Characterization of the prostanoid receptor profile of enprostil and isomers in smooth muscle and platelets *in vitro*

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1 Enprostil is composed, in approximately equal proportions, of 4 allenic isomers which are prostanoids structurally related to prostaglandin E₂ (PGE₂). The isomers are denoted as RS-86505-007, RS-86812-007 which are in the 'natural' R and S configuration (with respect to PGE₂) and RS-86505-008 and RS-86812-008 which are in the 'unnatural' R and S configuration. In the present study we have characterized their activity at prostanoid receptors, *in vitro*.

2 Enprostil acted as a highly potent ($-\log EC_{50} = 8.30 \pm 0.08$; mean $\pm$ s.e.mean, $n = 6$) EP₃ receptor agonist in the guinea-pig vas deferens, although no activity was observed at guinea-pig tracheal EP₂ receptors at concentrations up to and including 10 μ M. Attempts to study the action of enprostil at EP₁ receptors were complicated by a general increase in the spontaneous activity of the guinea-pig isolated ileum. This response was stereospecific (i.e. observed, with the 'natural' R and S isomers only) and was not mediated through EP₁, FP or TP receptors.

3 Enprostil also exhibited a potent agonist effect at FP and TP receptors in the rat colon and guinea-pig aorta ($-\log EC_{50}$ values = 7.34 ± 0.11 and 6.54 ± 0.07 , mean $\pm$ s.e.mean, $n = 4-8$ respectively). No activity at concentrations up to and including 10 μ M was observed at DP or IP receptors in the guinea-pig platelet mediating inhibition of ADP-induced aggregation.

4 A similar profile was observed with the 'natural' R and S allenic isomers of enprostil (RS-86505-007 and RS-86812-007, respectively); RS-86505-007 was between 4 and 10 fold more potent than the racemic enprostil. The 'unnatural' allenic R and S isomers of enprostil were much less potent than enprostil, with the latter being virtually inactive.

5 Enprostil and the 'natural' R and S isomers, therefore, were EP₃, FP and TP agonists, being most potent at the EP₃ receptor. The preferred configurations for these receptors appears to be the R, and to a lesser extent the S, form of the natural allenic isomer. The effect of enprostil at EP₁ receptors was not characterized in view of the presence of excitatory EP₃ receptors in the guinea-pig ileum. These data were in accordance with the pharmacological activity of enprostil, including inhibition of gastric acid secretion (possibly EP₃) and diarrhoea (possibly TP).

Introduction

A number of synthetic prostaglandins are in clinical development with regard to their use in the treatment of peptic ulcer disease (see Bauer *et al.*, 1987 for review). Enprostil (Gardrin: methyl ($\pm$)-7-[(1R*, 2R*, 3R*)-3-hydroxy-2-[(E)-3R*]-3-hydroxy-4-phenoxyl-1-butenyl]-5-oxocyclopentyl]-4,5-heptadecanoate) is a racemic mixture of 4 isomers structurally related to prostaglandin E₂ (PGE₂) (Carpio *et al.*, 1987). It has been shown to elicit potent inhibitory effects on gastric acid secretion, both *in vitro* (Soll *et al.*, 1986) and *in vivo* (Roszkowski *et al.*, 1986), and

possess efficacy in the treatment of ulcerative disorders of the gastrointestinal tract (McColl *et al.*, 1986; Thomas *et al.*, 1986; Lauritsen *et al.*, 1987). The exact mechanism by which these effects are mediated remains to be elucidated although, due to its structural similarity to PGE₂, an interaction at a PGE-sensitive receptor has been inferred (Roszkowski *et al.*, 1986).

In support of this hypothesis, enprostil has been previously shown to displace [³H]-PGE₂ specific binding from sites present on membranes from isolated parietal cells, which suggests that it may possess affinity for PGE-sensitive receptors (Woo *et*

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al., 1986). However, whilst a binding site is present which exhibits saturable, stereoselective, high affinity binding for the E series of prostaglandins (Soll *et al.*, 1986), the prostanoid receptors present on the parietal cell have yet to be classified on a functional basis. Competition radioligand binding studies (see Robertson, 1986 for review) with prostaglandins may be ambiguous with regard to degree of specific binding to physiologically relevant receptors (Coleman *et al.*, 1985). Beinborn *et al.* (1985) have shown that prostanoid binding to mucosal cell membranes can be simulated by experimental artifacts.

