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Prostaglandin E receptor subtypes in smooth muscle: agonist activities of stable prostacyclin analogues

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1 The agonist activities of a range of prostaglandin analogues on smooth muscle preparations sensitive to prostaglandin E_2 (PGE_2) have been investigated. When necessary thromboxane-like activity was eliminated using the thromboxane receptor antagonists EP 045 and EP 092.

2 On the bullock iris sphincter, rat stomach fundus and guinea-pig trachea, (±) ω-tetranor-16-*p*-chlorophenoxy PGE_2 (ICI 80205) and 16,16-dimethyl PGE_2 were more active contractile agents than PGE_2, whereas for relaxant activity on the cat trachea, guinea-pig trachea and dog hind limb arterial vessels *in vivo* the order of potency was reversed. 11-Deoxy PGE_1 exhibited greater relaxant than contractile activity when compared to PGE_2.

3 Iloprost and 6a-carba-$\Delta^{6,6a}PGI_1$ (potent mimetics of PGI_2) showed high contractile activity on the PGE-sensitive preparations. PGI_2 was less active and another potent PGI_2 mimetic, ZK 96480, showed only very weak activity. When tested, the dibenzoxazepines SC 19220 and SC 25191 blocked the contractile actions of iloprost and 6a-carba-$\Delta^{6,6a}PGI_1$ and those of PGE_2 and 16,16-dimethyl PGE_2 to similar extents. Each of the PGI_2 analogues showed weak activity on the relaxant systems.

4 On the proximal portion of the ascending colon of the rat, PGI_2, iloprost, 6a-carba-$\Delta^{6,6a}PGI_1$ and ZK 96480 always inhibited spontaneous activity at nanomolar concentrations. PGE_2 and PGE_1 showed weak contractile activity. The distal portion of the ascending colon was more responsive to the contractile action of PGE analogues: both iloprost and 6a-carba-$\Delta^{6,6a}PGI_1$ showed evidence of contractile activity, whereas PGI_2 and ZK 96480 always inhibited spontaneous activity.

5 Evidence was obtained that the rat stomach fundus also contains a PGF receptor; (±) ω-tetranor-16-*m*-trifluoromethylphenoxy $PGF_{2\alpha}$ (ICI 81008) acted as a specific agonist. $PGF_{2\alpha}$ and its ω-tetranor-16-*p*-fluorophenoxy analogue produced a higher maximum response that ICI 81008 probably due to their additional agonist action at the PGE receptor.

6 The data support the hypothesis that there are two subtypes of the PGE receptor. ZK 96480 has minimal activity on both receptor subtypes and appears to be a highly specific PGI_2 mimetic.

Introduction

The actions of prostaglandin E_2 (PGE_2) on living tissues are particularly numerous and it is of interest to determine the number and nature of the receptors involved. Bennett & Posner (1971) first suggested that two subtypes of the PGE receptor may exit. They showed that the dibenzoxazepine hydrazide SC 19220 (see Sanner *et al.*, 1973) and polyphloretin phosphate blocked the contractile action of PGE_2 on the longitudinal muscle of the guinea-pig ileum, whereas the relaxant action of PGE_2 on the circular muscle was unaffected. Later studies (Kennedy *et al.*, 1983) have shown that SC19220 blocks PGE_2-induced contractions of the guinea-pig ileum and guinea-pig and dog stomach fundus in a competitive manner (affinity constants $\simeq 4 \times 10^5 M^{-1}$), whereas PGE_2-induced contraction of the chick ileum and relaxation of the cat trachea are not inhibited. The Glaxo group have proposed that SC 19220 specifically blocks a subset of PGE receptors (usually associated with contractile responses) and can be used to identify these receptors in biological preparations.

Over the past five years we have attempted to consolidate this 'PGE receptor subtype hypothesis' by comparing the rank order of activity of prostanoid agonists, in particular synthetic PGE analogues, on different smooth muscle preparations. A major difficulty that we have encountered is the potent throm-

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boxane-like activity of certain PGE analogues. For example, both (±) ω-tetranor-16-*p*-chlorophenoxy PGE_2 (ICI 80205) (Figure 1) and 16,16-dimethyl PGE_2 contract the rabbit aorta strip and induce irreversible aggregation of human platelets (Jones *et al.*, 1979; 1982). Fortunately, the recent development of specific thromboxane receptor antagonists (see Jones *et al.*, 1984; Wilson & Jones, 1985) has allowed a true assessment of the PGE-like activities of these compounds and these data are presented here.

The bullock iris sphincter preparation contains a PGE-sensitive contractile system which is blocked by SC 19220 (Posner, 1973). Our recent studies (Dong & Jones, 1982) have revealed that iloprost, a stable and potent mimetic of PGI_2 (Casals-Stenzel *et al.*, 1983) (Figure 1), possesses remarkably high agonist activity on the iris preparation and appears to behave as a partial agonist at the PGE receptor. We have investigated the action of iloprost on several other PGE sensitive preparations and also examined two other potent PGI_2 mimetics, 6a-carba-$\Delta^{6,6a}PGI_1$ (Shibasaki *et al.*, 1983) and ZK 96480 (Mueller *et al.*, 1984) (Figure 1). The latter compound appears to have a much higher specificity than iloprost.

Methods

Isolated preparations

Full details of the bullock iris sphincter and guinea-pig trachea preparations are to be found in our previous publications (Dong & Jones, 1982; Jones *et al.*, 1982). Changes in muscle tension were recorded isometrically with a Grass FT03 force-displacement transducer linked to a Grass Polygraph.

Sections of trachea were obtained from cats under pentobarbitone anaesthesia. Each preparation, consisting of two rings joined at their cartilaginous sections, was suspended under 0.5 g load in Krebs solution at 37°C gassed with 95% O_2/5% CO_2. Tension changes were recorded isometrically as described above.

Rats of either sex were killed by a blow to the head and the stomach and ascending colon removed. Strips of stomach fundus and sections of colon were cut and suspended under 1 g and 0.5 g loads respectively in Krebs solution at 37°C gassed with 95% O_2/5% CO_2. Isotonic contractions of the stomach fundus were recorded using a Washington transducer linked to a Grass Polygraph. The spontaneous activity of the colon preparations was recorded with a Grass FT03 transducer and quantified by measuring the area under the trace over 1 min time periods using a Grass Integrator (Model 7P10B).

Dog hindlimb flow

Dogs of either sex (12–26 kg) were anaesthetized with pentobarbitone sodium via a foreleg vein. The trachea was cannulated and blood pressure was recorded from a carotid artery. Statham electromagnetic flow probes (3 or 4 mm) were placed around both femoral arteries and mean flows were recorded on a Grass Polygraph.

