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Potent contractile actions of prostanoid EP_3-receptor agonists on human isolated pulmonary artery

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1 In 13 of 15 experiments, prostaglandin E_2 (PGE_2) and sulprostone (a prostanoid EP_1/EP_3-receptor agonist) contracted isolated rings of human pulmonary artery at low concentrations ($\geqslant 5$ and $\geqslant 0.5$ nM respectively). Tissue was obtained from patients undergoing surgery mainly for carcinoma of the lung. Characterization of the receptors involved was complicated by loss of sensitivity to the contractile PGE action over the experimental period. In contrast, contractile responses to KCl, phenylephrine and the specific thromboxane (TP-) receptor agonist, U-46619, did not decrease with time.

2 The relative contractile potencies for seven PGE analogues, measured during the first few hours after setting up the preparations, were as follows: sulprostone > misoprostol = gemeprost $\geqslant$ PGE_2 $\geqslant$ GR 63799X > 17-phenyl-ω-trinor PGE_2 $\geqslant$ 11-deoxy PGE_1. This ranking indicates that an EP_3-receptor is involved.

3 The contractile action of sulprostone was not blocked by the TP-receptor antagonists, EP 169 and GR 32191, and the EP_1-receptor antagonist, AH 6809.

4 In two experiments, PGE_2 (50 nM) reduced basal tone and sulprostone was a weak contractile agent. Phenylephrine-induced tone was also inhibited by PGE_2 ($EC_{50} = 5-20$ nM), 11-deoxy PGE_1 and butaprost (a selective EP_2-receptor agonist); the latter prostanoids were about 2 and 4 times less potent than PGE_2 respectively. Interactions with phenylephrine were different in experiments where PGE_2 alone was contractile: PGE_2 induced contraction superimposed on the phenylephrine response and 11-deoxy PGE_1 induced either further contraction or had no effect. Butaprost produced relaxation at high concentrations; this may not be an EP_2 action since preparations were highly sensitive to relaxant actions of prostacyclin (IP-) receptor agonists (cicaprost and TEI-9063).

5 The study has shown that in the majority of experiments on the human isolated pulmonary artery, the contractile EP_3 system outweighed the relaxant EP_2 system. However, in two experiments the reverse was true. It is not clear to what extent these differences are due to disease processes affecting the tissues. The findings are discussed in relation to the adverse cardiovascular responses occasionally encountered during treatment of postpartum haemorrhage with sulprostone, and more generally to the clinical use of EP-receptor agonists in man.

Keywords: Prostanoid receptors; human pulmonary artery; prostaglandin E_2; sulprostone; misoprostol; gemeprost; cicaprost; TP-receptor antagonists; postpartum haemorrhage; obstetric complications

Introduction

Prostaglandin E_2 (PGE_2) and its analogues have been used therapeutically for their stimulant actions on the pregnant human uterus for some 25 years (Bygdeman *et al.*, 1968; Bygdeman, 1990). The work of Senior and colleagues (1993) clearly indicates that PGE analogues contract human myometrial smooth muscle by activating prostanoid EP_3-receptors. The division of EP-receptors into three subtypes is based largely on *in vitro* smooth muscle measurements and has been recently confirmed by molecular biology techniques (see *Trends Pharmacol. Sci.*, 1994). In general EP_1 and EP_3 subtypes mediate contraction of smooth muscle and EP_2-receptors relaxation.

Sulprostone, which is a highly potent EP_3 agonist, has been recommended for arrest of atonic postpartum haemorrhage due to its excellent tolerance (see Hoppe, 1986). However, the current study originates from observations of severe cardiovascular side effects in two patients receiving intravenous sulprostone for postpartum haemorrhage. Systemic hypotension occurred in the first patient; this is unlikely to have been due to peripheral vasodilatation since sulprostone is almost devoid of EP_2 relaxant activity on vascular smooth muscle (Coleman *et al.*, 1987a,b; Lawrence & Jones, 1992). In the second patient a chest X-ray showed pulmonary congestion (Stock *et al.*, 1994). In this context, the advice sheet on sulprostone (trade name Nalador) contains the warning: 'Do not inject as a bolus (the rapid occurrence of a high plasma level may lead to a critical increase of pressure in the pulmonary circulation)'. The mechanism of the pulmonary hypertension is not known and we therefore decided in the first instance to determine whether sulprostone and other PGE analogues have constrictor actions on human isolated pulmonary vessels, and if so, to identify the prostanoid receptor involved. A preliminary account of our studies has been presented to the British Pharmacological Society (Jones *et al.*, 1994).

