Br. J. Pharmac. (1982), **76**, 423–438

ANTAGONISM OF THE THROMBOXANE-SENSITIVE CONTRACTILE SYSTEMS OF THE RABBIT AORTA, DOG SAPHENOUS VEIN AND GUINEA-PIG TRACHEA

R.L. JONES, V. PEESAPATI & N.H. WILSON

Department of Pharmacology, University of Edinburgh Medical School, 1 George Square, Edinburgh EH8 9JZ

1 The thromboxane-sensitive contractile systems in spirally-cut preparations of the rabbit aorta, dog saphenous vein and guinea-pig trachea have been compared. The full or partial agonist activities of a range of bicyclic ring analogues were found to be remarkably similar on the three preparations. In addition, EP 045, a prostanoid with a phenylsemicarbazone ω-chain, blocked the action of both thromboxane A_2 (TXA_2) and the bicyclic ring analogues. Using 11,9-epoxymethano prostaglandin H_2 as the agonist, linear Schild plots with slopes close to unity were obtained on each preparation; this suggests a competitive type of antagonism.

2 Analogues of prostaglandin D_2 (PGD_2), PGE_2 and $PGF_{2\alpha}$ also contracted the three smooth muscle preparations; those analogues containing a 16-*p*-halophenoxy residue were highly active. On the rabbit aorta, EP 045 completely blocked the contractile actions of these agonists, perhaps indicating a single type of prostanoid receptor in this tissue. On the dog saphenous vein PGD_2, PGE_2 and 15-methyl PGE_2 exhibited relaxant activity when the tissue was partially contracted with either a thromboxane agonist or noradrenaline. On the guinea-pig trachea 16,16-dimethyl PGE_2 and the 16-*p*-chlorophenoxy analogue of PGE_2 were potent contractile agents whose action was not blocked by EP 045. PGE_2 and 15-methyl PGE_2 showed similar properties but exhibited relaxant activity with increasing concentrations in the organ bath. Our results indicate the presence of three types of prostanoid receptors in the guinea-pig trachea: thromboxane- and PGE-sensitive systems mediating contraction and a PGE-sensitive system mediating relaxation.

3 The similarity of the thromboxane-sensitive systems in the three smooth muscle preparations is discussed with particular reference to the differences in the equilibrium dissociation constants for EP 045.

Introduction

One of the products of enzyme action on the prostaglandin endoperoxides, prostaglandin G_2 (PGG_2) and PGH_2, is the highly unstable thromboxane A_2 (TXA_2) (Samuelsson, Hamberg, Malmsten & Svensson, 1976; Needleman, Moncada, Bunting, Vane, Hamberg & Samuelsson, 1976). This substance has a number of characteristic actions including aggregation of blood platelets and contraction of smooth muscle of vascular and respiratory origins (Svensson, Hamberg & Samuelsson, 1976; Svensson, Strandberg, Tuvemo & Hamberg, 1977). In these systems TXA_2 has greater potency than either of the prostaglandin endoperoxides or $PGF_{2\alpha}$, and it is now generally assumed that these effects result from the interaction of TXA_2 with discrete receptors, designated thromboxane receptors.

Chemically-stable derivatives of TXA_2 have recently been prepared and certain of these, for example 11a-carbathromboxane A_2 (Maxey & Bundy, 1980) and the 9a,11a-dicarba analogue, CTA_2 (Nicolaou, Magolda & Claremon, 1980), exhibit potent thromboxane-like activity on smooth muscle. Derivatives of PGH_2 in which the 9,11-peroxide bridge has been replaced by either epoxy-methano (Bundy, 1975), azo (Corey, Narasaka & Shibazaki, 1976) or etheno (Corey, Shibazaki, Nicolaou, Malmsten & Samuelsson, 1976) groups (Figure 1) also show thromboxane-like activity (see also the review by Nicolaou, Gasic & Barnette, 1978). However, high potency of this nature is not restricted to bicyclic compounds. We have shown that the 16-*p*-fluorophenoxy-17,18,19,20-tetranor derivatives of PGD_2 and $PGF_{2\alpha}$ exhibit potent thromboxane-like effects (Jones & Marr, 1977) whereas the 16-*m*-trifluoromethylphenoxy-17,18,19,20-tetranor derivative of $PGF_{2\alpha}$ is almost devoid of this type of activity (Jones, 1978). Combination of the 9,11-etheno PGH_2 ring with a 16-*p*-halophenoxy ω-chain results in compounds with potent and long lasting thromboxane-like actions *in vitro* and considerable toxicity *in vivo* (Jones, Wilson & Marr, 1979; Jones & Wilson, 1980).

0007-1188/82/070423-16 $01.00

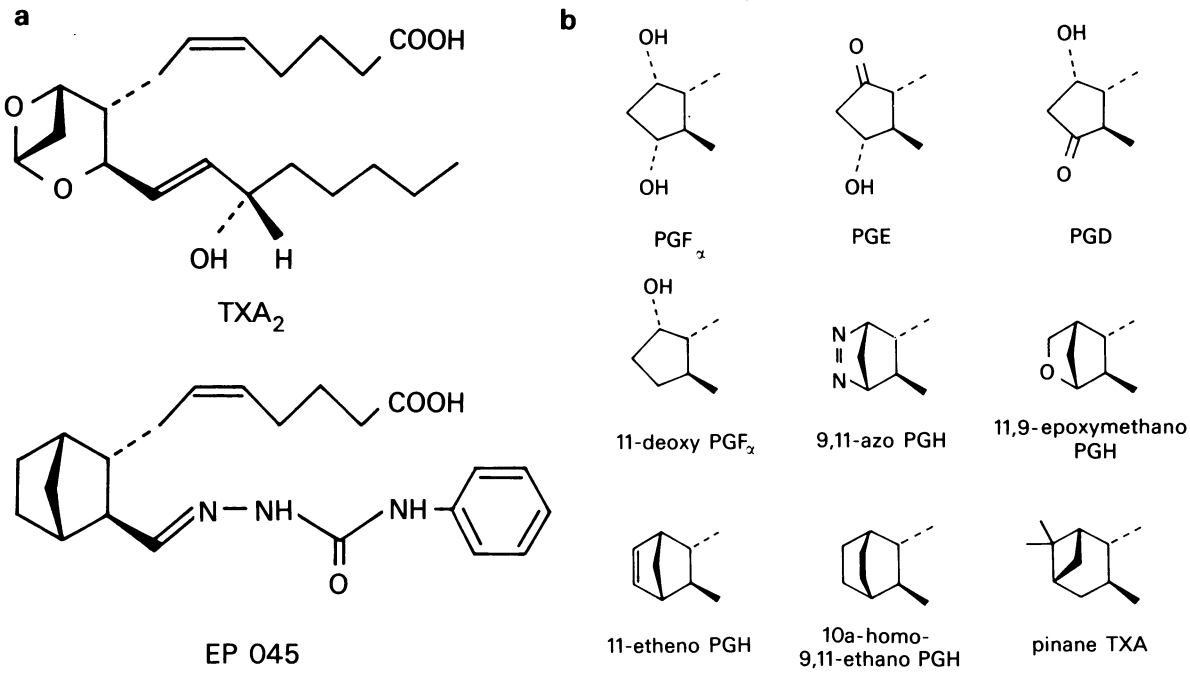

Figure 1 (a) Structures of thromboxane A_2 and the thromboxane antagonist EP 045 and (b) ring systems present in compounds described in the text. For convenience the trivial names only are given.

Careful examination of the actions of 9,11-etheno PGH_2 and 9,11-ethano PGH_2 revealed that they were both partial agonists on thromboxane-sensitive preparations (Jones & Wilson, 1980). In following up this observation we have synthesized a number of compounds, retaining the natural α-chain and the **bicyclo** [2,2,1] heptane ring but making considerable changes to the ω-chain. One of these compounds, coded EP 045 (Figure 1), antagonizes the action of TXA_2 and its stable mimics in a competitive manner. We have used EP 045, together with a large number of agonists, to compare the thromboxane receptor systems present in three isolated smooth muscle preparations, the rabbit aorta, dog saphenous vein and guinea-pig trachea. A preliminary account of the activity of EP 045 has been presented to the British Pharmacological Society (Jones & Wilson, 1981).

Methods

Isolated vascular preparations

Thoracic aortae from young male rabbits and tracheae from guinea-pigs of either sex were removed immediately after they had been killed. ous veins were obtained from pentobarbitone-anaesthetized dogs of either sex within 30 min of induction of anaesthesia.

