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The action of prostanoid receptor agonists and antagonists on smooth muscle and platelets

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1 Prostanoid receptors have been characterized in a range of guinea-pig and rat smooth muscle and platelets, according to the scheme of Coleman *et al.*, (1985a), using agonists (prostaglandin D_2 (PGD_2), PGE_1, PGE_2, 16,16 dimethyl PGE_2, $PGF_{2\alpha}$, PGI_2 and U46619) and putative selective antagonists (AH 6809 and SQ 29,548).

2 The ileum possesses EP_1- and IP-receptors; the trachea, EP_1-EP_2- and TP-receptors; the oesophageal muscularis mucosa, EP_1- and TP-receptors; the aorta and the portal vein TP-receptors. The rat colon possesses IP-, FP- and TP-receptors.

3 Guinea-pig platelets possess both IP and DP receptors mediating an inhibition of aggregation and TP receptors mediating proaggregation responses. No evidence was found for the presence of EP_1-, EP_2- or FP-receptors.

4 Misoprostol and fenprostalene were characterized using the above preparations. Misoprostol acts as a selective EP_1-agonist, and has little or no DP, FP, IP and TP activity. In the trachea precontracted with carbachol no evidence of EP_2-receptor stimulation was observed. Fenprostalene possesses FP and TP activity but no EP_1, EP_2, DP or IP activity.

Introduction

It has been proposed (Coleman *et al.*, 1985a) that distinct receptors exist for each of the 5 naturally occurring prostaglandins i.e. prostaglandin D_2 (PGD_2), PGE_2, $PGF_{2\alpha}$, PGI_2 and thromboxane A_2 (TxA_2). These receptors are denoted as DP, EP, FP, IP and TP respectively. Although primarily identified on the basis of rank orders of agonist potency, selective antagonists have been proposed (Coleman *et al.*, 1985a) for the TP- and a subtype of the EP-receptor (designated EP_1). In addition, smooth muscle preparations have been identified which exhibit a single response to a given prostaglandin when administered at low concentrations e.g., contractions of the guinea-pig ileum are elicited by low concentrations of PGE_2 and such responses are selectively antagonized by the EP_1-antagonist AH 6809 (Coleman *et al.*, 1985b). AH 6809 has also been shown to possess DP-antagonist activity at concentrations of 10^{-5} M and above (Keery & Lumley, 1985). However, AH 6809 is also strongly bound by plasma proteins, a feature which has been proposed to explain its lack of antagonism at EP receptors in the gastric mucosae (Coleman *et al.*, 1985c), and which may lead to an underestimate of potency at DP-receptors in platelet-rich plasma (Coleman *et al.*, 1985c). The absolute selectivity of AH 6809 for EP_1- or DP-receptors, therefore, remains equivocal. FP- and TP-receptors in the dog iris muscle and rabbit aorta, respectively, have, in contrast, been well characterized (Coleman *et al.*, 1985a) by use of selective agonists and antagonists.

However, in other tissues a more complicated response is observed. The guinea-pig trachea exhibits a biphasic concentration-response curve to PGE_1 or PGE_2 (Coleman & Kennedy, 1980) and only the initial contractile portion of the curve is antagonized by the EP_1-antagonist, SC-19920 (Coleman & Kennedy, 1985). It has been speculated that the relaxant response in this preparation to PGE_2 is mediated through a different receptor subtype. This receptor subtype is similar to that mediating responses of the cat trachea or chick ileum and has been denoted as EP_2 (Coleman *et al.*, 1986).

Smooth muscle preparations which exhibit responses to PGI_2 or PGD_2 which are specifically mediated through IP- and DP-receptors have not been well characterized. Contractions of the guinea-pig trachea in response to PGI_2 have been shown to be mediated by EP_1-receptors (Dong *et al.*, 1986). In addition, contractions of vascular smooth muscle

elicited by PGD_2 have been shown to be mediated by TP-receptors (Kennedy *et al.*, 1982; Dong & Jones, 1982). Inhibition of guinea-pig platelet aggregation has, however, been shown to be mediated by distinct IP- and DP-receptors (Coleman *et al.*, 1985b). The response to PGD_2 in this preparation is complicated by an interaction with TP-receptors, which results in an aggregation response and consequently a biphasic or bell-shaped concentration-response curve (Hamid-Bloomfield & Whittle, 1986).

The aims of the present study were as follows: firstly, we have attempted to characterize the receptor subtypes mediating prostanoid responses in the guinea-pig trachea, aorta, portal vein and oesophageal muscularis mucosae. These preparations, with the exception of the guinea-pig ileum (Coleman *et al.*, 1985b) and trachea (Coleman *et al.*, 1986) have yet to be characterized. Secondly, we have attempted to characterize the prostanoid receptors in the rat colon, a preparation that may be sensitive to the actions of FP-agonists. Finally, we have attempted to characterize the prostanoid receptor profile of some synthetic compounds i.e. fenprostalene and misoprostol, using the scheme proposed by Coleman *et al.* (1985a).

Methods

All experiments were conducted on isolated tissues, because of the problems associated with receptor classification using *in vivo* preparations. All tissues were removed from male Dunkin-Hartley guinea-pigs (250–350 g) with the exception of the colon which was removed from male Sprague-Dawley rats (200–250 g). In the case of the smooth muscle preparations all animals were killed by cervical dislocation.

