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Investigation of the prostaglandin E (EP-) receptor subtype mediating relaxation of the rabbit jugular vein

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1 Prostaglandin E_2 (PGE_2) relaxes circular smooth muscle of the rabbit isolated jugular vein at very low concentrations (mean pIC_{50} against histamine-induced contraction = 9.34). This effect is not blocked by the EP_1-receptor antagonist, AH 6809 (2 μM).

2 From a group of prostaglandin E analogues examined, 16,16-dimethyl PGE_2, misoprostol, 11-deoxy PGE_2-1-alcohol and 11-deoxy PGE_1 were highly potent relaxant agents, whereas 17-phenyl-ω-trinor PGE_2, MB 28767 and butaprost had low potency and sulprostone and oxoprostol were virtually inactive.

3 Comparison of the jugular vein data with published data for inhibitory agonist potencies on the cat trachea (EP_2 preparation) and the field-stimulated guinea-pig vas deferens (EP_3) indicates that the EP-receptor in the rabbit jugular vein is closest to the EP_2 subtype. However, the correlation is not entirely convincing. For example, butaprost, 16,16-dimethyl PGE_2 and 11-deoxy PGE_1 are of similar potency on the cat trachea, whereas butaprost is about 300 times less potent than the other two analogues on the jugular vein. The existence of more than one EP_2-receptor appears possible.

4 It was felt that the activity of butaprost required further investigation in view of the claim that it is a specific EP_2-receptor agonist. We have shown that butaprost has very low inhibitory activity on the guinea-pig vas deferens, a very sensitive EP_3-receptor containing preparation. However, on the chick ileum, the original EP_3 preparation, butaprost showed potent contractile activity (pEC_{25} ~8.0). In addition, its maximum response was lower than that of PGE_2; lower maxima were also found for sulprostone, MB 28767 and oxoprostol, but not for ICI 80205, 16,16-dimethyl PGE_2 and 17-phenyl-ω-trinor PGE_2. The maximal response to a combination of either sulprostone and butaprost or sulprostone and PGE_2 was similar to that achieved by PGE_2 alone. Analysis of the interaction between sulprostone and PGE_2 appears to exclude a partial agonist action for sulprostone. Furthermore neither sulprostone nor butaprost appear to have inhibitory activity on the ileum. AH 6809 at 2 μM produced only a small shift of the PGE_2 log concentration-response curve.

5 It is likely that contraction of the longitudinal smooth muscle of the chick ileum is mediated by (at least) two EP-receptor subtypes; activation of only one receptor system does not induce the maximum response (i.e. the acetylcholine maximum) of the preparation. One receptor could be an EP_3 subtype, at which sulprostone exerts a selective agonist action. The other receptor is unlikely to be an EP_1 subtype, because of the high agonist potency of butaprost, the low agonist potency of iloprost, and the low antagonist potency of AH 6809. An alternative hypothesis is that the chick ileum contains a novel EP-receptor subtype in addition to an EP_3-receptor.

Keywords: Synthetic prostaglandins E; EP-receptors; prostaglandin receptor antagonists; rabbit jugular vein; chick ileum; smooth muscle relaxation

Introduction

Prostaglandin E (EP-) receptors which are susceptible to block by SC 19220 and AH 6809 have been designated EP_1-receptors (Kennedy *et al.*, 1982). EP-receptors resistant to block by these agents have been divided into EP_2 and EP_3 subtypes on the basis of the relative agonist potencies of prostaglandin E (PGE) analogues on isolated smooth muscle preparations (Coleman *et al.*, 1987c). Thus sulprostone is a potent agonist on the chick ileum (contraction; EP_3 preparation), but has very low potency on the cat trachea (relaxation; EP_2 preparation). In contrast AY 23626 (*rac* 11-deoxy PGE_0) is of similar potency on both preparations.

We wished to study in detail the EP_2 agonist activities of a range of PGE analogues, but a regular supply of cat trachea was not available to us. In addition, the relaxant actions of PGE analogues on the guinea-pig trachea (presumed to be EP_2-receptor-mediated) are difficult to study owing to the presence of a very sensitive EP_1-receptor system mediating contraction (Dong *et al.*, 1986). We therefore investigated the suitability of other isolated smooth muscle preparations. One of these, the rabbit jugular vein, proved to be highly sensitive to the relaxant action of PGE_2 and the relative potencies of a range of prostanoid analogues are described here.

The PGE analogue butaprost was of particular interest to us since it is claimed to be a specific EP_2-receptor agonist (Gardiner, 1986). During studies to confirm its specificity we found it had potent contractile activity on the chick ileum, nominally an EP_3 preparation. Further investigations, described here, suggest that more than one EP-receptor subtype may be present in the preparation.

Methods

Rabbit jugular vein

Male New Zealand White rabbits (2–4 kg) were injected with heparin (1000 u) via a marginal ear vein prior to stunning and exsanguination. The external jugular veins were removed, cleared of fat and adherent connective tissue and cut into rings 4 mm wide. Each ring was suspended under a

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tension of 0.75 g between two Z-shaped hooks in a 10 ml organ bath containing Krebs solution (composition, mM: NaCl 118, KCl 5.4, $CaCl_2$ 2.5, $MgSO_4$ 1.0, NaH_2PO_4 1.1, $NaHCO_3$ 25, glucose 10). The bathing solution was aerated with 95% O_2/5% CO_2, maintained at 37°C, and contained indomethacin, 1 μM. Changes in tension were measured by means of Grass FT03 isometric transducers connected to a Grass Polygraph recorder. Preparations were allowed 1 h to equilibrate during which time the resting tension was re-adjusted to 0.75 g. Each preparation was initially contracted with histamine, 10 μM, to establish its maximum contractile response.