Spiral strips, 3 mm wide, were cut and suspended in 8 ml organ baths containing Krebs-Henseleit solution (NaCl 118, KCl 5.4, $MgSO_4$ 1.0, $CaCl_2$ 2.5, NaH_2PO_4 1.1, $NaHCO_3$ 25, dextrose 10 mmol/l), gassed with 95% O_2 and 5% CO_2 and maintained at 37°C. The bathing solution for the guinea-pig trachea experiments contained atropine sulphate (2×10^{-8} M) and indomethacin (10^{-6} M). Tension changes were recorded with a Grass FTO3 force displacement transducer linked to a Grass Polygraph. Each preparation was allowed 2 h to relax to a stable resting tension (2 g for rabbit aorta and dog saphenous vein and 0.2 g for guinea-pig trachea) during continuous upward displacement of the bathing fluid (one nominal bath clearance per minute). Two series of cumulative doses of the 11,9-epoxymethano PGH_2 analogue were then added with an intervening wash period of 60–90 min. For comparisons of agonist potency the test compound was added after a further 60–90 min wash period. When the response of the test compound decayed rapidly on washout of the organ bath, a further series of cumulative doses of the standard agonist was added. To study the an-

tagonistic action of EP 045, the second agonist dose series was washed out for 30 min and then a Krebs solution of EP 045 was pumped through the organ bath for 50 min. The flow was stopped and a third series of cumulative doses (suitably increased) of 11,9-epoxymethano PGH_2 was added to the organ.

Superfusion experiments

Pairs of rabbit aortic strips or dog saphenous vein strips were superfused (Watson-Marlow roller pump) in series with Krebs-Henseleit solution at 37°C at a flow rate of 10 ml/min. Tension changes were recorded as described previously. A combination of antagonists/inhibitors (atropine sulphate, final concentration of free base 5×10^{-7} M; mepyramamine maleate, free base 2.5×10^{-7} M; methysergide maleate, free base 1×10^{-6} M; phenoxybenzamine hydrochloride, free base 3×10^{-7} M; propranolol hydrochloride, free base 5×10^{-6} M; indomethacin 3×10^{-6} M) were added to the superfusate using a Braun infusion pump. Methysergide was omitted from the mixture when the dog saphenous vein was used since it caused a contractile response (see Apperley, Feniuk, Humphrey & Levy, 1980).

For generation of TXA_2 the effluent from an excised guinea-pig lung, perfused (10 ml/min) via the pulmonary artery with the Krebs solution, replaced the initial superfusion fluid. Sodium arachidonate (10–25 μg with dog saphenous vein and 20–50 μg with rabbit aorta) was infused for 1 min into the pulmonary artery inflow to generate TXA_2. In some experiments the second tissue in the cascade was exposed to EP 045, and in others the lung was exposed to either indomethacin or UK 37248. The fluid leaving the first tissue in the cascade could be delayed (2 min) by passage through a coil of silicone rubber tubing immersed in a water bath at 37°C.

Compounds

The following compounds (all having the natural prostane configuration) were gifts from the Upjohn Company, U.S.A.: $PGF_{2\alpha}$, 15S-15-methyl $PGF_{2\alpha}$, 16,16-dimethyl $PGF_{2\alpha}$, PGE_2, 15S-15-methyl PGE_2, 16,16-dimethyl PGE_2, PGD_2, 15S-hydroxy-9α, 11α-(epoxymethano) prosta-5Z, 13E-dienoic acid (U 44069), 15S-hydroxy-11α, 9α-(epoxy-methano) prosta-5Z, 13E-dienoic acid (U 46619), 15S-hydroxy-9α, 11α-azoprosta-5Z, 13E-dienoic acid (U 51093).

The following compounds (all racemic) were donated by ICI Pharmaceuticals Division, U.K.: the 16-*p*-fluorophenoxy (ICI 79939), 16-*p*-chlorophenoxy (ICI 79492), 16-*m*-chlorophenoxy (ICI 80996) and 16-*m*-trifluoromethylphenoxy (ICI 81008) derivatives of 17,18,19,20-tetranor $PGF_{2\alpha}$; the 16-*p*-chlorophenoxy (ICI 80205) and 16-*m*-chlorophenoxy (ICI 81563) derivatives of 17,18,19,20-tetranor PGE_2; 15δ-hydroxy-9α,11α-etheno-prosta-5Z,13E-dienoic acid (ICI 86841).

PGI_2 sodium salt was donated by Schering AG, Berlin-Bergkamen. On the day of use a stock solution (200 μg/ml) was made in 0.05 M Tris-HCl buffer, pH 9.0 and stored at 0°C. Immediately before use serial dilutions of the stock were made with ice-cold 0.9% NaCl solution.

The 11-deoxy $PGF_{2\alpha}$ analogues were prepared in this laboratory by sodium borohydride reduction of the corresponding PGA_2 compounds. The 9α- and 9β-hydroxy epimers were separated by liquid-gel partition chromatography systems akin to those described by Brash & Jones (1974). The 16-*p*-fluorophenoxy derivatives of 17,18,19,20-tetranor PGD_2 and 17,18,19,20-tetranor PGE_2 were prepared from the PGF analogue (ICI 79939) by the method of Nishizawa, Miller, Gorman, Bundy, Svensson & Hamberg (1975).

The following compounds were also prepared in our laboratory (Wilson, Peepsapati & Jones, 1982): (±)15S-hydroxy-9α, 11α-ethano-prosta-5Z, 13E-dienoic acid; (±)15S-hydroxy-9α, 11α-etheno-16-*p*-fluorophenoxy- 17,18,19,20-tetranor-prosta-5Z, 13E-dienoic acid (EP011) and its 9α, 11α-ethano (EP 031), 9α,11α-ethano-16-*p*-chlorophenoxy (EP 032) and 16-*p*-chlorobenzyl (EP 016) derivaives; pinane thromboxane A_2 (PTA_2) and its 16-*p*-fluorophenoxy derivative; (±)-15S-hydroxy-9α,11α-ethano-10a-homo-prosta-5Z, 13E-dienoic acid; Ep 045 – (±)5-**endo**-(6′-carboxyhex-2′Z-enyl-6-**exo**[N-(phenylcarbamoyl) hydrazonomethyl] -**becyclo** [2,2,1] heptane.

Stock solutions of the prostaglandin analogues were usually stored in ethanol at −20°C. For preparations of aqueous solutions a small volume of the ethanolic stock solution was evaporated using a nitrogen jet and the residue dissolved in 0.9% w/v NaCl solution (saline) with addition of small amounts of solid $NaHCO_3$ and warming to 50°C. Aqueous sodium arachidonate solutions (1–3 mg/ml) were prepared by adding 20 μl 1M NaOH to 6 mg arachidonic acid in 1 ml of ethanol. The solvent was evaporated using a nitrogen jet and the residue was dissolved in the appropriate volume of saline solution. A new solution was made for each experiment.

Indomethacin was kindly supplied by Merck, Sharpe & Dohme, Ltd. The thromboxane synthetase inhibitor, UK 37248, was a gift from Pfizer UK, Sandwich, Kent. It was dissolved in 0.8% NaCl solution and the pH adjusted to 5.0 with dilute NaOH solution.

Table 1 Activities of prostaglandin analogues on isolated preparations of rabbit aorta, dog saphenous vein and guinea-pig trachea

Parent compound and derivatives		*Equipotent molar ratios* (standard agonist, 11,9-epoxymethano PGH_2 = 1.0) *Rabbit aorta*	*Dog saphenous vein*	*Guinea-pig trachea*
$PGF_{2\alpha}$		p.a. 75–90%, 1220 nM	p.a. 47–61%, 2500 nM	29 *
15-methyl		145	360	not tested
16,16-dimethyl		8.7	15	0.71 *
16-*p*-fluorophenoxy	(ICI 79939)	3.1	4.0	0.90 *
16-*p*-chlorophenoxy		3.4	4.8	0.38 *
16-*m*-chlorophenoxy		(820)	not tested	92 *
16-*m*-trifluoromethylphenoxy	(ICI 81008)	(1220)	(3070)	257 *
PGE_2		(274)	↓	↑↓ **
15-methyl		(625)	↓	↑↓ **
16,16-dimethyl		30	55	[0.15] **
16-*p*-fluorophenoxy		4.3	5.0	not tested
16-*p*-chlorophenoxy	(ICI 80205)	7.3	8.5	[0.035]**
16-*m*-chlorophenoxy		(270)	not tested	not tested
PGD_2		(295)	↓	51 *
16-*p*-fluorophenoxy		11	16.5	1.7 *
16-*m*-chlorophenoxy		(450)	not tested	not tested
11-deoxy $PGF_{2\alpha}$		p.a. 69–87%, 440 nM	p.a. 43–72%, 104 nM	p.a. 72–78%, 118 nM
15-methyl		5.0	6.9	not tested
16,16-dimethyl		0.93	0.80	0.071 *
16-*p*-chlorophenoxy		0.42	0.60	0.083 *
9,11-azo PGH_2	(U 51093)	1.4	1.2	1.1
9,11-epoxymethano PGH_2	(U 44069)	p.a. 83–89%, 16 nM	p.a. 66–90%, 22 nM	p.a. 70–78%, 38 nM
9,11-etheno PGH_2		p.a. 63–69%, 110 nM	p.a. 72–83%, 29 nM	p.a. 23–40%, 12 nM
16-*p*-fluorophenoxy	(EP 011)	0.12	0.087	0.055
16-*p*-chlorobenzyl	(EP 016)	p.a. 44–65%, 190 nM	p.a. 12–43%, 410 nM	p.a. 8–16%, —
9,11-ethano PGH_2		p.a. 38–72%, 100 nM	p.a. 41–73%, 18 nM	p.a. 51–59%, 127 nM
16-*p*-flurorophenoxy	(EP 031)	0.26	0.13	not tested
16-*p*-chlorophenoxy	(EP 032)	0.39	0.31	0.30
10a-homo-9,11-ethano PGH_2		p.a. 81–90%, 6.9 nM	p.a. 71–75%, 5.1 nM	p.a. 61–80%, 9.6 nM
Pinane TXA_2		p.a. 47–60%, 210 nM	p.a. 24–30%, 21 nM	p.a. 4–13%, —
16-*p*-fluorophenoxy		p.a. 75–95%, 3.2 nM	p.a. 25–51%, 5.2. nM	p.a. 27–38%, 12 nM

Full agonists: unbracketed values are mean equipotent molar ratios (e.p.m.r.) for 3 or more determinations. Values in square brackets indicate that the compound has a pure PGE-like contractile action on the guinea-pig trachea and the log concentration-response curves are not parallel to that of the standard agonist: e.p.m.r. refer to the 50% maximum response of the standard agonist.

