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Prostanoid stimulation of anion secretion in guinea-pig gastric and ileal muscosa is mediated by different receptors

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1 The receptors that mediate stimulation of anion secretion by prostanoids in isolated preparations of guinea-pig gastric and ileal mucosa have been compared by use of selective prostanoid agonists and antagonists.

2 In gastric mucosa, the relative potency of agonists suggested that the control of anion secretion in this tissue was complex and may be mediated by EP_2, EP_3, and TP receptors. A role for TP receptors was confirmed with the TP-selective antagonist AH23848 which inhibited short circuit current responses to the TP receptor agonist U-46619 with a pA_2 value of 8.44 but was without effect on responses to prostaglandin E_2 (PGE_2) or the EP selective agonist, sulprostone.

3 In ileal mucosa, the relative potency of agonists differed from that observed in gastric mucosa and was consistent with the view that anion secretion in this region of intestine was controlled by DP and EP_2 receptors.

4 These studies suggest that anion secretion in gastric and ileal mucosa is controlled by different prostanoid receptor subtypes and so provide important informtion for the design of prostanoids which may protect gastric mucosa and that are free from side effects such as diarrhoea.

Introduction

Prostanoids exert many diverse actions in the body, and these effects have been rationalised through the classification of prostanoid receptors. Such classification has been carried out by examining the rank orders of agonist potencies of the natural prostaglandins, and this has resulted in the identification of receptors for thromboxane A_2 (TP receptors), E prostaglandins (EP receptors), F prostaglandins (FP receptors), D prostaglandins (DP receptors) and prostacyclin (IP receptors) (Kennedy *et al.*, 1982). This classification has been consolidated by use of synthetic agonists exhibiting receptor specificity including U-46619 (TP selective), BW245C (DP selective) and fluprostenol (FP selective; Coleman *et al.*, 1985) and selective antagonists such as AH23848 for TP receptors (Brittain *et al.*, 1985). Furthermore studies using the synthetic agonists sulprostone and AY23626 and the antagonists AH6809 and SC19220 have revealed that EP receptors are a heterogeneous population comprising EP_1, EP_2 and EP_3 subtypes (Coleman *et al.*, 1987).

Prostanoids stimulate the gastric secretion of a non-parietal fluid composed mainly of sodium and chloride ions (Miller *et al.*, 1983; Bunce & Clayton, 1987). This non-parietal secretion may be an important component of the mucosal protective properties of prostanoids in the stomach acting to reduce the absorption of noxious substances and diluting them in bulk solution (Moody & Zalewsky, 1981; Thomson, 1984; Pihan & Szabo, 1989). Prostanoids also stimulate electrolyte secretion in the intestine (Racusen & Binder, 1980; Musch *et al.*, 1987) that is responsible for the watery diarrhoea which has tended to limit the clinical use of prostanoids for treatment of peptic ulceration (Brand *et al.*, 1985; Lauritsen *et al.*, 1986).

Using an isolated preparation of guinea-pig gastric mucosa, we have previously demonstrated that gastric non-parietal secretion stimulated by prostaglandin E_2 (PGE_2) is the result of stimulation of electrogenic chloride secretion, a process that is independent of gastric acid secretion (Bunce & Spraggs, 1988). In the present study we have attempted to characterize the receptor types that mediate the secretory effect of prostanoids in gastric mucosa and compare them with the receptors that mediate stimulation of electrogenic anion secretion in the ileal mucosa of this species.

Portions of this work have been communicated to the British Pharmacological Society (Bunce & Spraggs, 1987) and the 13th International Congress of Gastroenterology (Spraggs & Bunce, 1988). These studies suggest that stimulation of electrogenic anion secretion by prostanoids in gastric and ileal mucosa is mediated by different prostanoid receptor types.

Methods

Male guinea-pigs of the Dunkin-Hartley strain weighing 250–350 g were used. For gastric mucosa, guinea-pigs were starved for 18 h to 24 h with free access to water and were anaesthetized by inhalation of a halothane/nitrous oxide/O_2 gas mixture. The muscle layers overlying the fundic (acid-secreting) region of the stomach were removed by a blistering technique as described by Main & Pearce (1978). Two pieces of gastric mucosa were obtained from each stomach and these were mounted in Ussing type chambers (window area 0.8 cm^2).

For ileal mucosa, guinea-pigs with free access to both food and water were killed by cervical dislocation. A 10 cm segment of ileum starting 20 cm from the ileo-caecal junction was removed. Up to four pieces of this segment, each approximately 1.5 cm long were opened along the mesenteric border and pinned, mucosal face down on a wax plate. The overlying muscle layers were removed by dissection with fine forceps and each segment was mounted in an Ussing chamber.

