Characterization of receptors involved in the direct and indirect actions of prostaglandins E and I on the guinea-pig ileum

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1 A study of the effects of prostaglandin E₂ (PGE₂) and eleven synthetic analogues on the guinea-pig isolated ileum preparation has revealed three distinct contractile actions, each associated with a different prostaglandin E (EP-) receptor subtype. In addition, PGI₂ (prostacyclin) and its stable analogues can activate prostaglandin I (IP-) receptors to elicit both contraction and relaxation of the ileum.

2 Two of the PGE actions involve direct stimulation of the smooth muscle, being unaffected by 1 μ M morphine treatment. One action is blocked by AH 6809 at micromolar concentrations and ICI 80205 and 16,16-dimethyl PGE₂ are particularly potent agonists. Activation of EP₁-receptors appears to be involved. The second action is unaffected by AH 6809; sulprostone and MB 28767 are potent agonists. Comparison with agonist potency rankings on the guinea-pig vas deferens indicates that EP₃-receptors may be involved.

3 The third PGE effect and the stimulant PGI effect are blocked by morphine, indicating enteric neurones and/or sensory nerve terminals as sites of action. EP₂-receptors may be involved in the PGE action, in view of the marked effect of morphine on the contractile actions of misoprostol, 11-deoxy PGE₂-1-alcohol, 11-deoxy PGE₁ and butaprost, all of which show some selectivity for EP₂-receptors. The PGI action is most easily studied with cicaprost (EC₂₅ = 1.3 nM), since iloprost, carbacyclin and to a lesser extent PGI₂ also have agonist activity at EP₁-receptors.

4 The contractile action of 17-phenyl- ω -trilor PGE₂ on the ileum is unaffected by morphine. Since this analogue shows only weak agonist activity on the rabbit jugular vein (EP₂ preparation) and guinea-pig vas deferens (EP₃), it may be a more useful standard agonist than PGE₂ in EP₁-receptor studies.

5 In the presence of morphine and AH 6809, cicaprost inhibits histamine-induced contractions (IC₂₅ = 22 nM). PGI₂ and iloprost show mixed inhibitory/potentiating actions, whereas carbacyclin only potentiates histamine contractions. This IP-receptor-mediated inhibition may account for the bell-shaped log concentration-response curve of cicaprost (no inhibitors present) and the very marked block of iloprost-induced contractions by AH 6809.

6 We have found no evidence for either IP-receptors mediating direct contraction or EP-receptors mediating inhibition of the ileum longitudinal smooth muscle, as has been suggested in the literature.

7 In view of the complexity of prostanoid action on the guinea-pig ileum we feel that the preparation must be used with caution to ascertain the EP₁ agonist or antagonist potencies of novel compounds.

Keywords: Prostaglandins E; prostacyclin analogues; prostaglandin antagonists; morphine; smooth muscle

Introduction

Contractions of the longitudinal muscle of the guinea-pig isolated ileum to prostaglandin E₁ (PGE₁) and PGE₂ are unaffected by hexamethonium but are partially inhibited by tetrodotoxin (TTX) and atropine (Horton, 1965; Bennett *et al.*, 1968). It has been suggested that two excitatory actions are involved, one on smooth muscle cells, the other on intrinsic cholinergic nerves (Bennett *et al.*, 1968). Further evidence for a neuronal component of PGE action stems from the finding that morphine inhibits the contractile action of PGE₁ by 60% and PGE₂ by 41% (Sanner, 1971).

In later studies directed towards characterizing prostaglandin E (EP-) receptors, Kennedy *et al.* (1982) showed that PGE₂ was the most potent of the natural prostanoids on the guinea-pig ileum, in the presence of atropine, indomethacin and phenoxybenzamine. They also found that the dibenzoxazepine SC 19220 caused parallel rightward shifts in the log concentration-response curves of PGE₁, PGE₂ and PGF_{2 α} (pA₂ = 5.4, 5.6 and 5.3 respectively). On the basis of these results they defined the guinea-pig ileum as an EP₁-selective preparation. More recently, the xanthone carboxylic acid AH 6809 has been shown to have a similar profile of activity to SC 19220 in the guinea-pig ileum (Coleman *et al.*, 1985). Its higher potency (pA₂ = 6.8, PGE₂ as agonist) and good water solubility are distinct advantages over SC 19220.

The neuronal EP-receptor in the ileum has never been fully characterized. Recent work by Poll *et al.* (1988a, b), in which potentiation of electrical field stimulation responses was measured, suggests that this receptor is unlikely to be the EP₁ subtype.

Finally, Gardiner (1986) has reported that the PGE analogue, butaprost, at high concentrations (3–10 μ M) produces an unsurmountable inhibition of histamine-induced contractions of the guinea-pig ileum. Gardiner (1986) has proposed that butaprost is a selective EP₂-receptor agonist (EP₂-receptors are associated with the smooth muscle relaxant actions of prostaglandins E) and consequently that EP₂ receptors mediate smooth muscle relaxation in the ileum.

In this study we have examined the actions of PGE₂ and 11 of its analogues (Figure 1) on the guinea-pig isolated ileum with a view to determining the number and nature of the EP-receptors present. At the start of the study, ICI 80205 and 16,16-dimethyl PGE₂ were known to be highly potent EP₁-receptor agonists, with some selectivity for EP₁- as opposed to EP₂-receptors (Dong *et al.*, 1986). Sulprostone was known to be a highly potent EP₃-receptor agonist (inhibition of the twitch response of guinea-pig vas deferens to electrical field stimulation), with moderate EP₁ agonist potency, but negligible EP₂ agonist activity (Coleman *et al.*, 1987). Misoprostol was claimed to be an EP₁-selective agonist (Eglen & Whiting, 1988), but concurrent work indicated potent EP₂ agonist activity (relaxation of cat trachea) (Coleman *et al.*, 1988).