An alternative approach is to use functional tissue responses to study prostanoid receptors. These have been classified (Kennedy *et al.*, 1982; Coleman *et al.*, 1985; Coleman, 1987) according to the scheme summarised in Table 1. The appropriate synthetic agonists and, where available, synthetic antagonists are also shown. The scheme has proved to be of use in our laboratory in the profiling of a number of synthetic prostanoids including misoprostol, fenprostalene, and RS-61756-007 (Eglen & Whiting, 1988; 1989). The latter two compounds are structurally similar to enprostil.

Enprostil consists of four epimers (which in this study are denoted as RS-86505-007, RS-86812-007, RS-86505-008 and RS-86812-008; Figure 1); two of which are in the 'natural' configuration and two are in the 'unnatural' configuration with respect to the structure of PGE₂. The aim of the present study was to characterize the profile of enprostil, and its constituent epimers at the prostanoid receptor subtypes as defined in Table 1. In this respect, Coleman *et al.* (1988) have obtained preliminary data showing that enprostil acted as a potent EP₁, EP₃, FP and TP agonist.

An abstract of this work has been previously presented to the British Pharmacology Society meeting, London, December, 1988 (Eglen *et al.*, 1989).

Figure 1 Structures of the 4 isomers of enprostil. (R) and (S) denote the absolute configurations.

Table 1 Classification of prostanoid receptors (after Kennedy *et al.*, 1982; Coleman *et al.*, 1985)

Receptor	Natural prostaglandin	Synthetic agonist	Synthetic antagonist
DP	PGD ₂	BW 245C	BW A868C
EP ^a	PGE ₁ /PGE ₂	Sulprostone	AH 6809
FP	PGF _{2α}	Fluprostenol	NA
IP	PGI ₂	Iloprost	NA
TP	TxA ₂	U46619	SQ 29,548

^a EP receptors presently form 3 subtypes, which are denoted as EP₁, EP₂ and EP₃ (Coleman *et al.*, 1987). These are characterized as shown below.

Receptor	Natural prostaglandin	Synthetic agonist	Synthetic antagonist
EP ₁	PGE ₁ /PGE ₂	Sulprostone	AH 6809
EP ₂	PGE ₁ /PGE ₂	Butaprost	NA
EP ₃	PGE ₁ /PGE ₂	Sulprostone	NA

NA – not available.

Methods

Smooth muscle preparations

All experiments were conducted on smooth muscles isolated from guinea-pigs (male Dunkin-Hartley, 300–350 g) or rats (male, Sprague-Dawley, 200–250 g) previously killed by CO₂ asphyxiation. Platelet-rich plasma was prepared from freshly obtained blood (withdrawn by cardiac puncture) from guinea-pigs previously anaesthetised with sodium pentobarbitone (60 mg kg⁻¹, i.p.). The preparations used to study each prostanoid receptor are summarised in Table 2 and detailed methodology has been described recently (Coleman, 1987; Eglen & Whiting, 1988). The tissues were transferred to warm (37°C) physiological salt solutions (pH 7.4) containing indomethacin (1 μM), for further preparation. Guinea-pig proximal ileum and rat ascending colon (Eglen & Whiting, 1988) were gently flushed intraluminally and suspended under 1.0 g tension in Tyrode physiological salt solution. Guinea-pig trachea, previously cut into zig-zag strips (Emmerson & MacKay, 1971), and rings of guinea-pig thoracic aorta were suspended under 1.0 g tension in Krebs physiological salt solution. Guinea-pig vas deferens were prepared according to the method of Hedquist & von Euler (1972) and suspended between parallel platinum electrodes in Krebs physiological salt solution, under 0.5 g tension.

All smooth muscles were allowed 60 min to equilibrate, during which time the bathing fluid was replaced every 15 min. After this period, KCl (50 mM) was added to all tissues, with the exception of the

Table 2 Prostanoid receptors and bioassays employed in the present study

Receptor	Bioassay
DP	Guinea-pig platelet
EP ₁	Guinea-pig ileum
EP ₂	Guinea-pig trachea
EP ₃	Guinea-pig vas deferens
FP	Rat colon
IP	Guinea-pig platelet
TP	Guinea-pig aorta

