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The effects of E and A prostaglandins on gastric mucosal blood flow and acid secretion in the rat

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Summary

1. The effects of prostaglandins E_1, E_2, A_1 and A_2 on gastric acid secretion and mucosal blood flow were studied by means of a [^{14}C]-aniline clearance technique in the anaesthetized rat.

2. During intravenous administration of these prostaglandins, in doses which almost completely inhibited pentagastrin- and histamine-induced acid secretion, a fall in clearance was observed.

3. Clearance per unit acid secretion increased during prostaglandin administration, precluding a primary reduction in mucosal blood flow as the mechanism of the antisecretory action.

4. Prostaglandins increased clearance during basal secretion, indicating a direct vasodilator effect on the gastric mucosa.

5. The possibility that endogenous prostaglandins contribute to functional vasodilatation in the gastric mucosa and that exogenous prostaglandins may be of clinical value in the treatment of peptic ulcer is discussed.

Introduction

Prostaglandins inhibit gastric secretion in the rat (Ramwell & Shaw, 1968), dog (Robert, Nezamis & Phillips, 1967) and man (Classen, Koch, Bickhardt, Topf & Demling, 1971). Although prostaglandin E_1 can also inhibit secretion in the isolated bullfrog mucosa (Way & Durbin, 1969) its mechanism of action *in vivo* is not yet fully understood. It was suggested that in the dog prostaglandin E_1, a potent vasodilator, acts primarily by reducing gastric mucosal blood flow (Wilson & Levine, 1969). However, Jacobson (1970) concluded that the observed fall in mucosal blood flow (MBF) was secondary to the inhibition of acid secretion.

Prostaglandins also inhibit ulcer formation in the rat (Robert, Nezamis & Phillips, 1968) and may be involved in the local control of gastric secretion by a negative feedback mechanism (Shaw & Ramwell, 1968). Using the [^{14}C]-aniline clearance technique, by which MBF can readily be studied in this species (Main & Whittle, 1972, 1973a), we have therefore investigated the effects of prostaglandins of the E and A series on the relationship between rat gastric acid secretion and MBF.

Methods

Female rats weighing 200–250 g were starved for 18 h but allowed water. Anaesthesia was induced with urethane (1·6 g/kg subcutaneously as a 25% solution) and the trachea was cannulated.

The gastric lumen was perfused with 0·9% w/v NaCl solution (saline), or in certain experiments during basal conditions, acidic saline (0·01 N HCl, pH 2), at a rate of 0·2 ml/min by a technique (Main & Whittle, 1973a) similar to that described by Ghosh & Schild (1958). Samples of perfusate were collected at intervals of 20 min and the acid content determined by titration of aliquots with 0·01 N NaOH using phenolphthalein as indicator. The volume of the cannula from the tip in the pylorus to the point of sample collection was 0·4 to 0·5 ml. The time lag between administration of 0·1 ml of 0·1 N HCl via the tip of the oesophageal cannula and detection of an increase in acid was 2 min with a peak acid output at 3 to 4 minutes.

Blood pressure was recorded and blood samples withdrawn via a cannula in a carotid artery. Drugs were administered intravenously in a constant volume not exceeding 3·3 ml/h via a cannula in a femoral vein. The dead space of the venous cannula was 0·1 ml and drugs administered via a rubber connector reached the circulation within 1–2 minutes. Body temperature was maintained at 34° C $\pm$ 0·5° by means of a rectal probe and a warming blanket.

Measurement of [^{14}C]-aniline clearance

One hour after surgery [C^{14}]-aniline was injected i.v. in a loading dose of 2·0 μCi/kg followed by a continuous infusion of 0·033 (μCi/kg)/min to maintain a steady plasma concentration. The loading dose of aniline was either 10 mg/kg or 6 μg/kg and was followed by a continuous infusion of either 170 (μg/kg)/min or 0·1 (μg/kg)/minute. The [^{14}C]-aniline content of arterial blood and gastric perfusate was determined by the method of Main & Whittle (1973a).