Smooth Muscle

Ileum Two cm portions of proximal ileum were gently flushed intraluminally and placed in Tyrode physiological salt solution (PSS), the composition of which is given below, under 1 g tension and maintained at 37°C, pH 7.4. After 60 min equilibration, concentration-response curves were constructed to the agonist on a non-cumulative basis, allowing 30 s exposure on a 5 min dose cycle.

Oesophageal muscularis mucosae The outer striated muscle layer was gently stripped away leaving an inner tube of muscularis mucosal tissue. The tissue was then suspended under 0.5 g tension in Tyrode PSS. After 60 min equilibration, concentration-response curves were constructed in a cumulative fashion.

Trachea The tissues were cut in a zig-zag fashion, placed in Krebs PSS (see below) and in experiments in which contractions were studied, agonists were added cumulatively. In experiments in which relaxations were studied, the tone of the preparation was raised by the addition of carbachol (10 μM). This agonist, added at a concentration that elicited 80% of the maximal response, produced a sustained contraction which was maintained for the duration of the experiment. In these experiments prostanoid agonists were added cumulatively.

Aortae and portal vein These preparations were cleaned of adhering connective tissue and cut helically to produce a strip. The tissues were suspended under 1.0 g tension in Krebs PSS (the composition of which is given below), and maintained at 37°C, pH 7.4. After 60 min equilibration, concentration-response curves were constructed in a cumulative fashion.

Colon A short length (1.5 cm) of ascending colon was gently flushed intraluminally and suspended under 1.0 g tension in Tyrode PSS. After 60 min equilibration concentration-response curves were constructed in a cumulative fashion.

Measurement of responses All responses were measured isometrically, with a Hugo Sachs transducer (k 30) and recorded with a Graphtec-Watanabe recorder. In experiments in which cumulative concentration-response curves were constructed, the response was allowed to reach a plateau, before the addition of the next concentration of agonist. Agonist concentrations were spaced at 0.5 log intervals. Only one agonist was applied to each tissue, the responses being normalized against a maximum contraction to a full prostanoid agonist i.e., DP–PGD_2 EP_1–16,16 dimethyl PGE_2: EP_2–PGE_2; FP–$PGF_{2\alpha}$; TP–U46619; IP–PGI_2. The concentration-response curves, where applicable, were repeated (2–3 times) until reproducible responses were obtained.

Analysis of results

Agonists The potencies were calculated as $-\log EC_{50}$ values. In experiments where the maximum response was smaller than that to the standard agonist, the EC_{50} was calculated as the concentration causing a response 50% of its own maximum.

Antagonists Concentration-response curves were obtained and repeated until reproducible, and a further curve was then obtained in the presence of antagonist, as previously described (Dong *et al.*,

1986), using an equilibration period of 60 min. One concentration of antagonist only was examined on each preparation. The affinity of the antagonist was then determined by the method of Arunlakshana & Schild (1959) to estimate the pA_2 value. The dose-ratios were calculated for each antagonist concentration, in a minimum of 4 preparations. The concentration-ratios were then pooled and a straight line and intercept with the abscissae was calculated by linear regression.

Physiological salt solutions All solutions contained phenoxybenzamine (7×10^{-7} M) and indomethacin (2.8×10^{-6} M). In most experiments, atropine (8×10^{-7} M) was added, except where tracheal relaxations were measured and the tone was increased by carbachol.

The composition of Krebs PSS was (mM): NaCl 118.4, KCl 4.9, $MgSO_4.7H_2O$ 1.2, KH_2PO_4 1.2, glucose 11.1, $NaHCO_3$ 25.0 and $CaCl_26H_2O$ 2.5.

The composition of Tyrode PSS was (mM): NaCl 136.9, KCl 2.7, $MgCl_2.6H_2O$ 1.1, $NaH_2PO_4.2H_2O$ 0.4, glucose 5.6, $NaHCO_3$ 11.9 and $CaCl_2.6H_2O$ 1.8.

Inhibition of platelet aggregation

Blood was withdrawn by cardiac puncture and placed in plastic tubes contained sodium citrate (3.8% w/v; the solutions were mixed 1 part sodium citrate: 9 parts blood). Platelet-rich plasma (PRP) was prepared by centrifuging at $200\,g_{av}$ for 100 min. The PRP was aspirated and the remaining blood was centrifuged at $9000\,g_{av}$ for 2 min to produce platelet-poor plasma (PPP). Platelet aggregation in a Chrono-log Whole Blood Aggregometer was studied at 37°C with continuously stirred (2000 r.p.m.) PRP. Aggregation was induced by 2×10^{-6} M ADP, which was just sufficient to elicit an irreversible response; i.e. 2×10^{-6} M was approximately the EC_{80} for the ADP concentration-response curve. PRP was allowed 2 min equilibration at each antagonist concentration, followed by addition of agonist. In experiments studying inhibition of aggregation, ADP was added 1 min after the agonist.

The aggregation response was measured as an increase in light transmission (arbitrary units), and determined within 2 min after addition of the inducer of aggregation. For a primary wave proceeding into a secondary wave this means that the measured response virtually corresponds to the magnitude of the primary wave only. The potency of each agonist was determined as that concentration eliciting 50% of the maximum inhibition of aggregation. The antagonist affinity was described in a similar manner to that described above for the smooth muscles.