For investigation of the relaxant actions of prostanoids, the TP-receptor antagonist GR 32191 (10 μM) was added 5 min before the addition of a dose of histamine (usually 1 μM) sufficient to induce a stable level of tone 50–60% of the maximum. Larger histamine responses were subject to a considerable degree of fade. Each preparation was exposed to two series of cumulative doses of PGE_2 (standard agonist) before the testing of a prostaglandin analogue. The preparations were washed frequently during the 30 min period between cumulative additions. Log concentration-response curves were plotted, taking response as the percentage of the histamine-induced tone remaining. IC_{50} values, defined as the concentration required to reduce the histamine response by 50%, were read by eye from the graph and converted into pIC_{50} values ($-\log IC_{50}$). Values from single preparations from 5 different animals were averaged and s.e.mean calculated. Equi-effective molar ratios (e.m.r.) were calculated in the following way: IC_{50} for the analogue/IC_{50} for PGE_2 (second curve) in the same preparation. A mean e.m.r. from the 5 preparations was calculated.

The antagonism of the contractile action of U-46619 and MB 28767 by GR 32191 was measured as described previously (Jones *et al.*, 1982). The antagonist was added 20 min before the first agonist dose.

Chick ileum

Chicks (5–20 days old) were killed by decapitation. The abdomen was opened, the ileum removed and adherent mesenteric tissue cut away. Segments about 20 mm long were mounted vertically in 10 ml organ baths under 0.5–0.75 g tension and tension changes were recorded as described above. The bathing solution was Tyrode solution of the following composition (mM): NaCl 136, KCl 2.7, $CaCl_2$ 1.4, $MgCl_2$ 0.49, NaH_2PO_4 0.32, $NaHCO_3$ 12 and glucose 5; it was bubbled with 95% O_2/5% CO_2 and maintained at 37°C. All preparations were exposed to several, almost maximal, doses of acetylcholine (bath concentration = 2 μM) during the first 1 h after setting up, followed by a single dose of PGE_2 (14 nM). Following one of the procedures described below, the maximal response to acetylcholine was obtained. Responses were calculated as a percentage of the acetylcholine maximum.

Concentration-response relationships for PGE_2 (one preparation) and for test prostanoids (3 preparations from the same animal) were obtained by use of non-cumulative (1, 3, 10, 30 or 1, 5, 10, 50) sequences. The drug contact time was 30–90 s and a minimum of 15 min was allowed between additions. For each analogue, a mean pEC_{25} value ($-\log$ of concentration producing 25% of the acetylcholine maximum response) was calculated from results obtained on single preparations from 5 animals.

To study the interaction between two agonists, a cumulative concentration-response curve to the first agonist was obtained on one preparation. A single dose of the second agonist was added to another preparation, followed 2–3 min later by cumulative doses of the first agonist.

To study the effect of potential inhibitors, cumulative concentration-response curves for PGE_2 were obtained on two preparations. Following wash-out, AH 6809 or the 'inhibitor cocktail' was added to one (test) preparation and 15 min later a second concentration-response curve to PGE_2 was obtained. A control curve to PGE_2 was obtained on the second preparation. A dose-ratio was calculated from the two EC_{50} values obtained on the test preparation. Dose-ratios from 5 preparations were averaged and the s.e.mean calculated.

Compounds

11-Deoxy PGE_2-1-alcohol was prepared in our laboratory from *nat* PGA_2. The following compounds were gifts: sulprostone, iloprost, PGI_2 sodium salt, carbacyclin and cicaprost from Prof. H. Vorbruggen, Schering AG, Berlin; ICI 80205 (*rac* 16-*p*-chlorophenoxy-ω-tetranor PGE_2) from Dr K. Gibson, ICI Pharmaceuticals, U.K.; MB 28767 (15S-hydroxy-9-oxo-16-phenoxy-ω-tetranorprost-13E-enoic acid) 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 (6-isopropoxy-9-oxoxanthen-2-carboxylic acid) and GR 32191 (9α-(biphenylyl)methoxy-17β-hydroxy-12β-(N-piperidinyl)-ω-octanorprost-4Z-enoic acid) from Dr R.A. Coleman, Glaxo, U.K. PGE_2, 16,16-dimethyl PGE_2, 17-phenyl-ω-trinor PGE_2 and 11-deoxy PGE_1 were purchased from Cayman Chemicals, U.S.A. The structural formulae of these analogues may be found in a previous publication (Lawrence *et al.*, 1991).

Ethanolic stock solutions of the prostanoids ($10^{-2} - 3 \times 10^{-2}$ M) were stored at −20°C and diluted with 0.9% NaCl solution for use. Because of the unexpected low potency of butaprost on the jugular vein and the known ease of dehydration of the β-ketol system in the PGE ring, the butaprost stock solution was chemically analysed at one month intervals. Thin layer chromatography showed a single spot with a mobility similar to the structurally similar misoprostol (both prostanoids are methyl esters). U.v. spectroscopy showed no evidence of a PGA chromophore (expected λ_{max} = 220 nm) and alkali conversion (0.1 M NaOH, 25°C, 30 min) to the corresponding PGB derivative (λ_{max} = 280 nm) confirmed the concentration of butaprost in the stock solution.