In general, PGI₂ relaxes smooth muscle preparations (see Gryglewski, 1987). However, Gaion & Trento (1983) have

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Figure 1 Structures of prostaglandin E (PGE) analogues used in this study.

shown that PGI₂ contracts the longitudinal muscle of the guinea-pig ileum and at a concentration of 20 nM ($\sim$ EC₅₀) these contractions are abolished by TTX, and markedly inhibited by atropine and hemicholinium-3. In addition, PGI₂ potentiates electrically-induced contractions whilst having no effect on responses to exogenous acetylcholine (ACh). On the basis of these results it was proposed that PGI₂-induced contractions are mediated by ACh released from cholinergic neurones as a consequence of increased excitability of the cell bodies. Further evidence for this mechanism of action derives from the observation (Gaion & Trento, 1984) that the contractile action of PGI₂ is inhibited by noradrenaline, morphine and the purinergic receptor agonist N⁶-phenylisopropyl adenosine (PIA). Gaion & Gambaratto (1987) later showed that TTX or atropine only partially inhibit contractions to higher concentrations of PGI₂ (1 μ M). The authors suggested that part of the contractile action of PGI₂ at high concentrations is due to stimulation of prostaglandin I (IP-) receptors present on the smooth muscle cells.

There have been no reports of the actions of stable analogues of PGI₂ on the neuronal IP-receptor in the guinea-pig ileum. We wished to investigate the action of cicaprost (Figure 2) since we have shown it to be a highly specific agonist for

IP-receptors (Dong *et al.*, 1986), in contrast to iloprost which has unexpectedly high EP₁-agonist potency (Dong & Jones, 1982; Dong *et al.*, 1986; Sheldrick *et al.*, 1988). We have also examined carbacyclin and 10,10-difluoro-13,14-didehydro PGI₂; the latter has greater aqueous stability than PGI₂ due to the electron-withdrawing effect of the fluoro substituents on the enol ether unit (Fried *et al.*, 1980).

Methods

Isolated preparations

Male guinea-pigs (300–500 g) were killed by a blow to the head followed by exsanguination. Preparations were mounted in 10 ml organ baths for recording of tension with Grass FT03 isometric transducers connected to a Grass Polygraph recorder. The bathing solution was aerated with 95% O₂ and 5% CO₂, maintained at 37°C and changed by upward displacement and overflow.

Guinea-pig ileum The terminal portion of the ileum was excised after discarding the 8–10 cm portion nearest the ileo-

Figure 2 Structures of prostaglandin I (PGI) analogues used in this study.

caecal junction. Segments about 2 cm long were bathed in Tyrode (composition, mM: NaCl 136, KCl 2.7, CaCl₂ 1.4, MgCl₂ 0.49, NaH₂PO₄ 0.32, NaHCO₃ 12 and glucose 5). The tissues were allowed to equilibrate for about 1 h prior to testing with a priming dose of PGE₂ (14 nM).

Agonist doses were added non-cumulatively in a 1, 3, 10, 30 or 1, 5, 10, 50 sequence. Sufficient time was allowed for the response to each dose to reach a maximum and a minimum of 15 min was allowed between additions. Responses were calculated as a percentage of the ACh maximum obtained in each preparation at the end of the experiment.

Concentration-response relationships for a particular prostanoid were established simultaneously in two preparations from the same animal, one being continuously exposed to the inhibitor, starting 20 min prior to the first agonist dose. Log concentration-response curves were constructed with results from five different animals. Mean pD₂₅ values ($-\log EC_{25}$) in the presence and absence of the inhibitor were calculated and compared by an unpaired Student's *t* test. A mean dose-ratio at the EC₂₅ level was calculated from the five individual experiments.

For the estimation of inhibitory potency, preparations were bathed in Tyrode solution containing 1 μ M morphine and 2 μ M AH 6809. The tissue maximum was established initially with 1.5 μ M histamine and subsequently, each preparation was exposed repeatedly at 2 min intervals to a dose of histamine producing a contraction 40–50% of the maximum. Inhibitors were added immediately after wash-out of histamine. Log concentration-inhibition curves were obtained and pIC₅₀ values calculated.

Guinea-pig trachea Tracheal rings were set up as described previously by us (Tymkewycz *et al.*, 1991). The Krebs solution (composition, mM: NaCl 118, KCl 5.4, MgSO₄ 1.0, CaCl₂ 2.5, NaHPO₄ 1.1, NaHCO₃ 25 and glucose 10) contained indomethacin (1 μ M) and atropine (20 nM); EP 092 (1 μ M) was added 15 min before each cumulative agonist sequence. 17-Phenyl- ω -trilor PGE₂ was used as the standard agonist.

Schild analysis of the antagonism of 17-phenyl- ω -trilor PGE₂ by AH 6809 (15 min pre-incubation) was carried out as described previously (Tymkewycz *et al.*, 1991).