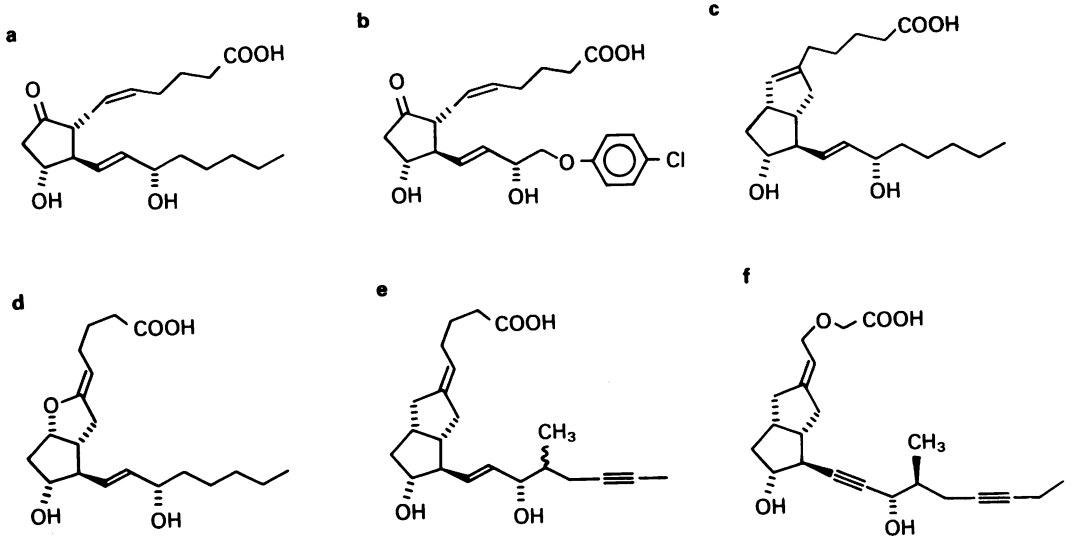

Figure 1 Structures of prostanoid agonists used in this study: (a) prostaglandin E_2 (PGE_2), (b) ICI 80205 (racemic), (c) 6a-carba-$\Delta^{6,6a}PGI_1$, (d) PGI_2, (e) iloprost and (f) ZK 96480.

A small side branch of each femoral artery near the inguinal ligament was cannulated for close-intra-arterial infusion of drugs. A continuous infusion of 0.9% w/v NaCl solution (saline) at 0.25 ml min^{-1} was maintained to each limb throughout the experiment. Each limb was challenged alternately at 15–20 min intervals with drug solution for 30 s. In some experiments the saline solution was replaced by a solution of EP 092 sodium salt (1.2 mg ml^{-1} with respect to the free acid).

Drugs

EP 045 (*rac* 5-*endo*(6'-carboxyhex-2'Z-enyl)-6-*exo*[N-phenylcarbamoyl)-hydrazonomethyl]-bicyclo[2.2.1] heptane), EP 092 (*rac* 5-*endo*(6'-carboxyhex-2'Z-enyl)-6-*exo*{1''-[N-(phenylthiocarbamoyl)hydrazono] ethyl}-bicyclo[2.2.1]heptane and ω-tetranor-16-*p*-fluorophenoxy $PGF_{2\alpha}$ were prepared in our laboratory. PGE_1, PGE_2, 16,16-dimethyl PGE_2, PGD_2 and $PGF_{2\alpha}$ were received as gifts or purchased from the Upjohn Co., U.S.A. ICI 80205 ((±) ω-tetranor-16-*p*-chlorophenoxy PGE_2) and ICI 81008 ((±) ω-tetranor-16-*m*-trifluoromethylphenoxy $PGF_{2\alpha}$) were gifts from ICI Pharmaceuticals Division, Alderley Park. PGI_2, iloprost and ZK 96480 were gifts from Schering AG, Berlin. 6a-Carba-$\Delta^{6,6a}PGI_1$ was a gift from the Sagami Chemical Research Centre, Kanagawa, Japan. SC 19220 (1-acetyl-2-[8-chloro-10, 11-dihydrodibenz (b,f) (1,4) oxazepine-10-carbonyl]-hydrazine) and SC 25191 (1-*n*-butanoyl-2-[8-chloro-10, 11-dihydrodibenz (b,f) (1,4) oxazepine-10-carbonyl]hydrazine) were gifts from Searle, U.S.A. Leukotriene C_4 was a gift from Merck Frosst Canada Inc.

Results

The relative activities of the prostanoids investigated are given in Table 1. PGE_2 was used as the standard agonist except for studies on the guinea-pig trachea where 16,16-dimethyl PGE_2 was used. Cumulative sequences of drug addition were used. Equipotent molar ratios (EPMR) are given where there is good evidence that the compound is a full agonist and that its log concentration-response curve is parallel (as judged by eye) to that of the standard agonist. Approximate values are quoted for PGI_2 since with each of the isolated preparations responses to individual doses were not sustained, presumably reflecting the instability of this compound in aqueous media at neutral pH.

Where affinity constants (K_B) are quoted for the thromboxane receptor antagonists EP 045 and EP 092, these were obtained from Schild plots using 11,9-epoxymethano PGH_2 as the agonist. Full details of the appropriate methods may be found in the publications by Jones *et al.* 1982 and Armstrong *et al.* 1985.

PGE-sensitive contractile systems

In all experiments the cyclo-oxygenase inhibitor indomethacin (1×10^{-6}M) was added to the bathing fluid to abolish any inherent tone and to maintain a stable resting tension.

(a) *Bullock iris sphincter* The major part of the data in Table 1 comes from our previously published work (Dong & Jones, 1982). The most important new finding is the very weak contractile activity of the potent PGI_2 mimetic, ZK 96480. This contrasts sharply with the high activity of two other stable PGI_2 analogues, iloprost (ZK 36374) and 6a-carba-$\Delta^{6,6a}PGI_1$. At a concentration of 1×10^{-6}M ZK 96480 produced no contraction. At 1×10^{-5}M ZK 95480 gave contractions equal to about 30% of the PGE_2 maximum. From a comparison of the magnitudes of responses to PGE_2 in the absence and presence of ZK 96480 (1×10^{-5}M) it appears that the interaction between the two agents is additive. Iloprost produces contractile responses at concentrations as low as 2.5×10^{-9}M and behaves as a partial agonist at the PGE receptor (Dong & Jones, 1982).

