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POTENCY AND SELECTIVITY OF METHYL ANALOGUES OF PROSTAGLANDIN E_2 ON RAT GASTROINTESTINAL FUNCTION

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1 The potency and selectivity of action of prostaglandin E_2 and its (15S)- or (15R)-15 methyl and 16,16 dimethyl analogues on gastrointestinal function have been studied in the rat.

2 The (15S)-15 methyl and 16,16 dimethyl analogues were 40 times as active as prostaglandin E_2 in inhibiting pentagastrin-stimulated acid secretion on intravenous administration to the anaesthetized rat, and 100 times as active on subcutaneous injection to the chronic fistula rat.

3 In antisecretory doses, the analogues, like prostaglandin E_2, caused bile reflux and, in higher doses, profuse diarrhoea.

4 The (15S)-15 methyl and 16,16 dimethyl analogues were at least 30 times as active as prostaglandin E_2 in causing changes in intestinal intraluminal pressure *in vivo*, but were equipotent on isolated smooth muscle.

5 In equivalent antisecretory doses, the methyl analogues had little effect on systemic arterial blood pressure and resting mucosal blood flow compared with prostaglandin E_2.

6 The (15R) methyl epimer administered parenterally had little effect on gastrointestinal function but brief acid incubation greatly increased its activity.

Introduction

Naturally-occurring prostaglandins of the E series inhibit gastric acid secretion in man (Classen, Koch, Bickhardt, Topf & Demling, 1971) and inhibit acid secretion and ulcer formation in animals (Robert, Nezamis & Phillips, 1968). However, their clinical usefulness is limited by side effects including cardiovascular effects and gastrointestinal motility changes (Classen *et al.*, 1971) and by lack of antisecretory effects when administered orally (Horton, Main, Thompson & Wright, 1968). Recently, orally administered methyl analogues of prostaglandin E_2 (15-methyl and 16,16 dimethyl prostaglandin E_2) have been shown to inhibit gastric acid secretion in man (Karim, Carter, Bhana & Ganasan, 1973a, b; Robert, Nylander & Andersson, 1974). We have therefore compared the potency and selectivity of action of these analogues with the parent prostaglandin on several aspects of gastrointestinal function.

A preliminary report of this work has been presented to the British Pharmacological Society (Main & Whittle, 1974a).

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Methods

Gastric acid secretion in the anaesthetized rat

The gastric lumen of the urethane-anaesthetized rat (200-250g body weight) was perfused with 0.9% w/v NaCl solution (saline, 0.2 ml/min) as previously described (Main & Whittle, 1973a). The acid output was determined in 0.5 ml aliquots of perfusate, collected at 20 min intervals, by titration to pH 7 with 0.01 M NaOH using an automatic titrator apparatus (Radiometer, Copenhagen), and expressed as output, μEquivalents/minute.

Gastric acid secretion in the unanaesthetized rat

The gastric lumen of the unanaesthetized rat was perfused with saline by a method similar to that described by Borella & Herr (1971). Following midline laparotomy in a pentobarbitone-anaesthetized (40 mg/kg s.c.) female rat, thin walled stainless-steel cannulae were implanted in the stomach, one in the pyloric antrum and one in the forestomach. The pyloric cannula was 15 mm long and 4.8 mm in diameter. The intra-abdominal portion had a 5 mm long polythene tip with a groove 1.5 mm from the end, into which the outer layer of gastric muscle was attached by a

purse-string suture. Two close-fitting rings (15 mm diameter) of small-mesh nylon gauze were attached to the cannula, one at the gastric wall and the other to a groove 6 mm from the internal end. Connective tissue which formed on and between the gauze rings provided support and allowed only one third of the cannula to protrude externally. An external stainless-steel washer attached to a groove 4 mm from the outer end prevented the cannula from moving inwards. The cannula in the forestomach had a diameter of 2.5 mm but was similar to the pyloric cannula in all other respects.

At least one month after surgery, a chronic fistula rat which had been starved for 18 h was placed in a Bollman-type restraining cage. After connecting the smaller cannula to a rubber catheter passed through adjustable slits in the cage floor and flushing the gastric lumen with warm saline to clear food particles, saline was perfused at 0.2 ml/min and the perfusate collected from the large cannula at 10 min intervals. Drugs were administered subcutaneously, or intravenously via a needle inserted into the tail vein.