Methods

Isolated pulmonary artery preparations

Lung tissue was obtained from 15 Chinese patients undergoing resection of the lung at the Prince of Wales Hospital, Shatin. Twelve of the patients (10 males, 13–76 years; 2 females, 43 and 62 years) had lung cancer and three (one female, 15 years; 2 males, 53 and 58 years) had pulmonary tuberculosis.

Within 10–20 min of lung removal, dissection of sections of pulmonary lobar artery (3–5 mm in diameter) from

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macroscopically healthy areas of a lung lobe was commenced. The tissue was placed in Krebs-Henseleit solution (composition, mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.18, $CaCl_2$ 2.5, KH_2PO_4 1.18, $NaHCO_3$ 25, glucose 10, pH 7.32) at 4–10°C for transport to the laboratory. Adherent tissue, especially that containing tar deposits, was carefully removed. Vessel rings (3–4 mm wide) were cut and suspended in 10 ml organ baths containing the Krebs solution aerated with 95% O_2/5% CO_2 and maintained at 37°C; indomethacin (1 μM) was present throughout the experiments. Washing was by upward displacement and overflow. Tension was recorded with isometric force-displacement transducers (Grass FT03) linked to MacLab recording systems (Chart software version 3.3, data sampling rate = 40 per min). Usually two pairs of matched preparations (i.e. from adjacent portions of an artery) were studied.

Experimental protocols

About 20 min after setting up in the organ bath, each preparation was exposed to a single dose of KCl (40 mM); about 5% of preparations gave weak contractions to KCl and were abandoned. About 1 h after set-up a single priming dose of PGE_2 (50 nM) was applied for 10–15 min.

Agonist potencies Initial experiments showed that sensitivity to the contractile actions of the PGE analogues decayed with time. However, it was not certain that sensitivity would decay in all experiments. Consequently a cross-over design was used in which a cumulative dose-response curve for either PGE_2 or sulprostone was obtained on one preparation concurrently with a cumulative dose-response curve for the test analogue on the matched preparation; these measurements took place between 1.5 and 3 h of setting up the preparations. Responses greater than 80% of the predicted maximum were not obtained in an attempt to reduce tachyphylaxis. After washout each analogue was then retested on the other preparation.

Relaxant effects A stable increase in tone of about 0.7 g was induced by phenylephrine (0.4–2 μM), U-46619 (5–10 nM) or KCl (40 mM). Responses to cumulative doses of prostanoids were then obtained.

Antagonist affinities In the first protocol, one of the matched preparations was treated with antagonist and the other with vehicle for 15 min. Cumulative dose-response curves to the same agonist were then obtained on both preparations. In the second protocol, stable submaximal responses to a particular agonist were obtained on two matched preparations. The antagonist was then added to one preparation and the vehicle to the other; the effects were observed for at least 15 min. All these measurements took place 1.5–3 h after setting up the preparations.