Both preparations were bathed bilaterally with 20 ml of Krebs-Henseleit solution, maintained at 37°C and gassed with 95% O_2:5% CO_2. The composition of this solution (in mM) was: NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 24.8, KH_2PO_4 1.2 and glucose 11.1. Indomethacin (1 μM) was added to the serosal bathing solution to inhibit endogenous prostanoid synthesis.

Tissues were continuously voltage-clamped at zero potential, with compensation for fluid resistance and differences in

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potential between calomel cells using a voltage clamp amplifier (DVC-1000, World Precision Instruments, Connecticut, U.S.A.) and the applied short circuit current (SCC) was continuously recorded as an index of electrogenic anion secretion (Bunce & Spraggs, 1988).

Compounds

Indomethacin (Sigma) was dissolved in 1% $NaHCO_3$ in saline at a concentration of 1 mM. PGE_2 and $PGF_{2\alpha}$ (Upjohn) were diluted for use from stock solutions of 10 mg ml^{-1} and 5 mg ml^{-1} respectively. Fluprostenol (ICI) was diluted from a stock solution of 5 mg ml^{-1}. BW245C (3-(3-cyclohexyl-3-hydroxypropyl)-2,5-heptenoic acid; Wellcome) was dissolved in 1% $NaHCO_3$ at a concentration of 10 mM. AY23626 (11-deoxy PGE_0; Ayerst), 16,16-dimethyl PGE_2 (Cayman), misoprostol (Searle) and enprostil (Syntex) were dissolved in 60% ethanol in distilled water at a concentration of 20 mg ml^{-1}. PGD_2, PGI_2, U-46619 (11,9 epoxymethano PGH_2) and sulprostone were synthesized at Glaxo and dissolved in 60% ethanol in distilled water at a concentration of 20 mg ml^{-1}, except for PGI_2 which was dissolved in Tris buffer (pH 9). AH23848 ([1α(z),2β,5α]-(±)-7-[5-[[(1,1-biphenyl)-4-yl] methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid) and AH6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid) were also synthesized at Glaxo and dissolved in 1% $NaHCO_3$ in saline at a concentration of 10 mM. All drugs (except for PGI_2, which was diluted in Tris buffer, pH 8) were diluted in saline and added to both bathing solutions in volumes of less than 0.6 ml. The vehicles used to dissolve these drugs did not modify SCC when added to bathing solutions at appropriate concentrations.

Expression of results

SCC was recorded as μA cm^{-2} and expressed as arithmetic mean ± s.e.mean. EC_{50} values were expressed as geometric mean with 95% confidence limits.

Preliminary studies showed that gastric mucosa exhibited tachyphylaxis to repeated administration of PGE_2 and therefore concentration-response curves were constructed by addition of single concentrations of agonist to individual mucosa using a randomised block design. Increases in SCC to agonists were measured as changes in SCC from basal values after a contact time of 60 min. For full agonists, with maximal responses not significantly different from that of PGE_2, EC_{50} values were defined as the concentration to produce an increase in SCC of 38 μA cm^{-2}, this being 50% of the maximal response to PGE_2 (77 ± 6 μA cm^{-2}, $n = 6$). For partial agonists EC_{50} values were calculated at 50% of their own maximum. EC_{50} values were determined from pooled data for individual agonists by least squares regression. For full agonists only equieffective molar ratios (EMR) were determined relative to PGE_2 (EMR = 1) by dividing the mean agonist EC_{50} by the EC_{50} value for PGE_2, and these data were used for receptor classification. In experiments where antagonists were tested, these compounds were added to both bathing solutions 30 min prior to the addition of agonist. Where employed, Schild analysis was performed by determination of concentration-ratios by dividing the EC_{50} for the agonist in the presence of antagonist by the EC_{50} value for the agonist alone. Concentration-ratios were then used to calculate pA_2 and Schild slope values from a plot of the Schild equation.

In ileal mucosa, it was possible to construct repeatable cumulative concentration-response curves for PGE_2. Therefore the effects of agonists on SCC in ileal mucosa were compared with the effects of PGE_2 in individual tissues. Increases in SCC (above basal levels) for agonists were expressed as percentages of the control PGE_2 maximal response in the tissue. EC_{50} and EMR values for agonists were determined graphically in individual tissues and subsequently pooled to provide geometric mean and 95% confidence limits. EC_{50} values for full agonists were determined from the PGE_2 maximum as described above for gastric mucosa. Again, EMRs were only calculated for full agonists (PGE_2 = 1).

Results

Effects of prostanoids on gastric mucosa

The naturally occurring prostanoids and the stable thromboxane A_2-mimetic, U-46619 stimulated increases in SCC in gastric mucosa. Two types of SCC response were observed and are illustrated in Figure 1.