See also Kennedy *et al.*, 1982; Coleman, 1987; Eglen & Whiting, 1988 for references and reviews

vas deferens, for a 5 min period in order to obtain an estimate of the viability of the tissues. The tissues were then washed and allowed to return to baseline tension levels. Phenoxybenzamine (1 μ M) was then added to all tissues with the exception of the trachea and vas deferens for a period of 15 min. The tissues were then washed twice over the following 10 min period. Atropine (1 μ M) was then added to all preparations, with the exception of the trachea and vas deferens, for the duration of the experiment. Contractile responses were determined in the guinea pig ileum (EP₁), rat colon (FP), and guinea-pig aorta (TP). Previous studies (Eglen & Whiting, 1988) have shown that the guinea-pig trachea, and rat colon also possess EP₁ and TP receptors through which contractile responses are mediated. Consequently, the EP₁ and TP antagonists, AH 6809 (3 μ M) and SQ 29,548 (1 μ M) respectively, were routinely added in studies with these tissues in order to exclude activity at these receptors. The pA₂ values for AH 6809 and SQ 29,548 at EP₁ and TP receptors have been shown (Eglen & Whiting, 1988) to be 7.4 and 8.3, respectively.

In studies with the trachea, relaxations due to EP₂ receptor stimulation were determined in tissues pre-contracted with carbachol (10 μ M). Agonists were added once a sustained contracture was attained. EP₃ receptor function was studied by determining the inhibition of the height of the 'twitch' contractions of the vas deferens, which were elicited by field stimulation. A period of 15 min was allowed in order to attain reproducible contractions to the stimulation. The stimulation-parameters were trains of pulses at 2 min intervals (supramaximal voltage, 5 Hz, 1 ms duration).

In some studies, the effects of the antagonists AH 6809 or SQ 29,548 were measured to determine the role of EP₁ or TP receptors, respectively. The equilibration period with these antagonists was 60 min. Only one concentration of antagonist was applied to each tissue, and control studies, undertaken in paral-

lel, were used to correct for potential changes in sensitivity of the preparations.

Platelet studies

The method used was that described by Eglen & Whiting (1988). Briefly, platelet rich plasma (PRP) was prepared from freshly obtained guinea-pig blood, by centrifugation at 200 *g* for 10 min. Platelet poor plasma (PPP) was prepared by centrifuging at 900 *g* for 2 min. The TP antagonist, SQ 29,548 (1 μ M) was added routinely to the PRP to exclude any proaggregant effects of agonists due to TP receptor stimulation (Eglen & Whiting, 1988). Adenosine 5'-diphosphate (ADP, 2 μ M) was then added to elicit reversible platelet aggregation. Agonists were added 1 min before the ADP in order to determine inhibitory effects on the subsequent aggregation response. Previous studies (Hamid-Bloomfield & Whittle, 1986; Eglen & Whiting, 1988) have shown that both IP and DP receptors mediate inhibition of ADP-induced aggregation in guinea-pig platelets. IP receptors were distinguished from DP receptors in this preparation, by using 10 μ M AH 6809. At this concentration, AH 6809 acts as a competitive antagonist of DP receptors, but not IP receptors, and exhibits a pK_B value of 5.7 (Eglen & Whiting, 1988).

Measurement and analysis of results

Smooth muscles All agonists were added on a cumulative basis, using incremental concentrations spaced at 0.5 log intervals. Successive concentrations were added once a sustained response was obtained in response to the previous agonist concentration. The exceptions to this protocol were the ileal studies, in which non-cumulative concentration-response curves were constructed in order to prevent desensitization. Only one agonist was applied to each tissue, the responses being normalized against the maximum response elicited by a full prostanoid agonist i.e. EP₁ – 16,16 dimethyl PGE₂, EP₂ – PGE₂, EP₃ – sulprostone, FP – PGF_{2 α} , TP – U46619.

All tissue responses were determined as changes in isometric tension (mg), recorded by either a Hugo Sachs K30 or Grass FT03 force transducer and displayed on either a Graphtec-Watanabe or Beckman Dynograph R311 recorder, respectively. The responses were calculated as percentage of the maximal response and the potencies were derived by calculation of the EC₅₀, by means of non-linear iterative curve fitting procedures (Parker & Waud, 1971). In the antagonist studies, the dissociation constants were estimated from a single concentration of

antagonist by use of the relationship: $-K_B = [\text{antagonist}]/\text{concentration-ratio} - 1$ (Furchgott, 1972).