Although the iris sphincter preparation contains a thromboxane-sensitive contractile system, EP 045 (2.5×10^{-6}M; $K_B = 4.9 \times 10^7 M^{-1}$) and EP 092 ($1 \times 10^{-6}$M; $K_B = 2.8 \times 10^8 M^{-1}$) have little effect on the equipotent molar ratios quoted in Table 1. This is true for ω-tetranor-16-*p*-fluorophenoxy $PGF_{2\alpha}$, which shows potent thromboxane-like activity on the rabbit aorta, dog saphenous vein and human platelets (Jones & Marr, 1977; Jones *et al.*, 1979). An e.p.m.r. of 1.8 for ω-tetranor-16-*p*-fluorophenoxy $PGF_{2\alpha}$ in the presence of EP 045 (Table 1) indicates that this analogue has considerable PGE-like contractile activity. The closely related analogue (±) ω-tetranor-16-*m*-trifluoromethylphenoxy $PGF_{2\alpha}$ (ICI 81008) has only very weak PGE-like activity.

Two dibenzoxazepines SC 19220 and SC 25191 were tested for blocking activity. SC 25191 (*n*-butyl group replaces the methyl group in SC 19220) was particularly difficult to work with owning to its tendency to crystallize rapidly from aqueous solution (e.g. saline substocks containing 2% ethanol). Warming to 60°C did not induce dissolution. It was therefore added to the organ bath in ethanol. The final concentration of ethanol (0.05%) did not affect responses to PGE_2 or 11,9-epoxymethano PGH_2. In control preparations responsiveness to both PGE_2 and 11,9-epoxymethano PGH_2 was well maintained; dose-ratios for comparison of the second cumulative agonist sequence with the first fell within the ranges 0.95–1.25 ($n = 7$) and 0.85–1.20 ($n = 6$) respectively. At 1×10^{-5}M (20 min pre-incubation) both SC 19220 and SC 25191 shifted

Table 1 Activities of prostanoids on prostaglandin E-sensitive preparations

A *Prostanoid*	*Equipotent molar ratios for contractile effects* *Bullock iris sphincter*	*Rat stomach fundus strip**	*Guinea-pig trachea**
PGE analogues			
PGE_2	1.0 (6 nM)	1.0 (5 nM)	↑↓
ICI 80205	0.16	0.048	0.23
16,16-Dimethyl PGE_2	0.44	0.27	1.0 (3 nM)
PGE_1	3.1	2.6	↑↓
11-Deoxy PGE_1	70	65	Contraction not seen
PGI analogues			
PGI_2	~135	~47	~250
6a-Carba-$\Delta^{6,6a}PGI_1$	17	17	66
Iloprost	p.a. 33–75% (27 nM)	p.a. 36–89% (16 nM)	p.a. 66–83% (27 nM)
ZK 96480	>2000	530	>1000
PGF analogues			
$PGF_{2\alpha}$	53	13	500
ω-Tetranor-16-*p*-fluorophenoxy $PGF_{2\alpha}$	1.1 (1.8*)	0.80	8.8
ICI 81008	1400	max = 20–75% (15 nM)	>1000
PGD analogues			
PGD_2	680	250	>700

B *Prostanoid*	*Equipotent molar ratios for relaxant effects* *Guinea-pig trachea*	*Cat trachea*	*Dog hindlimb blood flow*
PGE analogues			
PGE_2	1.0 (10 nM)	1.0 (40 nM)	1.0 (1 nM)
ICI 80205	Relaxation not seen	70	81†
16,16-Dimethyl PGE_2	14	9.4	45†
PGE_1	1.3	2.1	1.3
11-Deoxy PGE_1	12	13	11
PGI analogues			
PGI_2	>680	~200	120
6a-Carba-$\Delta^{6,6a}PGI_1$	103	74	104
Iloprost	-	>270	116
ZK 96480	>660	>300	34
PGF analogues			
$PGF_{2\alpha}$	>750	85	↑↓
ω-Tetranor-16-p-fluorophenoxy $PGF_{2\alpha}$	Relaxation not seen	>250	Constriction
ICI 81008	>500	>250	Constriction
PGD analogues			
PGD_2	>300	>300	1020

Equipotent molar ratios (e.p.m.r.) are the means of at least 3 determinations. Concentrations in brackets are EC_{50} values for contractile effects or EC_{30} values for relaxant effects (calculated with respect to the compound's own maximum response); see text for dog hindlimb blood flow. p.a. = partial agonist. *Indicates that the thromboxane receptor antagonist EP 045 (2.5×10^{-6} M) was present in the bathing fluid; †indicates that the thromboxane receptor antagonist EP 092 was continuously infused into the hind limb flow; ↑↓ indicates that both contractile and relaxant effects were evident and that e.p.m.r. could not be calculated.

the PGE_2 log concentration-response curve to the right in a parallel manner. Dose-ratios of 3.1, 3.4, 4.5, and 5.9, 11.5, 13.9, 14.8, 16.4 were obtained respectively, giving affinity constants of 2.6×10^5 and $1.2 \times 10^6 M^{-1}$ respectively. The contractile action of 11,9-epoxymethano PGH_2 was little affected by either agent (dose-ratio < 2.0, $n = 3$). In three experiments SC25191 (1×10^{-5}M) was also observed to block the effects of 16,16-dimethyl PGE_2 and iloprost. Dose-ratios calculated for PGE_2, 16,16-dimethyl PGE_2 and iloprost were 5.9, 4.0 and 5.7, respectively; 11.5, 13.2 and 27.8; 14.8, 12.1 and 11.5.

(b) *Rat stomach fundus* All preparations were treated with EP 045 (2.5×10^{-6}M; $K_B = 8.2 \times 10^6 M^{-1}$) throughout the experiment. Of the natural prostaglandins examined PGE_2 and PGE_1 were the most active, followed by $PGF_{2\alpha}$ and then PGI_2 and PGD_2 (Table 1). As with the bullock iris sphincter ICI 80205 and 16,16-dimethyl PGE_2 were more active than PGE_2.

Iloprost behaved as a partial agonist with a maximum response between 36 and 89% of the PGE_2 maximum ($n = 7$). It opposed the contractile action of PGE_2 whereas its interaction with both $PGF_{2\alpha}$ and carbachol was additive. PGI_2 produced a maximum response between 88 and 100% of the PGE_2 maximum, but its log concentration-response curve was shallower. ZK 96480 showed only weak contractile activity, being about 10,000 times less active than ICI 80205.

