate (BDH) methacholine (Sigma Chemical Co.), pentagastrin (Peptavlon, ICI), N^6-2'O-dibutyryl adenosine 3'-5'-monophosphate cyclic (Boehringer-Mannheim) were made up freshly when required. Prostaglandin E_2 and the prostacyclin analogues 6β-PGI_1 and (5α)-9-deoxy-5-9α-epoxy-16-phenoxy 17,18,19,20-tetranor prostaglandin F_1 which were synthesized as described elsewhere (Johnson, Lincoln, Smith, Ayer, Nidy, Thompson, Axen, Aiken, Gorman, Nishizawa & Honohan, 1979) were stored in ethanol (−40°C) and added directly to the serosal solution.

Statistical analysis

Results, calculated as Δ acid output (peak acid output above basal secretion) are expressed as the mean ± s.e. mean of (n) values. The significance of the difference between groups was assessed by Student's t test for unpaired data, and $P < 0.05$ was taken as significant.

Results

Rat isolated whole stomach

Histamine (16 to 261 μM; 5 to 80 μg/ml) produced a dose-dependent secretory response, which reached maximal secretory levels of 221 to 276 nmol/min (Figure 1). The acid-secretory response to histamine (65 μM) was not significantly different from that induced by histamine at concentrations of 130 and 261 μM ($P > 0.7$ and $P > 0.1$ respectively) and was taken as the maximal secretory response to histamine.

Peak secretory responses to doses of histamine were observed between 24 and 40 min after addition to the bath.

Effect of incubation with prostaglandin E_2 on the histamine secretory response

Prostaglandin E_2 (0.5 to 1.0 μg/ml; 1.4 to 2.8 μM) produced a dose-related and surmountable inhibition of histamine-induced acid secretion (Figure 2). The secretory response to a submaximal (16 μM) dose of histamine was $\Delta 90 \pm 11$ nmol/min ($n = 23$) which was significantly reduced to $\Delta 17 \pm 4$ nmol/min ($n = 6$; $P < 0.01$) by PGE_2 (1.4 μM) and to $\Delta 1 \pm 20$ nmol/min ($n = 11$; $P < 0.001$) by PGE_2 (2.8 μM). Likewise, with a maximal concentration of histamine (65 μM) producing an acid secretory response of $\Delta 221 \pm 16$ nmol/min ($n = 37$), PGE_2 (2.8 μM) produced a significant inhibition (to $\Delta 104 \pm 16$ nmol/min, $n = 27$, $P < 0.001$) whereas the lower concen-

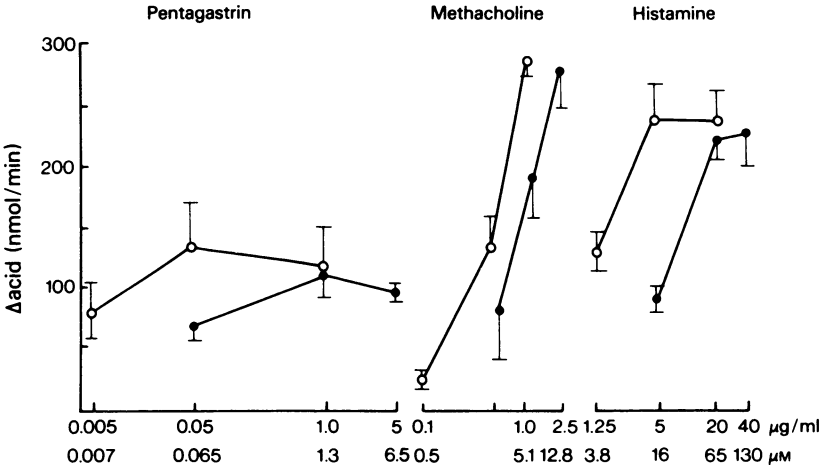

Figure 1 Stimulation of acid secretion from isolated lumen-perfused whole stomachs from rat (●) or mouse (○) by pentagastrin, methacholine and histamine. Results shown as Δ acid output (increase over basal secretion) in terms of nmol/min, are the mean of 10 to 50 experiments for each value; vertical lines show s.e. mean.

tration of PGE_2 produced no significant inhibition. With increasing supramaximal concentrations of histamine (up to 261 μM), the inhibition by PGE_2 (2.8 μM) was no longer significant.