Guinea-pig vas deferens Lengths (20 mm) of vas deferens were suspended in Krebs solution (no additions) between stainless steel electrodes for supra-maximal electrical field stimulation (60 V, 1 ms, 10 Hz for 1 s every 32 s). Prostanoid doses were added cumulatively and PGE₂ was used as the standard agonist. Log concentration-inhibition curves were constructed.

Compounds

11-Deoxy PGE₂-1-alcohol (starting material *nat* PGA₂) and EP 092 were prepared in our laboratory. The following compounds were gifts which we gratefully acknowledge: 10,10-difluoro-13,14-didehydro PGI₂ sodium salt from Prof. J. Fried, University of Chicago, U.S.A.; sulprostone, PGI₂ sodium salt, iloprost, carbacyclin and cicaprost from Prof. H. Vorbruggen, Schering AG, Berlin; ICI 80205 (racemic) from Dr K. Gibson, ICI Pharmaceuticals, U.K.; MB 28767 and oxoprostol (both racemic) from Dr M. Caton, Rhone-Poulenc, U.K.; misoprostol and enisoprost from Dr P. Collins, G.D. Searle, U.S.A.; butaprost from Dr P. Gardiner, Bayer, U.K.; AH 6809 from Dr R.A. Coleman, Glaxo, U.K. PGE₂, PGF_{2 α} , 16,16-dimethyl PGE₂, 17-phenyl- ω -trilor PGE₂, 11-deoxy PGE₁ and U-46619 were purchased from Cayman Chemicals, U.S.A.

Ethanol stock solutions of the prostanoids (10^{-2} – 3×10^{-2} M) were stored at -20°C and diluted with 0.9% NaCl solution for use. In the case of PGI₂ and 10,10-difluoro-13,14-didehydro PGI₂, the sodium salt was dissolved in 50 mM Tris-HCl buffer pH 9.0. Dilutions of the PGI₂ stock were kept on ice and used for one dose sequence only, whereas with the more stable 10,10-difluoro analogue dilutions were frozen to -20°C after use and re-used for several experiments.

Results

Contractile actions of prostaglandin E analogues on the guinea-pig ileum

No inhibitors present Several different types of contractile response were elicited by the PGE analogues tested. ICI 80205, 16,16-dimethyl PGE₂, PGE₂ and 17-phenyl- ω -trilor PGE₂ produced rapidly developing increases in tension with minimal increase in rhythmic activity. On washout of the organ bath the return to resting tension was rapid. In contrast, MB 28767, oxoprostol, misoprostol, enisoprost, 11-deoxy PGE₂-1-alcohol, 11-deoxy PGE₁ and butaprost produced more slowly developing responses which typically showed

a large degree of spiking. These responses were slower to wane on washout.

Log concentration-response curves for all the analogues are shown in Figure 3. Those for MB 28767, oxoprostol, enisoprost, 11-deoxy PGE₂-1-alcohol, 11-deoxy PGE₁ and butaprost were shallower than those of the other analogues and in the case of MB 28767, enisoprost and 11-deoxy PGE₂-1-alcohol maximum responses were lower. Control pD₂₅ values ($-\log$ of molar concentration required to elicit a contraction 25% of the ACh maximum) are given in Table 1, in order that the effects of the inhibitors on all analogues can be compared.

Effect of morphine Using parallel preparations from the same guinea-pigs, log concentration-response curves for the PGE analogues in the presence of 1 μ M morphine were obtained

Figure 3 Log concentration-response curves for contraction of the guinea-pig ileum by prostaglandin E (PGE) analogues, with no inhibitors (○), 1 μ M morphine (●) and 1 μ M morphine and 2 μ M AH 6809 (□) present. (a) ICI 80205, (b) 16,16-dimethyl PGE₂, (c) PGE₂, (d) 17-phenyl- ω -trilor PGE₂, (e) sulprostone, (f) MB 28767, (g) oxoprostol, (h) misoprostol, (i) enisoprost, (j) 11-deoxy PGE₂-1-alcohol, (k) 11-deoxy PGE₁, (l) butaprost. Each point is the mean of 4–6 experiments, with s.e.mean shown by vertical bars. Note the different concentration scale for ICI 80205.

Table 1 Agonist potencies of prostaglandin E (PGE) and prostaglandin I (PGI) analogues on the guinea-pig ileum, trachea and vas deferens