Gastric mucosal blood flow

The [^{14}C]-aniline clearance technique was used to measure mucosal blood flow in the anaesthetized rat (Main & Whittle, 1973a). [^{14}C]-aniline was infused (0.033 μCi kg^{-1} min^{-1}, i.v.) and the gastric output and blood concentrations determined as previously described. The ratio of these values (clearance) gives an estimation of mucosal blood flow, which was expressed as % of basal values. Systemic arterial blood pressure was recorded and blood samples removed via a cannula inserted into a carotid artery. Drugs were administered through a cannula in a femoral vein.

Diarrhoea

Rats (190-230 g) were starved for 18 h in individual cages but allowed water. Following subcutaneous administration of the prostaglandin or saline, the number of rats in each group (each having at least 5 rats) exhibiting a mucoid diarrhoea within 1 h was noted. In control experiments, no wet faecal pellets or diarrhoea were observed.

Intestinal motility

Changes in intraluminal pressure *in vivo* were recorded from segments of the small intestine. Thin water-filled rubber balloons made from latex solution (Gerrard & Haig Ltd, Sussex), attached to polythene catheters, were introduced into the intestinal lumen via a small incision and tied in place avoiding large blood vessels. Pressure changes were recorded on a Devices chart recorder (M2R) via a Statham pressure transducer (P23Db).

Isolated intestinal segments were suspended in oxygenated Tyrode solution at 37°C and contractions recorded isometrically via a Grass force displacement transducer (FTO3C).

Drugs

Prostaglandin E_2, (15S) or (15R)-15 methyl prostaglandin E_2 methyl ester and 16,16 dimethyl prostaglandin E_2 were dissolved in redistilled methanol and stored under nitrogen at −15°C. Aqueous solutions were prepared freshly when required after evaporation of the methanol, and adjusted to pH 6 with sodium bicarbonate before use.

Results

Inhibition of gastric acid secretion

Steady rates of submaximal gastric acid secretion were obtained in the urethane-anaesthetized rat after 60-80 min of an infusion of pentagastrin (0.3 μg kg^{-1} min^{-1} i.v.). From preliminary experiments, the nature of the dose-response relationship of the prostaglandins in inhibiting acid output was determined. Near-maximal inhibition of acid secretion was obtained when prostaglandin E_2 (2 μg kg^{-1} min^{-1}) was infused intravenously for 40 min (Figure 1). A similar degree of inhibition, but of longer duration was obtained with a 40 min intravenous infusion of much lower doses of (15S)-15 methyl prostaglandin E_2 or 16,16 dimethyl prostaglandin E_2, 0.05 μg kg^{-1} min^{-1} (Figure 1). In further experiments, the same total dose of the analogues (2 μg/kg) gave a similar inhibition when infused over 20 min (Figure 2). From the results shown in Figures 1 and 2, it can be seen that the (15S) and 16,16 dimethyl analogues were at least 40 times more active than the parent prostaglandin as inhibitors of acid secretion. In contrast, the epimer (15R)-15 methyl prostaglandin E_2 caused only 60% inhibition of pentagastrin-stimulated acid secretion (from 1.29 ± 0.13 to 0.52 ± 0.1 μEquiv/min; n = 3) when infused for 40 min at a dose of 40 μg kg^{-1} min^{-1}. Thus it was at least 20 times less active than prostaglandin E_2 and 800 times less active than the (15S) analogue.

The effects of subcutaneous administration of the methyl analogues in the chronic fistula rat during resting acid secretion (2.8 ± 0.2 μEquiv/min, n = 50) are shown in Table 1. After allowing at least 90 min of perfusion for acid secretion to reach steady levels, three control collections were