Results

Rabbit jugular vein

PGE_2, the standard agonist, produced complete relaxation of rabbit jugular vein preparations contracted by histamine, with pIC_{50} values falling between 8.82 and 9.96 (third cumulative sequence on 13 preparations). AH 6809 at a concentration of 2 μM did not block the relaxant action of PGE_2 (Figure 1a), the dose-ratio being 0.87 ± 0.03 (s.e.mean, n = 5). The relaxant action of PGE_2 was also unaffected by the TP-receptor antagonist GR 32191 at a concentration of 10 μM; control pIC_{50} = 9.21 ± 0.11, GR 32191-treatment pIC_{50} = 9.24 ± 0.08 (s.e.mean, n = 5).

Relaxant potencies of prostanoids Eleven PGE analogues were tested for relaxant activity in the presence of 10 μM GR 32191. With the exception of sulprostone, oxoprostol and MB 28767, log concentration-response curves for the analogues were parallel to that of PGE_2 and at least 90% relaxation was produced. pIC_{50} values and equi-effective molar ratios (e.m.r.) are given in Table 1. Sulprostone and oxoprostol were of very low potency: at the highest concentrations tested of 3.6 and 1.4 μM, the relaxations were 22 ± 8% and 14 ± 5% (s.e.mean, n = 5) respectively.

The three stable PGI analogues, cicaprost, iloprost and carbacyclin, also relaxed the jugular vein with log concentration-response curves parallel to that of PGE_2. However they were considerably less potent than PGE_2 (Table 1).

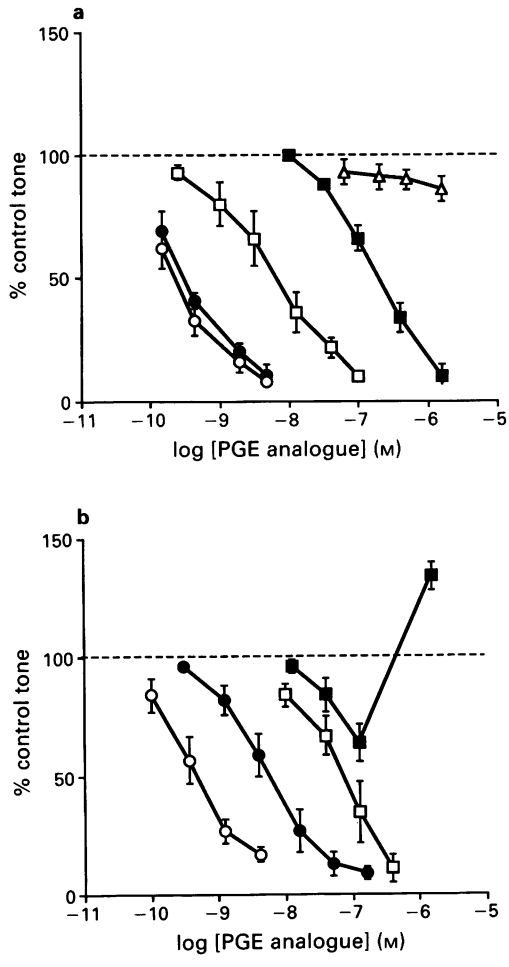

Figure 1 Log concentration-response curves for relaxation of the histamine-contracted rabbit jugular vein by prostaglandin E (PGE) analogues. (a) PGE_2 (●); PGE_2 in the presence of 2 μM AH 6809 (○); misoprostol (□); butosprost (■) and oxoprostol (△); (b) *nat* 11-deoxy PGE_1 (○); 11-deoxy PGE_2-1-alcohol (●); 17-phenyl-ω-trinor PGE_2 (□) and MB 28767 (■). The TP-receptor antagonist GR 32191 (10 μM) was present in all tests. Means for 5 experiments are shown; s.e.mean indicated by vertical bars.

Contractile/relaxant actions of MB 28767 In the presence of 10 μM GR 32191, MB 28767 relaxed the histamine-contracted jugular vein preparation, but the concentration-response curve was bell-shaped (Figure 1) with distinct contractile responses being seen at concentrations in excess of 100nM. The pIC_{25} for MB 28767 was 7.12, giving an e.m.r. of about 400 (Table 1).

In the absence of GR 32191 (and histamine), MB 28767 contracted the vessel rings and its log concentration-response curve was parallel to that of the TP-receptor agonist, U-46619 (Figure 2). The e.m.r. for MB 28767 relative to U-46619 was about 5.5. In the presence of 10 μM GR 32191, a large parallel rightward shift of the U-46619 curve was obtained; $pA_2 = 7.2 \pm 0.1$ (s.e.mean, $n = 5$). The contractile action of MB 28767 was blocked to a similar extent by 10 μM GR 32191, but a pA_2 value was not calculated since the highest concentration of MB 28767 tested (4.3 μM) produced only a 28% response.

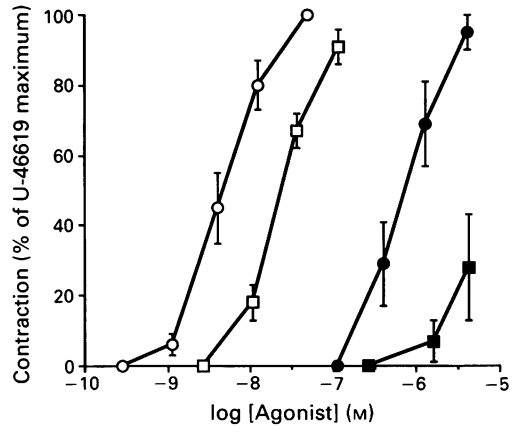

Figure 2 Log concentration-response curves for contraction of the rabbit jugular vein by U-46619 in the absence (○) and presence (●) of 10 μM GR 32191 and by MB 28767 in the absence (□) and presence (■) of 10 μM GR 32191. Each point is the mean of 5 experiments; vertical bars show s.e.mean.