Weak agonists showing no antagonism towards 11,9-epoxymethano PGH_2: values in curved brackets are e.p.m.r. at the 25% maximum response level (full concentration-response curve not established).

Partial agonists (p.a.): lower and upper values of the relative maximum response (11,9-epoxymethano PGH_2 = 100%) together with the concentration of the partial agonist required to produce a response 50% of its own maximum are given.

Relaxant activity: ↓ indicates that the compound shows relaxant activity only and ↑↓ that the compound has both contractile and relaxant properties.

EP 045 antagonism: on the three preparations, submaximal responses to all full agonists can be completely abolished by EP 045 (see text for concentrations), except for certain compounds acting on the guinea-pig trachea. * Denotes a partial block and ** minimal block indicating respectively that the contractile response has a significant PGE-like component or is completely the result of a PGE-like action.

Table 2 Classification of prostanoid actions on three isolated smooth muscle preparations

Type of agonist	*Rabbit aorta* (RA) *and dog saphenous vein* (DSV)	*Guinea-pig trachea* (GPT)
Full agonists of high potency		
Group A e.g. 11,9-epoxymethano PGH_2, 16-*p*-halophenoxy analogues of PGE_2 and $PGF_{2\alpha}$	Rapid onset of action. Rapid offset after washing. Cumulative dose-response curves obtainable.	Bicyclic compounds: similar to actions on RA and DSV.
Group B e.g. 9,11-azo PGH_2	Slower onset and offset. Cumulative curves	$PGF_{2\alpha}$ analogues: relative to standard agonist, more potent than on RA and DSV; incomplete antagonism by EP 045.
Group C e.g. EP 011	Slow onset (> 1 h). V. slow offset (> 10 h). No cumulative curves. Later, reduced sensitivity to standard agonist.	16,16-Dimethyl and 16-*p*-chlorophenoxy analogues of PGE_2: very potent contractile agents, dose-response curves not parallel to standard. Poorly antagonized by EP 045
Agonists of low potency		
e.g. PGD_2, PGE_2 and 15-methyl analogue	Rapid onset and offset. Maximal contraction not obtainable on RA. Some relax DSV.	Mixed contractile/relaxant effects. EP 045 does not block.
Partial agonists		
e.g. 11-deoxy $PGF_{2\alpha}$, 9,11-epoxymethano PGH_2 pinane TXA_2	Maxima smaller. Inhibitory to standard full agonist, but additive with noradrenaline. Both rapid and slow acting compounds found.	As with RA and DSV. Additive with histamine.

Unless otherwise stated, the thromboxane antagonist EP 045 was shown to inhibit completely the spasmogenic effects of the drugs on all three tissues.

Results

Comparisons of agonist potency on the rabbit aorta and dog saphenous vein

The spasmogenic action of each compound was compared with that of 11,9-epoxymethano PGH_2 (standard full agonist, equipotent molar ratio=1.0) on these two tissues. Doses were added cumulatively and the response was taken as the absolute increase in muscle tension above the resting tension. Log concentration-response curves were plotted. Initially each compound was examined to determine whether it was a full agonist or a partial agonist. It was easy to classify a compound as a partial agonist when its maximum response lay between 5 and 90% of the standard agonist maximum. However, it was more difficult to determine whether a compound with a maximum response similar in size to that of the standard agonist was truly a full agonist since there was usually a slight increase in the magnitude of the standard maximum response during the course of each experiment. In cases of doubt, a high concentration (1×10^{-5} M) of the standard agonist was added after establishment of the maximum response to the test compound: no further increase in tension within 20 min was taken to mean that the test compound was a full agonist.

Full agonists Log concentration-response curves for the standard agonist on different preparations of both the aorta and saphenous vein were highly reproducible. A 50% maximum response was achieved with a bath concentration of 13.3 ± 6.5 nM (mean $\pm$ standard deviation, $n = 67$) on the rabbit aorta and 7.0 ± 3.2 nM ($n = 55$) on the dog saphenous vein.

Three groups of compounds may be distinguished according to the nature of their responses (see Table 2).

Group A comprises the more active full agonists with rapid onset of action; following wash-out of the organ bath the muscle tension returned quickly to the resting level. The return to resting tension after a maximum contraction took less than 2 h, and subsequent testing of the standard agonist showed that responses were comparable to those obtained before addition of the test compound. Log concentration-response curves for the standard agonist and the test compound were parallel in each case. This group comprised 10 compounds in all: the standard agonist; the 15-methyl analogues of $PGF_{2\alpha}$ and 11-deoxy $PGF_{2\alpha}$; and all the 16,16-dimethyl, 16-*p*-fluorophenoxy and 16-*p*-chlorophenoxy analogues of $PGF_{2\alpha}$, PGE_2 and PGD_2 examined. In Table 1 equipotent molar ratios for these compounds are given as unbracketed values. The log concentration-response curves for the standard agonist and, as an example, the 16-*p*-fluorophenoxy analogue of $PGF_{2\alpha}$ (ICI 79939) are shown in Figure 2.

In group B the agonists had only slightly slower onset of action than compounds in group A so that a complete cumulative log concentration-response relationship could be obtained on one preparation. However, on continuous wash-out of the organ bath after a maximum response had been elicited, the response returned slowly to baseline over a period of 4 to 10 h. On retesting the standard agonist there was some loss of sensitivity. With these three compounds, 9,11-azo PGH_2 and the 16,16-dimethyl and 16-*p*-chlorophenoxy analogues of 11-deoxy $PGF_{2\alpha}$, equipotent molar ratios were calculated from the 50% maximum points on the test compound curve and the preceding standard agonist curve (again unbracketed values in Table 1).

In group C the agonists had slow onset of action. A single dose might require a contact time of 100 min for establishment of a stable increase in tension. This meant that a complete log concentration-effect relationship could not be established on a single preparation. Therefore, several preparations from the same animal with similar sensitivities to the standard agonist were used simultaneously, each being challenged with a different dose of the test compound. When a stable response had been produced a large dose of the test compound was added to establish the maximum response. Three compounds exhibit this type of action. They are the 16-*p*-fluorophenoxy analogues of 9,11-etheno PGH_2 (EP 011) and 9,11-ethano PGH_2 (EP 031) and the 16-*p*-chlorophenoxy analogue of 9,11-ethano PGH_2 (EP 032), and each is more active than the standard agonist (Table 1 and Figure 2). Removal of these agonists from the bathing fluid resulted in a very slow decay of the contractile response. With maximum or near maximum responses the tissue was washed overnight (15 h) and the tension had invariably declined to the original level by the next day. However, the preparation was then relatively unresponsive to the standard agonist and the maximum response was only 20 to 30% of the original maximum.