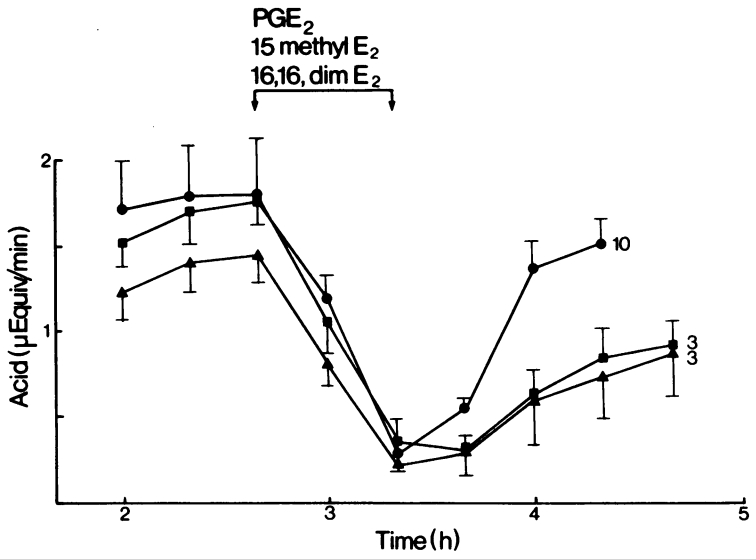

Figure 1 Effect of intravenously infused prostaglandin E_2 2 μg kg^{-1} min^{-1} (PGE_2) (●) (15S)-15 methyl prostaglandin E_2 0.05 μg kg^{-1} min^{-1} (■) and 16,16 dimethyl prostaglandin E_2 0.05 μg kg^{-1} min^{-1} (▲) on pentagastrin-stimulated gastric acid secretion in the anaesthetized rat. Results are the mean of the number of experiments indicated on each curve. Vertical lines show s.e. mean.

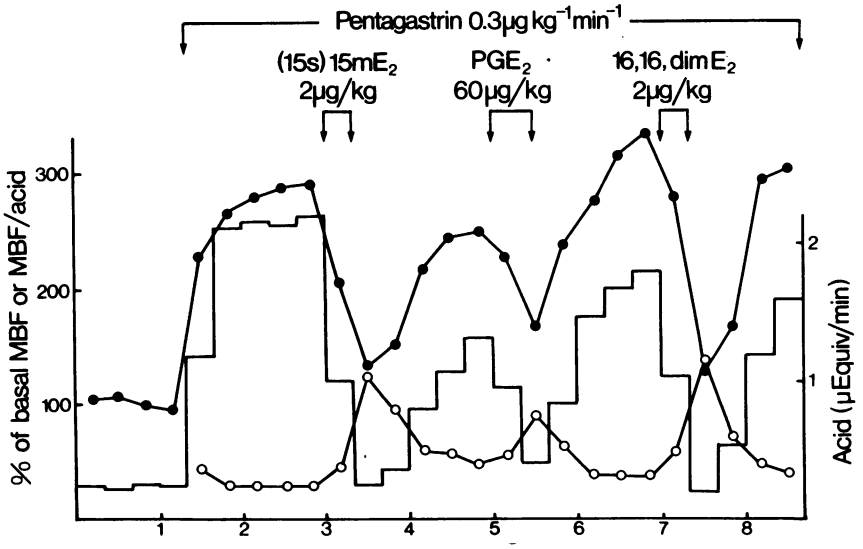

Figure 2 Effects of prostaglandin E_2 and methyl analogues on gastric acid secretion (⌐¬), mucosal blood flow (MBF ●) and the ratio of MBF to acid (○) during pentagastrin stimulation in the anaesthetized rat.

made prior to injection of saline or the prostaglandin (0.2-0.4 ml s.c.). The results in Table 1 were observed 20 min after injection and are expressed as % inhibition of resting acid output (the mean of the three control periods). The (15S) and 16,16 dimethyl analogues were approximately 100 times as active as prostaglandin E_2 when given subcutaneously. In two other experiments, the methyl analogues administered by intravenous injection had comparable effects.

In several experiments with high doses of prostaglandins, the gastric perfusate became alkaline (pH > 7). Furthermore, the clear perfusate became tinged yellow and the intensity and duration of this bile reflux was related to the dose of prostaglandin. The percentage of animals in each group in which bile reflux occurred is shown in Table 1.

The epimer, (15R)-15 methyl prostaglandin E_2 had only a slight inhibitory effect on acid output in subcutaneous doses of up to 4 mg/kg. However, after incubating this epimer at room temperature at pH 2 following the addition of 0.1 N HCl and readjusting to pH 6 with 0.1 N NaOH after 10 min, far smaller doses (10-25 μg/kg s.c.) caused a marked inhibition of acid secretion which was accompanied by bile reflux (Table 1) and diarrhoea.