Drugs

Stock solutions of prostanoids were prepared in ethanol (usually 3×10^{-2} M) and stored at −20°C. AH 6809 (6-isopropoxy-9-oxoanthene-2-carboxylic acid: Glaxo), carbachol (Sigma), fenprostalene (ICI), SQ 29,548([15 - [1α,2β(5Z),3β,4α] - 7 - [3 - [2 - (phenyl amino) - carbonyl]hydrazino]methyl] - 7 - oxobicyclo-[2.2.1] - hept - 2 - yl] - 5 - heptenoic acid]; Squibb), misoprostol (Searle), PGD_2, PGE_1, PGE_2, 16,16 dimethyl PGE_2, $PGF_{2\alpha}$, PGI_2, U46619 9,11-methanoepoxy PGH_2 Cayman Chemical Co) were used.

Results

All tissues responded to the prostanoid agonists by exhibiting concentration-dependent contractions. Relaxations were observed, only in the trachea when PGE_1 or PGE_2 was applied.

Agonists

Agonist potencies ($-\log EC_{50}$) and maximal responses (α) are given in Tables 1 and 2.

Ileum (Table 1) The most potent compound examined was 16,16 dimethyl PGE_2 whilst U46619 and PGD_2 were inactive at the concentrations studied. The rank order of agonist potency was 16,16 dimethyl $PGE_2 > PGE_1 > PGI_2 > PGE_2 > PGF_{2\alpha} \gg$ U46619 = PGD_2.

Oesophageal muscularis mucosae (Table 1) In contrast to the ileum, PGE_1 and 16,16 dimethyl PGE_2 were virtually equipotent in this preparation. The concentration-response curve to 16,16 dimethyl PGE_2 was biphasic (Figure 1) consisting of an initial portion which reached approx. 80% of the maximal contracture and a second component which attained 100% of the contracture. U46619 and PGD_2 were also active in this preparation. The rank order of agonist potency was $PGE_1 \geq$ 16,16 dimethyl $PGE_2 > PGE_2 =$ U46619 $> PGI_2 > PGF_{2\alpha} > PGD_2$.

Trachea (Table 1) The most potent compound eliciting contractions was 16,16 dimethyl PGE_2. In comparison, PGI_2 exhibited a lower maximum response than either 16,16 dimethyl PGE_2 or U46619. All compounds studied elicited a contractile response; however PGE_1 and PGE_2 elicited a relaxant response at higher concentrations. In tissues precontracted with carbachol, only PGE_1 and PGE_2 produced a relaxant response. The rank order of potency at receptors mediating contractions was 16,16 dimethyl $PGE_2 > PGE_2 >$ U46619 $> PGE_1 > PGF_{2\alpha} > PGD_2 > PGI_2$.

Table 1 Comparison of potencies of prostanoid agonists at receptors in guinea-pig ileum, oesophageal muscularis mucosae, trachea (contraction and relaxation), aorta, portal vein and rat colon

Agonist	*Ileum* ($-\log EC_{50}$)	α	*OMM* ($-\log EC_{50}$)	α	*Colon* ($-\log EC_{50}$)	α	*Trachea (contraction)* ($-\log EC_{50}$)	α	*Trachea (relaxation)* ($-\log EC_{50}$)	α	*Aorta* ($-\log EC_{50}$)	α	*Portal vein* ($-\log EC_{50}$)	α
PGD_2	No response		6.25 ± 0.03	1.0	7.13 ± 0.08[a]	1.0	6.05 ± 0.08	1.0	No response		6.43 ± 0.05	1.0	6.24 ± 0.12	0.6
PGE_1	7.81 ± 0.05	1.0	8.61 ± 0.05	1.0	6.43 ± 0.05[b]	1.0	7.19 ± 0.05	0.4	5.13 ± 0.08	1.0	No response		5.13 ± 0.08	1.0
PGE_2	6.84 ± 0.03	1.0	7.93 ± 0.08	1.0	7.05 ± 0.12[b]	1.0	7.82 ± 0.12	0.4	6.24 ± 0.12	1.0	No response		No response	
16,16 dimethyl-PGE_2	8.96 ± 0.08	1.0	8.51 ± 0.08	1.0	6.93 ± 0.05	1.0	8.26 ± 0.10	1.0	No response		6.92 ± 0.08	1.0	6.35 ± 0.03	0.5
$PGF_{2\alpha}$	6.21 ± 0.08	1.0	6.60 ± 0.10	1.0	8.02 ± 0.04[a]	1.0	6.21 ± 0.05	1.0	No response		5.21 ± 0.09	1.0	No response	
PGI_2	7.22 ± 0.05	1.0	7.05 ± 0.08	1.0	4.91 ± 0.08[b]	1.0	5.92 ± 0.08	0.6	No response		No response		No response	
U46619	No response		7.92 ± 0.05	1.0	5.93 ± 0.14[a]	1.0	7.64 ± 0.05	1.0	No response		7.32 ± 0.08	1.0	6.51 ± 0.05	1.0

Values are mean ± s.e. mean, $n = 4$–10. The potency is given by the $-\log EC_{50}$ value and the maximum response, relative to a standard is denoted by α.
All agonists were examined between 1×10^{-9} and 3×10^{-5} M.
OMM-oesophageal muscularis mucosae.
[a] Experiments undertaken in the presence of 3×10^{-7} M SQ 29,548.
[b] Experiments undertaken in the presence of 1×10^{-5} M AH 6809.