Clearance was calculated as the ratio of the gastric output to blood concentration of [^{14}C]-aniline. When clearance was expressed as percentage of basal (the basal value being the mean of the three values preceding stimulation), the results were similar with both the high and the low doses of aniline.

Statistical analysis

Results are shown as the mean $\pm$ standard error of the mean, where (n) is the number of values in the group. The significance of data was evaluated by Student's t test or paired data t test where appropriate. $P<0.05$ was taken as significant.

Drugs

Aniline hydrochloride, histamine acid phosphate (BDH, doses expressed as base); pentagastrin (Peptavlon, ICI); [^{14}C]-aniline hydrogen sulphate (50 mCi/mmol, Radiochemical Centre). Prostaglandins E_1, E_2, A_1 and A_2 were kindly supplied by Dr. J. E. Pike, Upjohn Company, Kalamazoo, U.S.A.

Results

Effects of prostaglandins during pentagastrin and histamine stimulation

Investigations were carried out on groups of four rats and in all cases the experimental sequence was identical. After a steady submaximal response to pentagastrin (0·33 (μg/kg)/min) or histamine (33 (μg/kg)/min) had been established, prostaglandin was infused for 40 minutes.

Prostaglandins E_1 and E_2

During the first 20 min period of prostaglandin E_2 administration (2·0 (μg/kg)/min), there was a significant inhibition of pentagastrin-induced acid secretion ($P<0{\cdot}02$ paired t test) but no significant change in clearance (Figure 1). In two experiments, where the level of secretion was relatively low, inhibition was

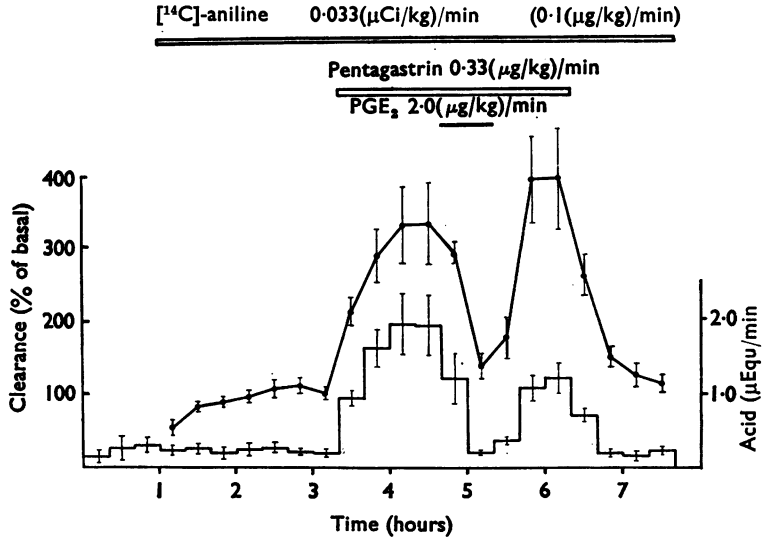

FIG. 1. Effects of prostaglandin E_2 (PGE_2) (2·0 (μg/kg)/min) on [^{14}C]-aniline clearance (upper curve) and acid secretion (lower curve) during pentagastrin (0·33 (μg/kg)/min) stimulation in the anaesthetized rat. Results expressed as the mean ± S.E. of four experiments.