PGE_2 (and PGI_2) stimulated a monophasic rise in SCC with an initial rapid increase followed by a slower rise to a plateau which was achieved approximately 30 min after drug addition. In contrast U-46619 (and PGD_2 and $PGF_{2\alpha}$) produced biphasic responses composed of an initial small reduction in SCC and followed by a slow increase to a plateau 60 min after drug addition. Figure 2 shows concentration-response curves for these prostanoids in gastric mucosa and their effects are summarised in Table 1. PGE_2 was the most potent agonist producing a maximum increase in SCC of 77 ± 6 μA cm^{-2} ($n = 6$). U-46619, PGD_2 and $PGF_{2\alpha}$ were full agonists, with maxima not significantly different from PGE_2 ($P > 0.05$, unpaired t test), but were less potent. PGI_2 was a less potent partial agonist with a maximum response significantly less than that for PGE_2 ($P < 0.05$, unpaired t test).

Since PGE_2 was the most potent of these agonists this suggests that EP receptors are involved in the SCC response in gastric mucosa (Kennedy *et al.*, 1982). The involvement of other receptor types was investigated using the TP receptor antagonist AH23848 and the DP and FP selective agonists BW245C and fluprostenol. AH23848 (30 to 300 nM) inhibited SCC responses to U-46619 with a pA_2 value of 8.44 and a Schild slope value of 0.98 (Figure 3). A higher concentration of AH23848 (1 μM) did not modify SCC responses to PGE_2 (data not shown). BW245C was a less potent stimulant of SCC with an EC_{50} value of 348 (97–853) nM ($n = 6$) and fluprostenol produced no changes in SCC at concentrations up to 1 μM ($n = 4$) (Table 1). The low potency of these selective agonists suggests that DP and FP receptors are not involved in the control of SCC in gastric mucosa.

The EP receptor types mediating the increase in SCC in gastric mucosa were investigated further by use of a range of

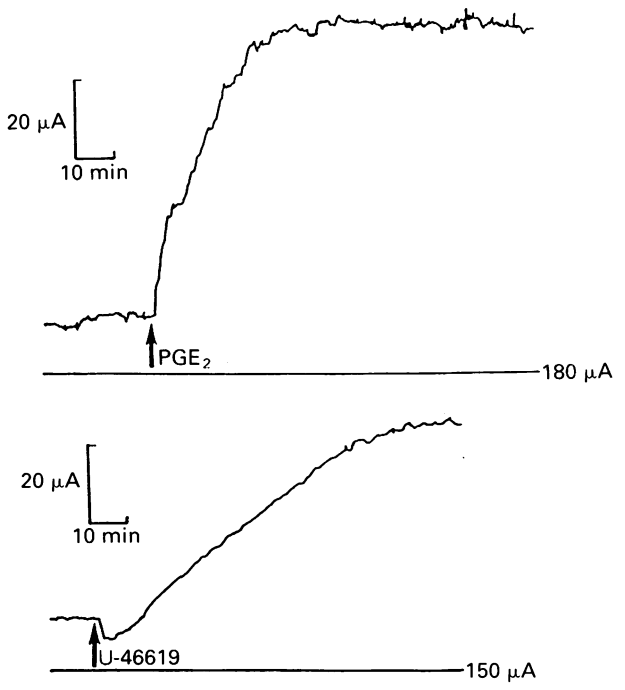

Figure 1 Examples of SCC recordings for prostaglandin E_2 (PGE_2) and U-46619 in guinea-pig isolated gastric mucosa. Records were obtained from 0.8 cm^2 areas of mucosa. Prostanoids were added to both bathing solutions (at arrow) at a concentration of 1 μM. Horizontal lines show SCC values of 180 and 150 μA respectively.

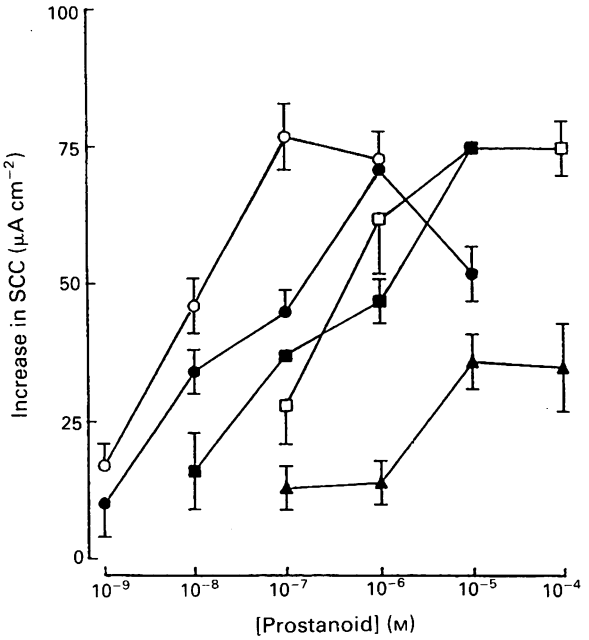

Figure 2 Concentration-response curves for stimulation of SCC by naturally occurring prostanoids and U-46619 in guinea-pig isolated gastric mucosa. Curves were constructed by addition of single concentrations of agonists to individual tissues. Each point is mean, vertical bars represent s.e.mean from 5 to 6 tissues: (○) prostaglandin E_2 (PGE_2); (●) U-46619; (■) PGD_2; (□) $PGF_{2\alpha}$ and (▲) PGI_2.