Thus a shift to the right of the dose-response curve to histamine, with no depression of the maximal response was achieved with PGE_2 in these doses (Figure 2).

The effect of prostacyclin analogues

The effects of the stable prostacyclin analogues on histamine stimulated acid secretion were determined as with PGE_2.

In the presence of 6β PGI_1, (2 μg/ml; 5.6 μM) there was a small shift in the histamine secretory response curve to the right with the responses to histamine (16 μM) being significantly ($P < 0.05$) reduced from Δ57 ± 11 to Δ25 ± 10 nmol/min (Figure 3).

Low doses of the stable prostacyclin analogue (5α)5,9-epoxy-16-phenoxy PGF_1 (0.026 μM; 0.01 μg/ml) also produced a displacement to the right of the histamine secretory response curve with significant inhibition of both rates of histamine stimulation (16 and 67 μM).

Effect of prostaglandin E_2 on methacholine-induced secretion

Methacholine at concentrations of 0.6 to 2.5 μg/ml (3.2 to 12.8 μM) produced a dose-related secretory response similar to that produced by histamine (Figure 1). Only the higher rate of secretion induced by methacholine was significantly ($P < 0.02$) reduced by PGE_2 (2.8 μM) (Figure 2).

Effect of prostaglandin E_2 on pentagastrin-induced secretion

The secretory responses of the rat whole stomach to pentagastrin (0.065 to 6.5 μM) were substantially smaller than those obtained with either histamine or methacholine and exhibited a shallow dose-response relationship (Figure 1). However, the onset of the acid secretory response to pentagastrin and the time for the peak secretory response to occur were more rapid than with either histamine or methacholine; there was also a rapid fade in the peak response, again in contrast to histamine and methacholine which both gave a maintained secretory response.

Only the acid secretory response to the intermediate dose of pentagastrin was reduced significantly ($P < 0.025$) by PGE_2 (2.8 μM) (Figure 2).

Effect of prostaglandin E_2 on dibutyryl cyclic AMP-induced secretion

As is shown in Figure 2, the stimulation of acid output by db cyclic AMP (51 to 204 μM) was significantly inhibited by PGE_2 (2.8 μM) only at the intermediate rate of stimulation. Thus, as with pentagastrin and methacholine-induced acid output, no consistent antisecretory effects of PGE_2 could be demonstrated.

Effect of prostaglandin E_2 on basal acid secretion

During the 12 min preincubation before addition of the agonist, PGE_2 (2.8 μM) significantly ($P < 0.05$) reduced basal acid secretion (by 36 ± 4 nmol/min, $n = 66$).