Unlike the bullock iris sphincter, the rat stomach fundus responded to low concentrations (threshold $= 2 \times 10^{-9}$M) of the $PGF_{2\alpha}$ analogue ICI 81008. However, ICI 81008 did not produce a maximum response equal to that produced by either PGE_2 or $PGF_{2\alpha}$. In 8 experiments the maximum ranged between 20 and 75%, and EC_{50} values between 8×10^{-9} and 2×10^{-8}M. ICI 81008 did not oppose the contractile action of either PGE_2 or $PGF_{2\alpha}$. Thus in the presence of a near maximal concentration of ICI 81008 (5×10^{-7}M), PGE_2 gave additional contractions at concentrations corresponding to submaximal responses in the control state; under these conditions PGE_2 was about 50 times more active than $PGF_{2\alpha}$. In

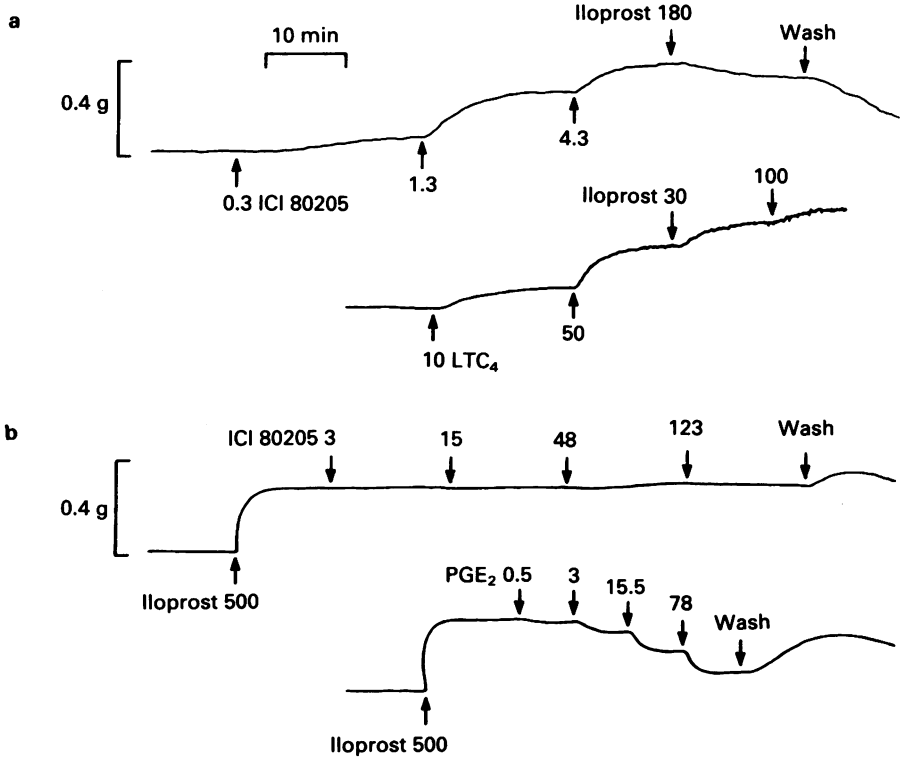

Figure 2 Guinea-pig isolated trachea: indomethacin (1×10^{-6}M), atropine (2×10^{-8}M) and EP 045 (2.5×10^{-6}M) were present in the bathing fluid. (a) Effect of iloprost on contractions induced by ICI 80205 and leukotriene C_4 (LTC_4). (b) Second preparation from the same guinea-pig. Effects of ICI 80205 and prostaglandin E_2 (PGE_2) on tone induced by iloprost. Final concentrations (nM) are shown.

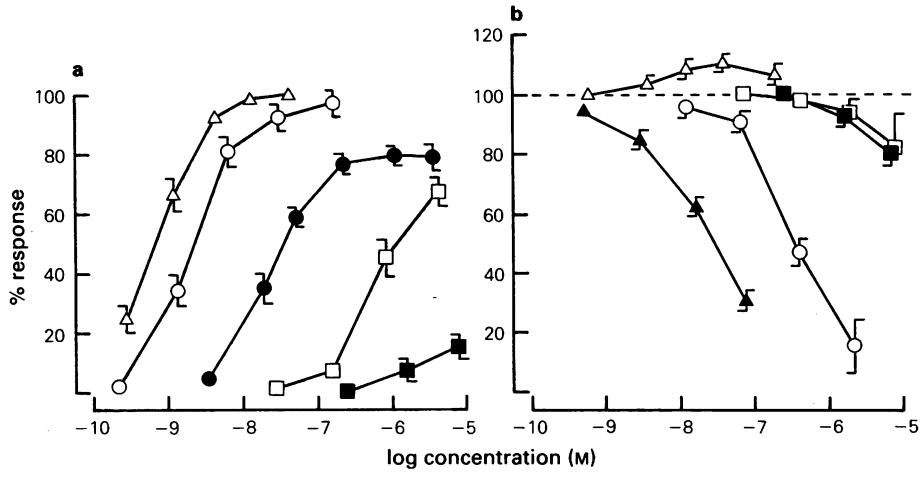

Figure 3 Log concentration-response curves of prostanoids on the guinea-pig isolated trachea. Indomethacin (1×10^{-6}M), atropine (2×10^{-8}M) and EP 045 (2.5×10^{-6}M) were present in the bathing fluid. (a) Contractile effects of ICI 80205 ($n = 8$ preparations) (△), 16,16-dimethyl prostaglandin E_2^* (16,16-dimethyl PGE_2) ($n = 8$) (○), iloprost ($n = 6$) (●), PGI_2 ($n = 5$) (□) and ZK 96480 ($n = 3$) (■). (b) Iloprost (5×10^{-7}M) was used to induce tone (= 100%) against which relaxant activity was assessed:- ICI 80205 ($n = 3$) (△), 16.16-dimethyl PGE_2 ($n = 6$) (○), PGE_2 ($n = 11$) (▲), PGI_2 ($n = 3$) (□) and ZK 96480 ($n = 3$) (■). Vertical lines indicate s.e.mean.

addition, near maximal contractions to either PGE_2 or $PGF_{2\alpha}$ were not inhibited following addition of ICI 81008 (5×10^{-7}M). ω-Tetranor-16-fluorophenoxy $PGF_{2\alpha}$ produced a maximum response equal to that obtained with PGE_2.