Platelet studies Agonists were added 1 min before the ADP and the extent of platelet aggregation was determined by measuring changes in light transmission (measured in arbitrary units) with a Chrono-log Whole-Blood aggregometer and recorded by a flat bed recorder. The responses were calculated as the percentage inhibition of the aggregation response to ADP, and the EC_{50} value was then calculated as described above.

Physiological salt solutions

The composition (in mM) of the physiological salt solutions used were as follows: Krebs solution: NaCl 118.4, KCl 4.9, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, glucose 11.1, $NaHCO_3$ 25.0 and $CaCl_2 \cdot 6H_2O$ 1.1.

Tyrod solution: NaCl 136.9, KCl 2.7, $MgCl_2 \cdot 6H_2O$ 1.1, $NaH_2PO_4 \cdot 2H_2O$ 0.4, glucose 5.6, $NaHCO_3$ 11.9 and $CaCl_2 \cdot 6H_2O$ 1.8.

All solutions were gassed with 5% CO_2 /95% O_2 and indomethacin (1 μM) was present throughout the experiments.

Drugs

The following drugs were used: PGD_2 , PGE_1 , PGE_2 , 16,16 dimethyl PGE_2 , PGI_2 , U46619 (9,11-methanoepoxy PGH_2), $PGF_{2\alpha}$ (Cayman Chemical Co.), BW 245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl) hydantoin; Burroughs Wellcome), sulprostone, enprostil (methyl $(\pm)$ -7-[(1R*, 2R*, 3R*)-3-hydroxy-2-[(E)-3R*-3-hydroxy-4-phenoxy-1-butenyl]-5-oxocyclopentyl]-4,5, heptienoate), and the isomers, RS-86505-007, RS-86505-008, RS-86812-007 and RS-86812-008 (IOC, Syntex Research, Palo

Alto); AH 6809 (6-isopropoxy-9-oxoanthene-2-carboxylic acid, Glaxo); SQ 29,548 (15-[1 α 2 β (5Z),3 β , H α]-7-[3-[2-(phenylamino)-carbonyl]hydrazino]-methyl]-7-oxobicyclo[2.2.1]-hept-2-yl]-5-heptenoic acid, Squibb); carbachol, indomethacin, atropine (Sigma) and phenoxybenzamine (SK & F).

Stock solution of prostanoids (usually 3×10^{-2} M) were prepared in ethanol and stored at $-20^\circ C$. Upon use, these solutions were diluted with H_2O . Specific details for the preparation and stability of the various compounds used in this study are described elsewhere (Coleman, 1987).

Results

In agreement with previously published data (Coleman, 1987; Eglen & Whiting, 1988; 1989) the majority of tissues responded to the prostanoid agonists by exhibiting concentration-dependent contractions. As previously shown (Eglen & Whiting, 1988; Hedquist & von Euler, 1972) the guinea-pig trachea and field stimulated vas deferens exhibited concentration-dependent relaxations and inhibition of twitch responses, respectively.

Agonists

The potencies, as denoted by the $-\log EC_{50}$ values, for both standard natural and synthetic prostanoids are shown in Table 3. These are also described below:

EP_1 receptors (guinea-pig ileum) The rank order of agonist potency was 16,16 dimethyl PGE_2 > PGE_1 > PGI_2 > PGE_2 > $PGF_{2\alpha}$ $\gg$ U46619 = BW 245C = 0. All these compounds, with the exception of U46619 and BW 245C, acted as full agonists. U46619 and BW 245C, at concentrations of up to 30

Table 3 Potencies ($-\log EC_{50}$) of standard prostaglandins at receptors in various tissues

	EP_1 (Ileum)	EP_2 (Trachea)	EP_3 (Vas deferens)	FP (Colon)	TP (Aorta)
PGE_1	7.81 ± 0.08	6.57 ± 0.06	8.46 ± 0.11	6.23 ± 0.07	NR
PGE_2	6.84 ± 0.12	7.43 ± 0.09	7.70 ± 0.06	7.21 ± 0.12	< 5.0
DME ₂	8.96 ± 0.03	5.45 ± 0.14	8.84 ± 0.03	6.93 ± 0.11	6.44 ± 0.08
$PGF_{2\alpha}$	6.21 ± 0.05	NR	6.16 ± 0.06	8.02 ± 0.09	6.20 ± 0.03
U46619	NR	NR	NR	NR	7.44 ± 0.12
Sulprostone	NR*	5.84 ± 0.05	9.32 ± 0.07	5.21 ± 0.11	NR
BW 245C	NR	NR	NR	NR	NR
PGI_2	7.34 ± 0.05	5.32 ± 0.08	5.52 ± 0.08	5.12 ± 0.13	NR

Values are mean $-\log EC_{50} \pm$ s.e.mean, $n = 4-8$. DME₂ = 16,16 dimethyl PGE_2 ; NR = no response at the concentrations studied (1 nM–30 μM).