Drugs

The following compounds were gifts: sulprostone and cicaprost (Schering AG, Berlin, Germany); misoprostol (G.D. Searle, U.S.A.); gemeprost (ONO 802; 16,16-dimethyl-*trans*-Δ^2 PGE_1-methyl ester) (Rhone-Poulenc, UK); TEI-9063 (17α,20-dimethyl-$\Delta^{6,6a}$-6a-carba PGI_1) (Teijin, Japan); GR 32191 (9α-(biphenylyl)methoxy-11β-hydroxy-12β-(N-piperidinyl)-ω-octanor-prost-4Z-enoic acid); GR 63799X (13-oxa-13,14-dihydro-16-phenoxy-ω-tetranor PGE_2-4-(benzoylamino) phenyl ester); AH 6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid) (Glaxo Group Research, U.K.). EP 169 (*rac* 4a,10a-dihomo-9α,11α-ethano-ω-heptanor-3-oxa-13-phenylthiocarbamoylhydrazino prostanoic acid) was synthesized by Dr N.H. Wilson of the Department of Pharmacology, University of Edinburgh. PGE_2, 17-phenyl-ω-trinor PGE_2, $PGF_{2\alpha}$, fluprostenol (ICI 81008) and U-46619 (15S)-hydroxy-11α,9α-(epoxymethano)prosta-5Z,13E-dienoic acid) were purchased from Cayman Chemicals, U.S.A., and (−)-phenylephrine hydrochloride and indomethacin from Sigma Chemical Co., U.S.A.

Results

Initial observations on the human pulmonary artery

The first three experiments showed that human pulmonary artery preparations were contracted by both PGE_2 and sulprostone (Figure 1a,b). Sensitivity was high initially with threshold effects observed at 1–5 and 0.2–0.5 nM respectively. However, there was a variable loss of sensitivity to the two PGE analogues throughout the experimental period (6–8 h). This took the form of both an increase in the EC_{50} value and a reduction in the maximum response. The phenomenon is illustrated for the whole series of experiments in Figure 2. Contractile responses to KCl, phenylephrine and the specific TP-receptor agonist U-46619 did not decay over the experimental period (data not shown).

Contractile potencies of PGE and PGF analogues

Log concentration-response curves for PGE_2 and five of its analogues, sulprostone, misoprostol, gemeprost, GR 63799X and 17-phenyl-ω-trinor PGE_2 are shown in Figure 3. Relative potencies, expressed as equi-effective molar ratios (PGE_2 = 1.0), are given in Table 1. The data derive only from the first cumulative sequence of doses on each preparation, since the variable loss of sensitivity meant that 'within preparation' estimates of relative potency (see Methods) were potentially subject to considerable error. Sulprostone was the most potent prostanoid and its rates of onset and return to resting tension on washout were always slower than those of the other prostanoids.

$PGF_{2\alpha}$ was only a weak agonist (Figure 3a) and the specific FP-receptor agonist, fluprostenol, produced no effect at concentrations up to 2 μM (2 experiments).

Antagonist studies

In a single experiment, the TP-receptor antagonist, EP 169 (200 nM), added before U-46619 blocked its contractile response, with parallel displacement of the log concentration-response curve to the right (dose-ratio = 41). Assuming reversible, competitive antagonism (Gaddum, 1957), a pA_2 value of 8.3 was obtained (single dose-ratio estimate). The log concentration-response curve to sulprostone was unaffected by this treatment. Similarly in two experiments, a second TP antagonist, GR 32191, at 200 nM failed to affect sulprostone but blocked U-46619 contractions with dose-ratios of 32 and 55, giving a mean pA_2 value of 8.3.

In three separate experiments, EP 169 (200 and 500 nM) and GR 32191 (200 nM) were added following establishment of submaximal responses to sulprostone (10 nM) and U-46619 (2–10 nM). The antagonists abolished the U-46619 response but had minimal or no effect on the sulprostone response; this is illustrated for EP 169 in Figure 4.

The interaction of sulprostone and the EP_1-receptor antagonist, AH 6809, was investigated in a similar manner to that of the TP antagonists. At 5 μM, AH 6809 had no effect on the log concentration-response curve to sulprostone (n = 1) and did not relax preparations already contracted with a submaximal concentration of sulprostone (n = 2). The same stock solutions of AH 6809 were effective in antagonizing the EP_1 contractile actions of 17-phenyl-ω-trinor PGE_2 and gemeprost on the guinea-pig isolated trachea (200 nM EP 169 present); with 2 μM AH 6809, dose-ratios of 50–60 were obtained (n = 3).