(c) *Guinea-pig trachea* All preparations were treated with atropine (2×10^{-8}M) and EP 045 (2.5×10^{-6}M; $K_B = 3.2 \times 10^7 M^{-1}$). PGE_2 stimulates both contractile and relaxant systems present in the trachea over the same concentration range ($3 \times 10^{-9} - 1 \times 10^{-6}$M) (see Jones *et al.*, 1982) and is therefore unsuitable for use as the standard agonist. Although ICI 80205 was found to be the most potent contractile agent (Table 1 and Figures 2 and 3) and did not show a relaxant effect, it was inconvenient to use because its effects were slow in onset and persisted after washout of the organ bath. 16,16-Dimethyl PGE_2 was suitable, however, since it was second in potency to ICI 80205 (Figure 3), washed out relatively quickly, and preparations were reproducibly sensitive to its action (EC_{50} values for 47 preparations lay between 1.5 and 5×10^{-9}M). Only at concentrations in excess of 1×10^{-7}M did 16,16-dimethyl PGE_2 show evidence of relaxant activity. Of the remaining PGE analogues, PGE_1 ($3 \times 10^{-9} - 3 \times 10^{-7}$M) showed dual activity similar to PGE_2 and 11-deoxy PGE_1 failed to produce a contractile effect at concentrations up to 1×10^{-5}M.

Iloprost was a potent contractile agent, typically producing an 80% maximal response (Figure 3); it induced no obvious reversal of its own contractile effect at concentrations up to 5×10^{-6}M. The contractile actions of ICI 80205 (Figure 2) and 16,16-dimethyl PGE_2 were antagonized by iloprost; those of histamine ($EC_{50} = 7 \times 10^{-7}$M) and leukotriene C_4 ($EC_{50} = 2 \times 10^{-8}$M) (Figure 2) were not. 6a-Carba-$\Delta^{6,6a}PGI_1$ was also a potent contractile agent; between response levels of 10 to 75% of maximum its log concentration-response curve was parallel to that of 16,16-dimethyl PGE_2 (e.p.m.r. = 66). Additional doses gave relatively small increases in tension (maximum = 80–85%) and above 1×10^{-6}M distinct relaxant effects were seen. PGI_2 was about 25 times less active than iloprost whereas ZK 96480 showed only weak contractile effects at concentrations in excess of 1×10^{-6}M. At 2×10^{-5}M ZK 96480 did not inhibit contractions produced by 16,16-dimethyl PGE_2.

Of the $PGF_{2\alpha}$ analogues, ω-tetranor-16-*p*-fluorophenoxy $PGF_{2\alpha}$ was a potent full agonist (e.p.m.r. = 8.8), $PGF_{2\alpha}$ was much less active and ICI 81008 showed only weak contractile activity.

In three separate experiments, individual responses (60% of maximum) were obtained to 16,16-dimethyl PGE_2 (5×10^{-9}M), iloprost (5×10^{-8}M), 6a-carba-$\Delta^{6,6a}PGI_1$ (1.5×10^{-7}M), ω-tetranor-16-*p*-fluorophenoxy $PGF_{2\alpha}$ (5×10^{-8}M) and histamine (1×10^{-6}M). Addition of SC 19220 (1×10^{-5}M) reduced the response to each prostanoid by 80–95% within 15 min. The inhibitory effect of SC 19220 is readily reversed on washout of the organ bath. After a 40 min period of continuous washing responses to the agonists were again established. Addition of SC 25191 (1×10^{-5}M) completely inhibited each prostanoid response. The action of histamine was unaffected by

the SC compounds. In the absence of EP 045, neither SC 19220 nor SC 25191 at 1×10^{-5}M inhibited an established contractile response to 11,9-epoxymethano PGH_2 (3×10^{-8}M) ($n = 3$).

PGE-sensitive inhibitory systems

(a) *Guinea-pig trachea* Iloprost (5×10^{-7}M) was added to the organ bath to establish a stable submaximal level of tone against which the relaxant effects of other prostanoids could be demonstrated. We considered that ilosprost was the best agent to use since its partial agonist action on the PGE-sensitive contractile system should oppose to a large degree the contractile action of a prostanoid without hindering its PGE-like relaxant activity. In practice we certainly find that the preparations treated in this manner are highly sensitive to the relaxant action of both PGE_2 ($EC_{30} = 5 \times 10^{-9}$–$2.5 \times 10^{-8}$M, 41 preparations) (Figure 3) and PGE_1 (e.p.m.r. = 1.3). 16,16-Dimethyl PGE_2 (Figure 3) and 11-deoxy PGE_1 produced relaxation at concentrations in excess of 1×10^{-8}M and had similar potencies (Table 1).

In contrast, ICI 80205 at concentrations up to 2×10^{-7}M caused small contractions presumably due to a combination of its weak relaxant activity and its powerful contractile activity overcoming the blocking action of iloprost. Higher concentrations were not used owing to lack of material.

Of the other PGI_2 analogues, 6a-carba-$\Delta^{6,6a}PGI_1$ showed relaxant activity at concentrations in excess of 5×10^{-7}M and this corresponded with reversal of its own contactile activity seen in the absence of iloprost. PGI_2 and ZK 96480 showed little relaxant activity (Figure 3), as did the $PGF_{2\alpha}$ analogues (Table 1).

(b) *Cat trachea* The cat trachea preparation does not generate inherent tone and does not contract to any prostanoids under investigation in this study including thromboxane mimetics such as 11,9-epoxymethano PGH_2. A stable submaximal contractile response to carbachol (2×10^{-7}–1×10^{-6}M) was induced and then cumulative doses of the prostanoid were added and the relaxations recorded. A wide variation in sensitivity to PGE_2 was observed and preparations which did not give a 30% relaxation with less than 2×10^{-7}M PGE_2 were abandoned. The relaxant action of PGE_2 was not inhibited by 2.5×10^{-6} EP 045 – dose-ratios of 0.68, 0.75, 0.86 and 0.89 were obtained on four separate preparations.

As with the guinea-pig trachea, the relaxant activity of PGE_1 approached that of PGE_2, whereas 16,16-dimethyl PGE_2 and 11-deoxy PGE_1 were an order of magnitude less active (Table 1). ICI 80205 was the least active of the PGE analogues (Table 1).

The response to the PGF analogues and to PGD_2 in the concentration range 1×10^{-7}M to 1×10^{-6}M usually took the form of a transient relaxation with no sustained loss of tone. We originally supposed that this was a specific effect mediated via PGF receptors which desensitized rapidly. However, this seems unlikely since in about 10% of preparations low concentrations (1×10^{-7}M) of PGE_2 induced a sharp loss of tone returning to a sustained level of reduced tone within 2 min, and in a few preparations spontaneous transient relaxations were seen. The EPMR values given in Table 1 relate to concentration-response relationships obtained from stable responses measured 4–5 min after drug addition.