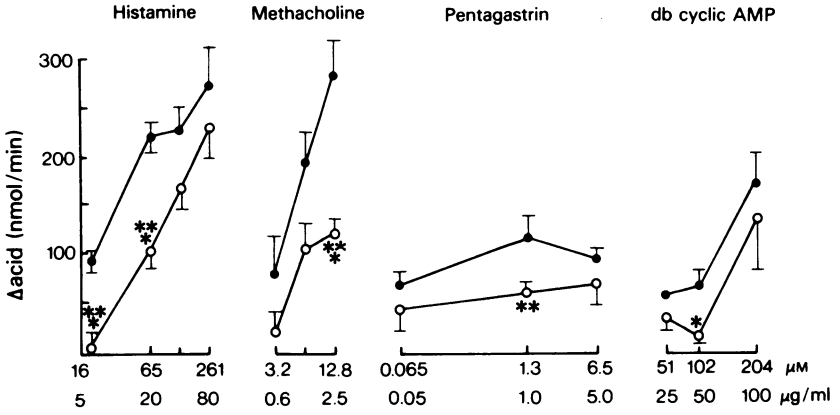

Figure 2 Inhibition of acid output from rat isolated stomachs by prostaglandin E_2 (PGE_2, 1 μg/ml; 2.8 μM) during stimulation with histamine, methacholine, pentagastrin and dibutyryl cyclic AMP. The results, which show Δ acid output (nmol/min) under control conditions (●) and following a 12 min incubation with PGE_2 (○) before stimulation are expressed as mean of 10 to 50 experiments for each value; vertical lines show s.e. mean. The level of statistical significance is shown by * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$.

The prostacyclin analogue, 6β PGI_1 (5.6 μM), did not significantly affect basal secretion whereas (5α)5,9-epoxy-16-phenoxy PGF_1 (0.026 μM) produced a marked reduction (65 ±7 nmol/min, $n = 24$) in basal acid secretion ($P < 0.05$).

Mouse isolated whole stomach

The mouse stomach had a basal acid secretion of 119 ±8 nmol/min ($n = 84$) and histamine caused a dose-dependent increase in acid secretion (Figure 1).

Effect of prostaglandin E_2 on histamine-induced secretion

In contrast to the rat stomach, PGE_2 (2.8 μM) had no antisecretory effect against the effects of histamine in the mouse stomach. Increasing the concentration of

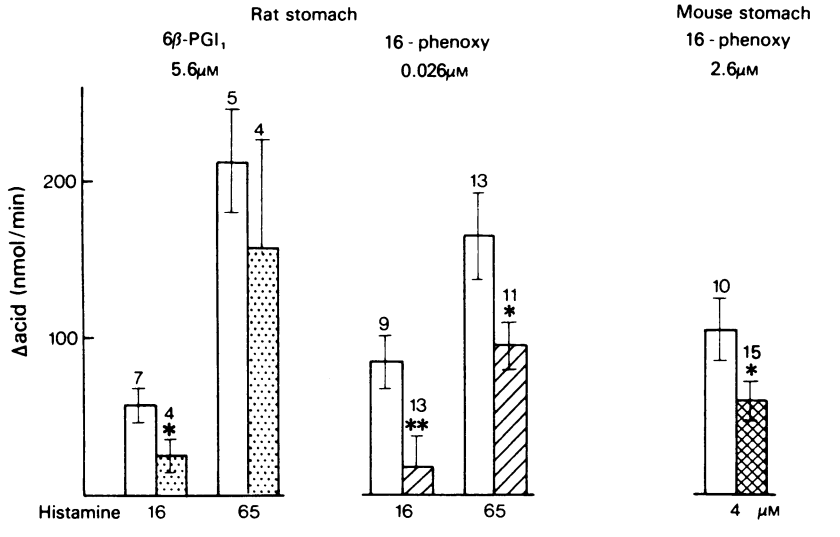

Figure 3 Effect of pre-incubation with the stable prostacyclin analogues, 6β-PGI_1 (5.6 μM) and (5α)5,9-epoxy-16-phenoxy PGF_1 (0.026 μM) in rat stomach; (2.6 μM in mouse stomach) on histamine-stimulated acid output. Results, shown as Δ acid (nmol/min) are mean of (n) values, where the open columns represent control values; vertical lines show s.e. mean. The statistical significance is represented by * $P > 0.05$; ** $P > 0.01$; *** $P > 0.001$.