		<i>Ileum</i>			
	<i>pD</i> ₂₅ for contraction (no inhibitors)	<i>Morphine</i> (1 μ M) vs no inhibitors	<i>Dose-ratio</i> <i>AH</i> 6808 (2 μ M) vs <i>Morphine</i> (1 μ M)	<i>Trachea</i> <i>pD</i> ₅₀ for contraction	<i>Vas deferens</i> <i>pIC</i> ₅₀ for inhibition
<i>Prostanoid</i>					
<i>PGE analogues</i>					
ICI 80205	9.54 $\pm$ 0.27	1.96 $\pm$ 0.19	12.6 $\pm$ 1***	10.08 $\pm$ 0.10	9.02 $\pm$ 0.10
16,16-Dimethyl PGE ₂	9.38 $\pm$ 0.11	1.62 $\pm$ 0.24	24.2 $\pm$ 5***	9.82 $\pm$ 0.03	9.62 $\pm$ 0.08
PGE ₂	8.96 $\pm$ 0.17	3.58 $\pm$ 0.38*	31.2 $\pm$ 7***	(8.0)↓	8.76 $\pm$ 0.03
17-Phenyl- ω -trilor PGE ₂	8.10 $\pm$ 0.10	0.94 $\pm$ 0.04	11.5 $\pm$ 2***	8.66 $\pm$ 0.15	7.96 $\pm$ 0.06
Sulprostone	7.92 $\pm$ 0.07	1.15 $\pm$ 0.03	3.5 $\pm$ 2	8.16 $\pm$ 0.08	9.69 $\pm$ 0.03
MB 28767	8.50 $\pm$ 0.11	5.2 $\pm$ 1.4*	1.8 $\pm$ 0.5	<6.0	8.97 $\pm$ 0.10
Oxoprostol	7.03 $\pm$ 0.16	4.0 $\pm$ 1.2	3.9 $\pm$ 1*	5.85 $\pm$ 0.16	8.35 $\pm$ 0.11
Misoprostol	8.10 $\pm$ 0.09	42 $\pm$ 8***	10 $\pm$ 4*	↓	9.12 $\pm$ 0.07
Enisoprost	7.37 $\pm$ 0.13	3.7 $\pm$ 1.0	4.1 $\pm$ 1.1	↓	9.22 $\pm$ 0.13
11-Deoxy PGE ₂ -1-alcohol	7.11 $\pm$ 0.14	>50	—	↓	7.78 $\pm$ 0.13
11-Deoxy PGE ₁	6.87 $\pm$ 0.22	>20	—	↓	—
Butaprost	6.67 $\pm$ 0.28	>10	—	↓	<6.0
<i>PGI analogues</i>					
Iloprost	8.62 $\pm$ 0.12	3.5 $\pm$ 1.2*	245 $\pm$ 57***	8.09 $\pm$ 0.13	6.79 $\pm$ 0.09
Carbacyclin	7.72 $\pm$ 0.08	1.6 $\pm$ 0.5	24.9 $\pm$ 8***	7.53 $\pm$ 0.04	6.96 $\pm$ 0.12
PGI ₂	8.12 $\pm$ 0.08	18.9 $\pm$ 0.5***	—	6.45 $\pm$ 0.09	—
Cicaprost	8.89 $\pm$ 0.11	>100	—	<6.0	<6.0
10,10-Difluoro-13,14-didehydro PGI ₂	8.05 $\pm$ 0.18	>100	—	—	—

Values are means $\pm$ s.e.mean ($n = 4-6$).

*** $P < 0.001$; * $P < 0.05$: significant rightward shift of the log concentration-response curve at the 25% maximum response level.

↓ Indicates that relaxant activity was observed.

(Figure 3). It had been established in preliminary experiments that 1 μ M morphine was sufficient to abolish the neuronal component of prostaglandin action on the ileum. The control and morphine curves for both 17-phenyl- ω -trilor PGE₂ and sulprostone were virtually superimposable. The corresponding curves for ICI 80205, 16,16-dimethyl PGE₂, oxoprostol, PGE₂ and MB 28767 were shifted to the right to a small extent, but only with the latter two agonists were the *pD*₂₅ values statistically significant. More marked shifts were seen for misoprostol, 11-deoxy PGE₂-1-alcohol, 11-deoxy PGE₁ and butaprost. Indeed butaprost showed no contractile activity in the presence of morphine.

Effect of morphine/AH 6809 Our original intention was to determine the effects of both AH 6809 and morphine/AH 6809 on the contractile actions of the PGE analogues. However we found that continuous exposure of the ileum preparations to AH 6809 alone often resulted in increased spontaneous activity; this was not seen when morphine was also present. Therefore in a separate series of experiments, log concentration-response curves in the presence of 1 μ M morphine/2 μ M AH 6809 were obtained (Figure 3) and compared with contemporaneous 1 μ M morphine curves (Table 1). For all analogues, morphine *pD*₂₅ values in the two series of experiments were not significantly different ($P > 0.05$). AH 6809 caused a parallel rightward shift in the log concentration-response curve of PGE₂ (dose-ratio = 31.2), consistent with a *pA*₂ of 7.08 $\pm$ 0.14 (95% confidence limits, $n = 5$). Similar shifts were found for 16,16-dimethyl PGE₂, ICI 80205, and 17-phenyl- ω -trilor PGE₂, while a smaller but significant shift was seen for oxoprostol. Misoprostol showed no contractile action in the presence of morphine/AH 6809. Over the 10–30% response level the curve for sulprostone was little affected by AH 6809 but at concentrations giving responses of 40–70% of maximum the shift was similar to that produced in the PGE₂ curve. The log concentration-response curve for MB 28767 was unaffected by AH 6809. In two experiments, 10 μ M AH 6809 caused a greater shift in the log concentration-response curve for PGE₂ (mean dose ratio = 52, *pA*₂ = 6.71). However, the curve for MB 28767 was not shifted.

Contractile actions of prostacyclin analogues on the guinea-pig ileum

No inhibitors present All the PGI analogues tested contracted the ileum, the rank order of potency at the 25% response level being: cicaprost $\geq$ iloprost $>$ PGI₂ = 10,10-difluoro-13,14-didehydro PGI₂ $\geq$ carbacyclin (Figure 4 and Table 1). The log concentration-response curve for cicaprost was bell-shaped and the maximum response was lower than those of PGI₂ and iloprost (Figure 4). 10,10-Difluoro-13, 14-didehydro PGI₂ showed a shallow log concentration-response curve, with threshold response at 2.5 nM and 60% maximal response at 800 nM (Figure 4).