Table 1 Potencies of prostanoids for relaxation of rabbit jugular vein and cat trachea

	Rabbit jugular vein		*Cat trachea*
Prostanoid	pIC_{50}	*e.m.r.*	*e.m.r.*
PGE analogues			
ICI 80205	7.25 ± 0.17	83	70*
16,16-Dimethyl PGE_2 (16,16 PGE)	9.21 ± 0.19	2.1	9.4*, 20**
PGE_2	9.34 ± 0.11	1.0	1.0
17-Phenyl-ω-trinor PGE_2 (17-Phe PGE)	7.21 ± 0.11	200	—
Sulprostone (Sul)	< 5.44	> 3000	> 7000**
MB 28767	see text	~400§	—
Oxoprostol (Oxo)	< 5.85	> 2000	—
Misoprostol (Miso)	8.21 ± 0.21	8.3	3.7**
11-Deoxy PGE_2-1-alcohol (PGE-1-alc)	8.27 ± 0.16	4.6	—
nat 11-Deoxy PGE_1	9.35 ± 0.12	1.4	—
rac 11-Deoxy PGE_1	9.22 ± 0.14	2.1	13*
Butaprost (Buta)	6.70 ± 0.10	685	17***
PGI analogues			
Cicaprost (Cica)	8.02 ± 0.19	—	> 300*
Iloprost (Ilo)	7.90 ± 0.08	—	> 270*
Carbacyclin	7.26 ± 0.19	—	—

Rabbit jugular vein: pIC_{50} valus are means ± s.e.mean of 5 experiments; prostaglandin E_2 (PGE_2) is the standard agonist. §Bell-shaped log concentration-response curve; e.m.r. calculated at IC_{25} level.
Cat trachea: published data, *Dong *et al.*, 1986 (PGE_2 is the standard agonist, $pIC_{30} = 7.4$); **Coleman *et al.*, 1988 ($pIC_{50} = 7.7$); ***Gardiner, 1986 ($pIC_{50} = 7.7$).
Abbreviation in parentheses used in Figure 5.

Chick ileum

Concentration-response curves All the PGE analogues tested contracted the chick ileum. However not all of them elicited the same maximum response (Figure 3). Using non-cumulative addition of doses, PGE_2, ICI 80205, 16,16-dimethyl PGE_2 (not shown), and 17-phenyl-ω-trinor PGE_2 elicited responses of at least 80–95% of the acetylcholine (ACh) maximum, whereas the maxima for sulprostone, butaprost and MB 28767 were 40 ± 4, 47 ± 3 and 56 ± 4% (s.e.mean, $n = 5$) respectively. Misoprostol and oxoprostol also showed a tendency towards a lower maximum. Iloprost showed weak contractile activity (Figure 3a) and cicaprost was even less active (0 and 20% of the ACh maximum at 0.13 and 1.3 μM respectively).

Interactions of agonists Cumulative log concentration-response curves to PGE_2 and butaprost in the presence of a supramaximally effective concentration of sulprostone (220 nM) were obtained (Figure 4a,b). In both cases the maximum response to the combination of agonists was very similar to that of PGE_2 alone. EC_{50} values (own maximum) for butaprost alone and in the presence of sulprostone were 38 and 51 nM respectively. The maximum response to butaprost by cumulative addition is higher than that obtained with ascending non-cumulative addition (Figures 3b and 4b). This appears to be related to the slow decay of butaprost contractions on washout, resulting in some desensitization by the non-cumulative technique.

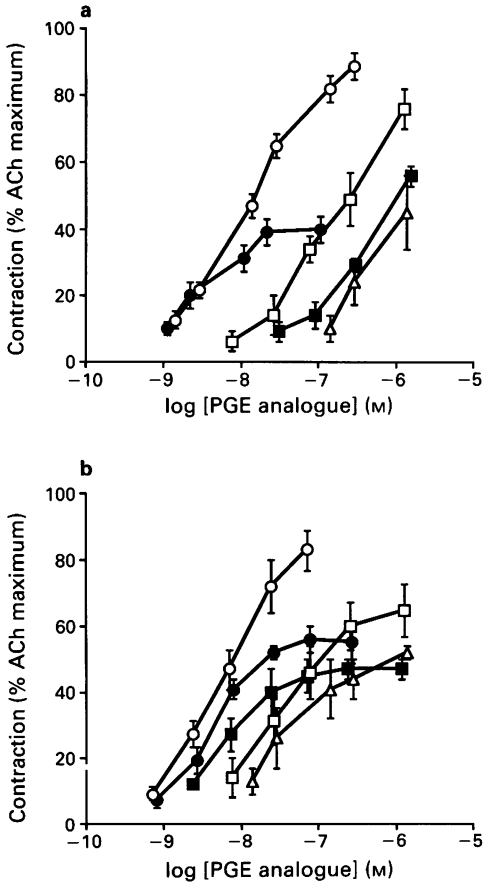

Figure 3 Non-cumulative log concentration-response curves for contraction of the chick ileum by prostanoids. (a) Prostaglandin E_2 (PGE_2) (○); sulprostone (●); 17-phenyl-ω-trinor PGE_2 (□); 11-deoxy PGE_2-1-alcohol (■) and iloprost (△). (b) ICI 80205 (○); MB 28767 (●); misoprostol (□); butaprost (■) and oxoprostol (△). Each point is the mean of 5 experiments; vertical bars show s.e.mean.