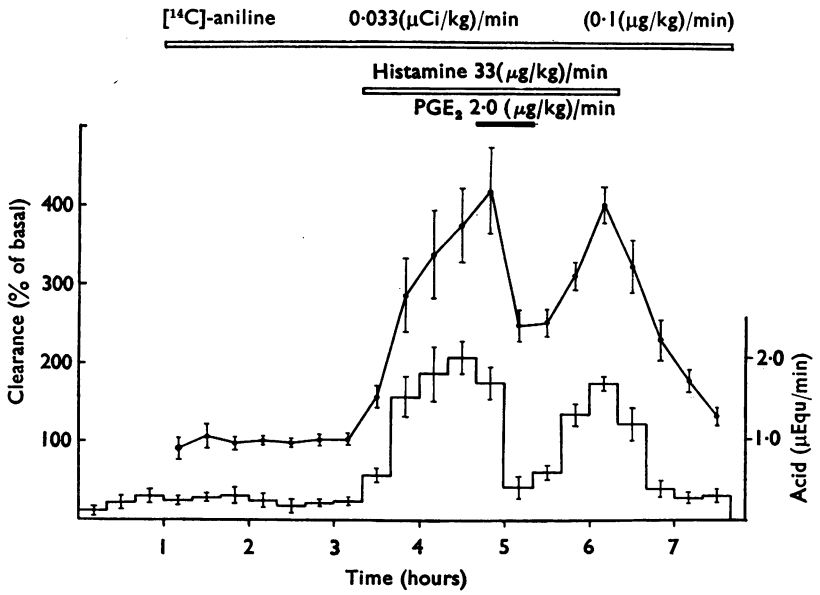

FIG. 2. Effects of prostaglandin E_2 (PGE_2) (2·0 (μg/kg)/min) on clearance (upper curve) and acid secretion (lower curve) during histamine (33(μg/kg)/min) stimulation. Results expressed as the mean ± S.E. of four experiments.

accompanied by an increase in clearance. The effects of prostaglandin E_2 during histamine stimulation are shown in Figure 2. The initial period of prostaglandin E_2 infusion again caused a significant fall in acid secretion ($P<0.05$) but in all four experiments clearance increased ($P<0.02$).

During the second 20 min period of prostaglandin E_2 administration, almost complete inhibition of the secretory response and a fall in clearance was observed in all experiments.

When the prostaglandin infusion was terminated, clearance rose to a level higher than that prior to prostaglandin administration at a time when acid secretion had not completely recovered (Figures 1 and 2).

The effects of prostaglandin E_1 (2·0 (μg/kg)/min) on clearance and acid secretion were found to be qualitatively and quantitatively similar to those of prostaglandin E_2.

Prostaglandins A_1 and A_2

In the first 20 min period of infusion, prostaglandins A_1 and A_2 (4·0 (μg/kg)/min) inhibited pentagastrin-stimulated acid secretion ($P<0.01$ for both) but increased clearance ($P<0.001$, $P<0.05$, respectively). During the second period, although acid secretion was inhibited by a mean of 59·5% for prostaglandin A_1, and 45·9% for A_2, clearance was not significantly different from the pre-inhibition plateau and only fell when the prostaglandin infusion was ended (Figure 3).

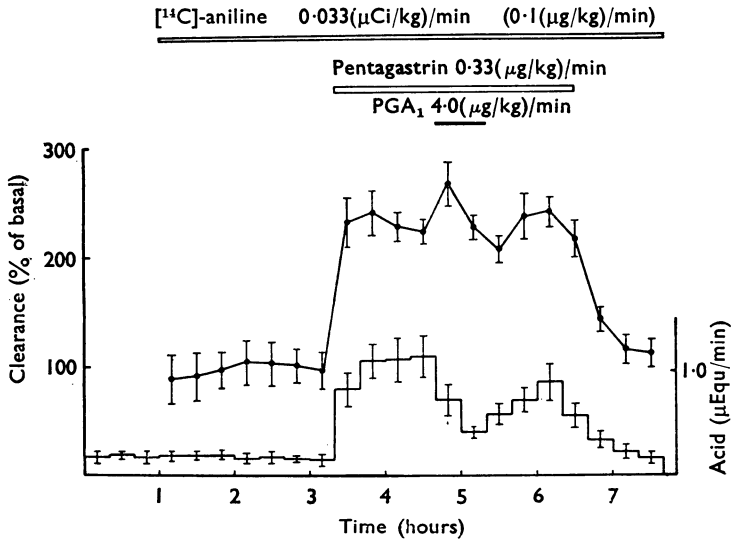

FIG. 3. Effects of prostaglandin A_1 (PGA_1) (4·0 (μg/kg)/min) on clearance (upper curve) and acid secretion (lower curve) during pentagastrin (0·33 (μg/kg)/min) stimulation. Results expressed as the mean ± S.E. of four experiments.