Effects of morphine The contractile actions of cicaprost and 10,10-difluoro-13,14-didehydro PGI₂ were abolished by morphine treatment (Figure 4). Indeed the higher concentrations of these two analogues produced an inhibitory response in those preparations which exhibited resting spontaneous activity. The log concentration-response curves for PGI₂ and iloprost were subject to a significant rightward shift in the presence of 1 μ M morphine. However the carbacyclin curve was virtually unaffected at the 25% response level and moved slightly to the right at the 60% level.

Effects of morphine/AH 6809 In the presence of morphine, AH 6809 (2 μ M) produced a roughly parallel shift to the right of the log concentration-response curve to carbacyclin, the dose ratio of 25 (Table 1) being similar to that for PGE₂. However, the inhibitory effect of AH 6809 on iloprost contractions was much greater (Figure 4).

Relaxant actions on the guinea-pig ileum

In the presence of 1 μ M morphine and 2 μ M AH 6809, butaprost (15–1500 nM) slightly potentiated (0–10%) submaximal contractile response to histamine in two preparations. In a further two preparations the histamine response was unaffected. More marked potentiation was seen with PGE₂ (14–1000 nM), often associated with small contractile responses to PGE₂ itself.

Cicaprost consistently inhibited histamine-induced contractions of the ileum; *IC*₂₅ = 22 $\pm$ 3 nM (mean $\pm$ s.e.mean, $n = 5$)

Figure 4 Log concentration-response curves for contraction of the guinea-pig ileum by prostaglandin I (PGI) analogues, with no inhibitors (○), 1 μ M morphine (●) and 1 μ M morphine and 2 μ M AH 6809 (□) present. (a) Iloprost, (b) carbacyclin, (c) PGI₂, (d) cicaprost, (e) 10,10-difluoro-13,14-didehydro PGI₂. Each point is the mean of 4–6 experiments, with s.e.mean shown by vertical bars.

(Figure 5). PGI₂ and iloprost (100–1000 nM) also inhibited histamine responses, but they also produced an initial contraction of the tissue prior to inhibition and the inhibitory effect did not reach a maximum until two or three histamine doses had been added. In some tissues (2/6 for iloprost and 2/5 for PGI₂) no inhibition was observed. In contrast, carbacyclin (10–1000 nM) did not inhibit histamine contractions but instead potentiated these responses.

Actions of other prostanoids on the guinea-pig ileum

The thromboxane A₂-mimetic, U-46619, did not contract the ileum at concentrations up to 3 μ M. PGF_{2 α} contracted the preparation (EC₂₅ ~ 200 nM); however, this prostanoid

Figure 5 Log concentration-response curve for cicaprost (○) as an inhibitor of histamine-induced contraction of the guinea-pig ileum (1 μ M morphine and 2 μ M AH 6809 present). Each point is the mean of 5 experiments, with the s.e.mean shown by vertical bars.

retained little activity in the presence of 2 μ M AH 6809. The PGF_{2 α} -mimetic, ICI 81008, was inactive as a contractile agent at concentrations up to 2 μ M.

Agonist potencies on EP-receptor systems in the guinea-pig trachea and vas deferens

pD₅₀ values for contraction of guinea-pig tracheal rings are shown in Table 1; the TP-receptor antagonist EP 092 (1 μ M) was present to block potential TP-agonist actions. Several of the PGE analogues (including PGE₂) relax the tracheal muscle and consequently estimates of their contractile potency cannot be obtained. The regression of guinea-pig ileum pD₂₅ values (morphine present) on guinea-pig trachea pD₅₀ values for ICI 80205, 16,16-dimethyl PGE₂, 17-phenyl- ω -trilor PGE₂, sulprostone, iloprost, carbacyclin and PGI₂ shows a highly significant dependence: slope = 1.46, r = 0.989, P < 0.001.

On the guinea-pig trachea, AH 6809 (0.2–5 μ M) shifted the log concentration-response curve to 17-phenyl- ω -trilor PGE₂ to the right in a parallel manner. The slope of the Schild plot (1.13) was not significantly different from unity and a pA₂ of 7.35 ± 0.15 (95% confidence limits) was obtained. AH 6809 also shifted the log concentration-response curves to sulprostone, iloprost and carbacyclin to the right in a parallel manner and its blocking potency was similar.

pIC₅₀ values for inhibition of the twitch response to electrical field stimulation of the guinea-pig vas deferens are also shown in Table 1. All the analogues with pIC₅₀ values greater than 7.0 were able to inhibit completely the twitch response. Comparison of guinea-pig ileum pD₂₅ values (morphine present) with guinea-pig vas deferens pIC₅₀ values for the above analogues, but substituting PGE₂ for PGI₂, does not result in a statistically significant correlation: r = 0.57, P > 0.05.

Discussion

The agonist specificities of the PGE and PGI analogues for the three EP-receptor subtypes and the IP-receptor are given in Table 2. The rankings are compiled from the present and previously published studies (see Table 2 legend). To simplify the analysis of our results we have assumed that PGE₂ and its analogues interact only with EP-receptors in the ileum. On human platelets, which are very sensitive to the inhibitory action of IP-receptor agonists (see Armstrong *et al.*, 1989), only PGE₂ and 11-deoxy PGE₁ show evidence of inhibitory activity; they are about 300 and 1000 times less potent than iloprost respectively (Jones unpublished observations). Furthermore, there is no evidence from these or other studies for DP-, FP-, or TP-receptors in the ileum (Welburn & Jones, 1978; Kennedy *et al.*, 1982).