Effects on gastric mucosal blood flow

Changes in mucosal blood flow ([^{14}C]-aniline clearance) during the stimulation and inhibition of gastric acid secretion are shown in Figure 2. Mucosal blood flow, which increased to a plateau during submaximal acid secretion stimulated by

Table 1 Effect of subcutaneously administered prostaglandin E_2 and its methyl analogues on gastric acid secretion and bile reflux in the conscious fistula rat

	Dose (μg/kg)	n	*Acid secretion (% inhibition)*	*Bile reflux (% incidence)*
Prostaglandin E_2	250	5	84 ± 7	100
	125	5	45 ± 13	80
(15S)-15 methyl E_2	10	3	82 ± 18	100
	2.5	5	80 ± 9	100
	1.25	5	46 ± 17	80
16,16 dimethyl E_2	2.5	5	84 ± 10	100
	1.25	6	35 ± 11	100
	0.625	4	19 ± 14	25
(15R)-15 methyl E_2	2500	4	11 ± 8	25
– after acid incubation	25	5	81 ± 19	100

Results, obtained from a colony of 12 rats used at intervals of at least one week, are expressed as mean with s.e., where *n* is the number of experiments.

Table 2 Ratio of mucosal blood flow (MBF) to acid secretion during pentagastrin-stimulated acid secretion, before and during administration of prostaglandin E_2 and its methyl analogues

		MBF/acid (% of basal)		
	Dose ($\mu g\ kg^{-1}\ min^{-1}$)	*Pentagastrin*	*Pentagastrin + prostaglandin*	n
Prostaglandin E_2	2.0	40 ± 6	140 ± 21**	4
(15S)-15 methyl E_2	0.05	33 ± 3	106 ± 24**	3
16,16 dimethyl E_2	0.05	39 ± 4	95 ± 26*	3
(15R)-15 methyl E_2	40	39 ± 8	68 ± 14*	3

Results are expressed as mean with s.e. where *n* is number of experiments.
* $P < 0.05$, ** $P < 0.01$ (paired data, Student's *t* test)

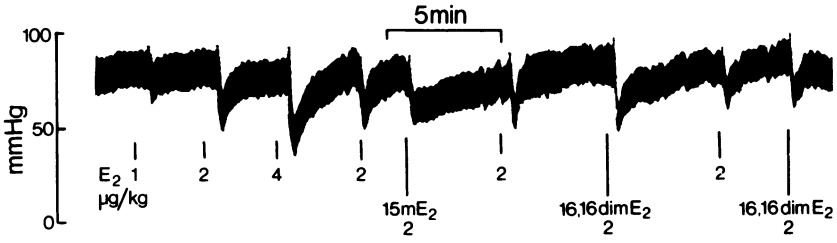

Figure 3 Effect of intravenous injection of prostaglandin E_2 (PGE_2), (15S)-15 methyl and 16,16 dimethyl analogues on systemic arterial blood pressure in the rat.

pentagastrin (0.33 $\mu g\ kg^{-1}\ min^{-1}$), fell during the inhibition of secretion by the prostaglandins. However, the ratio of mucosal blood flow to acid secretion, which reached steady values during pentagastrin stimulation (Figure 2), increased significantly during the administration of each prostaglandin (Table 2) indicating a primary inhibition of acid secretion.

During resting acid secretion and mucosal blood flow (acid saline, pH 2, being perfused to ensure adequate trapping of [^{14}C]-aniline) prostaglandin E_2 in a submaximal antisecretory dose (2 $\mu g\ kg^{-1}\ min^{-1}$ i.v.) caused a rise in mucosal blood flow (Table 3). The (15S)-15 methyl and 16,16 dimethyl analogues in doses sufficient to cause complete inhibition of maximally-stimulated acid secretion (0.2 $\mu g\ kg^{-1}\ min^{-1}$ i.v.) also caused an increase in resting mucosal blood flow, though of a lesser magnitude (Table 3).

Effects on systemic arterial blood pressure (BP)

Intravenous administration of an antisecretory dose of prostaglandin E_2 (2 $\mu g\ kg^{-1}\ min^{-1}$) caused a marked fall in BP which was maintained during the 40 min of infusion. In contrast, equivalent antisecretory doses of (15S)-15 methyl prostaglandin E_2 (0.05 $\mu g\ kg^{-1}\ min^{-1}$) caused only a small and transient fall in BP (Table 3). In four out of six experiments with 16,16 dimethyl prostaglandin E_2 there was little initial effect although BP subsequently rose gradually throughout the infusion. Intravenous infusion of the (15R) epimer (40 $\mu g\ kg^{-1}\ min^{-1}$) had no effect on BP (three observations).