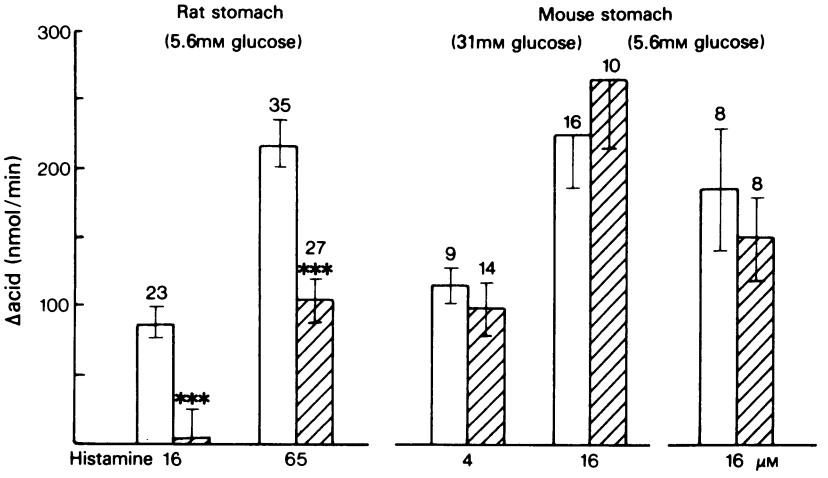

Figure 4 Effect of prostaglandin E_2 (PGE_2, 2.8 μM) on histamine-stimulated acid output (nmol/min) from rat and mouse isolated stomachs using different glucose concentrations. Results are mean of (*n*) values, where the open columns represent control values; vertical lines show s.e. mean. A statistically significant difference is represented by *** $P > 0.001$.

PGE_2 (to 14 μM) against the secretory response to histamine (4 μM) of Δ35 ± 9 nmol/min still did not result in any significant antisecretory action (Δ49 ±13 nmol/min). Lowering the glucose concentration to 5.6 mM (as used in the whole rat stomach) did not alter this insensitivity to PGE_2 (Figure 4).

The analogue, (5α)5,9-epoxy-16-phenoxy PGF_1 (0.026 μM) in the doses used in the rat stomach was also without significant effect on the acid output to histamine. The secretory response to histamine (4 μM) was Δ107 ± 20 nmol/min ($n = 10$) and in the presence of the 16-phenoxy analogue, the secretory response was Δ72 ± 24 nmol/min ($n = 8$; $P > 0.05$). However, when the dose of analogue was raised 100 fold (to 2.6 μM), the secretory response to histamine was significantly ($P < 0.05$) reduced to 61 ± 12 nmol/min ($n = 15$), as shown in Figure 3.

Effect of prostaglandin E_2 against methacholine- and pentagastrin-induced secretion

As with histamine, the sensitivity of the mouse stomach to both methacholine and pentagastrin was greater than found in the rat whole stomach (Figure 1). PGE_2 was without significant antisecretory effect against either secretagogue. Methacholine (2.6 μM) gave a secretory response of 132 ± 26 nmol/min ($n = 14$), which in the presence of PGE_2 (2.8 μM) was not significantly altered (Δ165 ± 25 nmol/min, $n = 16$). Pentagastrin (6.5 nM) induced a secretory response of Δ80 ± 24 nmol/min ($n = 9$) and in the presence of PGE_2 (2.8 μM) the secretory response was Δ76 ± 10 nmol/min ($n = 12$; $P > 0.05$).

In the mouse stomach, basal secretion was not changed significantly by PGE_2 (2.8 μM). However, at the highest concentration, the 16-phenoxy analogue (2.6 μM) significantly reduced ($P < 0.05$) basal acid secretion (by 23 ± 1 nmol/min, $n = 15$).

Discussion

Using isolated whole stomachs of the rat and mouse we have studied the characteristics of the antisecretory actions of PGE_2 and stable prostacyclin analogues on basal and stimulated acid secretion.

In the rat whole stomach, basal acid secretion was substantially higher than previously found by Bunce & Parsons (1976) using a comparable preparation. This may result from the lower Ca^{2+} concentration (1 mM) used in our experiments since Main & Pearce (1978b) demonstrated large increases in spontaneous acid output following reduction of the Ca^{2+} concentration in the rat stripped gastric mucosa.