Agonists of low potency On the rabbit aorta there were seven compounds, including for example PGE_2, of such low potency that the maximum contractile response relative to that of the standard agonist could not be determined. In order to avoid possible surfactant effects on the tissue these compounds were not used at concentrations in excess of 5×10^{-5} M (about 20 μg/ml). Addition of cumulative doses of 11,9-epoxymethano PGH_2 to the organ bath in the presence of sufficient test compound to give a 30–50% maximum response resulted in additional contractions, the magnitudes of which were equal to those

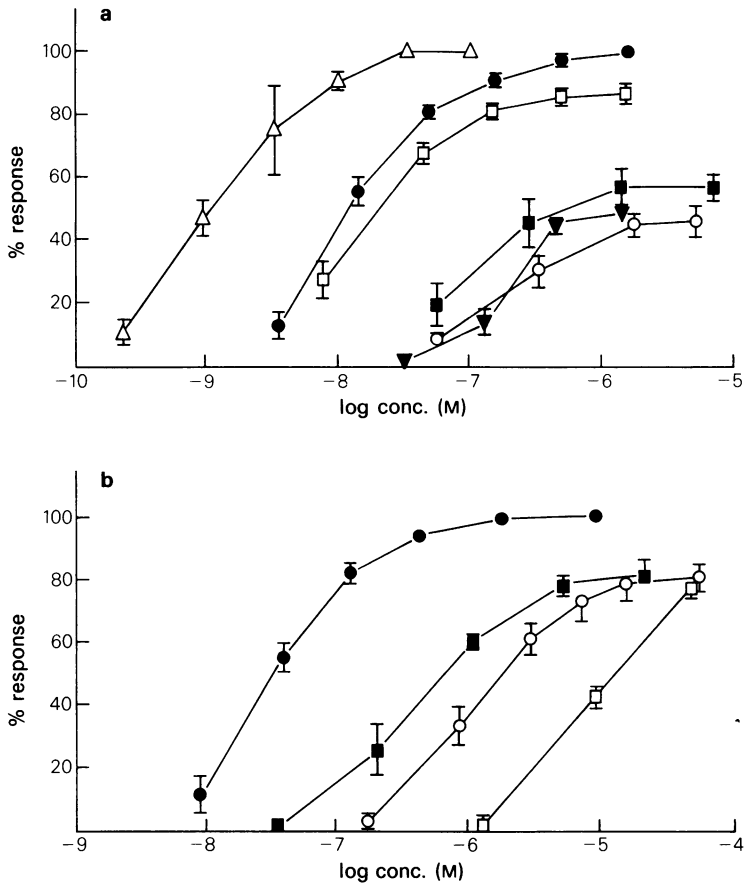

Figure 2 Log concentration-response curves on the rabbit isolated aorta. (a) Standard agonist, 11,9-epoxymethano PGH_2 (●) (number of preparations = 6); 9,11-epoxymethano PGH_2 (□) ($n = 3$); 9,11-etheno PGH_2 (■) ($n = 3$); 16-*p*-fluorophenoxy analogue of 9,11-etheno PGH_2, EP011, (△) (data derived from 15 different preparations, single dose + maximum dose on each); pinane TXA_2 (▼) ($n = 9$, single + maximum dose); 16-*p*-chlorobenzyl analogue of 9,11-etheno PGH_2 (○) ($n = 9$, single + maximum dose). (b) 16-*p*-Fluorophenoxy analogue of $PGF_{2\alpha}$, ICI 79939 (●) ($n = 6$); 11-deoxy $PGF_{2\alpha}$ (■) ($n = 4$); $PGF_{2\alpha}$ (○) ($n = 3$); 16-*p*-trifluoromethylphenoxy analogue of $PGF_{2\alpha}$, ICI 81008 (□) ($n = 3$). Error bars indicate s.e.mean.

calculated for the appropriate concentration increments on the previously established standard agonist log concentration-response curve. Thus there appeared to be no antagonism of the standard agonist by these compounds. In order to give some indication of their contractile activities, equipotent molar ratios at the 25% maximum level were calculated and these are shown in curved brackets in Table 1. With each of these compounds the tension declined rapidly on washout of the organ bath.

On the dog saphenous vein, PGE_2, 15-methyl PGE_2 and PGD_2 did not produce contractile effects but showed inhibitory activity towards both the 11,9-epoxymethano PGH_2 analogue and noradrenaline. This was investigated by first establishing a stable submaximal contractile response to the agonist and then adding the inhibitor in cumulative doses. PGD_2 showed the most consistent relaxant effect as shown in Figure 3 where the log concentration-response curves from six preparations (each from a different dog) have been combined. PGE_2 and 15-methyl PGE_2 gave more variable effects. On two preparations low concentrations of each agent caused complete relaxation (Figure 3) and PGE_2 was 5 and 7 times more active than 15-methyl PGE_2. On five other preparations (different dogs) PGE_2 was less active and did not produce complete relaxation (Figure 3). On these preparations 15-methyl PGE_2 produced relaxation in the concentration range 10^{-7} to 2×10^{-6} M but caused an overall contractile action at

concentrations of $5 = 10^{-6}$ M and above (Figure 3). On two further preparations, PGE_2 (10^{-8} to 10^{-6} M) produced no effect; 15-methyl PGE_2 also produced no relaxant effect but gave contractile responses between 10^{-6} and 10^{-5} M. 16,16-Dimethyl PGE_2 always produced a contractile response.

Partial agonists Compounds exhibiting partial agonist (p.a.) activity on the rabbit aorta and dog saphenous vein are indicated in Table 1. For each of the nine compounds in this group the maximum response relative to that of 11,9-epoxymethano PGH_2 is given together with the mean concentration which produced a response 50% of the partial agonist maximum (data from three or more preparations). Log concentration-response curves for six of the partial agonists on the rabbit aorta are shown in Figure 2. The bicyclic analogues showed slow onset of action and this was particularly marked with four compounds, the 16-*p*-chlorobenzyl analogue of 9,11-etheno PGH_2 (EP 016), 10a-homo-9,11-ethano PGH_2, pinane TXA_2 and its 16-*p*-fluorophenoxy analogue.

With these four compounds the single dose procedure described previously for the group C full agonists was employed.

In a separate series of experiments the interaction between each partial agonist and a full agonist, 11,9-epoxymethano PGH_2 or the 16-*p*-fluorophenoxy analogue of $PGF_{2\alpha}$ (ICI 79939), or noradrenaline was studied. All the compounds designated as partial agonists in Table 1 opposed the contractile action of 11,9-epoxymethano PGH_2 and ICI 79939, but acted additively with noradrenaline. Typical log concentration-response curves for 11,9-epoxymethano PGH_2 acting alone and in the presence of a fixed concentration of 9,11-ethano PGH_2 are shown in Figure 4.

Comparisons of agonist potency on the guinea-pig trachea

The bicyclic ring analogues (9,11-azo PGH_2 and below in Table 1) showed contractile activities on the guinea-pig trachea similar to those found on the rabbit aorta and dog saphenous vein. The standard agonist elicited a 50% maximum response at a mean bath concentration of $29 \pm$ s.d. 12 nM ($n = 119$). 9,11-Azo PGH_2, the 16-*p*-fluorophenoxy analogue of 9,11-etheno PGH_2 and the 16-*p*-chlorophenoxy analogue of 9,11-ethano PGH_2 were potent full agonists. The remaining bicyclic analogues examined were partial agonists. In the case of the 16-*p*-chlorobenzyl analogue of 9,11-etheno PGH_2 and pinane TXA_2 the low maximum response and the slow onset of action made the estimation of the concentration required for a 50% maximum response difficult, and no values are given in Table 1. The partial agonists opposed the contractile action of 11,9-epoxymethano PGH_2 but not that of histamine.

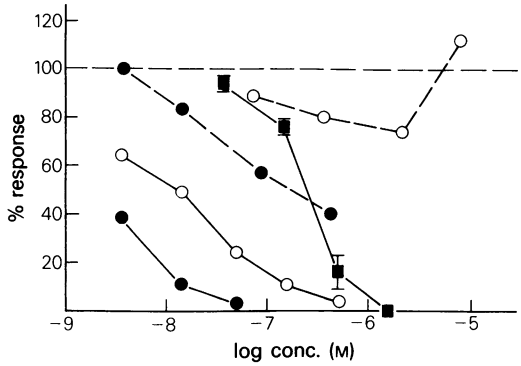

Figure 3 Concentration-response relationships for relaxant effects on the dog saphenous vein preparation. Each preparation was partially contracted with 11,9-epoxymethano PGH_2 (5.7 nM) and the inhibitors were added cumulatively with a contact time of 5 min for each dose. PGD_2(■———■) (mean of 6 preparations, error bars show s.e.mean); PGE_2 (●———●) and 15-methyl PGE_2 (○———○) on a single preparation; PGE_2 (●---●) and 15-methyl PGE_2 (○---○) on another preparation.

The $PGF_{2\alpha}$, PGD_2 and 11-deoxy $PGF_{2\alpha}$ analogues differed considerably in their actions on the guinea-pig trachea. All except 11-deoxy $PGF_{2\alpha}$ produced a maximum response similar in size to that produced by the 11,9-epoxymethano PGH_2 analogue, but there are considerable discrepancies between the equipotent molar ratios obtained on the guinea-pig trachea

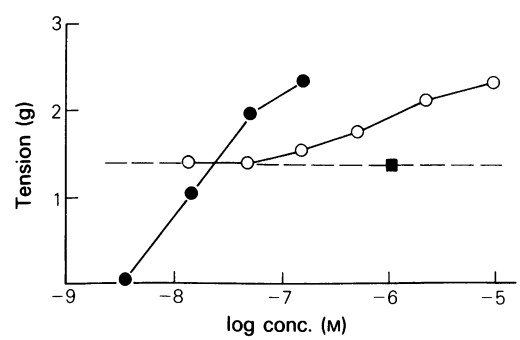

Figure 4 Partial agonist action of 9,11-ethano PGH_2 on the rabbit aorta. Log concentration-response curve for 11,9-epoxymethano PGH_2 acting alone (●); 9,11-ethano PGH_2 response at a concentration of 10^{-6} M (■); log concentration-response curve for 11,9-epoxymethano PGH_2 in the presence of 10^{-6} M 9,11-ethano PGH_2 (○). All doses were added cumulatively.

and those obtained on the rabbit aorta and dog saphenous vein (Table 1).