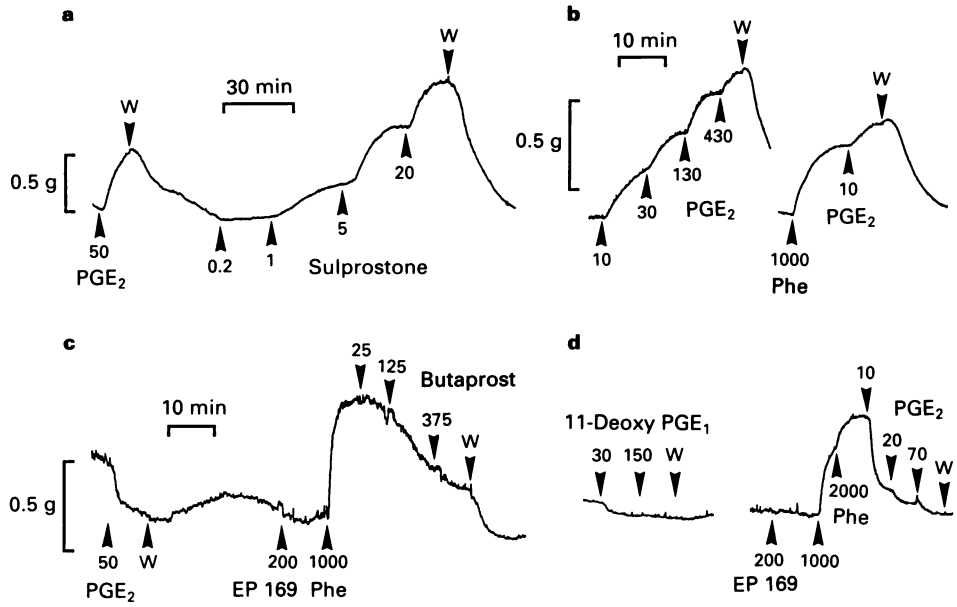

Figure 1 Experimental records showing the actions of prostaglandin E (PGE) analogues on the human isolated pulmonary artery. Records (a) and (b) are from preparations where the priming dose of PGE_2 (50 nM) induced contraction. Records (c) and (d) are from one of only two experiments in which preparations relaxed to the PGE_2 priming dose. W = wash; Phe = phenylephrine; EP 169 is a thromboxane receptor antagonist. Cumulative concentrations (nM) are shown.

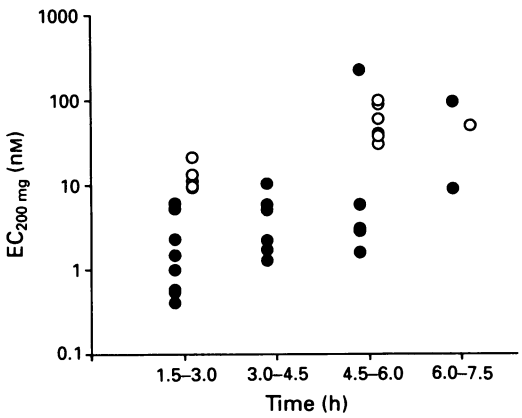

Figure 2 Changes in sensitivity to the contractile actions of sulprostone (●) and prostaglandin E_2 (PGE_2) (○) with time on the human isolated pulmonary artery. The ordinate scale is the concentration of prostanoid required to increase the tension in the preparation by 200 mg.