The vasodepressor activities of the prostaglandins were further studied following single intravenous injections in the urethane-anaesthetized rat in six experiments. As is shown in Figure 3 the (15S) and 16,16 methyl analogues were slightly less active than prostaglandin E_2 in lowering BP although the effects were often more prolonged and sometimes showed tachyphylaxis.

Diarrhoea

The incidence of diarrhoea in groups of rats injected with the methyl prostaglandins is shown in Figure 4. The ED_{50} (the dose producing diarrhoea within 1 h in 50% of the animals) was 7 $\mu g/kg$ s.c., for (15S)-15 methyl prostaglandin E_2 and 17 $\mu g/kg$ for 16,16 dimethyl prostaglandin E_2. Because of the large quantity of prostaglandin E_2 required for subcutaneous administration, only

Table 3 Effect of prostaglandin E_2 and its methyl analogues on resting mucosal blood flow (MBF) and systemic arterial blood pressure (BP)

	Dose ($\mu g\ kg^{-1}\ min^{-1}$)	*Initial fall in BP (mmHg)*	*Resting MBF (% of basal)*	n
Prostaglandin E_2	2.0	34 ± 3	235 ± 41	4
(15S)-15 methyl E_2	0.05	8 ± 3	–	3
	0.20	16 ± 6	149 ± 18	6
16,16 dimethyl E_2	0.05	2 ± 6	–	3
	0.20	8 ± 4	136 ± 8	3

Results are the mean with s.e., where *n* is the number of experiments.

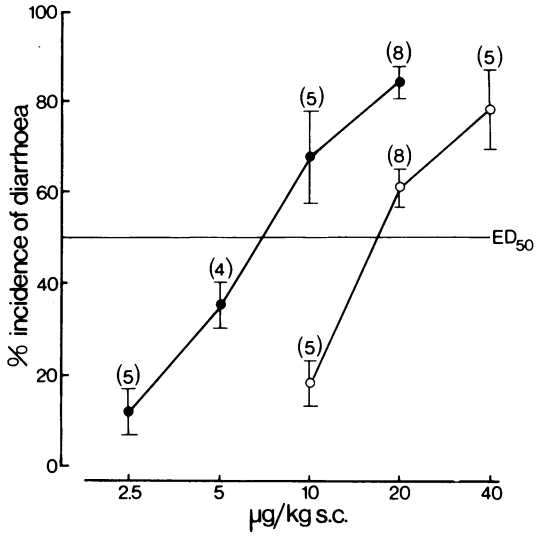

Figure 4 Incidence of diarrhoea in rats 1 h after subcutaneous injections of (15S)-15 methyl prostaglandin E_2 (•) and 16,16 dimethyl prostaglandin E_2 (○). Each point is the mean % incidence in several groups of rats when (*n*) is the number of groups. Vertical lines show s.e. mean.

three groups of rats at two dose levels (500 and 1000 μg/kg) were used; the ED_{50} was 820 μg/kg subcutaneously. The (15R)-15 methyl prostaglandin E_2 in subcutaneous doses of up to 5 mg/kg was inactive (five experiments) but after acid incubation as described above, doses of 10-20 μg/kg produced profuse mucoid diarrhoea (three observations).

Effects on intestinal motility

In a series of eight experiments *in vivo* intravenous administration of prostaglandin E_2 caused reproducible changes in intraluminal pressure of intestinal segments (upper and lower duodenum, jejunum and ileum) (Figure 5). The peak contraction occurred at a time when the fall in BP was returning towards resting levels. As shown in Figure 5a, the rapid rise in intraluminal pressure obtained with prostaglandin E_2 was also observed with the methyl analogues in comparable doses, but was followed by a second prolonged phase of intense motility. With lower doses, only the second phase of activity was observed (Figure 5b). Although comparison of potency with the parent prostaglandin is therefore difficult, these two methyl analogues were more than 30 times as active in inducing changes in intraluminal pressure.

The (15S) and 16,16 dimethyl analogues were approximately equipotent with prostaglandin E_2 on isolated gastrointestinal smooth muscle including rat forestomach strip, guinea-pig ileum and rabbit jejunum, as estimated by bracketing bioassay (two experiments on each tissue).