Figure 1 Concentration-response curves to 16,16 dimethyl PGE_2 (◆), PGE_1 (◇), U46619 (▲), $PGF_{2\alpha}$ (△) and PGD_2 (■) in guinea-pig isolated oesophageal muscularis mucosae. Values are mean, $n = 4$–6; s.e. mean shown by vertical lines.

Aorta (Table 1) PGE_1, PGE_2 and PGI_2 were inactive in this preparation. The most potent compound was U46619. The rank order of potency was U46619 > 16,16 dimethyl PGE_2 > PGD_2 > $PGF_\alpha \geqslant PGE_1 = PGE_2 = PGI_2$.

Portal vein (Table 1) As in the aorta, the most potent compound studied was U46619. PGD_2 and 16,16 dimethyl PGE_2 acted as partial agonists. No response was observed for PGE_1, PGE_2, $PGF_{2\alpha}$ and PGI_2. The rank order of potency was U46619 > 16,16 dimethyl PGE_2 > $PGD_2 \geqslant PGE_1 = PGE_2 = PGF_{2\alpha}$.

Rat colon (Table 1) All experiments were undertaken in the presence of either SQ 29,548 (4×10^{-7} M) or AH 6809 (1×10^{-5} M) to exclude interactions at TP- and EP_1-receptor activity respectively. The most potent compound studied was $PGF_{2\alpha}$ and the least potent was PGI_2. The rank order of potency was $PGF_{2\alpha}$ > PGD_2 > PGE_2 > U46619 > PGE_1 > PGI_2.

Inhibition of platelet aggregation (Table 2) The most potent compound examined was PGI_2. PGD_2 exhibited a biphasic concentration-response curve (Figure 2), and $PGF_{2\alpha}$ and PGE_2 were inactive at the concentrations studied; 16,16 dimethyl PGE_2 and U46619 did not inhibit aggregation. The order of potency was PGI_2 > PGE_1 > $PGD_2 \gg PGF_{2\alpha}$ = U46619 = 16,16 dimethyl PGE_2.

Aggregation of platelets (Table 2) U46619 was the most potent inducer of aggregation and apart from 16,16 dimethyl PGE_2, the remaining prostanoids did not promote aggregation.

Table 2 Comparison of potencies of prostanoid agonists at receptors in guinea-pig platelets

	Inhibition of aggregation				*Aggregation*			
	Control		+SQ, 29,548 3×10^{-7} M		*Control*		+SQ, 29,548 3×10^{-7} M	
Agonist	($-\log EC_{50}$)	α	($-\log EC_{50}$)	α	($-\log EC_{50}$)	α	($-\log EC_{50}$)	α
PGD_2	7.42 ± 0.08	0.41	8.45 ± 0.03	1.0	No response		No response	
PGE_1	8.73 ± 0.13	1.0	8.82 ± 0.05	1.0	No response		No response	
16,16 dimethyl PGE_2	No response		No response		6.03 ± 0.05	1.0	No response	
$PGF_{2\alpha}$	No response		No response		No response		No response	
PGI_2	9.92 ± 0.08	1.0	9.98 ± 0.12	1.0	No response		No response	
U46619	No response		No response		9.12 ± 0.08	1.0	6.52 ± 0.05	1.0

Values are mean ± s.e. mean, $n = 4$–6. The potency is given by the $-\log EC_{50}$ value and the maximum response, relative to a standard is denoted by α.

All agonists were examined between 1×10^{-12} and 3×10^{-5} M.

Inhibition of aggregation was determined with 2×10^{-6} M ADP as the aggregating agent.

Antagonists

In the ileum, trachea and oesophageal muscularis mucosae, AH 6809 antagonized responses to 16,16 dimethyl PGE_2 with similar pA_2 values in all 3 tissues (Table 3). In addition, the responses to PGE_1 and PGE_2 in the ileum and oesophageal muscularis mucosae were also similar to those obtained using 16,16 dimethyl PGE_2. Due to the low maximum response attained by PGE_1 and PGE_2 in eliciting a contractile response in the trachea, no Schild analysis was undertaken. However, the relaxant responses observed in the trachea elicited by either PGE_1 or PGE_2 were not antagonized by AH 6809. In addition AH 6809 was without effect on the contractions elicited in the trachea and oesophageal muscularis mucosae by U46619 or PGD_2.

AH 6809 did not antagonize the inhibitory responses to PGI_2 or PGE_1 in guinea-pig platelets. At 3×10^{-4} M, AH 6809 competitively antagonized the responses to PGD_2 (in the presence of 3×10^{-7} M SQ 29,548 to inhibit TP activity). The concentration-ratio was 200 ± 10.24 (mean ± s.e. mean, $n = 4$) and the calculated pK_B (Furchgott, 1972) was 5.7. These data are shown in Figure 3.