A maximally effective concentration of butaprost (500 nM) did not oppose the contractile action of ACh (Figure 4c); EC_{50} values (own maximum) for ACh alone and in the presence of butaprost were 165 and 140 nM respectively.

Effects of AH 6809 and the 'inhibitor cocktail' AH 6809 at 2 μM produced a parallel rightward shift in the log concentration-response curve of PGE_2 (Figure 4b). The respective pEC_{50} values show a statistically significant difference ($P < 0.05$, unpaired Student's t test); dose-ratio = 2.7 ± 0.8 (s.e.mean, $n = 5$), $pA_2 = 5.9$. However the cocktail of inhibitors (hyoscine 0.1, mepyramine 0.1, phenoxybenzamine 0.1, propranolol 3, methysergide 0.2 μg ml^{-1} and indomethacin 3 μM) used by Gardiner (1986) had no effect on the log concentration-response curves of either PGE_2 or butaprost.

Discussion

The results we have obtained for the PGE analogues on the rabbit jugular vein would suggest that their relaxant actions are mediated through EP_2-receptors. Thus the relaxant action of PGE_2 was not blocked by the EP_1-receptor antagonist AH 6809 (pA_2 for block of EP_1-receptors = 6.8–7.5, Coleman *et al.*, 1987c; Eglen & Whiting, 1988; Lawrence *et al.*, 1991). Furthermore, the highly potent EP_3-receptor agonist, sulprostone (agonist potencies: $EP_3 > EP_1 >> EP_2$) (Coleman *et al.*, 1987a,b) was only a very weak relaxant agent on the jugular vein. EP_3-receptors appear to mediate contraction in some vascular preparations, for example the rabbit renal artery (Ahluwalia *et al.*, 1988). In the present studies MB 28767, which also has potent EP_3 agonist activity (Jones & Wilson, 1990), contracted the rabbit jugular vein. However, MB 28767 is also a potent TP-receptor agonist (Banerjee *et al.*, 1985) and the present experiments with the TP-receptor antagonist GR 32191 (Lumley *et al.*, 1989) indicate that the contractile action of MB 28767 on the rabbit jugular vein is due to activation of TP-receptors.

Although we have used GR 32191 in the rabbit jugular vein experiments to suppress potential TP-receptor agonist actions of the PGE analogues, its affinity on the vein is lower than its affinities on other preparations (see Lumley *et al.*, 1989) and a concentration of 10 μM is barely sufficient when the PGE analogue has high TP agonist potency and low EP_2 agonist potency (e.g. MB 28767). We have recently reported low affinities for several TP-receptor antagonists (e.g. EP 092, EP 169 and ONO 11120) on two other rabbit isolated preparations, the thoracic aorta ring and blood platelets (Tymkewycz *et al.*, 1991). In early experiments on the jugular vein it was found that none of the three antagonists showed greater blocking potency than GR 32191 (unpublished observations).

Before discussing the potencies of the other PGE analogues as EP_2-receptor agonists on the jugular vein, it is necessary to consider possible interference from other prostanoid receptors mediating relaxation. Using the potent and specific DP-receptor antagonist BW A868C, Giles and coworkers (1989) showed that the rabbit jugular vein contains a DP-receptor which mediates relaxation. Low concentrations of BW A868C shifted the log concentration-response curves to PGD_2 and the DP-receptor agonist, BW 245C, to the right. However little further shift was seen with higher concentrations of BW A868C, since both agonists activate a second receptor (presumably the EP_2-receptor) which is not blocked by the antagonist. We do not know whether any of our PGE analogues activate the DP-receptor in the jugular vein since BW A868C was not available to us at the time of the experiments. However, by comparing concentration-response relationships in the Giles study with those presented here, it would appear that the DP-receptor relaxant system is considerably less sensitive than the EP_2-receptor system.