Our antagonism experiments with AH 6809 confirm the existence of an EP₁-receptor mediating contraction of the ileum longitudinal smooth muscle. AH 6809 at 2 μ M significantly shifted the log concentration-response curves of ICI 80205, 16,16-dimethyl PGE₂, PGE₂ and 17-phenyl- ω -trilor PGE₂ to the right in a roughly parallel manner: the pA₂ values agree with previously published data on the guinea-pig ileum (pA₂ = 6.8, PGE₂ as agonist, Coleman *et al.*, 1985) and the present measurements on the guinea-pig trachea (pA₂ = 7.35, 17-phenyl- ω -trilor PGE₂ as agonist). Of the PGI analogues investigated, iloprost and carbacyclin showed potent contractile actions which were blocked by AH 6809. A similar profile was seen on the guinea-pig trachea. This extends our original finding that the contractile actions of iloprost and $\Delta^{6,6a}$ -6a-carba PGI₁ (an isomer of carbacyclin) on the guinea-pig trachea and bullock iris sphincter were blocked by SC 19920 and its analogue SC 25191 (Dong *et al.*, 1986). The very pronounced block by AH 6809 of the contractile action of iloprost on the ileum is probably due to a combination of EP₁-receptor antagonism and the IP-receptor-mediated inhib-

Table 2 Specificity of prostaglandin E (PGE) and prostaglandin I (PGI) analogues as agonists at EP- and IP-receptors

Prostanoid	EP ₁	EP ₂	EP ₃	IP
<i>PGE analogues</i>				
ICI 80205	++++	+	+++	
16,16-Dimethyl PGE ₂	++++	++(+)	++++	
PGE ₂	+++	+++	+++	
17-Phenyl- ω -trilor PGE ₂	+++	+	++	
Sulprostone	++	0	++++	
MB 28767	(+)	(+)	+++	
Oxoprostol	(+)	0	++	
Misoprostol	+	++(+)	+++	
Enisoprost	+	+(+)	+++	
11-Deoxy PGE ₂ -1-alcohol	(+)	++	++	
11-Deoxy PGE ₁	+	++(+)	++	
Butaprost	(+)	++*	0	
<i>PGI analogues</i>				
Iloprost	+++	(+)	+	++++
Carbacyclin	++		+	++
PGI ₂	+	+	+	+++(+)
Cicaprost	(+)	(+)	0	++++

One + corresponds to a potency difference of approximately one order of magnitude. EP₁ ranking from measurements on guinea-pig fundus (Coleman *et al.*, 1988) and guinea-pig trachea and ileum (this study); EP₂ ranking from cat trachea (Coleman *et al.*, 1988), guinea-pig trachea (Dong *et al.*, 1986) and rabbit jugular vein (Lawrence *et al.*, 1989); EP₃ rankings from guinea-pig vas deferens (this study); IP rankings from human platelets (Armstrong *et al.*, 1989).

* Potency of butaprost is low on rabbit jugular vein.

itory action of iloprost (see later) becoming more effective as its concentration is increased.

There is a very good correlation between contractile potencies on the guinea-pig ileum (morphine present) and guinea-pig trachea (analogues with relaxant activity excluded), consistent with EP₁-receptor activation in both preparations. Furthermore, there is no significant correlation between agonist potencies on guinea-pig ileum (morphine present) and guinea-pig vas deferens, the latter being the standard EP₃ preparation. Both ICI 80205 and 17-phenyl- ω -trilor PGE₂ show some selectivity for EP₁- as opposed to EP₃- (this study) and EP₂-receptors (Dong *et al.*, 1986; Lawrence *et al.*, 1989). However, 17-phenyl- ω -trilor PGE₂ would have a potential advantage over ICI 80205 in studies to characterize EP₁-receptors owing to its very low TP agonist potency. 17-Phenyl- ω -trilor PGE₂ may be a useful lead compound in the development of more selective EP₁-receptor agonists.

In our earlier studies the potent prostacyclinmimetic, cicaprost, showed only very weak EP₁-receptor agonist activity (Dong *et al.*, 1986). This also appears to be the case in the ileum. However, it is possible (cf. iloprost) that an inhibitory action operating through an IP-receptor is masking a weak EP₁ contractile component. The 10,10-difluoro-13,14-didehydro analogue of PGI₂, again a potent prostacyclin mimetic, also appeared to have low EP₁ agonist activity. The presence of the 13,14-acetylenic bond in cicaprost and the difluoro analogue extends the rigidity of the C4-C13 structure to C15 and this could prevent a favourable interaction of the 15 α -hydroxyl with the agonist binding site of the EP₁-receptor.

The finding of a morphine/AH 6809-resistant contractile effect for sulprostone, MB 28767, oxoprostol and enisoprost suggests the existence of a second EP-receptor on the smooth muscle cells. Whether the more potent EP₁-receptor agonists also exhibit this type of activity is impossible to determine without a considerably greater block of EP₁-receptors than that achieved with 2 μ M AH 6809; higher concentrations of AH 6809 produce low antagonist specificity in other systems (Keery & Lumley, 1988). It is unlikely that this second direct contractile component is mediated via an EP₂-receptor since both sulprostone (Coleman *et al.*, 1987) and MB 28767 (Lawrence *et al.*, 1989) have very weak activity in this respect. They are however highly potent EP₃-agonists as judged by their ability to inhibit the twitch response of the guinea-pig vas deferens to electrical field stimulation (inhibition of trans-

mitter release) (Coleman *et al.*, 1987). Thus the ileum receptor may be an EP₃ subtype. Against this proposal are the low potencies of enisoprost and 11-deoxy PGE₂-1-alcohol and the complete inactivity of misoprostol. At this stage it is tempting to suggest yet a further EP-receptor subtype. However, it must be remembered that the sensitivity of the vas deferens to sulprostone is about two orders of magnitude greater than that of the AH 6909-resistant contractile system of the ileum. Differences in receptor density and/or efficiency of receptor-effector coupling between the two systems could dramatically alter relative agonist potencies.