SQ 29,548 acted as a competitive antagonist against contractions of the oesophageal muscularis mucosae, aorta, portal vein and trachea induced by U46619. The antagonist exhibited similar pA_2 values at receptors in the aorta when either U46619, PGD_2 or 16,16 dimethyl PGE_2 was used. These data are shown in Table 4. Tracheal contractions elicited by 16,16 dimethyl PGE_2 were, however, unaffected by

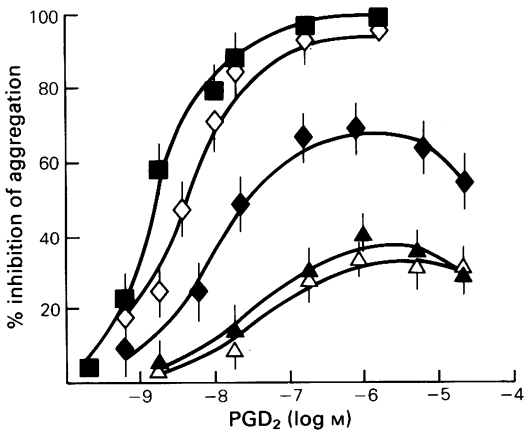

Figure 2 Effect of SQ 29,548 on inhibitory response curves to PGD_2 in guinea-pig isolated platelets. Values are mean, $n = 4$–6; s.e. mean shown by vertical lines. The control curve is denoted by (△), which was repeated in the presence of 4×10^{-9} M (▲), 1.2×10^{-8} M (◆), 4×10^{-8} M (◇) and 4×10^{-7} M (■) SQ 29,548.

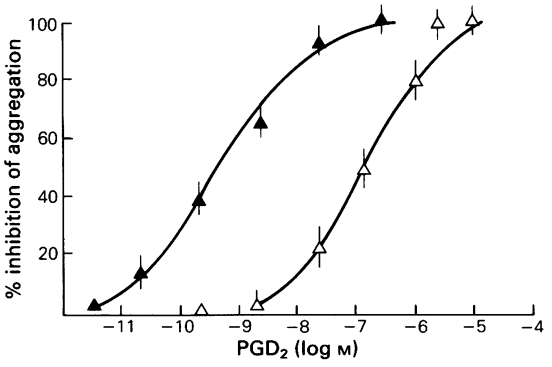

Figure 3 Effect of AH 6809 (3×10^{-4} M) on inhibition of platelet aggregation in response to PGD_2. Control responses (▲); responses in AH 6809 (△). All experiments were conducted in presence of 3×10^{-7} M SQ 29,548 to inhibit TP-mediated effects. Values are mean, $n = 6$; s.e. mean shown by vertical lines.

Table 3 AH 6809 pA_2 values and Schild slopes at receptors mediating contractions of guinea-pig ileum, oesophageal muscularis mucosae, trachea, aorta and portal vein

	Ileum		*OMM*		*Trachea* (contraction)		*Aorta*		*Portal vein*	
Agonist	pA_2	*Slope*	pA_2	*Slope*	pA_2	*Slope*	pA_2	*Slope*	pA_2	*Slope*
PGE_1	7.42 ± 0.05	0.85 ± 0.13	7.51 ± 0.08	0.89 ± 0.15	a		—		—	
PGE_2	7.39 ± 0.08	0.91 ± 0.18	7.49 ± 0.09	0.91 ± 0.08	a		—		—	
16,16 dimethyl PGE_2	7.59 ± 0.05	0.92 ± 0.15	7.29 ± 0.15	0.83 ± 0.05*	7.48 ± 0.09	0.86 ± 0.91	No effect at 1×10^{-5} M		No effect 1×10^{-5} M	
U46619	—		No effect at 1×10^{-5} M		No effect of AH 6809 at 1×10^{-5} M		No effect at 1×10^{-5} M		No effect 1×10^{-5} M	
PGD_2	—		No effect at 1×10^{-5} M		No effect of AH 6809 at 1×10^{-5} M		No effect at 1×10^{-5} M		No effect 1×10^{-5} M	

Values are mean ± s.e. mean, $n = 4$–12.
* undertaken in the presence of SQ 29,548 (3×10^{-7} M).
—indicates no response to the agonist in this tissue (see Table 1).
OMM-oesophageal muscularis mucosae.
[a] Not possible to undertake analysis due to low maximum response attained by agonist.

Table 4 SQ 29,548 pA_2 values and Schild slopes at receptors mediating contractions of guinea-pig ileum, oesophageal muscularis mucosae trachea, aorta and portal vein

	Ileum		*OMM*		*Trachea* (contraction)		*Aorta*		*Portal vein*	
Agonist	pA_2	*Slope*	pA_2	*Slope*	pA_2	*Slope*	pA_2	*Slope*	pA_2	*Slope*
PGE_1	No effect at 1×10^{-6} M		No effect at 1×10^{-6} M		b		—		—	
PGE_2	No effect at 1×10^{-6} M		No effect at 1×10^{-6} M		b		—		—	
16,16 dimethyl PGE_2	No effect at 1×10^{-6} M		a		No effect at 1×10^{-6} M		8.59 ± 0.08	0.93 ± 0.08	b	
U46619	—		8.43 ± 0.08	1.0 ± 0.05	8.49 ± 0.05	0.89 ± 0.12	8.40 ± 0.09	0.82 ± 0.12	8.35 ± 0.12	0.95 ± 0.08
PGD_2	—		8.51 ± 0.12	0.98 ± 0.08	8.32 ± 0.10	0.92 ± 0.15	8.46 ± 0.12	0.73 ± 0.15	b	

Values are mean ± s.e. mean, $n = 5$–12.
[a] Second portion of biphasic concentration-response curve only blocked at 3×10^{-7} M SQ 29,548.
[b] Experiment not undertaken since prostaglandin acted as a partial agonist.
—indicates no response to agonist.
OMM-oesphageal muscularis mucosae.