* An increase in spontaneous activity was observed which persisted after washout. The variability of this response was difficult to quantify (Figure 2), and no $-\log EC_{50}$ value was calculated.

Figure 2 Responses of guinea-pig isolated ileum to prostaglandin E_2 (PGE_2) (a), sulprostone (b) and enprostil (c). Agonists were added for 30 s on a 5 min dose cycle in a non-cumulative fashion.

μM had no agonist activity. The contractile responses, with the exception of sulprostone, were qualitatively similar, in that clearly defined contractions were observed which did not persist when the bathing solution was replaced (Figure 2). However, the responses to sulprostone consisted of an increase in the spontaneous activity of the preparation and these responses did persist after the bathing solution was replaced. The magnitude of this activity appeared to be concentration-dependent, but due to the extreme variability of the responses, no accurate measurements could be performed and the $-\log EC_{50}$ values were not calculated. These responses are shown in Figure 2, together with responses to PGE_2 for comparative purposes. The responses to sulprostone were unaffected by the inclusion of either the EP_1 antagonist, AH 6809 ($3 \mu M$) or the TP antagonist, SQ 29,548 ($1 \mu M$; data not shown). No contractile responses were observed in this preparation to either U46619 or BW 245C at the concentrations studied (1 nM – $30 \mu M$).

EP_2 receptors (guinea-pig trachea) The rank order of agonist potency at receptors mediating relaxation of this preparation was $PGE_2 > PGE_1 > \text{sulprostone} > 16,16 \text{ dimethyl } PGE_2 = PGI_2$. All compounds acted as full agonists. No relaxant response was observed in this preparation to either BW 245C, $PGF_{2\alpha}$ or U46619 at the concentrations studied (1 nM – $30 \mu M$).

EP_3 receptors (guinea-pig vas deferens) The rank order of agonist potency at receptors inhibiting the twitch response to electrical stimulation was sulprostone $> 16,16 \text{ dimethyl } PGE_2 > PGE_1 > PGE_2 > PGE_{2\alpha} > PGI_2$. All compounds acted as full agonists. The responses to all these agonists were unaffected by inclusion of $3 \mu M$ AH 6809 (data not shown). No inhibitory responses were observed to U46619 or BW 245C at the concentrations studied (1 nM – $30 \mu M$).

FP receptors (rat colon) The rank order of agonist potency was $PGF_{2\alpha} > PGE_2 > 16,16 \text{ dimethyl } PGE_2 > PGE_1 > \text{sulprostone} = PGI_2$. These compounds acted as full agonists whilst U46619 and BW 245C were inactive at the concentrations used (1 nM – $30 \mu M$).

TP receptors (guinea-pig aorta) The rank order of potency observed in this preparation was U46619 $> 16,16 \text{ dimethyl } PGE_2 > PGF_{2\alpha} > PGE_2$. All compounds, with the exception of PGE_2 , acted as full agonists. At $3.2 \times 10^{-5} M$ PGE_2 elicited a contractile response which was $28 \pm 3\%$ (mean $\pm$ s.e.mean, $n = 6$) of the maximal response. PGE_1 , sulprostone PGI_2 and BW 245C were inactive at the concentrations studied (1 nM – $30 \mu M$).

The rank order of potency of the above compounds are in accordance with the hypothesis that the responses observed are due to EP_1 , EP_2 , EP_3 , FP and TP stimulation, respectively (Coleman *et al.*, 1987; Eglen & Whiting, 1988).

Enprostil and isomers

The potencies of enprostil and the 4 epimers at the various prostanoid receptors are shown in Table 4. Enprostil exhibited responses in the ileum that were clearly different in nature from those of other EP_1 agonists such as PGE_1 or PGE_2 but most closely resembled those observed to sulprostone (see above). These responses are shown in Figure 2 and in Table 4 have been denoted as 'no response' for the reasons described below. The increase in spontaneous activity was also highly variable, and persisted after washout. Some degree of concentration-dependence was observed, but no accurate quantitation could be performed and, therefore, the interaction of enprostil at ileal EP_1 receptors was difficult to quantify. The responses in the ileum were also observed with the 'natural' R and S isomers but were not observed with the 'unnatural' R and S isomers. The increase in spontaneous activity was unaffected by the inclusion of either the TP antagonist, SQ 29,548 ($1 \mu M$) or the EP_1 antagonist, AH 6809 ($3 \mu M$; data not shown).