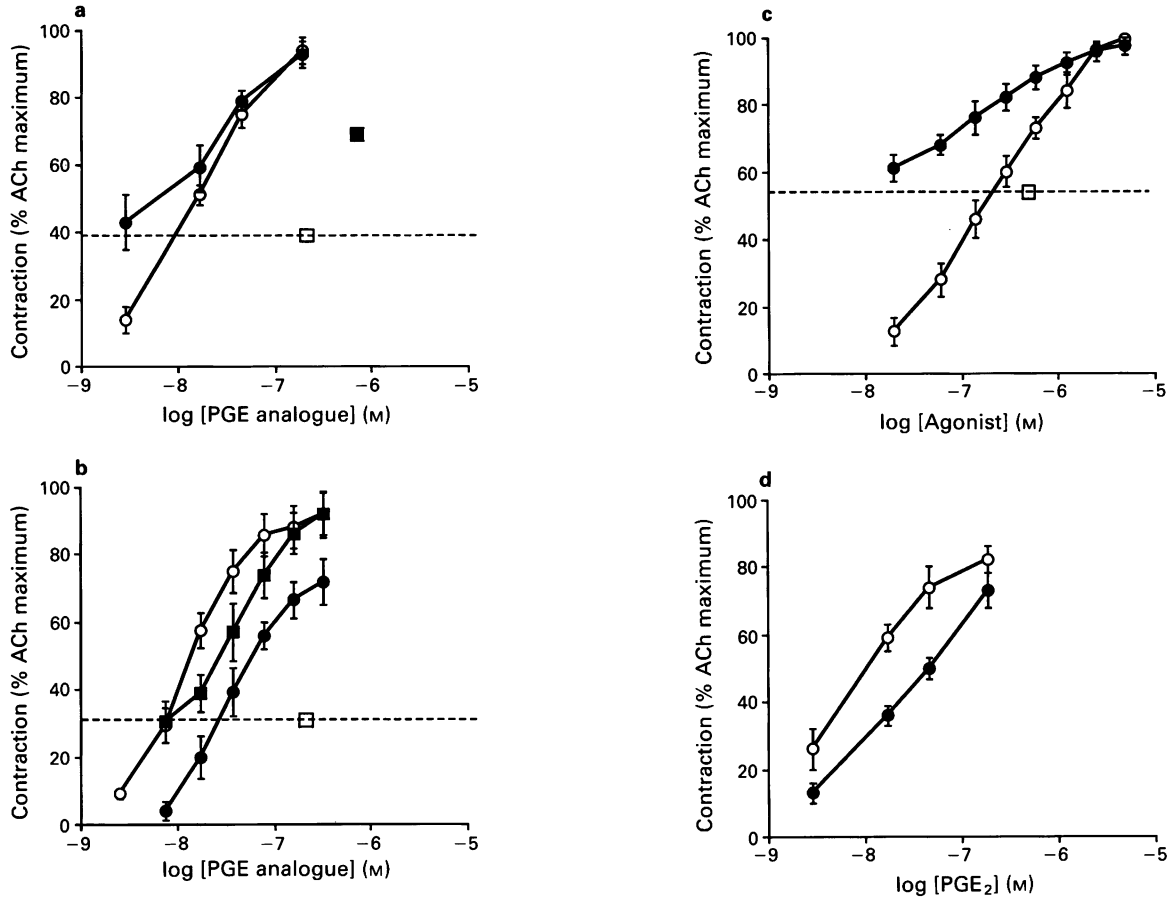

Figure 4 Log concentration-response curves for contraction of chick ileum. (a) Prostaglandin E_2 (PGE_2) alone (○) and PGE_2 in the presence of 220 nM sulprostone (●); this concentration of sulprostone elicits its own maximum response (□). The predicted EC_{50} for the PGE_2 curve assuming sulprostone is a partial agonist with a K_d of 5 nM is also shown (■); the PGE_2 maximum is assumed to equal the acetylcholine maximum. (b) PGE_2 alone (○); butaprost alone (●) and in the presence of 220 nM sulprostone (■); the response to sulprostone is also shown (□). (c) Acetylcholine alone (○) and in the presence of 500 nM butaprost (●); the response to butaprost is also shown (□). (d) PGE_2 alone (○) and in the presence of 2 μM AH 6809, an EP_1-receptor antagonist. (●). Each point is the mean of 5 experiments; vertical bars show s.e.mean.

In a preliminary report Giles *et al.* (1990) have also shown that prostacyclin, iloprost and carbacyclin relax the rabbit jugular vein, with pIC_{50} values of 7.36, 7.42 and 6.57 respectively. Our preparations were slightly more sensitive to the relaxant actions of iloprost and carbacyclin. In addition cicaprost, which shows high specificity for IP-receptors (Dong *et al.*, 1986) was found to be marginally more potent than iloprost. However, PGE_2 is at least 20 times more potent than these PGI_2-mimetics in relaxing the jugular vein and since none of the PGE analogues examined here are potent agonists on the very sensitive IP-receptor system of the human platelet, we feel that their relaxant activities are unlikely to be due to activation of IP-receptors.

The relaxant activity of butaprost is unexpectedly low on the rabbit jugular vein ($pIC_{50} = 6.70$, e.m.r. = 685, Table 1). Gardiner (1986) reported that butaprost induces relaxation of guinea-pig and cat trachea with pIC_{50} values of about 6.5, whereas higher concentrations are required on the cat lung strip ($pIC_{50} = 5.4$) and human bronchiole (5.1). We have confirmed the absolute potency of butaprost on the guinea-pig trachea ($pIC_{50} = 6.5-7.0$, unpublished observations), and hence in combination with physico-chemical tests (see Methods) have established the integrity of our sample of butaprost. However an accurate estimate of the potency of butaprost relative to PGE_2 can be obtained only on the cat trachea because of the dual contractile/relaxant activity of PGE_2 on the other preparations. Table 1 shows that there appears to be a marked difference between the e.m.rs for butaprost on the rabbit jugular vein and cat trachea. The discrepancy is even greater if the potencies of butaprost relative to either 16,16-dimethyl PGE_2 or 11-deoxy PGE_1 are compared. On the cat trachea the three PGE analogues have similar potencies whereas on the rabbit jugular vein butaprost is about 300 times less potent. The argument (see Kenakin, 1984) that, in preparations containing similar receptors, agonist efficacies influence relative potencies through differences in 'post-receptor gains' between the preparations is unlikely to explain our data. The rabbit jugular vein, on which butaprost has a low relative potency, is by far the more sensitive preparation; pIC_{50}s for PGE_2 on rabbit jugular vein and cat trachea are 9.34 and 7.7 respectively. If butaprost were a low efficacy EP_2 agonist, we would expect it to be much weaker on the cat trachea and also to act as a partial agonist; this is not evident in the concentration-response curves presented by Gardiner (1986). We conclude therefore that the EP-receptors mediating relaxation in the rabbit jugular vein and the cat trachea appear to be different; further studies are obviously required.