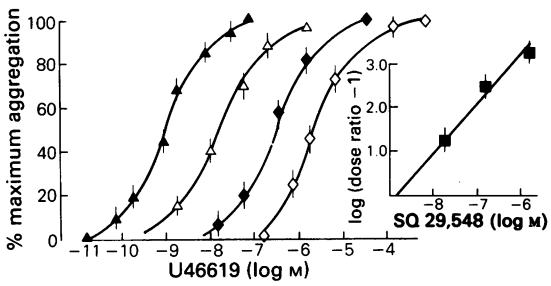

Figure 4 Effect of SQ 29,548 on aggregation responses to U46619 in guinea-pig platelets. Values are mean, $n = 6$; s.e. mean shown by vertical lines. Control curve (▲); curve repeated in the presence of 2.4×10^{-8} M (△), 4×10^{-7} M (◆) and 2.4×10^{-6} M (◇) SQ 29,548. The resulting Schild plot from these data is shown in the inset.

SQ 29,548. Relaxations of the trachea elicited by PGE_1 or PGE_2 were also unaffected by SQ 29,548. The second portion of the biphasic concentration-response curve to 16,16 dimethyl PGE_2 at receptors in the oesophageal muscularis mucosae was abolished in the presence of SQ 29,548 (4×10^{-7} M). This antagonist was inactive against contractions to PGE_1, PGE_2 or PGI_2 in the ileum, trachea or oesophageal muscularis mucosae (Table 4).

SQ 29,548 did not antagonize the inhibition of platelet aggregation induced by PGI_2 or PGE_1. However, it did reverse, in a concentration-dependent manner, the second portion of the biphasic curve to PGD_2 (Figure 3). In addition, SQ 29,548 competitively antagonized aggregation responses to U46619 and the pA_2 value was 8.77 ± 0.05, slope $= 0.99 \pm 0.08$ (mean ± s.e. mean, $n = 4$; Figure 4). At 3×10^{-7} M, SQ 29,548 inhibited the aggregation induced by 16,16 dimethyl PGE_2.

Misoprostol

Misoprostol exhibited concentration-dependent contractions of the guinea-pig ileum, oesophageal muscularis mucosae, trachea and rat colon. No relaxations to this agonist were observed in the trachea. The compound was without effect on the aortic and portal vein preparations. These data are shown in Table 5.

Misoprostol did not induce platelet aggregation except at the highest concentration studied (4×10^{-5} M). At this concentration a slight aggregation response was seen (20 ± 3%; mean ± s.e. mean, $n = 4$, of the U46619 response). This response was antagonized by SQ 29,548 (4×10^{-7} M). At very high concentrations (4×10^{-6}–4×10^{-5} M), misoprostol did inhibit platelet aggregation in the presence of 4×10^{-7} M SQ 29,548.

Fenprostalene

Fenprostalene was without effect on the ileum but elicited contractions of the oesophageal muscular mucosae, trachea and rat colon. In addition, the compound elicited contractions of the aorta and portal vein. Fenprostalene induced aggregation of platelets, and these responses were antagonized by SQ 29,548 (4×10^{-7} M). These data are shown in Table 5.

Table 5 Potency ($-\log EC_{50}$) and maximum response (α) of misoprostol and fenprostalene at prostanoid receptors in guinea-pig and rat smooth muscle or guinea-pig platelets

Preparation	*Misoprostol* ($-\log EC_{50}$)	α	*Fenprostalene* ($-\log EC_{50}$)	α
Ileum	6.83 ± 0.06	1.0	No response	—
OMM	7.23 ± 0.09	1.0	6.73 ± 0.02	—
Trachea (contraction)	6.51 ± 0.12	1.0	6.82 ± 0.05	1.0
Trachea (relaxation)	No response	—	No response	—
Aorta	No response	—	6.70 ± 0.08	1.0
Portal vein	No response	—	5.82 ± 0.12	0.8
Colon	No response	—	7.40 ± 0.07	1.0
Aggregation	No response[a]		9.31 ± 0.05	1.0
Inhibition of aggregation	4.71 ± 0.05[b]	0.9	No response	

Values are mean ± s.e. mean, $n = 5$. All agonists examined between 1×10^{-9} and 3×10^{-5} M.
[a] At 4×10^{-5} M, a slight aggregation response was seen (20 ± 3%, of U46619 response).
[b] -in presence of 3×10^{-7} M SQ 29,548.
OMM-oesophageal muscularis mucosae.

Discussion

Prostanoid receptors are currently classified into at least 5 subtypes, as described in the Introduction. The evidence for this classification is derived primarily from rank orders of agonist potencies, although selective antagonists are available for the EP_1- and TP-class (Coleman *et al.*, 1985a). The aim of the present study was to assess the usefulness of this classification in analyzing (a) the receptor population of guinea-pig and rat smooth muscles and (b) the receptor profiles of misoprostol and fenprostalene in these isolated tissues.