Neither enprostil nor any isomers acted as EP_2 receptor agonists, since no relaxations of the precon-

Table 4 Potencies ($-\log EC_{50}$) of enprostil and isomers at prostanoid receptors in various tissues

	EP ₁ (Ileum)	EP ₂ (Trachea)	EP ₃ (Vas deferens)	FP (Colon)	TP (Aorta)
Enprostil	NR*	NR	8.30 ± 0.08	7.34 ± 0.11	6.54 ± 0.07
RS-86505-007	NR*	NR	8.86 ± 0.03	7.41 ± 0.10	7.43 ± 0.05
RS-86812-007	NR*	NR	8.18 ± 0.05	7.57 ± 0.08	6.86 ± 0.12
RS-86505-008	NR	NR	6.76 ± 0.11	6.46 ± 0.03	NR
RS-86812-008	NR	NR	<6.0	NR	5.39 ± 0.14

Values are mean $-\log EC_{50} \pm$ s.e.mean, $n = 4-8$. PGE₂ and sulprostone were standard agonists at the EP₃ receptor ($-\log EC_{50}$ values = 7.70 and 9.32), PGF_{2 α} was the standard agonist at FP receptors ($-\log EC_{50} = 8.02$), whilst U46619 was the standard agonist at TP receptors ($-\log EC_{50} = 7.44$). NR = no response.

* Indicates no clear sustained contractile response at the concentrations studied (1 nM–10 μ M), although an increase in spontaneous activity was observed.

tracted guinea-pig tracheal preparations were observed at the concentrations studied (10 nM–10 μ M). However, potent inhibitory effects on the twitch response in the vas deferens were observed. The rank order of potency at EP₃ receptors in this tissue was RS-86505-007 > enprostil = RS-86812-007 > RS-86505-008 > RS-86812-008.

At FP receptors mediating contractions of the rat colon, enprostil, RS-86505-007 and RS-86812-007 were equipotent. In contrast, RS-86505-008 was approximately 10 fold less potent, whilst no response was observed to RS-86812-008 at the concentrations studied (1 nM–10 μ M).

At TP receptors mediating guinea-pig aortic contractions, the order of potency was RS-86505-007 > RS-86812-007 $\geq$ enprostil $\gg$ RS-86812-008. RS-86505-008 was inactive at the concentrations studied (1 nM–10 μ M). The responses to these agonists at TP receptors were sustained for 30 min after addition, and persisted after extensive washing of the tissue.

Platelet studies

PGI₂ and BW 245C acted as potent and selective IP and DP receptor agonists ($-\log EC_{50}$ values = 9.3 ± 0.08 and 8.6 ± 0.13, mean ± s.e.mean, $n = 8$, respectively). The responses to BW 245C, but not PGI₂, were shifted in a parallel fashion to the right in the presence of AH 6809 and the calculated $-\log K_B$ was 5.3. Neither enprostil nor any of the isomers studied (under the conditions of TP blockade by 1 μ M SQ 29,548) inhibited ADP-induced aggregation, at the concentrations studied (1 nM–10 μ M).

Discussion

Enprostil is a 4,5 allenic 16 phenoxy prostaglandin, with structural similarities to PGE₂ (Carpio *et al.*, 1987). It has been previously shown to displace

[³H]-PGE₂ from binding sites in isolated parietal cells (Woo *et al.*, 1986), although the activity at prostanoid receptors in functional bioassays has not been fully studied. We have undertaken such a study and also assessed the activity of each of the 4 isomers of enprostil. Preliminary data by Coleman *et al.* (1988) and Reeves *et al.* (1988) have shown that it exhibits EP₁, EP₃, FP and TP agonist activity.