The PGE_2 analogues also showed different properties on the guinea-pig trachea. PGE_2 produced a contractile response at concentrations of 4×10^{-9} to 10^{-7} M; at higher concentrations a reversal of the contractile action was seen (Figure 5). 15-Methyl PGE_2 (10^{-8} to 10^{-6} M) produced similar effects but the contractile action was greater relative to the 11,9-epoxymethano PGH_2 maximum before reversal took place. 16,16-Dimethyl PGE_2 and the 16-*p*-chlorophenoxy analogue of PGE_2 (ICI 80205) were potent contractile agents on the guinea-pig trachea (Figure 5). The maximum response with both compounds always lay between 65 and 95% of the 11,9-epoxymethano PGH_2 maximum. Addition of small doses of 11,9-epoxymethano PGH_2 to the organ bath following establishment of a maximum response to either of the PGE_2 analogues gave additional contractions and there was no apparent antagonism between the two agonists. When PGE_2 was added to the bath in the presence of 5×10^{-9} M ICI 80205 a concentration-dependent relaxation (2×10^{-9}–10^{-7} M) was seen. The equipotent molar ratios for 16,16-dimethyl PGE_2 and ICI 80205 given in Table 1 relate to the 50% maximum response level of the standard agonist, 11,9-epoxymethano PGH_2. The molar ratios will of course vary depending on the response level chosen and to distinguish them from values obtained for compounds having log concentration-response curves parallel to that of the standard agonist they have been placed in square brackets in Table 1.

Effect of EP 045 on responses to thromboxane A_2

Pairs of either the rabbit aorta or the dog saphenous vein, arranged in series, were challenged with TXA_2 by infusing sodium arachidonate into the pulmonary artery of guinea-pig isolated lung and allowing the venous effluent to superfuse the tissues. The assay tissues were treated with a mixture of blocking agents to reduce or abolish the actions of acetylcholine, histamine, 5-hydroxytryptamine (5-HT) and catecholamines. Indomethacin (3×10^{-6} M) was also added to prevent thromboxane and prostaglandin production in the assay tissues themselves. Sodium arachidonate (100 μg for 1 min), infused to the tissues only, produced no contractile effect. Before the lung was introduced into the cascade system, a dose-response relationship for 11,9-epoxymethano PGH_2 was established. A dose of 300 ng with the rabbit aorta and 100 ng with the dog saphenous vein, infused for 1 min (flow = 10 ml/min), usually gave a response between 70 and 80% of the maximum. With the lung in position, sufficient sodium arachidonate was infused into it to give a response from the assay tissues which matched that produced by the above doses of the stable analogue; this was 20–40 μg for 1 min with the rabbit aorta and 10–20 μg for 1 min with the dog saphenous vein in the cascade.

The identification of the generated component as TXA_2 was based firstly on the ability of indomethacin and the thromboxane synthetase inhibitor UK 37248 to inhibit its biosynthesis in the lung. Concentrations of indomethacin and UK 37248 of 1×10^{-6} M and

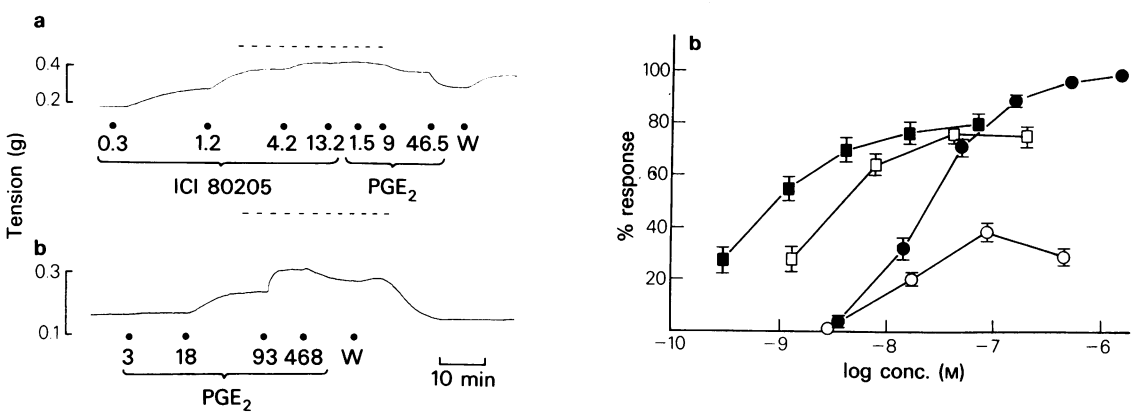

Figure 5 (a) Guinea-pig isolated trachea: the bathing solution contained atropine sulphate (2×10^{-8} M) and indomethacin (10^{-6} M). The upper record shows contractile responses to ICI 80205 and relaxant responses to PGE_2. The lower record (second preparation from the same guinea-pig) shows contractile responses to PGE_2 with reversal of this effect at the highest dose level. The dashed lines represent the maximum responses of each tissue to 11,9-epoxymethano PGH_2. Organ bath concentrations (nM) are shown. W = start of continuous wash. (b) Log concentration-response relationships on the guinea-pig isolated trachea: 11,9-epoxymethano PGH_2 (●) ($n = 11$); 16-*p*-chlorophenoxy analogue of PGE_2, ICI 80205 (■) ($n = 6$); 16,16-dimethyl PGE_2 (□) ($n = 4$) and PGE_2 (○) ($n = 4$). Error bars indicate s.e.mean.

1×10^{-5} M respectively in the lung perfusate almost completely abolished the response to the arachidonate challenge; with three times these concentrations no response was seen. However, the tissue response to the 11,9-epoxymethano PGH_2 analogue was unaffected by these agents. The second piece of evidence concerns the short half-life of the active component in aqueous solution at pH 7.6 and 37°C. An estimate of the half-life was obtained by comparing responses of the second tissue in the cascade when the superfusate from the first tissue was allowed to drip immediately onto the second tissue and when the superfusate was pumped through a delay coil (2 min) before reaching the second tissue. The first tissue showed that the release of the active component was reproducible. The 2 min delay markedly reduced the response of the second tissue and by reference to the dose-response relationship for the 11,9-epoxymethano PGH_2 analogue on the second tissue it was possible to make an estimate of the loss of activity. In three experiments the half life of the active component was found to be 35, 50 and 50 s.

Treatment of the second tissue in the cascade with EP 045 inhibited responses to both the generated TXA_2 and the 11,9-epoxymethano PGH_2 analogue. Typical experiments using the rabbit aorta are shown in Figure 6. The concentration dependence of this blocking action is illustrated in Figure 7. It can be seen that EP 045 blocks the action of TXA_2 and 11,9-epoxymethano PGH_2 to a similar extent and that lower concentrations are required on the dog saphenous vein compared with the rabbit aorta.

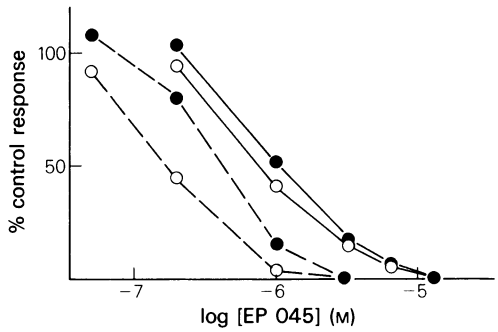

Figure 7 Inhibition by EP 045 of responses to TXA_2 (●) and 11,9-epoxymethano PGH_2 (○) on superfused preparations of the rabbit aorta (———) and dog saphenous vein (— — —). The ordinate scale represents the contractile response in the presence of EP 045 expressed as a percentage of the control response. At each concentration of EP 045, the % control values for TXA_2 and the stable analogue are single results obtained in the same experiment. TXA_2 was generated by infusing sodium arachidonate into guinea-pig isolated lung.

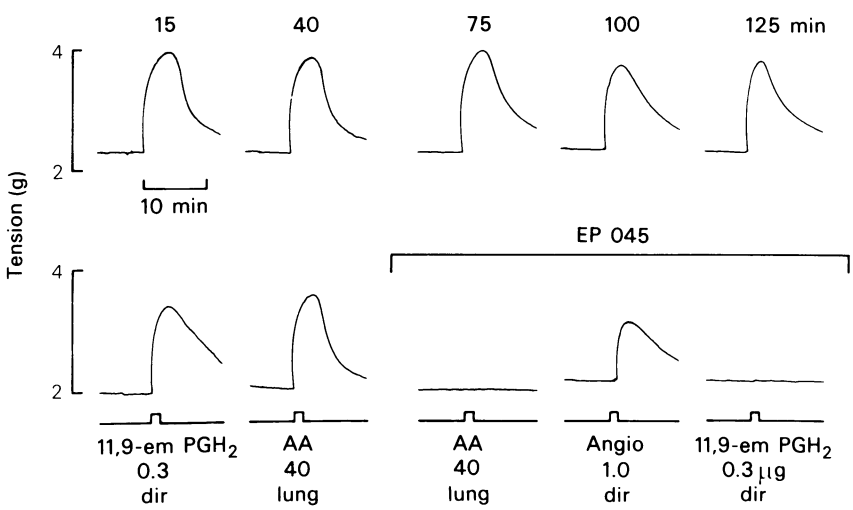

Figure 6 EP 045 antagonism of TXA_2 and 11,9-epoxymethano PGH_2. The records show tension changes in two rabbit aorta strips superfused in series with the effluent from a guinea-pig isolated lung. dir = direct application of the compound to the detecting tissues; lung = infusion of the compound into the lung via the pulmonary artery. The sodium salt of arachidonic acid (AA) was infused into the lung to generate TXA_2. EP 045 (1.3×10^{-5} M) was applied to the second tissue in the cascade (lower record). The application of angiotensin amide (Angio) demonstrates that a non-specific depression of the second tissue has not occurred. The times relate to the introduction of the lung into the cascade system.