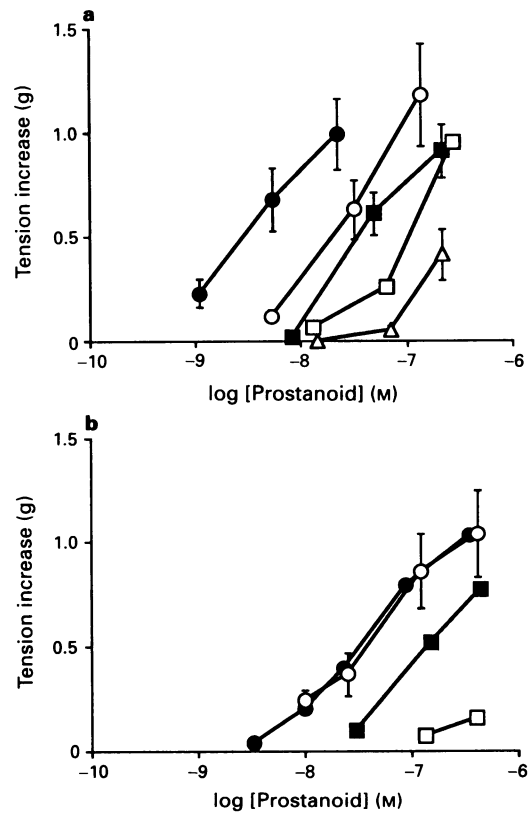

Figure 3 Log concentration-response curves for contractile effects of prostanoids on the human isolated pulmonary artery. Data were obtained between 1.5 and 3 h in experiments where the priming dose of prostaglandin E_2 (PGE_2) produced contraction. Values are mean ± s.e.mean. (a) Sulprostone (●, $n = 8$), misoprostol (○, $n = 4$), GR 63799X (■, $n = 6$), 17-phenyl-ω-trinor PGE_2 (□, mean curve, $n = 2$), $PGF_{2\alpha}$ (△, $n = 3$). (b) PGE_2 (○, $n = 4$), gemeprost (●, mean curve, $n = 2$), 11-deoxy PGE_1 (■, □, individual experiments).

Relaxant actions of prostanoids

The two prostacyclin analogues, cicaprost and TEI-9063, potently inhibited agonist-induced tone on the pulmonary artery preparations (Figure 5a). Relaxant potencies against equi-effective concentrations of phenylephrine (1–2 μM) and U-46619 (5–10 nM) were similar: e.g. cicaprost $IC_{50} = 2.6 \pm 0.5$ nM ($n = 4$) and 2.5 ± 0.5 nM ($n = 3$) respectively. However, the maximum relaxation achieved against KCl was only 65–85%; this is shown for TEI-9063 in an experiment in which the prostacyclin-mimetics were particularly potent (Figure 5a).

There was no evidence from the first 8 experiments that any of the PGE analogues examined were exerting a relaxant EP_2 action. However, we felt it was possible that a strong contractile EP action could mask a weak relaxant action. We therefore addressed this situation in the next 5 experiments by determining the effects of butaprost and 11-deoxy PGE_1 (both of which show moderate potency and selectivity for EP_2-receptors) on phenylephrine-induced tone. In three of the experiments, the PGE_2 priming dose (50 nM) increased tension by about 0.5 g, as found previously. Butaprost had no effect on basal tone at 25 nM but gave slight relaxations

Table 1 Prostanoid EP_3 agonist potencies and EP-receptor selectivities of prostaglandin E (PGE) analogues

PGE analogue	*Equi-effective molar ratio* *Contraction of human pulmonary artery*	*Inhibition of cyclic AMP in human platelets*	*Inhibition of guinea-pig vas deferens*	*EP-receptor selectivity*
PGE_2	1.0 (EC_{50} = 50 nM)	1.0 (IC_{50} = 5.5 nM)	1.0 (IC_{50} = 1.5 nM)	$EP_1 = EP_2 = EP_3$
Sulprostone	0.11	0.40	0.14	$EP_3 > EP_1 \gg EP_2$
Misoprostol	0.70	4.1	0.51	$EP_2 = EP_3 > EP_1$
Gemeprost	0.84*	–	1.0	$EP_3 = EP_1 > EP_2$
GR 63799X	1.5	8.0	0.24	$EP_3 > EP_1 \gg EP_2$
17-Phenyl-ω-trinor PGE_2	3.3*	15	6.3	$EP_1 > EP_3 > EP_2$
11-Deoxy PGE_1	≥4.3*	–	18	$EP_2 > EP_3 > EP_1$
Butaprost	Relaxation only	>140	>1000	$EP_2 \gg EP_1$ / EP_3

Human pulmonary artery data were obtained in the present study; *two experiments only. Human platelet cyclic AMP data are from Matthews & Jones (1993) and guinea-pig vas deferens data from Lawrence *et al.* (1992) .