The guinea-pig ileum has been suggested as a tissue which contains a preponderance of EP_1-receptors, since responses to prostanoids are selectively antagonised by the EP_1-antagonists, AH 6809 (Coleman *et al.*, 1985b) or SC-19920 (Coleman & Kennedy, 1985). Similar results were also obtained in our studies, and the pA_2 values obtained using AH 6809 are similar to those reported previously. The ability of 16,16 dimethyl PGE_2, PGI_2 and $PGF_{2\alpha}$ to induce a response in this tissue indicates that these agonists also possess the ability to stimulate EP_1-receptors. 16,16 dimethyl PGE_2 was the most potent agonist examined at EP_1-receptors in the ileum or trachea. These data are in agreement with previous reports (Coleman *et al.*, 1986) which suggest that it can act as a selective EP_1 agonist. PGI_2 has also been shown to act as an EP_1-agonist (Dong *et al.*, 1986) in the trachea and the present study suggests it can also act as an EP_1-agonist in the ileum. Gaion & Trento (1983) have reported that the guinea-pig ileum also contains IP-receptors mediating contractile responses. However, this response is abolished in the presence of tetrodotoxin and atropine. Since atropine was present in the bathing fluid in the present study, it is likely that the contractile response to PGI_2 was mediated through EP_1-receptors. The lack of activity of the TP-agonist, U46619 (Coleman *et al.*, 1981) or PGD_2 indicates the lack of TP- and DP-receptors in this tissue. This finding is also in accord with the lack of antagonism exhibited by SQ 29,548 against responses to either PGE_1 or PGE_2 in the ileum.

It should be noted that in the present study PGE_2 is much less active at contractile receptors in the guinea-pig ileum than 16,16 dimethyl PGE_2 (130 fold) or PGE_1 (10 fold). These data are in contrast to those reported by Coleman *et al.* (1987) in which PGE_2 was marginally less active than 16,16 dimethyl PGE_2 (13 fold) and more active than PGE_1 (12 fold). These disparities in the two studies clearly warrant further investigation and underline the problems associated with attempting to classify prostanoid receptors on the basis of agonist potencies alone (see Coleman *et al.*, (1985a) for review). It is suggested that the definitive characterization should arise from development of selective antagonists.

The trachea also appears to possess EP_1- receptors, since (a) the most potent compound studied was 16,16 dimethyl PGE_2 and (b) the responses were antagonized by AH 6809 with similar affinites (as estimated by the pA_2) to those observed at EP_1-receptors in the ileum. These data are in agreement with previous studies using the less potent EP_1-antagonist, SC-19920 (Kennedy *et al.*, 1982). The tissue, however, appears to contain other receptor types including the TP-receptor, since (a) a response was elicited by U46619 and (b) such responses were antagonized by the TP-antagonist SQ 29,548 (Ogletree *et al.*, 1985) with pA_2 values consistent with TP-receptor stimulation. The responses to 16,16 dimethyl PGE_2 in the trachea were not antagonized by SQ 29,548, which indicates that this agonist acted primarily at EP_1-receptors in this tissue. These data are in agreement with previous studies using other TP-antagonists (Ogletree *et al.*, 1985). The responses to PGI_2 in this preparation have been previously ascribed to EP_1-receptor stimulation (Dong *et al.*, 1986), whilst those to $PGF_{2\alpha}$ are reported to be mediated by both EP_1 and TP receptors (Coleman & Kennedy, 1980). The responses to PGD_2, in view of its low potency could not be definitively characterized, although as discussed below PGD_2 has the ability to act at TP receptors.

Of all the agonists studied, only PGE_1 and PGE_2 exhibited relaxant responses in the trachea. These responses were not antagonized by AH 6809 or SQ 29,548, indicating that they were not mediated by EP_1- or TP-receptors. In this respect these receptors mediating tracheal relaxation resemble those mediating relaxations of the cat trachea or contractions of the chick oesophagus, which have been designated as EP_2 (Coleman *et al.*, 1986).

In the present study, no relaxant response was observed to 16,16 dimethyl PGE_2 in the trachea precontracted with carbachol. This result is in contrast to data reported by Dong *et al.* (1986) in which a relaxation response was observed to this agonist in the trachea precontracted with iloprost, albeit at concentrations 10 fold greater than PGE_2. It is possible therefore that the expression of relaxant responses due to EP_2-receptor stimulation vary according to the agent used to raise the resting tone of the preparation.

The oesophageal muscularis mucosae also appears to possess more than one receptor population. EP_1-receptors appear to be present since they are stimulated by PGE_1 and PGE_2 and are antagonized by AH 6809 with pA_2 values consistent with EP_1-receptor stimulation. TP-receptors also are present and mediate responses to U46619 and PGD_2

Table 6 Proposed classification of prostanoid receptors mediating smooth muscle contraction and platelet aggregation/inhibition of aggregation

Tissue	*Receptors*
Guinea-pig ileum	EP_1, IP
Trachea	EP_1, EP_2, TP
OMM	EP_1, TP
Aorta	TP
Portal Vein	TP
Platelet	DP, IP and TP
Rat Colon	FP and TP

Terminology is that proposed by Coleman *et al.* (1985).
OMM = oesophageal muscularis mucosae.

since the pA_2 values for SQ 29,548 are consistent with TP stimulation. However, unlike the trachea, the oesophageal muscularis mucosae exhibited a biphasic concentration-response curve to 16,16 dimethyl PGE_2. The second portion of the curve was abolished in the presence of SQ 29,548 indicating that this was mediated through TP-receptors, whilst the initial portion of the curve was antagonized by AH 6809, indicating stimulation through EP_1-receptors. The oesophageal muscularis mucosae, therefore, contains both EP_1- and TP-receptors. Unlike the trachea, however, no EP_2-receptors were evident.