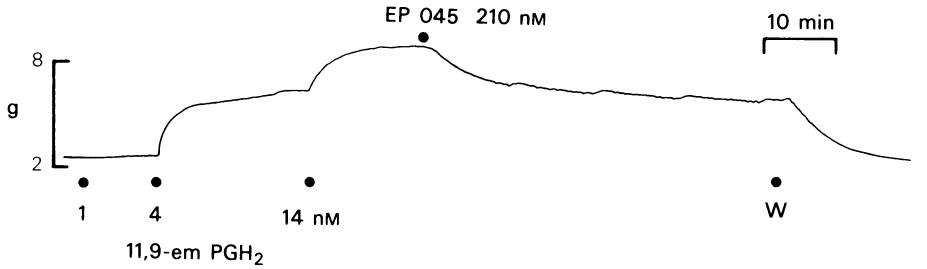

Figure 8 Time-course of the inhibitory action of EP 045 on an established response to 11,9-expoxymethano PGH_2 on the dog saphenous vein.

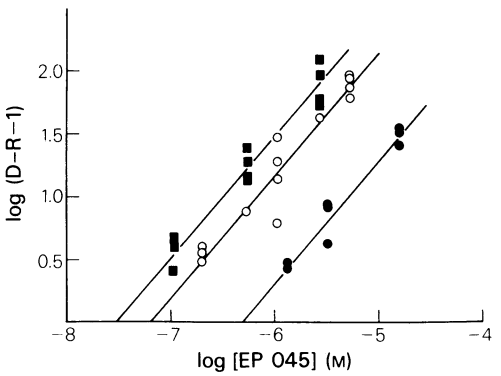

Figure 9 Schild plots for antagonism of 11,9-epoxymethano PGH_2 action by EP 045 on the guinea-pig trachea (■, GPT), dog saphenous vein (○, DSV) and rabbit aorta (●, RA). DR = dose-ratio. Each point derives from a single dose-ratio determination on a separate preparation. Regression lines were fitted by the method of least squares.

Measurement of equilibrium dissociation constants for EP 045 using 11,9-epoxymethano PGH_2 as agonist

For accurate measurement of the equilibrium dissociation constant of an antagonist, responses must be measured when both agonist and antagonist receptor occupancies have reached a steady state. This may clearly be impossible with an agonist as unstable as TXA_2 acting on tissues such as the rabbit aorta which give characteristically slow responses. We therefore chose to make estimations of the equilibrium dissociation constants for EP 045 on the three smooth muscle preparations using 11,9-epoxymethano PGH_2 as agonist in conventional organ bath systems.

The basic procedure was to establish a cumulative dose-response relationship for the standard agonist, wash out the agonist, expose the tissue to a fixed concentration of EP 045 for 50 min and then establish a further dose-response relationship for the agonist. The 50 min exposure time was decided upon from experiments in which the tissue was challenged with standard agonist in sufficient concentration to produce about an 80% maximum response and then EP 045 was added to the organ bath. The time course of the decay of the contractile response was observed (Figure 8). With the lowest concentration of EP 045 a stable level of contraction and hence of receptor blockade was always established well within the 50 min period. It was assumed that with the higher concentrations of EP 045 used, equilibrium blockade would be established at least as fast as with the lowest concentration. Three or four preparations were exposed to a single concentration of the antagonist to obtain a mean dose-ratio: three concentrations of the antagonist (1:5:25) were used.

On each tissue EP 045 caused a parallel shift to the right of the log concentration-response curve for the standard agonist with no reduction in the maximum response attainable. Plots of log (dose-ratio − 1) versus log molar concentration were constructed (Figure 9). The slope of the lines fitted by least squares regression were 0.98 on the rabbit aorta, 0.97 on the dog saphenous vein and 0.97 on the guinea-pig trachea; each value was not significantly different from unity (Student's t distribution, $P > 0.05$).

Equilibrium dissociation constants were found to be 5.0×10^{-7} M for rabbit aorta, 6.6×10^{-8} M for dog saphenous vein and 3.1×10^{-8} M for guinea-pig trachea.

EP 045 at the highest concentrations tested on each type of preparation did not affect the contractile action of noradrenaline on the aorta and saphenous vein or histamine and acetylcholine (atropine excluded from the bathing medium) on the guinea-pig trachea.

Effect of EP 045 on the action of other prostaglandin analogues

It was of interest to know whether EP 045 had a similar blocking action against the other prostaglandin agonists used in this study. However, it was clearly not practicable to construct Schild plots for EP 045 antagonism of a large number of analogues. As a compromise we investigated the ability of EP 045 to inhibit an established submaximal response to each full agonist. A cumulative dose sequence resulting in about an 80% maximum response was carried out. EP 045 (0.5 and 2.5 μM for dog saphenous vein and guinea-pig trachea and 2.5 and 12.5 μM for rabbit aorta) was added to the bath and the decay of the response observed. The lower concentration of the antagonist markedly reduced or completely abolished the response to each agonist on the dog saphenous vein and rabbit aorta; complete abolition of response was always seen with the higher concentration.

On the guinea-pig trachea a similar situation was found only with the standard agonist and the other bicyclic analogues. With the $PGF_{2\alpha}$ analogues, PGD_2 and its 16-*p*-fluorophenoxy analogue, and the 16,16-dimethyl and 16-*p*-chlorophenoxy analogues of 11-deoxy $PGF_{2\alpha}$, 0.5 μM EP 045 caused a 30–70% reduction in the contractile response: 2.5 μM EP 045 caused a further small inhibition only. It thus appears that the submaximal contractile responses to these analogues (indicated by one asterisk in Table 1) are due to interaction with two distinct receptors in the trachea. Although we have quoted equipotent molar ratios using 11,9-epoxymethano PGH_2 as the standard agonist, these values overestimate the true potencies of the analogues on the thromboxane-sensitive system. Concentration-response curves for 11,9-epoxymethano PGH_2 and ICI 79939 in the absence and the presence of EP 045 are shown in Figure 10.

EP 045 (2.5 μM) produced less than 10% inhibition of the established contractile response to 16,16-dimethyl PGE_2 and ICI 80205 on the trachea (indicated by two asterisks in Table 1). Further experiments were performed in which concentration-response relationships were obtained for 11,9-epoxymethano PGH_2 and either 16,16-dimethyl PGE_2 or ICI 80205 on two trachea preparations from the same guinea-pig. Each tissue was then exposed to EP 045 (2.5 μM) for 50 min and the agonist sequence of doses repeated. Typical results are shown in Figure 10. EP 045 caused only a small shift of the log concentration-response curves for the PGE_2 analogues (dose-ratios < 1.5, $n = 3$), whereas the curve for the 11,9-epoxymethano PGH_2 analogue was shifted considerably to the right. It thus appears likely that the PGE_2 analogues exert their contractile effects on guinea-pig trachea via a receptor other than the thromboxane receptor. Similar experiments using PGE_2 as agonist revealed EP 045 (2.5 μM) to have no significant effects on the concentration-response relationship.

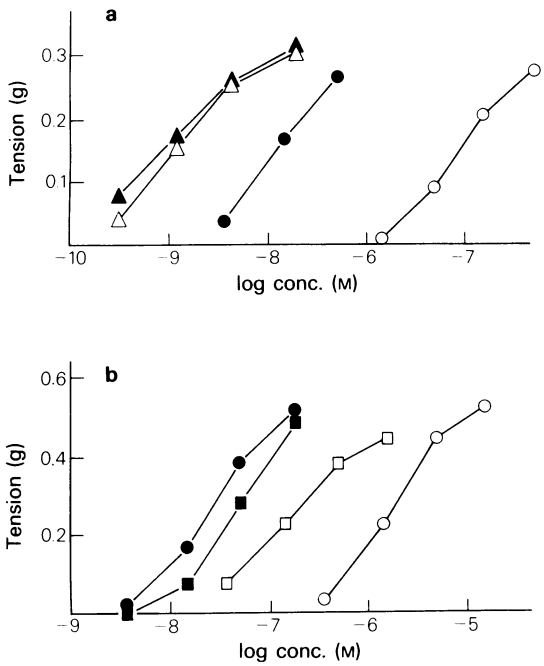

Figure 10 Blocking action of EP 045 on the guinea-pig isolated trachea; the bathing solution contained atropine sulphate (2×10^{-8} M) and indomethacin (10^{-6} M). (a) Log concentration-response curves for 11,9-epoxymethano PGH_2 acting alone (●) and in the presence of 2.5 μM EP 045 (○); ICI 80205 acting alone (▲) and in the presence of 2.5 μM EP 045 (△); two preparations from the same guinea pig were used. (b) Log concentration-response curves for 11,9-epoxymethano PGH_2 acting alone (●) and in the presence of 2.5 μM EP 045 (○); ICI 79939 acting alone (■) and in the presence of 2.5 μM EP 045 (□). Again two preparations from the same guinea-pig were used.