The concentrations of histamine stimulating acid secretion were comparable to those of Bunce & Parsons (1976) although in our studies the secretory responses were greater; this may again reflect the differences in Ca^{2+} concentration or in experimental design. In our studies no anaesthetic was used during removal of the stomach and only a single period of stimulation was used. Cholinergic- and pentagastrin-stimulated acid secretion has previously been demonstrated in the whole stomach and isolated stripped gastric mucosa of the rat (Bunce, Parsons & Rollings,

1976; Main & Pearce, 1978a) although tachyphylaxis was observed. This was avoided in the present study by use of each preparation for a single treatment only. The secretory response to methacholine in our experiments was similar to that induced by histamine, which contrasts with the low secretory responses to pentagastrin with its shallow dose-response relationship. Again, the rates of acid output were greater than previously reported (Bunce *et al.*, 1976).

In previous studies, where prostaglandin E_2 (PGE_2) has been used to inhibit histamine-stimulated acid secretion in the rat isolated stripped mucosa (Main & Pearce, 1978a) and whole stomach of the rat (Whittle *et al.*, 1978), only a single concentration of histamine was used. In the present study, pre-incubation with PGE_2 produced a displacement of the histamine dose-response curve to the right, whilst the inhibitory action of PGE_2 was surmountable by high doses of histamine. Using an isolated parietal-cell system from dog mucosa, Soll (1978a, 1980) has also shown that the inhibition of histamine stimulation by low doses of PGE_2 was reversed by increasing the concentration of histamine. Furthermore, PGE_2 did not inhibit either carbachol or gastrin-stimulated parietal-cell activity. We too have found that, in contrast to histamine stimulation, PGE_2 produced inconsistent effects on acid secretion stimulated by pentagastrin or methacholine.

These results are also similar to those in the rat stripped gastric mucosa where histamine-stimulated acid secretion was readily inhibited by PGE_2 but the secretion stimulated by methacholine and pentagastrin only became susceptible to inhibition by PGE_2 after a long incubation period (Main & Pearce, 1978a). Interestingly, Soll (1978b, 1980) has observed that a background of histamine stimulation not only greatly enhanced the isolated parietal-cell responses to carbachol and gastrin but also made these responses susceptible to inhibition by PGE_2. In isolated whole stomach or in stripped mucosal preparations, there is a mixture of cell types, some of which are capable of mediator release, including histamine. This could therefore provide a background for additional stimulation by methacholine or pentagastrin and possibly explain the inconsistent inhibitory effects of PGE_2 on the secretory responses to secretagogues other than histamine.

The analogues 6β-PGI, and the 16-phenoxy derivative likewise produced a shift to the right of the dose-response curve for histamine stimulation, with the inhibition of acid output being surmountable. Previously these compounds, like PGE_2, have been shown to inhibit gastric acid secretion in the isolated whole stomach of the rat when administered during stable submaximal rates of histamine stimulation (Whittle & Boughton-Smith, 1979). In the present experiments where the prostaglandins were pre-incubated with the tissue before stimulation, a comparable degree of inhibition was achieved.

In our studies on the isolated mouse stomach, basal secretion was similar to that described previously (Wan, 1977; Angus & Black, 1979; Angus, Black & Stone, 1980). Histamine, methacholine and pentagastrin all stimulated acid secretion at lower doses than required in the rat stomach, although the maximal rates of acid output in response to these secretagogues were similar to those observed for the rat. In contrast to the increased sensitivity to secretagogues, the mouse stomach was relatively insensitive to the inhibitory actions of the prostaglandins tested. Even at the higher concentrations, PGE_2 did not inhibit histamine-stimulated acid secretion. However, by increasing the concentration of the more potent stable 16-phenoxy prostacyclin analogue by a 100 times that used in the rat, a significant inhibition of histamine-stimulated acid secretion was achieved. The differences in sensitivity to the inhibitory effects of PGE_2 on histamine-stimulated acid secretion in the rat and mouse preparation are difficult to explain but may be linked to age, the rat being an immature weanling of approx. 3 weeks old, compared with the adult mice used. Studies on histamine H_2-receptor antagonists, however, gave comparable effects in the mouse stomach (Angus & Black, 1979; Angus *et al.*, 1980) or the immature rat stomach (Bunce & Parsons, 1976). It is thus not yet apparent whether differences in the penetration of the muscular layers or in the uptake or metabolism of the prostaglandins by the rat or mouse stomach *in vitro* could explain the marked difference in sensitivities to these compounds. In all other species so far reported, PGE_2, and more recently, the prostacyclin analogues have been potent antisecretory agents either *in vitro* or *in vivo*.