Prostaglandin A_1 (8·0 (μg/kg)/min) caused almost complete inhibition of pentagastrin-stimulated secretion accompanied by a fall in clearance in the second period. Thus, prostaglandins of both the A and E series, in doses which produced a comparable degree of secretory inhibition, had similar effects on clearance.

Effects of prostaglandins on the ratio of clearance to acid output ($R_{c/a}$)

As reported previously (Main & Whittle, 1973a) the ratio of clearance to acid output ($R_{c/a}$) fell during the initial period of pentagastrin or histamine administration to a value which remained constant throughout the rising phase and plateau of the secretory response. Figure 4 illustrates that, at a time when both clearance and acid secretion had been reduced by prostaglandin E_2, $R_{c/a}$ values were significantly increased ($P<0.001$ for both groups).

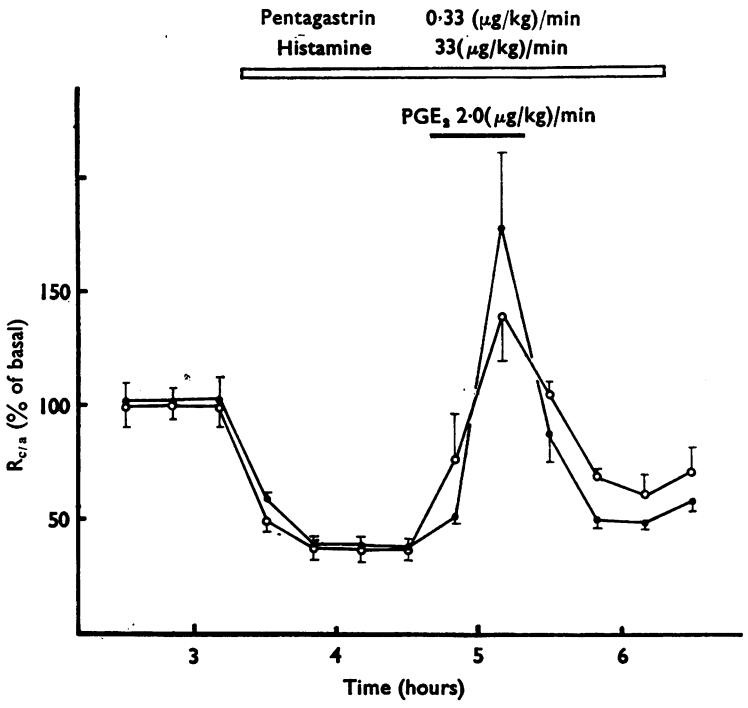

FIG. 4. Effects of prostaglandin E_2 (PGE_2) (2·0 (μg/kg)/min) on the ratio of clearance to acid output ($R_{c/a}$) during inhibition of pentagastrin (○——○) (0·33 (μg/kg)/min) and histamine (●——●) (33 (μg/kg)/min) stimulation. Results are expressed as the mean ± S.E. of four experiments for each series.

A significant increase ($P<0.001$) in $R_{c/a}$ was also observed during inhibition of pentagastrin-induced secretion by prostaglandins E_1, A_1 and A_2 (Table 1).

TABLE 1. *Ratio of clearance to acid output ($R_{c/a}$) during pentagastrin (0·33 (μg/kg)/min) administration (a) before, (b) during the second 20 min period of prostaglandin infusion and (c) after prostaglandin infusion.*

Drugs	PGE_2 2·0 (μg/kg)/min	PGE_1 2·0 (μg/kg)/min	PGA_2 4·0 (μg/kg)/min	PGA_1 4·0 (μg/kg)/min	PGA_1 8·0 (μg/kg)/min
(a) Pentagastrin	40±6	49±8	38±3	38±8	41±6
(b) Pentagastrin + Prostaglandin	140±21	180±13	78±6	87±19	180±38
(c) Pentagastrin	59±9	63±7	73±2	60±10	55±11

Results are expressed as a percentage of the basal value (100%) and shown as the mean±S.E. of four experiments.