synthetic PGE analogues; as described above for the naturally occurring prostanoids two types of SCC response were observed. AY23626 and misoprostol produced monophasic rises in SCC, similar to PGE_2, whilst enprostil, 16,16-dimethyl PGE_2 and sulprostone increased SCC in a biphasic manner similar to U-46619. Figure 4 shows concentration-response curves for these agonists in gastric mucosa and their effects are summarised in Table 1. All of the compounds were potent full agonists for stimulation of SCC with equieffective molar ratios (EMR) ranging from 0.4 to 15 (PGE_2 = 1). The possibility that the biphasic responders were acting solely at TP receptors to increase SCC was unlikely since AH23848 (1 μM) did not modify SCC responses to sulprostone (data not shown). In addition sulprostone induced increases in SCC were not inhibited by the EP_1 receptor antagonist AH6809 (10 μM) suggesting that EP_1 receptors did not mediate changes in SCC (data not shown).

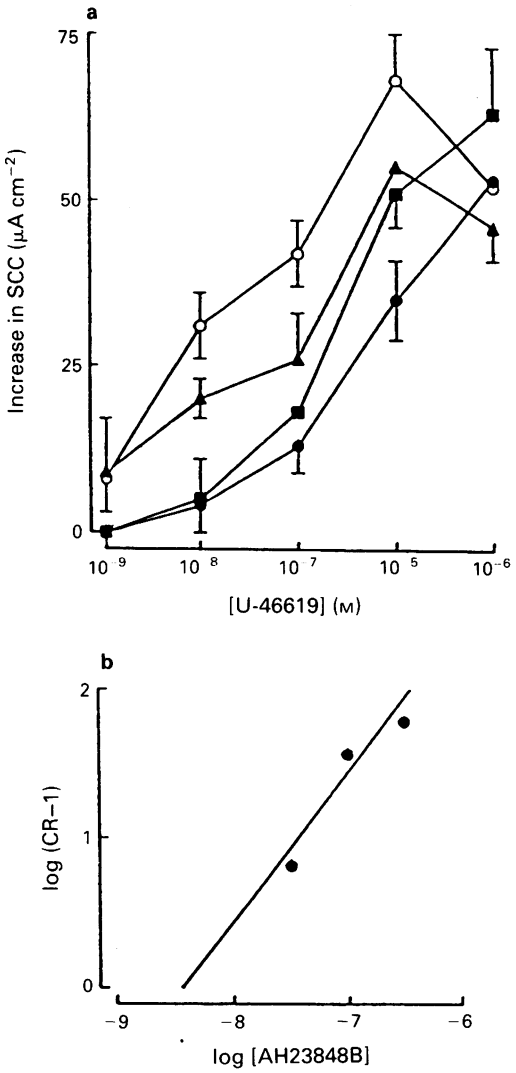

Figure 3 Effect of AH23848 on SCC responses to U-46619 in guinea-pig isolated gastric mucosa. In (a) are shown concentration-response curves for U-46619 alone (○) and in the presence of AH23848, 30 (▲), 100 (■) and 300 nM (●). Concentration-response curves were constructed in the manner described for Figure 2. Each point is the mean, vertical bars represent s.e.mean from 5 to 6 tissues. (b) Schild plot derived from these data. Linear regression analysis produced a pA_2 value of 8.44 and a slope of 0.98.

Table 1 Effects of prostanoid agonists on SCC in guinea-pig isolated gastric mucosa

Agonist	EC_{50}	*EMR* (PGE_2 = 1)	E_{max} (μA cm^{-2})
PGE_2	5 (2–11)	1	77 ± 6
PGD_2	162 (42–523)	31	75 ± 10
$PGF_{2\alpha}$	186 (37–510)	36	75 ± 10
PGI_2	2300 (900–8230)†	ND	36 ± 5*
U-46619	38 (16–96)	7	71 ± 8
BW245C	348 (97–853)	70	47 ± 5 (at 3 μM)‡
Fluprostenol	>1000†	ND	0 ± 0*
AY23626	58 (8–599)	11	65 ± 19
16,16 Dimethyl PGE_2	2 (0.3–19)	0.4	62 ± 4
Enprostil	4 (1–9)	0.8	94 ± 4
Misoprostol	12 (3–65)	2	67 ± 8
Sulprostone	76 (20–293)	15	65 ± 7

EC_{50} (in nM) values are geometric mean (95% confidence limits) and E_{max} values are arithmetic mean ± s.e.mean for 4 to 6 observations at each concentration. Equieffective molar ratios (EMR; PGE_2 = 1) were calculated for full agonists as the ratio of mean EC_{50} values of agonist and PGE_2.