PGE_2 is likely to exert its anti-secretory action by suppressing the formation of cyclic AMP, a proposed intracellular mediator of gastric acid secretion. In early *in vitro* studies on the amphibian gastric mucosa, PGE_2 inhibited the secretory response to histamine but not to directly applied cyclic AMP (Way & Durbin, 1969). In studies in the rat *in vivo*, PGE_2 inhibited the secretory responses to histamine yet failed to reduce the small secretory response to db cyclic AMP alone (Main & Whittle, 1974). Similar findings have been reported with methyl analogues of PGE_2 in the rat isolated stripped mucosa (Main & Whittle, 1976; Main & Pearce, 1978a) and we have likewise shown in the present study on the rat isolated whole stomach, that PGE_2 had inconsistent actions on the stimulation by db cyclic AMP. Further support has come from studies on parietal-cells isolated from dog gastric mucosa, where the secretory response to histamine but not db cyclic AMP was inhbited by PGE_2 (Soll, 1980).

All these findings support the concept that antisec-

retory prostaglandins act at a stage prior to the production of cyclic AMP via a direct inhibitory action on adenylate cyclase. Thus, Soll (1978a, 1980) has shown that the low concentrations of PGE_2 which inhibit histamine-induced activity in isolated parietal-cells, also markedly inhibit the elevation of cyclic AMP induced by histamine. Major & Scholes (1979) have likewise shown that PGE_2 and its analogues can prevent the stimulation of cyclic AMP formation by histamine in isolated parietal-cells.

Our findings suggest that the interaction of PGE_2 with the adenylate cyclase system in rat stomach is surmountable although our data are insufficient to analyse the nature of the prostaglandin and histamine actions at the receptor level. Similar results with low concentrations of PGE_2 during histamine stimulation have been found using isolated canine parietal-cells (Soll, 1980). The histamine H_2-receptor antagonist, metiamide, has previously been shown to exhibit competitive characteristics on the histamine dose-response curve in the rat isolated stomach (Bunce & Parsons, 1976). However, it is known that under certain conditions, non-competitive antagonists may exhibit such characteristics at low concentrations and in the current study, it is unlikely that PGE_2 and histamine interact at the same site. These findings may therefore reflect the nature of the inhibitory interaction of PGE_2 with adenylate cyclase at one site and the ability of histamine in higher concentrations to overcome the secretory inhibition by further stimulating adenylate cyclase at another. Whether such effects would occur at much higher concentrations of prostaglandins, when non-specific effects may also be elicited, has not been investigated in the present study.

The present findings with the isolated perfused whole stomach of the rat reinforce its usefulness in the pharmacological investigation and evaluation of drugs affecting gastric acid secretion. We have shown that PGE_2 and stable prostacyclin analogues can potently inhibit histamine-stimulated gastric acid secretion, and thus this model provides a useful technique for the study and analysis of the interaction of prostaglandins with the acid secretory processes in mammalian gastric mucosa.

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References

ANGUS, J.A. & BLACK, J.W. (1979). Analysis of anomalous pK_B values for metiamide and atropine in the isolated stomach of the mouse. *Br. J. Pharmac.*, **67**, 59–65.

ANGUS, J.A., BLACK, J.W. & STONE, M. (1980). Estimation of pK_B values for histamine H_2-receptor antagonists using an *in vitro* acid secretion assay. *Br. J. Pharmac.*, **68**, 413–423.