Discussion

EP 045 inhibits the contractile action of TXA_2 on both the rabbit aorta and dog saphenous vein and probably achieves this effect by occupation of thromboxane receptors. The use of a stable thromboxane agonist considerably simplifies the investigation of compounds with thromboxane blocking activity and we have used the 11,9-epoxymethane analogue of PGH_2 (U 46619), originally described by Bundy (1975), for this purpose. Recent studies have shown that U 46619 has the same profile of activity as TXA_2 on smooth muscle (Coleman, Humphrey, Kennedy, Levy & Lumley, 1981). EP 045 also blocks the action of 11,9-epoxymethano PGH_2 on the rabbit aorta, dog saphenous vein and guinea-pig trachea: log concentration-response curves for the agonist are shifted to the right in a parallel manner and the maximum responses are not suppressed. The Schild plots are linear and the slopes are close to unity for each preparation. These sorts of observations are usually interpreted to mean competition between agonist and antagonist for occupation of a common receptor.

The three preparations differ in their responses to the natural prostaglandins and this may indicate the existence on the smooth muscle cells of several types of prostanoid receptors. Table 2 summarizes the actions of the protanoids we studied. The rabbit aorta is the simplest of the preparations: it has no inherent tone, gives responses which do not fade over periods of 1–2 h, and responds by contraction to all the compounds we have tested. PGD_2, PGE_2, $PGF_{2\alpha}$ and PGI_2 have weak agonist activity and their actions are completely blocked by EP 045. In addition $PGF_{2\alpha}$ behaves as a partial agonist and antagonizes the action of the 11,9-epoxymethano PGH_2 analogue. These data lead us to propose tentatively a single type of prostanoid receptor mediating contraction in the rabbit aorta.

The dog saphenous vein preparation exhibits consistent contractile responses to low concentrations of thromboxane-like agents, but is more complex than the rabbit aorta since prostaglandin-induced relaxation can occur. PGD_2 gives highly reproducible effects on the saphenous vein whereas PGE_2 and its 15-methyl analogue show considerable differences in sensitivity when individual preparations are compared. Relaxant responses to prostaglandins of the E series on vascular smooth muscle both *in vivo* and *in vitro* are widely documented (see review by Malik & McGiff, 1976). Responses to PGD_2 on vascular smooth muscle tend to be more variable. In the sheep, dog and pig, PGD_2 is a pressor agent owing to its direct peripheral arteriolar constrictor action (Jones, 1976; 1978). Structure-activity data related to this effect provide good evidence that the receptor is different from that mediating thromboxane-like vascular constriction. However, when the PGE_2-sensitive peripheral dilator system in the above animals is highly responsive then depressor responses to PGD_2 may also be seen. This suggested to us that PGD_2 may mimic to a small extent the inhibitory action of PGE_2 on vascular smooth muscle. With respect to experiments described here on the dog saphenous vein *in vitro* it is probable that PGD_2 and PGE_2 act as agonists on separate receptors to produce their relaxant effects. The possession of significant relaxant activity by other compounds, for example the PGD_2 and PGE_2 analogues listed in Table 1, may affect the estimation of their thromboxane-like activity on the saphenous vein. With the 16,16-dimethyl, 16-*p*-fluorophenoxy and 16-*p*-chlorophenoxy analogues of PGE_2 and the 16-*p*-fluorophenoxy analogue of PGD_2 we can find no evidence that this is the case. Each compound produces a maximum response equivalent to that produced by the standard agonist and their log concentration-response curves are parallel to that of the standard agonist (cf. PGE_2 action on the guinea-pig trachea). Like the rabbit aorta, the contractile actions of all the analogues on the dog saphenous vein can be abolished by EP 045.

The guinea-pig isolated trachea was one of the first preparations on which pure prostaglandins were tested. Main (1964) showed that PGE_1 at low concentration (10^{-8} M) would inhibit both the inherent tone of the preparation and the contractile responses to acetylcholine and other spasmogens. Later it was observed that PGE_2 relaxed and $PGF_{2\alpha}$ contracted an *in vivo* preparation (James, 1969). Following the discovery of TXA_2 the high potency of this substance in contracting guinea-pig isolated trachea was demonstrated (Svensson *et al.*, 1977).

The inherent tone of the guinea-pig isolated trachea can be inhibited with a combination of atropine and indomethacin, indicating contributions from muscarinic and cyclo-oxygenase systems (Farmer, Farrar & Wilson, 1974). However, Puglisi (1973) in a rarely-quoted paper reported that indomethacin potentiated the contractile action of $PGF_{2\alpha}$ whilst inhibiting the relaxant action of PGE_1. In some preparations indomethacin actually reversed the relaxant action of PGE_1. More recently Gardiner & Collier (1980) have confirmed this action of indomethacin and have proposed the existence of two opposed receptors (contractile and relaxant) on guinea-pig tracheal and human bronchial smooth muscle.

We have chosen to treat the guinea-pig trachea preparation with an atropine/indomethacin combination for the purposes of producing a stable resting tension and removing any influence of endogenous prostaglandins and thromboxanes on the action of

exogenously-applied analogues. A dual action of PGE_2 was readily apparent in our preparations, but neither the contractile nor relaxant components were affected by EP 045. Thus we may conclude that the contractile action of PGE_2 on the trachea is not mediated via the same receptor with which the bicyclic analogues interact. Indeed, even with the relaxant effect operating, the concentrations of PGE_2 which caused tracheal contraction were much lower than those required for contraction of the rabbit aorta; both preparations had similar sensitivities to 11,9-epoxymethano PGH_2. 16,16-Dimethyl PGE_2 and the 16-*p*-chlorophenoxy analogue of PGE_2 (ICI 80205) were potent contractile agents which showed no evidence of a dual action and whose effects were not blocked by EP 045.

Thus there appear to be three receptor systems responsive to prostanoids in the guinea-pig trachea. The first is activated by thromboxane-like agents to produce contraction and is readily blocked by EP 045; the second mediates a relaxant effect and is sensitive to PGE_2 and its 15-methyl analogue; the third gives rise to a contractile effect and PGE_2 analogues are potent agonists, but it is not blocked by EP 045. This latter system may be similar to that identified recently by us in the bullock iris sphincter muscle (Dong & Jones, 1982), on which PGE_2 is a potent contractile agent and the measurements are not complicated by any relaxant action of PGE_2. 16,16-Dimethyl PGE_2 and ICI 80205 are several times more active than PGE_2 whereas $PGF_{2\alpha}$, PGD_2 and PGI_2 show only weak activity. EP 045 does not block the agonist action of the PGE_2 analogues on the iris preparation.

The partial block of the contractile action of $PGF_{2\alpha}$ and PGD_2 and their analogues on the guinea-pig trachea by EP 045 is perhaps indicative of a simultaneous interaction of these agonists with the thromboxane-sensitive and PGE-sensitive contractile systems. The marked difference in potency between 16,16-dimethyl PGE_2 and 11,9-epoxymethano PGH_2 in the presence of EP 045 demonstrates the low activity of the PGH_2 analogue on the PGE-sensitive contractile system.

A question which requires careful consideration is whether the thromboxane/EP 045-sensitive receptor systems in the rabbit aorta, dog saphenous vein and guinea-pig trachea preparations are truly identical. Comparison of the absolute potency of the standard agonist and of the relative potencies of the full agonists (excluding those compounds which show PGE-like contractile activity on the guinea-pig trachea) reveals good agreement especially when one takes into account the difficulties of working with the slow-acting analogues, for example EP 011. It is quite easy to underestimate the potency of a slow-acting analogue relative to the standard agonist and indeed our earlier equipotent molar ratios (Jones *et al.*, 1979; Jones & Wilson, 1980) should be disregarded because in many cases, in an attempt to construct a complete log concentration-response curve on a single preparation, insufficient time was allowed for achievement of a stable increase in tension by each dose.

There is also good correlation between the tissues in terms of the partial agonist activity of the different analogues. Of particular interest is the partial agonist action of 9,11-epoxymethano PGH_2 in contrast to the full agonist action of the isomeric 11,9-epoxymethano PGH_2; this difference has not been reported previously. The finding that the **bicyclo** [2,2,2] octane analogue, 10a-homo-9,11-ethano PGH_2, was a partial agonist was hardly surprising, but its high agonist activity on the three preparations was unexpected (threshold responses elicited at 1×10^{-9} M). Pinane TXA_2 is a partial agonist on all three preparations. Substitution of a 16-*p*-fluorophenoxy group in the ω-chain increases its agonist activity but unlike the 9,11-etheno PGH_2/EP 011 and 9,11-ethano PGH_2/EP 031 pairs does not result in full agonist activity.