* denotes E_{max} values significantly less than PGE_2 ($P < 0.05$, unpaired t test); for partial agonists EC_{50} values (†) were calculated from their own maximum.

ND denotes that EMR estimates were not determined for these partial agonists.

‡ Maximal response for BW245C was not achieved at the highest concentration tested.

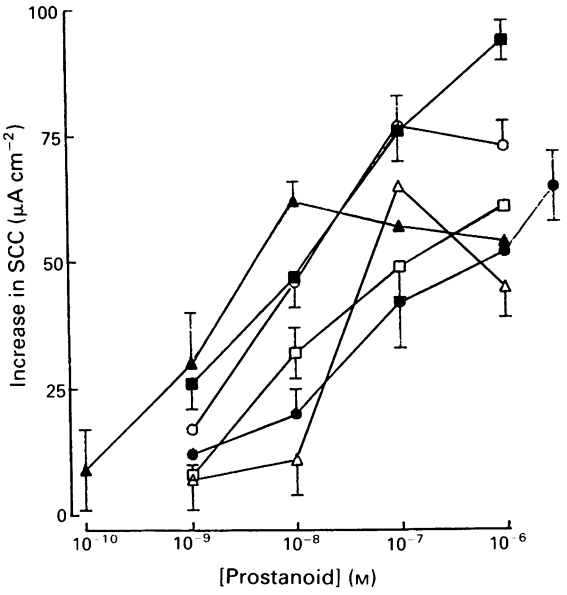

Figure 4 Concentration-response curves for stimulation of SCC by prostaglandin E (PGE) analogues in guinea-pig isolated gastric mucosa. Curves were constructed in the manner described in Figure 2. Each point is mean, vertical bars represent s.e.mean for 6 tissues: (○) PGE_2; (▲) 16,16-dimethyl PGE_2; (■) enprostil; (□) misoprostol; (△) AY23626 and (●) sulprostone.

Effects of prostanoids on ileal mucosa

PGE_2 was a potent stimulant of SCC in ileal mucosa with an EC_{50} value of 18 (5–54) nM and a maximum increase in SCC of $125 \pm 14\,\mu A\,cm^{-2}$ ($n = 6$). This curve could be repeated in tissues after washing with the second PGE_2 concentration-response curve producing an EC_{50} value of 20 (8–49) nM and a maximum increase in SCC of $120 \pm 9\,\mu A\,cm^{-2}$ ($n = 6$) at 100 nM. Therefore test prostanoids were compared with PGE_2 in individual tissues. Figure 5 compares the effects of the naturally occurring prostanoids and U-46619 on SCC and Table 2 summarizes the relative potencies of full agonists in ileal mucosa. In contrast to its activity in gastric mucosa, U-46619 in concentrations up to 1 μM produced a maximum increase in SCC of 14% of the PGE_2 maximum and did not modify subsequent responses to PGE_2 (data not shown). In addition, PGD_2 was 20 times more potent than PGE_2. PGI_2 and $PGF_{2\alpha}$ were only slightly less potent than PGE_2 but displayed non-sigmoid concentration-response relationships. The marked potency of PGD_2 suggested that DP receptors controlled SCC in ileal mucosa and this was confirmed with a selective DP agonist BW245C, which although producing a smaller maximum response than PGE_2 was a potent stimulant of SCC (Table 2).

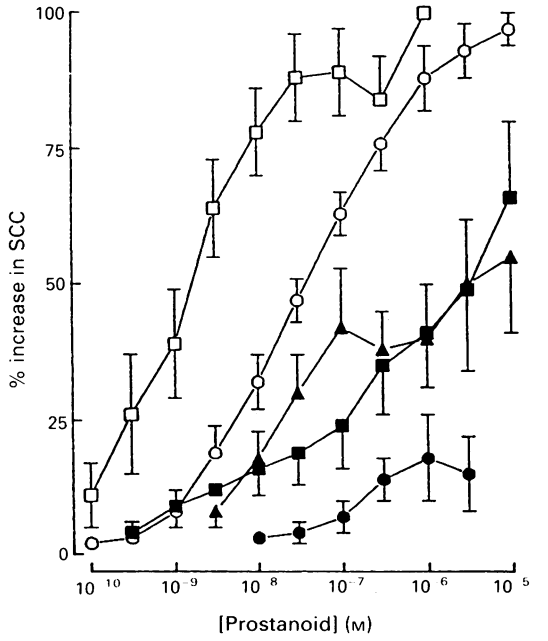

Figure 5 Concentration-response curves for stimulation of SCC by natural prostanoids and U-46619 in guinea-pig isolated ileal mucosa. Data are expressed as percentage of the maximal response to prostaglandin E_2 (PGE_2) in individual tissues. Curves are mean, vertical bars represent s.e.mean from 5 to 6 tissues: (□) PGD_2; (○) PGE_2; (▲) PGI_2; (■) $PGF_{2\alpha}$ and (●) U-46619. Mean EC_{50} values for PGE_2 in this series of experiments ranged from 35 to 57 nM.