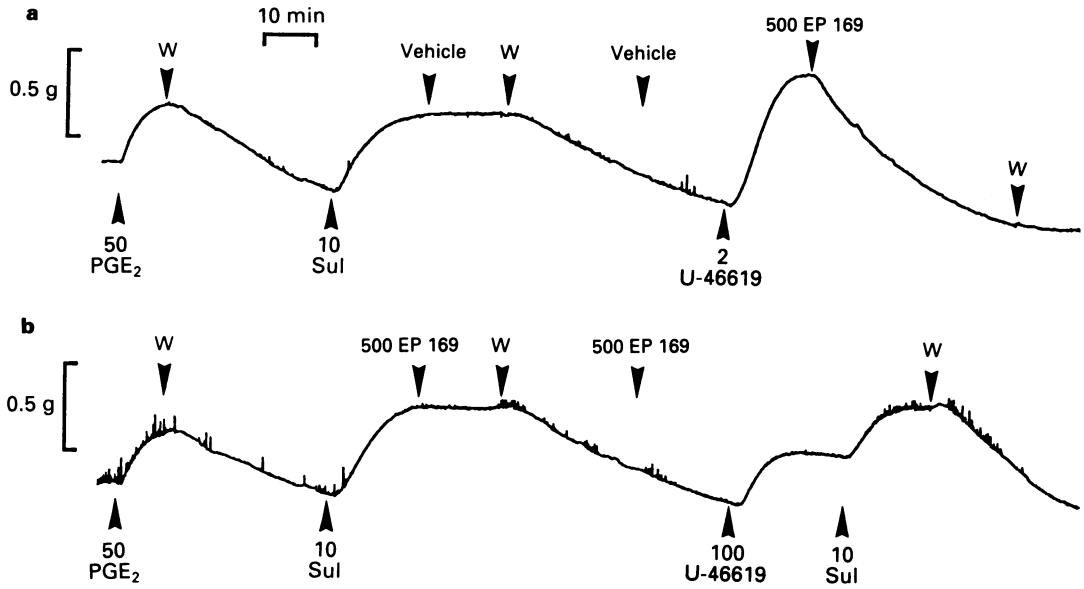

Figure 4 Experimental records showing that the TP-receptor antagonist, EP 169, blocks the contractile action of U-46619, but not that of sulprostone, on the human pulmonary artery. Records (a) and (b) are from matched preparations. Sul = sulprostone, W = wash. Concentrations are in nanomolar.

(0.05–0.15 g) at 125–375 nM. 11-Deoxy PGE_1 (30–450 nM) induced contraction, but its effects were variable (Figure 3b). After production of tone with phenylephrine, PGE_2 gave additional contractions (Figure 1b). Butaprost inhibited tone although the sensitivity of this effect was low and varied to some degree. Relaxation was not seen at 25 nM; on the most sensitive preparation, the IC_{50} value was 350 nM; on the least sensitive preparation, 375 nM butaprost induced 20% relaxation. 11-Deoxy PGE_1 (30–450 nM) had essentially no effect on preparations that were more sensitive to butaprost, whereas in butaprost-insensitive preparations additional contractions were obtained (Figure 5a).

In the other two experiments a quite different picture was seen. PGE_2 (50 nM priming dose), butaprost (125 nM) and 11-deoxy PGE_1 (30 and 150 nM) reduced the resting tone, an effect which was reversed on washing (Figure 1c,d). Phenylephrine-induced tone was also markedly inhibited by PGE_2, butaprost and 11-deoxy PGE_1 (Figures 1c,d and 5b). Butaprost was 3.1 and 4.7 times and 11-deoxy PGE_1 2.4 and 1.9 times less potent than PGE_2. The presence of 200 nM EP 169 had no effect on the response profiles. In both these experiments sensitivity to sulprostone was low, with $EC_{200\,mg}$ values ranging from 32–130 nM early in the experiments.