In relation to the morphine-sensitive contractile actions of prostanoids on the ileum, it would seem sensible to postulate the presence of both EP- and IP-receptors on enteric neurones and/or sensory nerve terminals. From the previous argument, the lack of effect of morphine on the contractile actions of 17-phenyl- ω -trilor PGE₂ and sulprostone would suggest that the EP-receptor is neither the EP₁ nor EP₃ subtype. A large component of the actions of misoprostol, 11-deoxy PGE₂-1-alcohol, 11-deoxy PGE₁ and butaprost is due to activation of the neuronal receptor. Since these analogues show some selectivity for EP₂-receptors which mediate relaxation of vascular and respiratory smooth muscle (Table 2), it seems likely that the neuronal receptor may be of the EP₂ subtype. The pD₂₅ values for these analogues in the absence of inhibitors give rough estimates of potency at the neuronal EP-receptor (Table 1). The ranking is misoprostol > 11-deoxy PGE₂-1-alcohol $\geq$ 11-deoxy PGE₁ $\geq$ butaprost: PGE₂ is likely to be at least as potent as misoprostol. However, the activity of MB 28767 is difficult to reconcile with the presence of an EP₂-receptor subtype and further studies are required. The actions of butaprost and 11-deoxy PGE₂-1-alcohol are entirely or almost entirely due to activation of the neuronal EP-receptor and it is of interest that the maximum contraction induced by these analogues is only about 40% of the maximum contraction produced by PGE₂, which probably activates all three EP-receptor-mediated systems. EP₁-selective analogues elicit contractions of the ileum which are more rapid in onset and less prone to spiking than those elicited by analogues with pronounced EP₂/EP₃ activity. The difference is similar to that usually seen with ACh and nicotine on the ileum and probably reflects direct (EP₁) as opposed to neuronal (EP₂) activation of the ileum smooth muscle; why slow, spiky contractions should also be typical of EP₃-receptor activation is not clear however.

The neuronal excitatory (morphine-sensitive) action of PGI₂ and its analogues on the guinea-pig ileum is unusual since IP-receptor activation is typically associated with inhibition of smooth muscle tone, including rhythmic activity of intestinal smooth muscle (see Skuballa *et al.*, 1987). With the exception of carbacyclin, most of the 25% maximum contractile response appears to be due to activation of neuronal IP-receptors and hence the pD₂₅ values in the absence of inhibitors give estimates of agonist potency: cicaprost ≥ iloprost > PGI₂ = 10,10-difluoro-13,14-didehydro PGI₂. Carbacyclin would appear to have the lowest potency. This ranking is similar to those found for other PGI-sensitive systems in smooth muscle and platelets (Armstrong *et al.*, 1989).

In the presence of morphine and AH 6809, PGI₂, cicaprost and iloprost, but not carbacyclin, inhibited contractile responses to histamine. However, only cicaprost was devoid of accompanying contractile activity. This stimulant action may be due to surmountability of the EP₁-receptor block and/or an agonist action at the AH 6809-resistant EP-receptor. The ileum is more sensitive to the neuronal contractile action of cicaprost (pD₂₅ = 8.89) than the direct inhibitory action (pIC₂₅ = 7.66) and this probably accounts for the bell-shaped nature of the cicaprost log concentration-response curve.

Gardiner (1986) found that the log concentration-response curve for butaprost on the guinea-pig ileum was bell-shaped,

with the maximum response being <20% of the tissue maximum at 1 μM. Gardiner also reported that butaprost inhibited histamine-induced contractions at concentrations of 3 and 10 μM. In contrast, we found a linear log concentration-response curve (36% response at 1 μM) and no inhibition of histamine. It is impossible to prepare concentrated aqueous solutions of butaprost (a methyl ester) without initial dissolution in an organic solvent. We therefore suspect a depression of smooth muscle contractility due to the organic solvent (nature unknown) used in Gardiner's experiments. In our experiments, ethanol was used, resulting in a maximum concentration in the bathing fluid of 20 mM.

In conclusion, the data presented demonstrate that the guinea-pig longitudinal muscle/enteric neurone system contains EP- and IP-receptors additional to the EP₁ subtype. Thus, claims about the EP₁-selectivity of a compound based on its contractile potency on this preparation should be interpreted with caution. In order to determine unequivocally the receptor subtype involved the effect of both morphine and AH 6809 should be investigated. With the use of these inhibitors, however, the guinea-pig ileum remains a useful preparation for assessing the receptor selectivity of PGE and PGI analogues.