The aorta and portal vein appear to be sensitive to the action of U46619 and the responses are antagonized by SQ 29,548. U46619 has been proposed as an aspecific TP-agonist (Coleman *et al.*, 1981). The pA_2 values obtained are indicative of TP-receptor function, and were similar to values obtained in the rat or rabbit aorta (Ogletree *et al.*, 1985). The responses to PGD_2 and 16,16 dimethyl PGE_2 were also antagonized by SQ 29,548, indicating that in these preparations these agonists can act at TP-receptors. Similar results using these agonists have been observed previously (Jones *et al.*, 1982) and agree with data obtained in this study using the trachea. It is interesting to note that in the portal vein PGD_2 acted as a partial agonist indicating that either the receptor number or efficiency of stimulus response-coupling is less in this tissue, in comparison to the aorta.

The ascending rat colon exhibited contractile responses to all the prostaglandins examined. All experiments were conducted in either the presence of AH 6809 or SQ 29,548 to exclude EP_1- and TP-receptor activity respectively. Preliminary experiments had shown that responses to U46619 were reduced in the presence of SQ 29,548, indicating the presence of TP-receptors. The low potency of PGI_2 and PGD_2 in this tissue, relative to their effects at IP- or DP-receptors in the platelet, argues against the presence of these receptors in this tissue. It should be noted that Dong *et al.* (1986) have reported that analogues of PGI_2 exhibit an inhibitory action in this preparation, which may provide an explanation for the low potency of PGI_2 at receptors eliciting a contractile response observed in the present study. The potent action of $PGF_{2\alpha}$ and fenprostalene (see below) which was unaffected by AH 6809 or SQ 29,548 provides reasonable evidence for the presence of FP-receptors. However, the lack of specific FP-antagonists precludes any definitive conclusion.

The ability of PGI_2 and PGE_1 to inhibit platelet aggregation is in agreement with previous results. It has been proposed (Miller & Gorman, 1979) that PGE_1 acts on IP-receptors to mediate this response. The inability of the EP_1-antagonist (AH 6809) to inhibit the effect of PGE_1 in this preparation is in accord with this suggestion. PGD_2 exhibited a biphasic concentration-response curve, the second portion of which was antagonized by SQ 29,548, indicating TP interaction. This has been reported previously (Hamid-Bloomfield & Whittle, 1986). In the presence of TP blockade, PGD_2 acted as a full agonist at inhibiting aggregation, an effect that was selectively antagonized by a high concentration (relative to its affinity at EP_1-receptor, see above) of AH 6809. Those data are also in agreement with previous studies (Keery & Lumley, 1985). The lack of effect of $PGF_{2\alpha}$ is in agreement with previous findings (Armstrong *et al.*, 1985), and indicates the absence on FP-receptors in this preparation.

The pro-aggregant responses of U46619 and 16,16 dimethyl PGE_2 were antagonized by SQ 29,548, indicating action of TP-receptors. The pA_2 calculated using U46619 as the agonist was similar to that observed at TP-receptors in the aorta portal vein and trachea indicating similar receptors in the platelet and these tissues. The higher potency of U46619 at TP-receptors in the platelets, in comparison to the aorta, may indicate the presence of a higher effective receptor reserve.

The receptor subtypes proposed to exist in these tissues are summarised in Table 6.

Misoprostol and fenprostalene

A secondary aim of the present study was to classify the actions of misoprostol and fenprostalene. Misoprostol is a potent antisecretory prostaglandin, structurally related to PGE_1 (Collins *et al.*, 1985). This compound acted as a potent agonist at the EP_1-receptors in the ileum, trachea, and oesophageal muscularis mucosae, but was devoid of activity at

EP_2-receptors in the trachea or TP-receptors in the aorta. As described above, relaxations of the trachea due to EP_2-receptor stimulation, vary according to the agent used to raise the initial tone. However, preliminary experiments (Eglen, unpublished observations) in which the trachea was precontracted with U46619 also showed a lack of relaxation by misoprostol. It should also be noted that misoprostol was studied at EP_1-receptors in the ileum, trachea and oesophageal muscularis mucosae. Additional studies using misoprostol at EP_2-receptors in the cat trachea or chick oesophagus are also required before one can state that misoprostol is a selective EP_1-agonist. Misoprostol did not induce platelet aggregation except at high concentrations, also indicating a lack of TP agonism. A very weak inhibition of platelet aggregation was observed at high concentrations. Little or no action was observed at receptors in the rat colon, which may indicate a lack of FP agonism. Misoprostol has been reported to exhibit fewer side effects than PGE_1 (Bauer, 1985) and this may be related to its selective EP_1 action. It has been shown that the prostanoid receptors inhibiting gastric secretion resemble EP_1-receptors (Reeves & Stables, 1985) with regard to the order of agonist potency.

Fenprostalene is used as a luteolytic agent in cattle, and previous studies have indicated that it is a TP agonist since it contracted the guinea-pig trachea and rabbit aorta (Jackson & Jessup, 1984). In the present study the results using the smooth muscles and platelets are in agreement with these data, and further suggest that fenprostalene lacks EP_1 and EP_2 activity. In addition, the ability of fenprostalene to contract the rat colon may indicate FP agonism. Cloprostenol, is structurally related to fenprostalene but has been reported to possess much less TP activity (Jackson & Jessup, 1984). Both of these agents are however, potent agonists at FP-receptors and this may be related to their luteolytic activity (Coleman *et al.*, 1985a).

In conclusion, the data obtained with prostanoid agonists and selective antagonists provide further evidence for the classification of prostanoid receptors as defined by Coleman *et al.* (1985a). In addition, the use of the above compounds has enabled (a) the classification of receptors in the preparations examined and (b) the profiles of misoprostol and fenprostalene to be obtained.

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