For a meaningful comparison of the partial agonists on the three tissues it is essential to estimate their equilibrium dissociation constants. Colquhoun (1973) has described a method for achieving this whereby for matching responses several pairs of concentrations of the full agonist acting alone and in the presence of a fixed concentration of the partial agonist are obtained. When these concentrations are plotted against each other a linear relationship is found if classical occupation theory holds. From the slope of the line the equilibrium dissociation constant can be found. We attempted to use this method in the first instance on the dog saphenous vein using the 16-*p*-fluorophenoxy analogue of $PGF_{2\alpha}$ (ICI 79939) as the full agonist. Linear plots were obtained in about two-thirds of the experiments and equilibrium dissociation constants were recorded as follows: $PGF_{2\alpha}$ (fixed concentration of 2×10^{-5} M) 1.4, 1.8 and 2.7×10^{-6} M; 9,11-ethano PGH_2 (2×10^{-7} M) 0.7 and 1.5×10^{-7} M, (1×10^{-6} M) 0.38 and 2.4×10^{-7} M; EP 016 (3×10^{-6} M) 2.3, 8.4 and 9.9×10^{-8} M. The considerable variation in the results prompted us to examine the accuracy of the method in more detail. It was apparent that a small change in the concentration-response relationship for the full agonist during the course of the determination could make a marked difference to the calculated value for the dissociation constant. With the slow onset/slow offset partial agonists it was not possible to determine whether such a change had taken place. We therefore decided that in our experimental situation this method would not give sufficiently accurate values of the equilibrium dissocia-

tion constants to be of use in comparing the characteristics of the thromboxane receptors in the three smooth muscle preparations.

Equilibrium dissociation constants for the pure antagonist EP 045 could, however, be obtained with reasonable accuracy. The value obtained on the rabbit aorta (5×10^{-7} M) is about an order of magnitude higher than the values obtained on the dog saphenous vein and guinea-pig trachea (6.6×10^{-8} M and 3.1×10^{-8} M respectively). These values were obtained using the 11,9-epoxymethano PGH_2 analogue as agonist, but the greater blocking activity of EP 045 on the dog saphenous vein compared with the rabbit aorta is also seen when TXA_2 is used. EP 045 is not unique in this respect and we have found similar differences with other semicarbazone and thiosemicarbazone analogues related to EP 045. It seems premature on the basis of the EP 045 experiments alone to put forward the idea that the thromboxane receptor in the rabbit aorta is different from those in the dog saphenous vein and guinea-pig trachea; further studies are obviously required.

EP 045 blocks the aggregation of human platelets induced by either 11,9-epoxymethano PGH_2 or arachidonic acid but has little effect on ADP responses (Jones & Wilson, 1981). We are at present investigating the nature of this effect in detail with the intention of comparing the results with those described here for the three smooth muscle preparations.

Gifts of prostaglandins from ICI Pharmaceuticals, U.K., Schering AG, Berlin-Bergkamen, and the Upjohn Company, U.S.A. are gratefully acknowledged. We thank the Pfizer Company, U.K. for the sample of UK 37248. These studies were supported by the Medical Research Council (programme grant) and the National Research Development Corporation. The excellent technical assistance of Mr C.G. Marr and Mrs Lynne Fell is highly appreciated.

References

APPERLEY, E., FENIUK, W., HUMPHREY, P.P.A. & LEVY, G.P. (1980). Evidence for two types of excitatory receptor for 5-hydroxytryptamine in dog isolated vasculature. *Br. J. Pharmac.*, **68,** 215–224.

BRASH, A.R. & JONES, R.L. (1974). Straight phase separation of prostaglandin methyl esters on lipophilic gels. *Prostaglandins*, **5,** 441–454.

BUNDY, G.L. (1975). Synthesis of prostaglandin endoperoxide analogs. *Tetrahedron Lett.*, 1957–1960.

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.

COLQUHOUN, D. (1973). The relation between classical and co-operative models for drug action. In *Drug Receptors.* ed. Rang, H.P. pp. 149–182. London: Macmillan.

COREY, E.J., NARASAKA, K. & SHIBAZAKI, M. (1976). A direct stereo-controlled total synthesis of the 9,11-azo analogue of the prostaglandin endoperoxide, PGH_2. *J. Am. chem. Soc.*, **98,** 6417–6418.

COREY, E.J., SHIBAZAKI, M., NICOLAOU, K.C., MALMSTEN, C. & SAMUELSSON, B. (1976). Simple stereocontrolled total synthesis of a biologically active analogue of the prostaglandin endoperoxide (PGG_2, PGH_2). *Tetrahedron Lett.*, 737–741.

DONG, Y.G. & 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-19220. *Br. J. Pharmac.*, **52,** 559–565.

GARDINER, P.J. & COLLIER, H.O.J. (1980). Specific receptors for prostaglandins in airways. *Prostaglandins*, **6,** 819–841.

JAMES, G.W.Z. (1969). The use of the 'in vivo' trachea preparation of the guinea pig to assess drug action on lung. *J. Pharm. Pharmac.*, **21,** 379–386.

JONES, R.L. (1976). Cardiovascular actions of prostaglandins D and E in the sheep: evidence for two distinct receptors. *Adv. Prostaglandin Thromboxane Res.*, **1,** 221–230.

JONES, R.L. (1978). Definition of prostaglandin-sensitive arterial contractor systems. *Acta. biol. med. Germ.*, **37,** 837–844.

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

JONES, R.L. & WILSON, N.H. (1980). Partial agonism of prostaglandin H_2 analogs and 11-deoxy-prostaglandin $F_{2\alpha}$ to thromboxane sensitive preparations. *Adv. Prostaglandin Thromboxane Res.*, **6,** 467–475.

JONES, R.L. & WILSON, N.H. (1981). Thromboxane receptor antagonism shown by a prostanoid with a **bicyclo** [2,2,1] heptane ring. *Br. J. Pharmac.*, **73,** 220–221P.

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

MAIN, I.H.M. (1964). The inhibitory action of prostaglandins on respiratory smooth muscle. *Br. J. Pharmac. Chemother.*, **22,** 511–519.

MALIK, U.K. & McGIFF, J.C. (1976). Cardiovascular actions of prostaglandins. In *Prostaglandins: Physiological, Pharmacological and Pathological Aspects.* ed. Karim, S.M.M. pp. 103–200. England: MTP Press.

MAXEY, K.M. & BUNDY, G.L. (1980). The synthesis of 11a-carbathromboxane A_2. *Tetrahedron Lett.*, **21,** 445–448.

NEEDLEMAN, P., MONCADA, S., BUNTING, S., VANE, J.R., HAMBERG, M. & SAMUELSSON, B. (1976). Identification of an enzyme in platelet microsomes which generates thromboxane A_2 from prostaglandin endoperoxides. *Nature*, **261**, 558–560.

NICOLAOU, K.C., GASIC, G.P. & BARNETTE, W.E. (1978). Synthesis and biological properties of prostaglandins, thromboxanes and prostacyclins. *Angewandte Chemie*, **17**, 293–312.

NICOLAOU, K.C., MAGOLDA, R.L. & CLAREMON, D.A. (1980). Carbocyclic thromboxane A_2. *J. Am. chem. Soc.*, **102**, 1404–1409.

NISHIZAWA, E.E., MILLER, W.M., GORMAN, R.R., BUNDY, G.L., SVENSSON, J. & HAMBERG, M. (1975). Prostaglandin D_2 as a potential antithrombotic agent. *Prostaglandins*, **9**, 109–121.

PUGLISI, L. (1973). Opposite effects of prostaglandins E and F on tracheal smooth muscles and their interaction with calcium ions. *Adv. Biosci.*, **9**, 219–227.

SAMUELSSON, B., HAMBERG, M., MALMSTEN, E. & SVENSSON, J. (1976). The role of prostaglandin endoperoxides and thromboxanes in platelet aggregation. *Adv. Prostaglandin Thromboxane Res.*, **2**, 737–746.

SVENSSON, J., HAMBERG, M. & SAMUELSSON, B. (1976). On the formation and effects of thromboxane A_2 in human platelets. *Acta physiol. scand.*, **98**, 285–294.

SVENSSON, J., STRANDBERG, K., TUVEMO, T. & HAMBERG, M. (1977). Thromboxane A_2: effects on airway and vascular smooth muscle. *Prostaglandins*, **14**, 425–436.

WILSON, N.H., PEESAPATI, V. & JONES, R.L. (1982). Synthesis of prostanoids with **bicyclo** [2,2,1] heptane, **bicyclo** [3,1,1] heptane and **bicyclo** [2,2,2] octane ring systems. Activities of 15-hydroxy epimers on human platelets. *J. med. Chem.*, (in press).

(*Received December 8, 1981.*
Revised March 11, 1982.)