The data obtained with the standard prostanoids were in good agreement with those in the literature (Kennedy *et al.*, 1982; Coleman *et al.*, 1985; 1988; Coleman, 1987; Eglen & Whiting, 1988). Sulprostone was the most potent agonist studied, at EP₃ receptors, but was less potent at both EP₁ and EP₂ receptors in agreement with previous findings (Coleman *et al.*, 1988). AH 6809 has been shown to antagonise responses to all of these agonists (with the exception of sulprostone) at ileal EP₁ receptors, but not at EP₃ receptors in the vas deferens (Coleman *et al.*, 1987; Eglen & Whiting, 1988; this study). However, definitive evidence of the three putative subtypes awaits the development of selective EP₂ and EP₃ antagonists.

In the presence of EP₁ and TP blockade, the rat colon was highly sensitive to FP receptor stimulation by PGF_{2 α} as previously shown (Eglen & Whiting, 1988). Finally, the guinea-pig aorta has been shown to possess TP receptors (Eglen & Whiting, 1988; 1989) and in accordance with these studies, it was highly sensitive to the effects of the TP agonist, U46619 (Coleman *et al.*, 1981).

The high potency of the selective DP agonist BW 245C (Hamid & Whittle, 1985; Giles *et al.*, 1989) at receptors inhibiting guinea-pig platelet aggregation is in accordance with the presence of DP receptors in this tissue (Hamid-Bloomfield & Whittle, 1986; Eglen & Whiting, 1989). The similarity in pK_B values for AH 6809 when either PGD₂ (5.7, Eglen & Whiting, 1988) or BW 245C (5.3, this study) was used, also suggested that these agonists interacted at the same receptor. The lack of TP activity of BW

245C renders this a more useful pharmacological tool than PGD₂ with which to classify DP receptors, and the development of BW A868C (Giles *et al.*, 1989) as a potent and selective DP antagonist has allowed further characterization of this receptor subtype. The high potency of PGI₂ and lack of antagonism by AH 6809 also indicate the presence of IP receptors in this tissue (Hamid-Bloomfield & Whittle, 1986; Eglen & Whiting, 1988), although no selective IP antagonist has been identified to date.

Enprostil potently inhibited twitch responses in the field stimulated vas deferens, indicating that it possessed EP₃ agonist properties (Coleman *et al.*, 1987). Since no relaxations of the precontracted guinea-pig trachea were observed, it probably lacks EP₂ receptor activity. It was not possible to characterize definitively the activity of enprostil on EP₁ receptors with the guinea-pig ileum. Enprostil did not exhibit single uniphasic contractile responses of the guinea-pig ileum, although these characteristics were observed with EP₁ agonists such as PGE₁, PGE₂ or 16,16-dimethyl PGE₂ (Eglen & Whiting, 1988; this study). A series of spontaneous contractions were observed which persisted even after washout of enprostil. Since this activity was not observed with either U46619 or PGF_{2α}, it is unlikely that they can be ascribed to TP or FP receptor stimulation, respectively. In addition, the lack of effect of the EP₁ and TP antagonists, AH 6809 and SQ 29,548, suggested that the response was not mediated through either EP₁ or TP receptors respectively. Coleman *et al.* (1988) have shown an interaction of enprostil with EP₁ receptors in the guinea-pig fundus. Attempts to duplicate these data in our hands proved problematic due to the additional presence of FP and TP receptors (Dong *et al.*, 1986; R.M. Eglen unpublished observations).

The lack of a response in the ileum to the 'unnatural' R and S isomers of enprostil indicated a stereospecific interaction at a receptor mediating these responses. Sulprostone, which was also a potent EP₃ receptor agonist, elicited similar responses and similar findings have been observed with two prostanoids structurally related to enprostil, RS-61756-007 (Eglen & Whiting, 1989) and fenprostalene (Eglen & Whiting, 1988). One possible explanation is that these responses may be due to the presence of excitatory EP₃ receptors in this tissue. Similar conclusions have been reached by Poll *et al.* (1988) and Lawrence & Jones (1989) with the guinea-pig ileum. In agreement with these workers, we conclude that the guinea-pig ileum is an unsuitable preparation with which to study agonists with mixed EP₁ and EP₃ receptor activity, such as sulprostone and enprostil. Enprostil, therefore, possesses activity at EP₃ receptor, but not at EP₂ receptors. Its ability to stimulate EP₁ receptors (as found by Coleman *et al.*,

1988) remains to be studied further. Enprostil also acted as an agonist at both TP and FP receptors, a finding in agreement with the work of Coleman *et al.* (1988). The compound was approximately 10 fold less potent than U46619 at TP receptors, and approximately 3 fold less potent than PGF_{2α} at FP receptors. Enprostil lacked activity at platelet DP and IP receptors. Enprostil, therefore, possesses the following rank order of activity at prostanoid receptors: EP₃ > FP > TP ≥ EP₂ = DP = IP. A structurally related prostanoid to enprostil, RS-61756-007 (Eglen & Whiting, 1989), has recently been shown to possess a highly potent and selective action at TP receptors. Fenprostalene, which is also structurally related to enprostil, also acts as a FP and TP receptor agonist (Eglen & Whiting, 1988). No data are available with regard to the EP₃ activity of fenprostalene and RS-61756-007.