Discussion

Our studies demonstrate that PGE_2 can either contract or relax the circular smooth muscle of human pulmonary artery preparations. In the majority of experiments the contractile system predominated, with sulprostone being a highly potent contractile agent and butaprost showing only weak relaxant activity. However, in two experiments the balance was reversed; sulprostone was weak and butaprost showed greater relaxant potency. It must be emphasized that all our tissue came from patients with severe lung disease. Clearly there is a possibility that disease processes could have modified the contributions of the opposing receptor systems from those operative in normal lung tissue. However, there is no obvious association between the *in vitro* profile obtained and the patient data available to use.

The contractile PGE action is unlikely to be mediated by FP-receptors, since the potent and specific FP-receptor agonist, fluprostenol (Crossley, 1975; Welburn & Jones, 1978) was inactive. Although $PGF_{2\alpha}$ contracted the preparations, it achieves this by activation of TP-receptors (Norel *et al.*, 1991). Activation of TP-receptors also seemed unlikely since sulprostone has only weak TP-agonist activity on standard

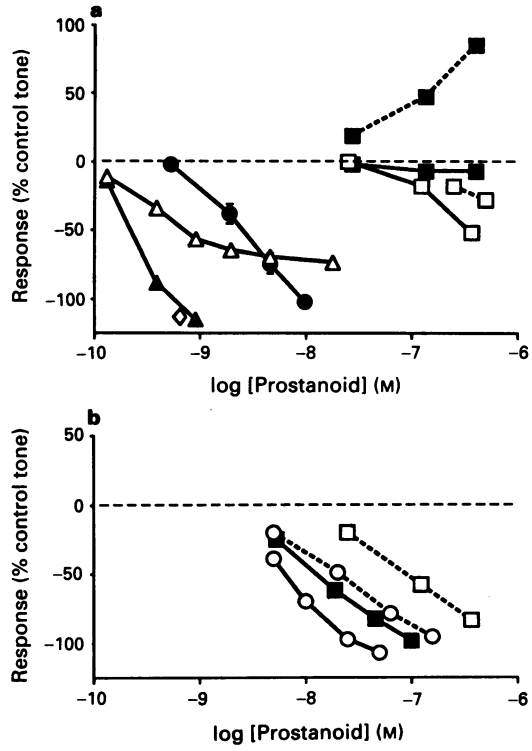

Figure 5 Relaxant effects of prostanoids against induced tone on human isolated pulmonary artery. (a) All preparations gave a contractile response to the prostaglandin E_2 (PGE_2) priming dose. Lower left: cicaprost against phenylephrine (●, $n = 4$) and TEI-9063 against phenylephrine (◇), U-46619 (▲) and KCl (△) in a single experiment. Upper right: 11-deoxy PGE_1 (■) and butaprost (□) against phenylephrine on two preparations (——, - - -) in the same experiment; EP 169 (200 nM) was present. (b) Single experiment in which the priming dose of PGE_2 relaxed basal tone. PGE_2 (○), 11-deoxy PGE_1 (■) and butaprost (□) against phenylephrine in two preparations (——, - - -); EP 169 (200 nM) was present.

TP preparations such as rat aorta (Coleman *et al.*, 1988). Two direct pieces of evidence support this view. First, two potent and specific TP-receptor antagonists did not block the contractile action of sulprostone; EP 169 has a pA_2 range of 8.3–8.8 on standard TP preparations (excluding rabbit tissues which are relatively insensitive to TP antagonists) (Tymkewycz *et al.*, 1991). GR 32191 has been examined previously for block of U-46619 on the human pulmonary artery (Lumley *et al.*, 1989); the reported pA_2 of 8.2 is in agreement with observations in this study. Secondly cross-tachyphylaxis between sulprostone and the TP-receptor agonist, U-46619, was not found. Indeed responses to KCl and phenylephrine were also well maintained throughout the experimental period, thus indicating that the preparations were not generally deteriorating. In general, the agonist actions of PGE analogues are not prone to tachyphylaxis, and this applies to inhibitory (EP_3) actions on the guinea-pig vas deferens (see later).