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References

- ARMSTRONG, R.A., LAWRENCE, R.A., JONES, R.L., WILSON, N.H. & COLLIER, A. (1989). Functional and ligand binding studies suggest heterogeneity of platelet prostacyclin receptors. *Br. J. Pharmacol.*, **97**, 657–668.
- BENNETT, A., ELEY, K.G. & SCHOLES, G.B. (1968). Effects of prostaglandins E₁ and E₂ on human, guinea-pig and rat isolated small intestine. *Br. J. Pharmacol.*, **34**, 630–638.
- COLEMAN, R.A., HUMPHRAY, J.M., SHELDRIK, R.L.G. & WHITE, B.P. (1988). Gastric antisecretory prostanoids: actions at different prostanoid receptors. *Br. J. Pharmacol.*, **95**, 724P.
- COLEMAN, R.A., KENNEDY, I. & SHELDRIK, R.L.G. (1985). AH 6089, a prostanoid EP₁ antagonist. *Br. J. Pharmacol.*, **85**, 273P.
- COLEMAN, R.A., KENNEDY, I. & SHELDRIK, R.L.G. (1987). New evidence with selective agonists for the subclassification of PGE₂-sensitive receptors. *Adv. Prostaglandin Thromboxane Leukot. Res.*, **17A**, 467–470.
- DONG, Y.J. & JONES, R.L. (1982). Effects of prostaglandins and thromboxane analogues on the bullock iris and the dog iris sphincter preparations. *Br. J. Pharmacol.*, **76**, 149–155.
- DONG, Y.J., JONES, R.L. & WILSON, N.H. (1986). Prostaglandin E receptor subtypes in smooth muscle: agonist activity of stable prostacyclin analogues. *Br. J. Pharmacol.*, **87**, 97–107.
- EGLEN, R.M. & WHITING, R.L. (1988). The action of prostanoid receptor agonists and antagonists on smooth muscle and platelets. *Br. J. Pharmacol.*, **94**, 591–601.
- FRIED, J., MITRA, D., NAJARAAN, M. & MEHROTRA, M.M. (1980). 10,10-Difluoro-13,14-didehydroprostacyclin: a chemically and metabolically stabilized potent prostacyclin. *J. Med. Chem.*, **23**, 234–237.
- GAION, R.M. & GAMBARATTO, L. (1987). Target sites for the inhibition of prostacyclin effects in the guinea-pig ileum. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **336**, 445–452.
- GAION, R.M. & TRENTO, M. (1983). The role of prostacyclin in modulating cholinergic neurotransmission in the guinea-pig ileum. *Br. J. Pharmacol.*, **80**, 279–286.
- GAION, R.M. & TRENTO, M. (1984). The role of adrenergic, purinergic and opiate receptors in the control of prostacyclin-induced contraction in the guinea-pig ileum. *Arch. Int. Pharmacodyn.*, **271**, 33–44.
- GARDINER, P.J. (1986). Characterization of prostanoid relaxant/inhibitory receptors using a highly selective agonist, TR 4979. *Br. J. Pharmacol.*, **87**, 45–56.
- GRYGLEWSKI, R.J. (1987). The impact of prostacyclin studies on the development of its stable analogues. In *Prostacyclin and its Stable Analogue Iloprost*. pp. 3–15. ed. Gryglewski, R.G. & Stock, G. Berlin: Springer-Verlag.
- HORTON, E.W. (1965). Biological activity of pure prostaglandins. *Experientia*, **21**, 113–118.
- KEERY, R.J. & LUMLEY, P. (1988). AH 6809, a prostaglandin DP-receptor blocking drug on human platelets. *Br. J. Pharmacol.*, **94**, 745–747.
- KENNEDY, I., COLEMAN, R.A., HUMPHREY, P.P.A., LEVY, G.P. & LUMLEY, P. (1982). Studies on the characterisation of prostanoid receptors. *Prostaglandins*, **24**, 667–689.
- LAWRENCE, R.A., JONES, R.L. & WILSON, N.H. (1989). Relaxant properties of prostaglandin E analogues on the rabbit jugular vein. *Br. J. Pharmacol.*, **98**, 796P.
- POLL, C., GRIX, S. & COLEMAN, R.A. (1988a). Do excitatory neuronal prostanoid receptors exist in the guinea-pig ileum. *Br. J. Pharmacol.*, **94**, 332P.
- POLL, C., GRIX, S. & COLEMAN, R.A. (1988b). Further evidence for the existence of neuronal prostanoid receptors which enhance neurotransmission in the guinea-pig ileum. *Br. J. Pharmacol.*, **95**, 15P.
- SANNER, J. (1971). Prostaglandin inhibition with a dibenzoxazepine hydrazine derivative and morphine. *Ann. N.Y. Acad. Sci.*, **180**, 396–405.
- SHELDRIK, R.L.G., COLEMAN, R.A. & LUMLEY, P. (1988). Iloprost – a potent EP₁ and IP receptor agonist. *Br. J. Pharmacol.*, **94**, 334P.
- SKUBALLA, W., RADUCHEL, B. & VORBRUGGEN, H. (1987). Chemistry of Stable Prostacyclin Analogues: Synthesis of Iloprost. In *Prostacyclin and its Stable Analogue, Iloprost*, pp. 17–24. ed. Gryglewski, R.J. & Stock, G. Berlin: Springer-Verlag.
- TYMKEWYCZ, P.M., JONES, R.L., WILSON, N.H. & MARR, C.G. (1991). Heterogeneity of thromboxane (TP-) receptors: evidence from antagonist but not agonist potency measurements. *Br. J. Pharmacol.*, **102**, 607–614.
- WELBURN, P.J. & JONES, R.L. (1978). A comparison of PGF_{2α} and three 16-aryloxy analogues on the isolated rabbit jejunum. *Prostaglandins*, **15**, 287–296.

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