Effects of prostaglandins on resting clearance

Since the administration of antisecretory prostaglandins would abolish the pH gradient across the mucosa and hence make clearance measurements invalid, it was necessary to perfuse acidic saline (pH 2) through the gastric lumen. This procedure did not significantly alter resting clearance. Prostaglandins, in doses used to inhibit acid secretion, increased resting clearance ($P<0.001$ in all series) as shown in Table 2. The magnitude of this effect was proportional to the initial fall in systemic arterial blood pressure, prostaglandins of the E series being the more potent.

TABLE 2. *Comparison of effects of prostaglandins on resting clearance (taken as 100%) and systemic arterial blood pressure.*

	PGE_1 2·0 (μg/kg)/min	PGE_2 2·0 (μg/kg)/min	PGA_1 4·0 (μg/kg)/min	PGA_2 4·0 (μg/kg)/min
Clearance (% of basal)	251±35	235±41	167±13	145±5
Initial fall in B.P. (mmHg)	36±6	34±3	25±4	23±3

Values expressed as the mean±S.E. of four experiments.

Discussion

Vasoactive drugs which inhibit gastric secretion may act either directly on the secretory mechanism, or by a primary reduction in MBF which limits the process of secretion. The latter effect, which could be brought about by a direct constriction of mucosal blood vessels, by shunting blood away from the mucosa or by a fall in blood pressure causing a reduction in perfusion pressure, might result in ischaemic damage to the mucosa. The measurement of MBF in the anaesthetized rat by the [^{14}C]-aniline clearance technique (Main & Whittle, 1973a) provides a satisfactory and economical means of investigating the mode of action of vasoactive antisecretory drugs of potential clinical value.

Prostaglandins of the E and A series are potent vasodilators in most vascular beds, and in doses which inhibit gastric secretion in dog and rat cause a fall in arterial blood pressure. In the dog, Wilson & Levine (1969) and Jacobson (1970) showed that prostaglandin E_1 reduced MBF (as measured by aminopyrine clearance) an effect which the former authors considered to be responsible for the concurrent secretory inhibition. In the rat, prostaglandins E_1 and E_2 likewise caused a fall in clearance during inhibition of acid secretion although in some experiments clearance rose initially at a time when acid output was already decreasing. It is therefore unlikely that the inhibitory action on acid secretion is mediated by a fall in MBF.

Theoretically, drugs which inhibit acid secretion by a primary effect on the microcirculation should reduce the ratio of clearance to acid ($R_{c/a}$) whereas direct inhibition should result in unchanged or increased values. In the dog, the ratio of clearance to volume secretion (R) did not change during inhibition of pentagastrin-induced secretion by prostaglandin E_1, but rose significantly during inhibition of histamine stimulation (Jacobson, 1970). More recently, Wilson & Levine (1972) also reported an increase in R during inhibition by prostaglandin E_1 of maximum histamine stimulation in the dog, although an initial fall in R was observed.

From the results of Jacobson (1970) it can be shown that $R_{c/a}$ also rose during prostaglandin E_1 inhibition of both stimuli, lending further support to his conclusion that inhibition cannot be secondary to a reduction in MBF. In the rat, $R_{c/a}$ increased markedly during inhibition of pentagastrin- and histamine-induced secretion by prostaglandin E_1 and E_2. This increase is incompatible with a vasoconstrictor action or primary reduction in MBF.