(c) *Dog hindlimb blood flow* The arterial vessels of the dog hindlimb dilated when exposed to low plasma concentrations (1×10^{-9}M) of PGE_1 and PGE_2. PGI_2 and its stable analogues were much less active (Table 1). At doses 100 times larger than those of PGE_2, ICI 80205 produced either a mixed response, namely a

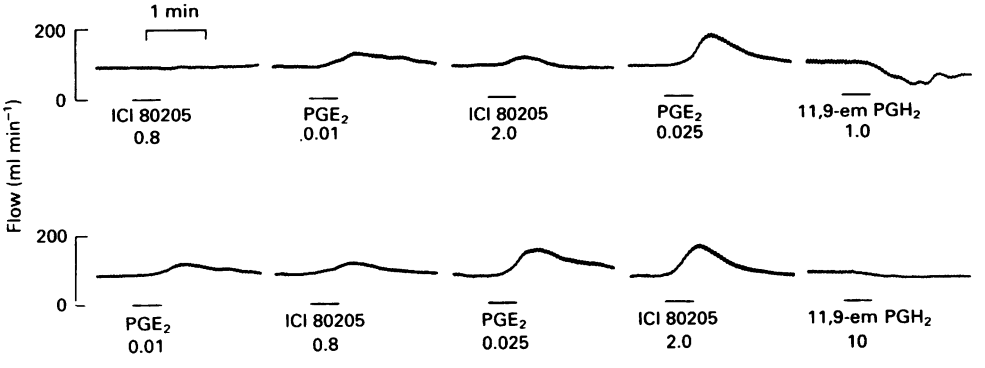

Figure 4 Hindlimb blood flow in an anaesthetized dog (22 kg). Prostanoids were infused into the femoral artery for 30 s. Doses shown are in nmol. In the lower trace the thromboxane receptor antagonist EP 092 was continuously infused into the femoral artery at a rate of 300 μg min^{-1}. There was an interval of about 15 min between consecutive portions of this recording. PGE_2 = prostaglandin E_2 and 11,9-em PGH_2 = 11,9,epoxymethano PGH_2.

moderate vasodilatation which quickly reversed to give a smaller vasoconstriction (Figure 4), or vasodilatation associated with a very shallow dose-response relationship. Since the hindlimb also responds with vasoconstriction to thromboxane-like agonists (e.g. 11,9-epoxymethano PGH_2 produces theshold falls in flow at 1×10^{-8}M in plasma), we surmised that ICI 80205 was producing a balanced effect through simultaneous activation of PGE and thromboxane A (TXA) receptors. Continuous infusion of EP 092 (300 $\mu g\ min^{-1}$) into the hindlimb flow blocked the constrictor action of 11,9-epoxymethano PGH_2 (dose-ratios ≥ 20 in 5 experiments), did not effect the dilator action of PGE_2, and inhibited the constrictor action of ICI 80205 (Figure 4) such that the PGE_2 and ICI 80205 log dose-response curves became parallel. Under these circumstances ICI 80205 was 50–100 times less active than PGE_2. A similar procedure was adopted for 16,16-dimethyl PGE_2; EP 092 halved the e.p.m.r. value indicating that the thromboxane-like vasoconstrictor action of 16,16-dimethyl PGE_2 was affecting the estimation of its vasodilator activity.

PGD_2 always gave a dilatation at doses 1000 times greater than those of PGE_2 used. $PGF_{2\alpha}$ gave variable responses – either a weak dilatation or a weak vasoconstriction. ICI 81008 always produced vasoconstriction ($\geq$ 25% fall in flow) and its action was unaffected by infusion of EP 092. In contrast ω-tetranor-16-*p*-fluorophenoxy $PGF_{2\alpha}$ was a much more powerful constrictor, equal in potency to 11,9-epoxymethano PGH_2, and like 11,9-epoxymethano PGH_2 could reduce hindlimb flow to 10% of the resting level. Its action was markedly suppressed by EP 092, but reversal to produce a dilatation was not seen. PGI_2, iloprost and 6a-carba-$\Delta^{6,6a}PGI_1$ were about 100 times less active than PGE_2; ZK 96480 was slightly more active (Table 1).

The PGI-sensitive inhibitory system of the rat colon

It was observed that the proximal and distal portions of the ascending colon of the rat responded differently to PGE and PGI analogues. The spontaneous rhythmic activity of proximal segments could always be completely inhibited by PGI_2, iloprost, 6a-carba-$\Delta^{6,6a}PGI_1$ and ZK 96480 (e.g. Figure 5). ZK 96480 was the most potent compound ($IC_{50} = 1.5–2.5$ nM, $n = 12$) followed by iloprost and PGI_2 and then 6a-carba-$\Delta^{6,6a}PGI_1$. EPMR are given in Table 2. PGE_2 and 16,16-dimethyl PGE_2 induced weak contractile responses with the dimethyl analogue being about 5 times more active than the parent compound. PGE_1 (5–50 nM) gave a contractile response (Figure 5) or a mixed response – a rise in baseline tension coupled with a reduction in the frequency and size of contractions. Mixed responses could be mimicked using suitable combinations of PGE_2 and ZK 96480.

Distal segments were only marginally less sensitive to the inhibitory action of ZK 96480 ($IC_{50} = 1.5–5$ nM, $n = 12$) but gave more pronounced contractile responses to PGE_2 and 16,16-dimethyl