BOUGHTON-SMITH, N.K. & WHITTLE, B.J.R. (1979). Some characteristics of the gastric antisecretory actions of prostaglandins in mammalian perfused whole-stomachs *in vitro*. *Br. J. Pharmac.*, **68**, 153–154P.

BUNCE, K.T. & PARSONS, M.E. (1976). A quantitative study of metiamide, and histamine H_2-antagonists on the isolated whole rat stomach. *J. Physiol.*, **258**, 453–465.

BUNCE, K.T., PARSONS, M.E. & ROLLINGS, N. (1976). The effect of metiamide on acid secretion stimulated by gastrin, acetylcholine and dibutyryl cyclic adenosine 3',5'-monophosphate in the isolated whole stomach of the rat. *Br. J. Pharmac.*, **58**, 149–156.

HOLTON, P. & SPENCER, J. (1976). Acid secretion by guinea-pig isolated stomach. *J. Physiol.*, **255**, 465–479.

JOHNSON, R.A., LINCOLN, F.H., SMITH, H.W., AYER, D.E., NIDY, E.G., THOMPSON, J.L., AXEN, U., AIKEN J.W., GORMAN, R.R., NISHIZAWA, E.E. & HONOHAN, T. (1979). Biological effects of stable prostacyclin analogs. In *Prostacyclin*. ed. Vane, J.R. & Bergstrom, S. pp. 17–28. New York: Raven Press.

MAIN, I.H.M. & PEARCE, J.B. (1978a). 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. & PEARCE, J.B. (1978b). Effect of calcium on acid secretion from the rat isolated gastric mucosa during stimulation with histamine, pentagastrin, methacholine and dibutyryl cyclic adenosine-3',5',-monophosphate. *Br. J. Pharmac.*, **64**, 359–368.

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.

MAIN, I.H.M. & WHITTLE, B.J.R. (1976). The role of prostaglandins in gastric acid secretion. In *Stimulus-Secretion Coupling in the Gastrointestinal Tract*. ed. Case, R.M. & Goebell, H. pp. 147–155. Lancaster, Great Britain: MTP Press.

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

SOLL, A. (1978a). Prostaglandin inhibition of histamine-stimulated aminopyrine uptake and cyclic AMP generation by isolated canine parietal cells. *Gastroenterology*, **74**, 1146.

SOLL, A. (1978b). The interaction of histamine with gastrin and carbamylcholine on oxygen uptake by isolated mammalian parietal cells. *J. clin. Invest.*, **61**, 381–389.

SOLL, A. (1980). Specific inhibition by prostaglandins E_2 and I_2 of histamine-stimulated [^{14}C]-aminopyrine

accumulation and cyclic AMP generation by isolated canine parietal cells. *J. clin. Invest.* **65**, 122–1229.

WAN, B.Y.C. (1977). Metiamide and stimulated acid secretion from the isolated non-distended and distended mouse stomach. *J. Physiol.*, **266**, 327–346.

WAN., B.Y.C., ASSEM, E.K. & SCHILD, H.O. (1974). Inhibition of *in vitro* stimulated gastric acid secretion by a histamine H_2-receptor antagonist, metiamide. *Eur. J. Pharmac.*, **29**, 83–88.

WAY, L. & DURBIN, R.P. (1969). Inhibition of gastric acid secretion *in vitro* by prostaglandin E_1. *Nature*, **221**, 874–875.

WHITTLE, B.J.R. & BOUGHTON-SMITH, N.K. (1979). 16-Phenoxy prostacyclin analogs—potent, selective antiulcer compounds. In *Prostacyclin*. ed. Vane, J.R. & Bergstrom, S. pp. 159–171. Raven Press: New York.

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., BOUGHTON-SMITH, N.K., MONCADA, S. & VANE, J.R. (1978b). The relative activity of prostacyclin (PGI_2) and a stable analogue 6β-PGI_1, on the gastrointestinal and cardiovascular systems. *J. Pharm. Pharmac.*, **30**, 597–599.

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