A similar pattern of activity was observed with both the R and S 'natural' isomers of enprostil, in that both stimulated EP₃, FP and TP receptors, whilst lacking activity at EP₂, DP and IP receptors. No definitive conclusions could be made with regard to EP₁ receptor activity. The most potent compound evaluated in the present study was the natural R epimer of enprostil (RS-86505-007) which was approximately 4 and 10 fold more potent than racemic enprostil at the EP₃ and TP receptors respectively. The activity of the R and S unnatural epimers was much less than enprostil, with the S epimer being virtually devoid of any activity. These data clearly suggest that it is the R and to a lesser extent the S configuration of the 'natural' epimer which is preferred for stimulation of the EP₃ and TP receptor.

How does the prostanoid receptor profile of enprostil relate to its gastric antisecretory activity? The rank order of affinity of prostanoid agonist binding to porcine mucosal cell membranes has been shown (Beinborn *et al.*, 1988) to be similar to the rank order of agonist potency defined in functional assays by Coleman *et al.* (1985) for the presence of an EP receptor. Similar findings have been obtained in functional studies by Reeves & Stables (1985) who used isolated parietal cells and gastric muscosal sheets, respectively. However, further characterization of the EP receptor subtype present on the parietal cells is complicated by the existence of three putative subtypes of this receptor, denoted as EP₁, EP₂ and EP₃ (Coleman *et al.*, 1987; Table 1). Reeves *et al.* (1988) have shown that the rank order of agonist potencies at EP receptors inhibiting acid secretion in rat isolated gastric mucosa, was similar to the rank order of potency at EP₃ receptors inhibiting electrically evoked twitch contractions of the guinea-pig vas deferens. The postulate that EP₃ receptors mediate inhibition of gastric acid secretion

is also supported by data showing that a number of synthetic prostaglandins, which are potent inhibitors of acid secretion, also appear to be potent EP₃ agonists (W.L. Smith & R.M. Eglén, unpublished observations).

Some workers have proposed that TP receptors may also play a role in the inhibition of gastric acid secretion (see Reeves & Stables, 1985 for references), although part of the effect of TP agonists appears to be indirect (Reeves & Stables, 1987). However, the evidence is controversial since other studies have shown that TP agonists are either ineffective (Tao & Wilson, 1984) or, when given by close injection into the gastric artery, can cause gastric mucosal damage (Espulugues & Whittle, 1988). Endogenous thromboxane A₂ may, therefore, be a local mediator of gastric injury. U68215 is a PGI₂ analogue which has been shown to inhibit acid secretion *in vivo* (Robert *et al.*, 1985). U68215 is an IP receptor agonist (R.M. Eglén, unpublished observations) which may suggest a role for IP receptors in the inhibition of gastric acid secretion (Beinborn *et al.*, 1985). However, PGI₂ and U68215 can also act as EP receptor agonists (Dong *et al.*, 1986; R.M. Eglén, unpublished

observations) and this property may mediate their actions in the stomach. Taken together, it is likely that the activity of enprostil at EP₃ receptors is responsible for its effects on gastric acid secretion (Roszkowski *et al.*, 1986), although the properties mediated through TP receptors may play a role in the diarrheogenic activity (Clayton *et al.*, 1988; Smith *et al.*, 1988).

In conclusion, the data confirm preliminary findings by Coleman *et al.* (1988) and Reeves *et al.* (1988) showing that enprostil is a potent EP₃ agonist, but additionally possesses activity at FP and TP receptors. It lacks activity at EP₂, DP and IP receptors. The 'natural' R isomer is more potent than the racemic enprostil, whilst both 'unnatural' isomers lack significant activity. The effect of enprostil and epimers, at EP₁ receptors is difficult to characterize, in view of the possible presence of excitatory EP₃ receptors in the ileum.

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