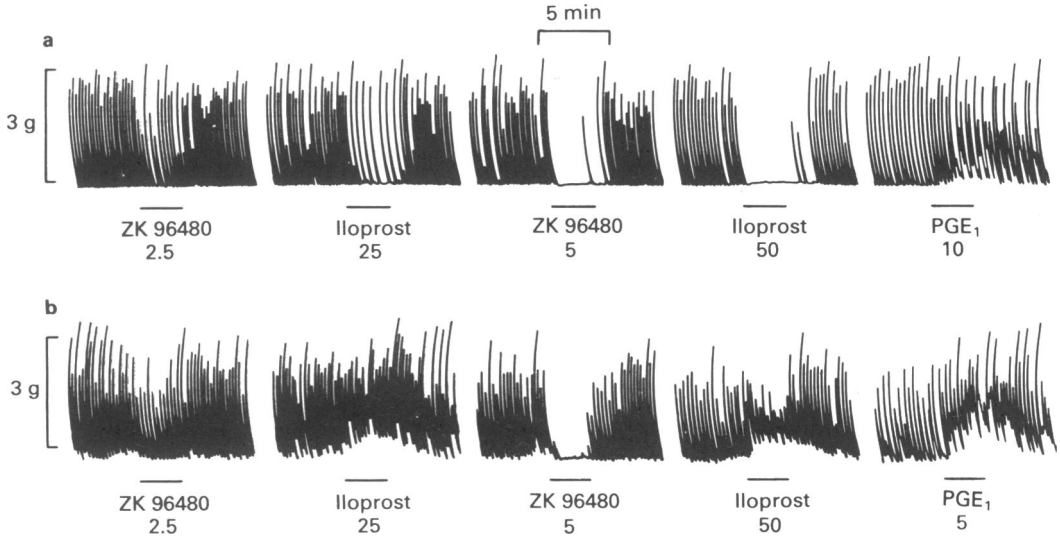

Figure 5 Rat isolated ascending colon. (a) Proximal section; inhibitory responses to iloprost and ZK 96480 are shown. (b) Distal section; ZK 96480 inhibits the spontaneous activity whereas iloprost (50 nM) shows a mixed response. Bath concentrations (nM) are given.

Table 2 Inhibition of the spontaneous activity of the proximal segment of the ascending colon of the rat by prostaglandin I (PGI) analogues

Prostanoid	*Equipotent molar ratio*
ZK 96480	1.0 (IC_{50} = 2 nM)
PGI_2	6.2
Iloprost	6.0
6a-Carba$\Delta^{6,6a}PGI_1$	19.3

Each value is the mean of at least four determinations.

PGE_2. PGE_1 always produced contractile responses similar to PGE_2, and PGI_2 always produced inhibition of spontaneous activity. Responses to iloprost and 6a-carba-$\Delta^{6,6a}PGI_1$ were variable. On preparations which were highly responsive to PGE_2 and PGE_1 mixed responses were seen (e.g. the response to 50 nM iloprost in Figure 5), whereas on preparations less responsive to PGE_2 complete inhibition was produced.

On the proximal segments SC 19220 (10^{-5}M) had only a very slight inhibitory effect on the contractile response to PGE_2 and failed to modify the response to PGE_1. Higher concentrations of SC 19220 suppressed spontaneous activity.

Discussion

The experiments presented here support the idea of two subtypes of PGE receptor. On the three contractile systems the PGE_2 analogue ICI 80205 exhibits very high agonist potency ($EC_{50} \sim 1 \times 10^{-9}$M) and is more active than PGE_2 whereas its effects on the PGE-sensitive relaxant systems are much weaker than those of PGE_2. At the other extreme, 11-deoxy PGE_1 shows more relaxant than stimulant PGE-like activity. There is some evidence in the literature to suggest that prostanoids with a greater selectivity than 11-deoxy PGE_1 can be synthesized. For example, the 16-hydroxy PGE analogue, TR 4752 (Gardiner & Collier, 1980), shows relaxant activity on the guinea-pig trachea at concentrations as low as 10^{-10}M and shows no evidence of contractile activity.

From the simplest viewpoint, the high stimulant activity of iloprost and 6a-carba-$\Delta^{6,6a}PGI_1$ on the bullock iris sphincter, rat stomach strip and guinea-pig trachea may be the result of agonist actions at either PGE or PGI receptors. We favour an agonist action at the PGE receptor for the following reasons: (a) the activities of PGI_2 and its stable analogues relative to PGE_2 and/or 16,16-dimethyl PGE_2 are similar on the three preparations, and the rank order of activity iloprost > 6a-carba-$\Delta^{6,6a}PGI_1$ > PGI_2 >> ZK 96480 is quite different from that seen on PGI-sensitive tissues. Indeed ZK 96480 is a potent mimetic of all the actions of PGI_2 including inhibition of platelet aggregation and relaxation of vascular smooth muscle (Mueller *et al.*, 1984) and is of similar potency to iloprost. (b) Iloprost behaves as a partial agonist, specifically opposing the contractile action of PGE-like full agonists. (c) SC 19220 and SC 25191, in the limited areas examined, block the activity of iloprost to the same extent as PGE_2 and 16,16-dimethyl PGE_2.

On the PGE-sensitive relaxant systems, PGI_2 and its three stable analogues are much less active than PGE_2. In the case of the dog hindlimb blood flow measurements it is possible that dilatation can also be induced through a specific PGI_2-sensitive mechanism since ZK 96480 is only about 30 times less active than PGE_2. We have found a quite different situation in the mesenteric arterial bed of the dog (unpublished observations). PGE_2 is equipotent with ZK 96480 and only about 3 times more active than PGI_2, iloprost and 6a-carba-$\Delta^{6,6a}PGI_1$. This strongly suggests the presence of both PGE and PGI receptors on the mesenteric arterial vessels: this has been proposed previously by Lumley *et al.* (1982) from studies with the natural prostanoids.

The inhibitory actions of PGI_2 (Gryglewski *et al.*, 1976) and related prostanoids on the rat ascending colon appear to be mediated via a PGI receptor. ZK 96480 was found to be the most potent agonist of those tested and showed no evidence of contractile activity on the distal portion of the ascending colon which is more responsive to PGE_2 and its structural analogues. In contrast, iloprost and 6a-carba-$\Delta^{6,6a}PGI_1$, showed distinct PGE-like agonist activity on the distal ascending colon and this corresponds with their activity on the other PGE-sensitive contractile systems. On the basis of its high potency and specificity, ZK 96480 could be a useful standard agonist in studies designed to compare PGI_2-sensitive systems in different tissues.

We have used the specific agonist action of the $PGF_{2\alpha}$ analogue ICI 81008 to indicate the presence of PGF receptors in the smooth muscle preparations. On the cat and dog iris sphincter muscles, where it is reasonable to suppose the existence of only PGF receptors, ICI 81008 is a full agonist and is 2–10 times more active than $PGF_{2\alpha}$ (Coleman *et al.*, 1981; Dong & Jones, 1982). In our studies on the rat stomach fundus ICI 81008 shows high agonist activity, whereas its effects on the other PGE-sensitive preparations are minimal. ICI 81008 produces a lower maximum response than either PGE_2 or $PGF_{2\alpha}$ and does not oppose their contractile actions. We suggest that ICI 81008 is a specific full agonist at the PGF receptor in the rat fundus and that the higher maximum responses achieved with $PGF_{2\alpha}$ and ω-tetranor-*p*-fluorophenoxy

$PGF_{2\alpha}$ are due to their combined action at both PGE and PGF receptors. Indeed the high contractile activity of the 16-*p*-fluorophenoxy $PGF_{2\alpha}$ analogue on a wide selection of smooth muscle preparations reflects its potent agonist activity at PGE, PGF and TXA receptors.