Eglen & Whiting (1988) reported that misoprostol at 300 nM contracted the guinea-pig trachea and there was no evidence of relaxation; from this and other data on the guinea-pig ileum and oesophageal muscularis mucosae, they proposed that misoprostol is a selective EP_1-receptor agonist. In our experience (Jones, unpublished observations) misoprostol (10–1000 nM) does not contract the indomethacin-treated guinea-pig trachea, whereas PGE_1 and PGE_2 show

contractile (EP_1) effects which reverse to relaxant (EP_2) effects as the concentration is raised. Furthermore when the tone of the trachea is raised by a TP-receptor agonist (e.g. U-46619, EP 171, Jones *et al.*, 1989), or an EP_1-receptor agonist (17-phenyl-ω-trinor PGE_2, iloprost) or histamine, misoprostol demonstrates potent relaxant activity. These latter results would agree with the potent relaxant action of misoprostol on the rabbit jugular vein reported here and on the cat trachea as reported by Coleman *et al.* (1988) (Table 1). Misoprostol is thus a potent agonist at EP_2-receptors. It is also a highly potent agonist at EP_3-receptors mediating inhibition of transmitter release (guinea-pig vas deferens, Lawrence *et al.*, 1991; guinea-pig atria, Mantelli *et al.*, 1991). Finally both EP_3 (initial contractile) and EP_2 (secondary relaxant) actions are seen on the non-pregnant human myometrium *in vitro* (Senior *et al.*, 1991). Although misoprostol undoubtably does have some agonist activity at EP_1-receptors (see Lawrence *et al.*, 1991), it is incorrect to label this prostanoid as a selective EP_1-receptor agonist.

11-Deoxy PGE_2-1-alcohol also has high relaxant potency on the rabbit jugular vein and is of particular interest because of the presence of a 1-alcohol group. It would appear that an ionised carboxyl function at the α-chain terminus is not a prerequisite for high EP_2 agonist potency. This cannot be implied from the high activity of methyl esters such as misoprostol, since one can never be certain that enzymatic de-esterification has not occurred within the tissue. 1-Alcohol PGE analogues are worthy of further investigation since they may lead to highly selective EP_2-receptor agonists. For example, 11–deoxy PGE_2-1-alcohol has low EP_1 agonist potency and only moderate EP_3 agonist potency (Lawrence *et al.*, 1991). Another 1-alcohol PGE analogue which has undergone considerable investigation is rioprostil (Kluender & Woessner, 1979). It has high EP_2 (cat trachea) and EP_3 (guinea-pig vas deferens) agonist potencies, but very low EP_1-agonist potency (guinea-pig fundus) (Coleman *et al.*, 1988; Reeves *et al.*, 1988). Finally the 1-alcohol PGE analogue oxoprostol has very low relaxant activity on the rabbit jugular vein. This is almost certainly related to the combination of 15-oxo and 16-phenoxy-17,18,19,20-tetranor groups in its ω-chain. Firstly, a 15(S)-15-hydroxyl group has been shown to be an important determinant of dilator potency in the dog hind limb (Nakano, 1972). Secondly a 16-phenoxy substituent accentuates EP_1 whilst reducing EP_2 agonist activity (Dong *et al.*, 1986). It is of interest that oxoprostol still retains considerable EP_3 agonist potency; e.m.r. on guinea-pig vas deferens = 3.1 (Lawrence *et al.*, 1991).

Our studies show that 17-phenyl-ω-trinor PGE_2 has only weak relaxant activity on the rabbit jugular vein (e.m.r. = 200). However it is a potent EP_1-receptor agonist on the guinea-pig ileum in the presence of morphine (e.m.r. relative to PGE_2 = 1.8). It is also a potent EP_1 agonist on the guinea-pig trachea, but its e.m.r. relative to PGE_2 is difficult to estimate since the latter has relaxant actions which oppose EP_1-receptor mediated contractions; its e.m.r. with respect to sulprostone (which lacks relaxant activity) = 0.32. 17-Phenyl-ω-trinor PGE_2 also has moderate EP_3 agonist potency on guinea-pig vas deferens; its e.m.r. relative to PGE_2 = 6.3 and relative to sulprostone = 49 (Lawrence *et al.*, 1991). Thus in combination with sulprostone it could be usefully employed to distinguish between EP_1- and EP_3-receptors. It may also be a lead to more selective EP_1-receptor agonists.

During our corroborative studies on butaprost, we observed that it had potent contractile activity on the chick ileum, but very little inhibitory activity on the guinea-pig vas deferens; both preparations are designated as EP_3-receptor containing preparations. The chick ileum finding is surprising, since Gardiner (1986) reported that butaprost was inactive on this preparation over a wide concentration range (2 nM–200 μM). In Gardiner's experiments the chick ileum was simultaneously exposed to several receptor antagonists (hyoscine, mepyramine, phenoxybenzamine, propranolol, methysergide) and the cyclo-oxygenase inhibitor indomethacin. We have found that this combination of agents does not affect the contractile activity of either butaprost or PGE_2.