The effects of the synthetic PGE analogues on SCC in ileal mucosa are shown in Figure 6. All of these analogues were less potent than PGE_2 and whilst AY23626 and misoprostol were full agonists, enprostil, 16,16-dimethyl PGE_2 and sulprostone were virtually devoid of activity (Table 2, Figure 6). Sulprostone produced no change in SCC and did not display antagonist activity to subsequent additions of PGE_2 at concentrations up to 10 μM (data not shown), showing that it had no measurable affinity for the prostanoid receptor in ileal mucosa.

Discussion

Prostanoids stimulate electrogenic chloride secretion in gastric mucosa and this is reflected by an increase in SCC (Bunce & Spraggs, 1988). In addition it is well established that prostanoid-stimulated anion secretion results in an increase in SCC across ileal mucosa (Musch *et al.*, 1987). In the present study we have attempted to identify the prostanoid receptor types that mediate the stimulation of anion secretion (increases in SCC) in gastric and ileal mucosa. Few selective antagonists for prostanoid receptors exist and receptor classification relies greatly on the relative potencies of selective agonists (Coleman *et al.*, 1985). Receptor classification based on rank order of agonist potency must meet established criteria and may be influenced by factors not related to receptor

Table 2 Effects of prostanoid agonists on SCC in guinea-pig isolated ileal mucosa

Agonist	*EMR* ($PGE_2 = 1$)	E_{max} (%)
PGE_2	1	100
PGD_2	0.05 (0.04–0.08)	98 ± 1
$PGF_{2\alpha}$	ND	66 ± 3 (at 10 μM)‡
PGI_2	ND	55 ± 14 (at 10 μM)‡
U-46619	ND	14 ± 9*
BW245C	ND [EC_{50} = 10 (3–36)]†	65 ± 9*
AY23626	48 (28–86)	94 ± 6
16,16-dimethyl PGE_2	ND	10 ± 8*
Enprostil	ND	15 ± 5 (at 3 μM)‡
Misoprostol	10 (4–23)	80 ± 2
Sulprostone	ND	0 ± 0 (at 10 μM)‡

EMR values are geometric mean (95% confidence limits) calculated from EC_{50} ratios in individual tissues as described in Methods. E_{max} values are % increases in SCC, as arithmetic mean ± s.e.mean in individual experiments, where the maximal response to PGE_2 equalled 100%. Values are means from 4 to 8 tissues. Unlike PGE_2, $PGF_{2\alpha}$ and PGI_2 produced non-sigmoid concentration-response relationships.

* denotes E_{max} values significantly less than PGE_2 ($P < 0.05$, paired t test).

ND denotes that EMR estimates were not determined for these partial agonists.

† EC_{50} value given for BW245C is relative to its maximal response.

‡ Maximal responses for these compounds were not achieved at the highest concentration tested.

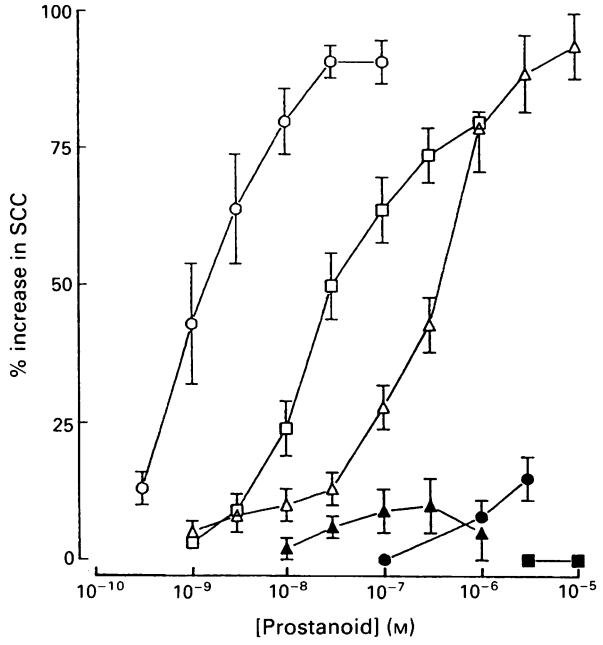

Figure 6 Concentration-response curves for stimulation of SCC by prostaglandin E (PGE) analogues in guinea-pig isolated ileal mucosa. Data are expressed as percentage of the maximal response to PGE_2 in individual tissues. Curves are mean, vertical bars represent s.e.mean from 4 to 8 tissues: (○) PGE_2; (□) misoprostol; (△) AY23626; (▲) 16,16 dimethyl PGE_2; (●) enprostil and (■) sulprostone. Mean EC_{50} values for PGE_2 in this series of experiments ranged from 1 to 30 nM.

differences (Furchgott, 1972). In the present studies some prostanoids were partial agonists or produced concentration-response curves whose slopes differed from PGE_2 and where this occurred, the compounds could not be used to compare responses in the two tissues. Nevertheless, in these studies the different activities of prostanoid agonists in these two tissues were striking and may be suggestive of a number of prostanoid receptor types involved in the control of gastrointestinal secretion.