The presence of two PGE-sensitive systems with opposing actions in the guinea-pig trachea is of some interest. In the case of our experiments with indomethacin-treated preparations we have presumed that the relaxant activity of PGE_2 prevents its true contractile activity from being expressed. Thus if PGE_2 had about one-third of the contractile activity of 16,16-dimethyl PGE_2, as on the rat stomach fundus and bullock iris sphincter, then threshold contractile effects would be seen at 1×10^{-9}M and the log concentration-response curve would be a mirror image of its log concentration-response curve for inhibition of iloprost-induced tone. What is the relationship of these two PGE-sensitive systems in the absence of cyclo-oxygenase inhibition, that is, in a preparation which generates inherent tone? Both indomethacin and SC 19220 inhibit inherent tone (Farmer *et al.*, 1974) whereas thromboxane receptor antagonists are usually ineffective (Jones, unpublished observations). It is possible that PGE_2 synthesized within the tracheal tissue is responsible for the production of the tone. PGE receptor antagonists with higher affinity than the SC analogues will be crucial to the resolution of this problem.

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References

ARMSTRONG, R.A., JONES, R.L., PEESAPATI, V., WILL, S.G. & WILSON, N.H. (1985). Competitive antagonism at thromboxane receptors in human platelets. *Br. J. Pharmac.*, **84**, 595–607.

BENNETT, A. & POSNER, J. (1971). Studies on prostaglandin antagonists. *Br. J. Pharmac.*, **42**, 584–594.

CASALS-STENZEL, J., BUSE, M. & LOSERT, W. (1983). Comparison of the vaso-depressive action of ZK 36374, a stable prostacyclin derivative, PGI_2 and PGE_1, with their effect on platelet aggregation and bleeding time in rats. *Prostaglandins, Leukotrienes and Med.*, **10**, 197–212.

COLEMAN, R.A., HUMPHREY, P.P.A., KENNEDY, I., LEVY, G.P. & LUMLEY, P. (1981). Comparison of the actions of U-46619, a prostaglandin H_2-analogue, with those of prostaglandin H_2 and thromboxane A_2 on some isolated smooth muscle preparations. *Br. J. Pharmac.*, **73**, 773–778.

DONG, Y.J. & JONES, R.L. (1982). Effects of prostaglandins and thromboxane analogues on bullock and dog iris sphincter preparations. *Br. J. Pharmac.*, **76**, 149–155.

FARMER, J.B., FARRAR, D.G. & WILSON, J. (1974). Antagonism of tone and prostaglandin mediated responses in a tracheal preparation by indomethacin and SC 19920. *Br. J. Pharmac.*, **52**, 559–565.

GARDINER, P.J. & COLLIER, H.O.J. (1980). Specific receptors for prostaglandins in airways. *Prostaglandins*, **19**, 819–841.

GRYGLEWSKI, R.J., BUNTING, S., MONCADA, S., FLOWER, R.J. & VANE, J.R. (1976). Arterial walls are protected against deposition of platelet thrombi by a substance (Prostaglandin X) which they make from prostaglandin endoperoxides. *Prostaglandins*, **12**, 685–713.

JONES, R.L. & MARR, C.G. (1977). Actions of 16-aryloxy analogues of prostaglandin $F_{2\alpha}$ on preparations responsive to prostaglandin endoperoxides. *Br. J. Pharmac.*, **61**, 694–696.

JONES, R.L., PEESAPATI, V. & WILSON, N.H. (1982). Antagonism of the thromboxane-sensitive contractile systems of the rabbit aorta, dog saphenous vein and guinea-pig trachea. *Br. J. Pharmac.*, **76**, 423–428.

JONES, R.L., WILSON, N.H., ARMSTRONG, R.A. & DONG, Y.J. (1984). Receptors for thromboxane and prostaglandins. In *Proceedings of the 9th International Congress of Pharmacology*, Vol. 2, ed. Paton, W., Mitchell, J. & Turner, P. pp. 293–301. London: MacMillan Press.

JONES, R.L., WILSON, N.H. & MARR, C.G. (1979). Thromboxane-like activity of prostanoids with aromatic substituents at C16 and C17. In *Chemistry, Biochemostry and Pharmacological Activity of Prostanoids*, ed. Roberts, S.M. & Scheinmann, F. pp. 210–220. Oxford: Pergamon Press.

KENNEDY, I., COLEMAN, R.A., HUMPHREY, P.P.A. & LUMLEY, P. (1983). Studies on the characterization of prostanoid receptors. In *Advances in Prostaglandin, Thromboxane and Leukotriene Research*. Vol. 11. ed. Samuelsson, B., Paoletti, R. & Ramwell, P. pp. 327–332, New York: Raven Press.

LUMLEY, P., HUMPHREY, P.P.A., KENNEDY, I. & COLEMAN, R.A. (1982). Comparison of the potencies of some prostaglandins as vasodilators in three vascular beds of the anaesthetised dog. *Eur. J. Pharmac.*, **81**, 421–430.

MUELLER, B., MAASS, B., STUERZEBECHER, S. & SKUBALLA, W. (1984). Antifibrillatory action of the stable orally active prostacyclin analogs iloprost and ZK 96480 in rats after coronary ligation. *Biomed. biochim. Acta.*, **43**, 175–178.

POSNER, J. (1973). Prostaglandin E_2 and the bovine sphincter pupillae. *Br. J. Pharmac.*, **49**, 415–427.

SANNER, J.H., MUELLER, R.A. & SCHULZE, R.H. (1973). Structure – activity relationships of some dibenzoxazepine derivatives as prostaglandin antagonists. *Adv.*

Biosci., **9**, 139–148.

SHIBASAKI, M., TORISAWA, Y. & IKEGAMI, S. (1983). Synthesis of 9(0)-methano-$\Delta^{6(9\alpha)}$PGI$_1$: the highly potent carbon analogue of prostacyclin. *Tetrahedron Lett.*, **24**, 3493–3496.

WILSON, N.H. & JONES, R.L. (1985). Prostaglandin endoperoxide and thromboxane A_2 analogs. In *Advances in Prostaglandin, Thromboxane and Leukotriene Research*, Vol. 14. ed. Pike, J.E. & Morton, D.R. Jr., pp. 393–425. New York: Raven Press.

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