Further investigations on the chick ileum have revealed that several PGE analogues, including sulprostone and butaprost, give smaller maximum responses than PGE_2. We first considered the possibility of partial agonism at an EP_3-receptor system in the chick ileum, particularly since the chick ileum is less sensitive than the guinea-pig vas deferens to PGE_2 (pEC_{50} = 7.8 and pIC_{50} = 8.8 respectively). If sulprostone is a partial agonist on a single receptor system in the chick ileum, it should shift the log concentration-response curve for PGE_2 (full agonist) to the right and it should be possible to predict the EC_{50} of this curve for any single concentration of sulprostone in the following manner. First the dissociation constant (K_d) of the partial agonist is estimated by comparison of its log concentration-response curve with that of the full agonist acting on the same receptors (Roberts, 1984). Applied to the data for sulprostone and PGE_2 in Figure 3, this method gives a K_d of about 5 nM for sulprostone. In the interaction experiments shown in Figure 4, sulprostone was used at a concentration of 220 nM. This is some 44 times its estimated K_d; therefore from van Rossum (1963) (also see Jenkinson, 1979) the concentration of PGE_2 required to produce a 70% maximum response (i.e. corresponding to half of the increment between the sulprostone response and the tissue maximum) is calculated to be 770 nM. The actual value in our experiments was 28 nM (Figure 4a). Clearly sulprostone does not antagonize the contractile action of PGE_2, as would be expected for a partial agonist present at a concentration in which it occupies a large proportion of the receptor pool. Furthermore, it is unlikely that the lower maximum of sulprostone is due to a second action of sulprostone opposing its contractile action (for example an agonist action on a separate receptor mediating smooth muscle relaxation), since inhibition of PGE_2 action would also be expected. In addition, sulprostone does not appear to oppose the contractile action of butaprost. Finally, there is no inhibition by butaprost of the contractile action of ACh as would be expected if it activated EP-receptors to produce an inhibitory response.

We feel that the most likely explanation of our data at this stage is that the chick ileum contains at least two EP-receptor subtypes which mediate contraction, and that maximal activation of only one receptor system cannot produce a maximum response of the preparation. One of these may be similar to the EP_3-receptor found in the guinea-pig vas deferens. A comparison of IC_{50} values for prostanoids on the vas deferens (Lawrence *et al.*, 1991) with pEC_{25} values on chick ileum from the present study is shown in Figure 5. We suggest that certain analogues (sulprostone, misoprostol and oxoprostol) elicit responses of the chick ileum in the 0–50% response range solely by activating EP_3-receptors; they lie close to the broken line in Figure 5. Prostanoids with points to the right of the line have potencies on the chick ileum which are greater than would be predicted from their EP_3 agonist potencies on the vas deferens and could therefore act on a second EP-receptor. In the case of PGE_2, ICI 80205, 16,16-dimethyl PGE_2, 17-phenyl-ω-trinor PGE_2 and perhaps 11-deoxy PGE_2-1-alcohol, both EP-receptors are activated giving rise to higher maxima, but the EC_{50} values for the two log concentration-response curves are not sufficiently different to give rise to biphasic curves. Butaprost lies well to the right of the broken line and probably acts solely on a second receptor. An estimate of the relative potencies of PGE_2 and butaprost on the second receptor system can be obtained from the log concentration-response curves for the two prostanoids in the presence of the supramaximally effective concentration of sulprostone; PGE_2 is about twice as potent as butaprost.

Since PGE_2, ICI 80205, 16,16-dimethyl PGE_2 and 17-phenyl-ω-trinor PGE_2 all have potent EP_1 agonist activity,

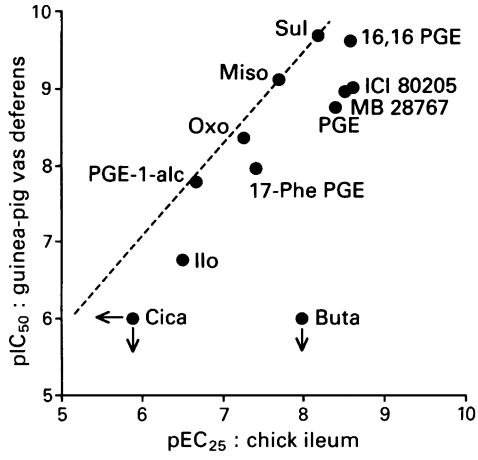

Figure 5 Correlation of agonist potencies of prostanoids on the guinea-pig vas deferens and the chick ileum (the full names of the analogues are given in Table 1). The broken line indicates a hypothetical relationship between EP_3-receptor agonist potencies on the vas deferens and the ileum. Prostanoids situated to the right of this line have greater agonist potency (in the 0–50% response range) on the ileum than would be expected from their EP_3 potency on the vas deferens; they may be agonists at another EP-receptor subtype in the ileum. The arrows by the cicaprost and butaprost points indicate that potency is less than that represented by the position of the point.

the second receptor may be an EP_1-receptor. However, selective block of EP_1-receptors by AH 6809 would be expected to give a biphasic log concentration-response curve for PGE_2 with a dose ratio of about 20 for the EP_1 component. This profile was not observed in our experiments. However we have observed this type of behaviour for AH 6809 on the guinea-pig ileum where sulprostone acts on both EP_1- and EP_3(?)-receptors to produce contraction (Lawrence *et al.*, 1991). Secondly, the potency of iloprost, which has potent EP_1-agonist activity (Dong & Jones, 1982; Dong *et al.*, 1986), is rather low on the chick ileum and this does not support the presence of an EP_1-receptor (the low potency of cicaprost also excludes IP-receptor-mediated contraction). However at this stage we cannot entirely rule out the presence of EP_1-receptors in the chick ileum.

The presence of both EP_1- and EP_3-receptors is unlikely to explain the quite potent contractile activity of butaprost, since this analogue has no detectable EP_1-agonist action (guinea-pig ileum) and only minimal EP_3 agonist action (guinea-pig vas deferens) (Lawrence *et al.*, 1991). Butaprost could be acting as an EP_2-agonist on the chick ileum. However this does not correlate with the low EP_2 agonist potency of ICI 80205 (Dong *et al.*, 1986; this study). An alternative explanation for our findings is that the chick ileum contains an EP_3-receptor and a novel EP-receptor, the latter being activated by PGE_2, ICI 80205, 16,16-dimethyl PGE_2, 17-phenyl-ω-trinor PGE_2 and butaprost. Further studies are in progress to investigate this possibility.

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