Tables 1 and 2 show that the relative potencies of the naturally occurring prostanoids and U-46619 for stimulation of SCC in gastric and ileal mucosa are different. Comparison of these with rank orders described for a variety of smooth muscle preparations and platelets (Kennedy *et al.*, 1982) suggests that the effects of prostanoids on SCC may be mediated by multiple prostanoid receptor types in both mucosal tissues.

In gastric mucosa, PGE_2 was the most potent naturally occurring agonist, suggesting an involvement of EP receptors. However U-46619 was more potent in gastric muscosa compared with tissues reported to contain a single population of EP receptors suggesting that TP receptors were also involved in stimulation of SCC (Bunce & Spraggs, 1987). The TP receptor antagonist AH23848 confirmed an involvement of TP receptors. AH23848 inhibited SCC responses to U-46619 in gastric mucosa with a pA_2 value consistent with that for competitive antagonism of TP receptors (Brittain *et al.*, 1985). In contrast, a concentration of AH23848 approximately 300 times its dissociation constant for TP receptors (1 μM) did not modify SCC responses to PGE_2 further suggesting that EP receptors also mediated this response. The stimulation of SCC by PGD_2 and $PGF_{2\alpha}$ could not be explained by the presence of DP or FP receptors since the low potencies of the selective agonists BW245C and fluprostenol excluded an involvement of DP or FP receptors (Coleman *et al.*, 1985). It is possible that PGD_2 and $PGF_{2\alpha}$ stimulate SCC by acting at both EP and TP receptors. In this context, other studies have suggested that PGD_2 (Jones *et al.*, 1982) and $PGF_{2\alpha}$ (Kennedy *et al.*, 1982) display activity at TP receptors in various tissues. These results are consistent with the view that prostanoids stimulate electrogenic chloride secretion in gastric mucosa by actions at EP and/or TP receptors and this may explain the different SCC response profiles encountered with agonists (Figure 1).

In ileal mucosa PGD_2 was more potent than PGE_2, a finding similar to previous observations in this tissue (Baird *et al.*, 1984). These results differ from reports *in vivo* where PGD_2 (and also PGI_2) were weak stimulants of enteropooling in the rat (Robert *et al.*, 1979b). These prostanoids may have different secretory effects across species although PGI_2 has been reported to be a potent stimulant of SCC in rat isolated colonic mucosa (Georg *et al.*, 1984). Since enteropooling *in vivo* reflects net electrolyte and fluid balance, the present studies may not account for additional actions of PGD_2 and PGI_2, such as effects on intestinal blood flow or electroneutral sodium chloride absorption (Bunce *et al.*, 1986). BW245C was also a potent stimulant of SCC, suggesting that stimulation of SCC in ileal mucosa was mediated by DP receptors (Coleman *et al.*, 1985). In contrast to gastric mucosa, U-46619 was virtually inactive as both an agonist and an antagonist in ileal mucosa suggesting that TP receptors were not involved in this response *in vitro*. DP receptors are poorly characterized since tissues that contain them, such as human platelets, also contain other prostanoid receptor types (e.g. IP receptors) which mediate the same response (Miller & Gorman, 1979). However, in ileal mucosa PGE_2 was more potent than expected if the responses were mediated solely by DP receptors. Therefore it is likely that the stimulation of SCC in ileal mucosa by prostanoid receptor agonists is mediated by DP and/or EP receptors.