Prostaglandins of the A series share the vasodilator actions of the E series, but lack many of their metabolic and smooth muscle stimulatory or inhibitory properties. Nevertheless, prostaglandin A_1 is as potent an inhibitor of food-induced secretion as prostaglandin E_1 in the dog (Robert *et al.*, 1967). In the rat, both prostaglandins A_1 and A_2 inhibit pentagastrin- or histamine-stimulated acid secretion (Main, 1973) although as the present results show, they are less potent than prostaglandins E_1 or E_2. During the initial period of prostaglandin A_1 or A_2 administration, acid output was significantly reduced whereas clearance was increased (Fig. 3) as seen in some experiments with prostaglandins E_1 and E_2. In another study (Main & Whittle, 1973b) orally perfused prostaglandins E_1 and E_2 also caused an increase in clearance although pentagastrin-induced acid secretion was inhibited. The results indicate that these prostaglandins are capable of maintaining or increasing MBF at a time when the vasodilator drive associated with the secretory process is reduced. Under basal conditions, where direct vasodilator effects on the mucosal circulation can be more readily demonstrated, prostaglandins of the E and A series caused a significant increase in clearance (Table 2).

These findings, together with the previously reported increase in prostaglandin release from the rat mucosa during stimulation (Ramwell & Shaw, 1968) raise the possibility that prostaglandins may have a physiological role, not only as negative feedback inhibitors of acid secretion, but also as mediators of functional vasodilatation in the gastric mucosa. This hypothesis would receive further support if drugs which antagonize the actions, or inhibit the synthesis or release of prostaglandins, reduce $R_{c/a}$ values and MBF. It is of interest that indomethacin, a potent inhibitor of prostaglandin synthesis (Vane, 1971) which increases pentagastrin-stimulated secretion, reduces basal mucosal blood flow without altering acid secretion (Main & Whittle, 1973c). Reduction of mucosal prostaglandin synthesis by aspirin-like drugs has been implicated in the formation of gastric erosions associated with the use of these anti-inflammatory drugs (Vane, 1971). Studies on both acid secretion and MBF may help to identify the mechanism underlying these clinically important toxic effects (Augur, 1970; Main & Whittle, 1973c). It is of interest to note that aminopyrine, used to measure MBF in the dog, shares many of the pharmacological properties of aspirin-like drugs. Although aminopyrine did not alter the volume of secretion or hydrogen-ion concentration in the studies of Jacobson, Linford & Grossman (1966), it has been found, like aspirin, to promote back diffusion of hydrogen ions from the gastric lumen (Rudick, Werther, Chapman, Dreiling & Janowitz, 1971). The possibility that aminopyrine inhibits mucosal prostaglandin synthesis has not yet been entirely excluded.

Prostaglandins are of potential value in the treatment of peptic ulceration. In the rat, they inhibit gastric and duodenal ulcer formation induced by a variety of methods (Robert *et al.*, 1968; Robert, Stowe & Nezamis, 1971), an effect which may be related to the inhibition of acid secretion. In man, orally administered

prostaglandin E_1 did not inhibit pentagastrin-stimulated secretion, at least in doses which had marked effects on gastrointestinal motility (Horton, Main, Thompson & Wright, 1968). However, parenterally injected prostaglandin E_1 (0·17–0·23 (μg/kg)/min) caused 61% inhibition of pentagastrin-induced secretion (Classen *et al.*, 1971) and prostaglandin A_1 (0·5–1·25 (μg/kg)/min) caused a 25% reduction in histamine-stimulated secretion (Wilson, Phillips & Levine, 1971). Though few side effects were observed, higher doses of prostaglandins which would be required to inhibit secretion completely would cause pronounced cardiovascular changes including a fall in blood pressure (Carlson, Ekelund & Orö, 1969).

It is apparent, therefore, that if prostaglandins are to be used clinically, an analogue which is orally effective and has a more selective action on gastric secretion is required. Although our results in the rat with naturally-occurring prostaglandins suggest a parallelism between their potency in causing inhibition of acid secretion, mucosal vasodilatation and a fall in blood pressure, the demonstration that their effects on the microcirculation and acid secretion are not causally related emphasizes that the search should continue for a suitable prostaglandin. The 15-methyl and 16,16-dimethyl analogues of prostaglandin E_2 which are orally effective and have a longer duration of action (Robert & Magerlein, 1973 ; Karim, Carter, Bhana & Ganesan, 1973) may prove to be of clinical value.

B. J. R. W. is an MRC scholar.

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