Discussion

Methylation at C-15 or C-16 protects prostaglandin E_2 from 15-hydroxy-prostaglandin

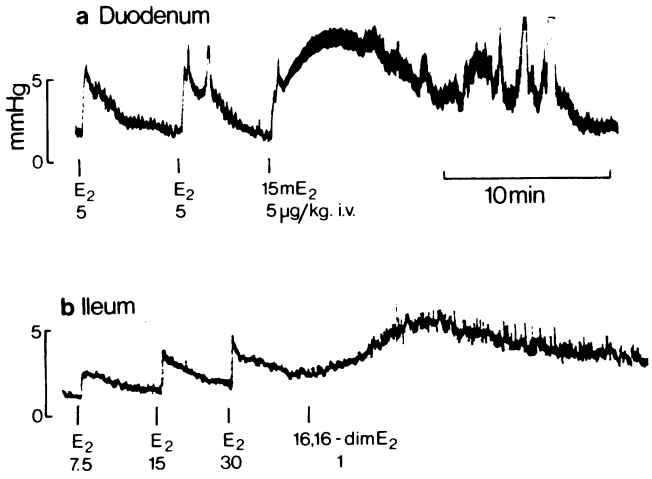

Figure 5 Effect of intravenous injections of prostaglandin E_2 and (a) its (15S)-15 methyl and (b) 16,16 dimethyl analogues on rat intestinal motility *in vivo*.

dehydrogenase (Weeks, Du Charme, Magee & Miller, 1973), an enzyme of prime importance in the metabolism and biological inactivation of prostaglandins (Samuelsson, Granström, Gréen & Hamberg, 1971). Thus the oral effectiveness and increased potency of the 15 methyl and 16,16 dimethyl analogues as inhibitors of gastric acid secretion in man (Karim *et al.*, 1973b; Robert *et al.*, 1974) may be due to decreased inactivation during and after absorption. Such an increase in potency does not, in itself, imply a change in the intrinsic activity of these analogues or in their spectrum of pharmacological actions. We therefore compared the potency and selectivity of these analogues with prostaglandin E_2 on several aspects of gastrointestinal function in the rat.

The (15S)-15 methyl and the 16,16 dimethyl analogues, administered intravenously, were approximately 40 times more active than prostaglandin E_2 in inhibiting pentagastrin-stimulated acid secretion in the anaesthetized rat, and their antisecretory effects were markedly prolonged. These results are comparable to those obtained in the Heidenhain-pouch dog after intravenous injection of these analogues (Robert & Magerlein, 1973). When administered subcutaneously to chronic fistulae rats, the methyl analogues were 100 times more potent than prostaglandin E_2 in inhibiting spontaneous secretion. This reduction in potency of prostaglandin E_2 on subcutaneous compared with intravenous injection may reflect metabolism during subcutaneous absorption.

In doses which inhibited gastric acid secretion by 50% or more, prostaglandin E_2 and the analogues caused a high incidence of duodenal reflux as indicated by the presence of bile in the gastric perfusate. Such reflux was unlikely to have contributed significantly to the reduction in acid output, which often returned towards control levels despite the continued presence of bile; moreover, inhibition was sometimes unaccompanied by bile reflux. The mechanism by which prostaglandins cause bile reflux has not yet been fully investigated but is likely to involve changes in duodenal motility and intraluminal pressure, as observed in the present investigation, coupled with changes in pyloric sphincter tone (Bertaccini, Impicciatore & De Caro, 1973). Evacuation of the gall-bladder cannot be a factor contributing to bile reflux in the rat, though it may be following prostaglandin administration in other species including man (Horton *et al.*, 1968).

The increased potency of the analogues in causing inhibition of acid secretion and bile reflux was also accompanied by increased potency on intestinal motility *in vivo* but not *in vitro*. It remains to be determined, whether this increase in potency and the biphasic nature of the intestinal response to the methyl analogues can both be attributed solely to a decreased rate of inactivation. Our results on intestinal smooth muscle *in vitro*, which agree with those of other workers (Weeks *et al.*, 1973; Strand, Miller & McGiff, 1974) who compared the (15S)-15 methyl analogue to prostaglandin E_2, support the suggestion that the increased potency *in vivo* reflects decreased inactivation rather than a change in intrinsic activity.