Since EP receptors appeared to mediate the stimulation of SCC by prostanoids in both gastric and ileal mucosa, more information regarding the EP receptor subtypes that control the effects was obtained with a range of synthetic PGE analogues. The activities of these compounds in the two mucosal preparations may be compared in Tables 1 and 2. In gastric mucosa all of these prostanoids were potent stimulants of SCC. Some of the agonists (sulprostone, enprostil and 16,16-dimethyl PGE_2) produced biphasic SCC response profiles similar to U-46619 (Figure 1). However, although sulprostone produced a similar response profile to U-46619, a high concentration of AH23848 (1 μM) did not modify the stimulation of SCC produced by sulprostone, thus excluding an involvement of TP receptors in this response. In contrast AY23626 and misoprostol produced monophasic increases in SCC similar to that observed for PGE_2 (Figure 1). The differential activities of sulprostone and AY23626 in isolated smooth muscle preparations have suggested a division of EP receptors into 3 subtypes (Coleman *et al.*, 1987). In gastric mucosa, the similar potencies of both sulprostone and AY23626 tended to exclude an involvement of EP_1 receptors. This lack of EP_1 receptor activity was confirmed by the lack of effect of the EP_1 receptor antagonist AH6809, which at 100 times its dissociation constant for EP_1 receptors (Coleman *et al.*, 1985) did not modify SCC responses to sulprostone. In addition, the rank order of agonist potency of the PGE analogues in gastric mucosa was not consistent with those observed for preparations reported to contain a single population of either EP_2 (Coleman *et al.*, 1988) or EP_3 receptors including inhibition of acid secretion in rat isolated gastric mucosa (Reeves *et al.*, 1988). Recent studies have shown that 16,16-dimethyl PGE_2 and enprostil are potent agonists at EP_3 receptors but weak at EP_2 receptors whilst misoprostol is equipotent at both EP_2 and EP_3 receptors (Coleman *et al.*, 1988). The possibility therefore exists that prostanoid agonists stimulate SCC in gastric mucosa by interaction with both EP_2 and EP_3 receptors.

In addition to stimulation of mucosal blood flow (Pihan *et al.*, 1986) and mucus secretion (Allen & Garner, 1980) a prostanoid-stimulated secretion of electrolyte and fluid may have a role in the protective actions of prostanoids in gastric mucosa, as suggested previously (Moody & Zalewsky, 1981; Thomson, 1984). Such a secretion would wash noxious agents away from the mucosal surface. Indeed the promotion of an

unstirred water layer at the surface of an epithelium by fluid secretion results in a reduction in solute absorption (Thomson, 1984; Pihan & Szabo, 1989) and may therefore prevent the absorption of substances damaging to gastric mucosa. In this context prostanoids that stimulate gastric non-parietal secretion such as 16,16 dimethyl PGE_2 (Robert *et al.*, 1979a), enprostil (Rozkowski *et al.*, 1986), misoprostol (Gana *et al.*, 1989) and U-46619 (Bunce & Clayton, 1987) have been shown to protect the gastric mucosa from damage.

The relative potencies of PGE analogues in ileal mucosa differed markedly from their relative potencies in gastric mucosa (Tables 1 and 2), consistent with the view that different EP receptor subtypes control secretion in these two regions of the gastrointestinal tract. The rank order of agonist potencies for these prostanoids in ileal mucosa was very similar to that encountered in cat trachea, a tissue reported to contain EP_2 receptors (Coleman *et al.*, 1988) and therefore it is likely that prostanoid-stimulated SCC in ileal mucosa is mediated by EP_2 receptors. It is perhaps not coincidental that prostanoids displaying potent full agonist activity in ileal mucosa (PGE_2, AY23626 and misoprostol) produced the same shape of response (monophasic) in gastric mucosa, whilst those compounds with little activity on ileal mucosa (sulprostone, enprostil and 16,16 dimethyl PGE_2) were biphasic responders in gastric mucosa. These observations support the contention that SCC in gastric mucosa is controlled by both EP_3 and EP_2 receptors. In view of the high diarrheogenic potency of 16,16 dimethyl PGE_2 and enprostil in rats *in vivo* (Robert *et al.*, 1979b; Rozkowski *et al.*, 1986), the lack of effects of these prostanoids upon SCC in ileal mucosa was surprising. This discrepancy may be related to the high metabolic stability of these analogues *in vivo*. In addition these prostanoids potently inhibit electroneutral NaCl absorption in the rat small intestine (Clayton *et al.*, 1988) and this effect of 16,16 dimethyl PGE_2 on fluid secretion in the colon may be a more important determinant of diarrhoea (Sernka *et al.*, 1982).

This classification of prostanoid receptors that mediate electrogenic anion secretion in the stomach and intestine has been based on the activities of a range of agonists with differing selectivities for prostanoid receptors. The classification has been complicated by the presence of multiple receptors controlling secretion in these different regions of the gastrointestinal tract. The antagonist activity of AH23848 provided unequivocal evidence of an involvement of TP receptors in the stimulation of gastric mucosal electrolyte secretion whilst the differing relative potencies of prostanoids in these tissues provided evidence of an involvement of other receptors. The definitive role of EP and DP receptors in these secretory responses requires the identification of antagonists with selectivity for these receptor types.

In summary, these studies of prostanoid-stimulated electrogenic chloride secretion in gastric mucosa suggest that the control of this secretion is complex. Furthermore comparisons of the effects of prostanoids in gastric and ileal mucosa suggest that secretion by these two regions of gastrointestinal tract may be controlled by different combinations of prostanoid receptors. This information may be important in the development of mucosal protective prostanoids that are free from side effects, such as diarrhoea.

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