In contrast, the methyl analogues were no more potent than prostaglandin E_2 as vasodepressors, as found by others (Weeks *et al.*, 1973; Strand *et al.*, 1974) using the (15S) analogue. In equivalent antisecretory doses, administered by intravenous infusion, the analogues had little detectable effect on the cardiovascular system compared with prostaglandin E_2, indicating a change in their selectivity of action. It has been suggested that the lack of increased vasodepressor potency of the methyl analogues may result from inactivation by enzymes other than 15-hydroxy-prostaglandin dehydrogenase (Weeks *et al.*, 1973). This would imply, however, that the resultant metabolites, while inactive on blood pressure, retain potent and long-lasting effects on gastro-intestinal function which we have observed in the present study.

The rise in mucosal blood flow per unit acid secretion during inhibition of secretion by the methyl analogues indicates that the changes in mucosal blood flow are secondary to changes in acid secretion. Furthermore, when acid secretion is completely inhibited by these methyl analogues, the vasodilator secretagogue, histamine, still increases mucosal blood flow (Main & Whittle, 1974b). During resting acid secretion the analogues caused a transient increase in mucosal blood flow indicating a direct vasodilator action on the mucosa, which is also seen with the parent prostaglandin. This agrees with our previous results using prostaglandins of E and A series (Main & Whittle, 1973b), though with the methyl analogues of prostaglandin E_2, there is no longer any parallelism between their effect on acid secretion and mucosal blood flow and their effects on blood pressure. It seems likely that the methyl analogues and the parent prostaglandins have the same mechanism of antisecretory action, possibly involving the adenyl cyclase-cyclic AMP system (Main & Whittle, 1974c).

The diarrhoea observed with low doses of the methyl analogues is likely to result from changes in intestinal motility; altered intestinal water and electrolyte secretion may also contribute to its fluid nature. Comparable effects were observed in other species including cat, guinea-pig and mouse (Main & Whittle, unpublished results). Although both analogues have greatly increased selectivity of

action on gastric secretion compared with blood pressure, the results summarized in Table 4 show that in the rat, the increased potency of the (15S) analogue on acid secretion is associated with a comparable increase in potency on gastro-intestinal motility, as indicated by diarrhoea. The 16,16 dimethyl analogue appears to possess some selectivity of action, but its effects on motility are still very marked in doses which inhibit acid secretion. It is of interest that a high incidence of side effects, including diarrhoea, accompanied the termination of pregnancy by repeated administration of the (15S) and 16,16 dimethyl analogues (Ballard & Quilligan, 1974; Karim, Sivasamboo & Ratnam, 1974) in doses similar to those used to inhibit acid secretion in other studies (Karim *et al.*, 1973a b; 1974; Robert *et al.*, 1974).

The epimer (15R)-15 methyl prostaglandin E_2, is a potent and apparently selective inhibitor of gastric acid secretion in man when administered orally, but is inactive when injected intravenously (Karim *et al.*, 1973b). In the rat, the (15R) epimer is likewise a very weak inhibitor of acid secretion when administered intravenously or subcutaneously, being approximately 1000 times less active than the (15S) analogue. The observed activity, may, however, be accounted for by as little as 0.1% conversion to or contamination with the (15S) epimer. Brief incubation of the (15R) epimer in acid caused a very marked increase in potency on systemic administration, not only in inhibiting acid secretion, but in causing changes in gastrointestinal motility leading to bile reflux and diarrhoea. This result suggests that acid incubation converts the (R) to the (S) epimer and that this process, taking place in the acid environment of the stomach, can account for the oral effectiveness of the (R) epimer.

Our results indicate that, in the rat, the greatly increased potency of 16,16 dimethyl and (15S)-15 methyl prostaglandin E_2 in inhibiting acid secretion is accompanied by little or no increase in selectivity with respect to intestinal motility. If these analogues are to be of value in the treatment of peptic ulcer in man, very careful regulation of the dosage regimen will be required to avoid unacceptable gastro-intestinal effects on prolonged administration. The (15R) epimer, on activation by acid incubation, acquires the range of pharmacological actions characteristic of the (15S) analogue. Its therapeutic value compared with the other analogues will depend, therefore, on its site of action on the gastric mucosa and on the extent to which systemic absorption occurs or is an essential accompaniment to its antisecretory action.

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Table 4 Comparison of the potency of prostaglandin E_2 and its methyl analogues in causing diarrhoea and inhibiting acid secretion

	ED_{50} (μg/kg, s.c.)		
	Diarrhoea	*Acid secretion*	*Selectivity ratio*
Prostaglandin E_2	820	138	5.9
(15S)-15 methyl E_2	6.8	1.4	4.8
16,16 dimethyl E_2	17.0	1.5	11.3

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