The most probable mechanism underlying the potent contractile actions of PGE_2 and its analogues is therefore activation of EP_1- and/or EP_3-receptors. The EP_1-receptor can be excluded since the EP_1 antagonist, AH 6809 at 5 μM, did not block the contractile action of sulprostone; given a pA_2 of 6.8 (Coleman *et al.*, 1985) to 7.35 (Lawrence *et al.*, 1992), a dose ratio of 30–110 would be expected. Support for the presence of an EP_3-receptor subtype in the pulmonary artery derives from our measurements of the agonist potencies of the eight PGE analogues examined (Table 1). In particular, sulprostone was found to be more potent than 17-phenyl-ω-trinor PGE_2. This is the situation in the guinea-pig vas deferens, the archetypal EP_3 preparation, whereas the 17-phenyl analogue is more potent on EP_1 preparations such as the guinea-pig trachea and ileum (Lawrence *et al.*, 1992). However it is necessary to emphasize that there is some discrepancy between relative agonist potencies obtained on the guinea-pig vas deferens and those obtained on another EP_3 system, namely inhibition of adenosine 3':5'-cyclic monophosphate (cyclic AMP) accumulation in human washed platelets (Matthews & Jones, 1993). The latter mechanism appears to be involved in the potentiating action of PGE analogues on platelet aggregation induced by ADP, PAF or U-46619. In particular GR 63799X (Bunce *et al.*, 1990) was relatively less potent on the human platelet compared to the vas deferens. Similarly in the present experiments, GR 63799X is somewhat less potent than sulprostone.

Of the other PGE analogues with potent contractile activity on the pulmonary artery, gemeprost (Cervagem) is used for induction of second trimester abortion and for cervical softening prior to surgical termination of first trimester pregnancy (Crowshaw, 1983; Cameron & Baird, 1984b; see Dollery, 1991). Like its close relative, 16,16-dimethyl PGE_2, it is a potent EP_3-agonist on the guinea-pig vas deferens (Table 1) and a potent EP_1-receptor agonist on the guinea-pig trachea ($EC_{50} = 3.8$ nM, 200 nM EP 169 present). Due to its unavailability at the time, gemeprost was not tested on the human platelet cyclic AMP system.

With respect to prostanoid-induced relaxation of the pulmonary artery, we have confirmed earlier reports (Hadhazy *et al.*, 1985; Haye-Legrand *et al.*, 1987) of the potent action of cicaprost, a chemically stable analogue of prostacyclin (Sturzebecher *et al.*, 1986). TEI-9063, an isocarbacyclin analogue with potent inhibitory effects on human platelets (Negishi *et al.*, 1991), was also a potent relaxant agent. Since cicaprost is the most specific of the prostacyclin-mimetics examined to date (Dong *et al.*,1986; Lawrence *et al.*, 1992), we presume these agents are acting on IP-receptors in the pulmonary artery.

On preparations where PGE_2 was contractile, butaprost, a selective EP_2 agonist (Gardiner, 1986), produced relaxation at relatively high concentrations. This effect may be due to activation of EP_2-receptors, but in view of the very high sensitivity of the artery preparations to prostacyclin mimetics, a contribution from IP-receptor activation cannot be ruled out. Stronger evidence for the presence of relaxant EP_2-receptors was obtained in just two experiments. PGE_2 inhibited phenylephrine-induced tone at concentrations of 5 nM and above, and 11-deoxy PGE_1 and butaprost were only about 2 and 4 times less potent. The variable activity of 11-deoxy PGE_1 appears to be due to its ability to activate both contractile EP_3 (Table 1) and relaxant EP_2 systems, coupled with differing sensitivities of the two systems in different preparations in the same experiment and in different experiments. In contrast, butaprost did not contract preparations which contracted well to PGE_2 and sulprostone, since it is essentially devoid of EP_3 agonist activity (Lawrence *et al.*, 1992).

There have been several reports of cardiovascular collapses associated with the use of PGE_2 for obstetric purposes (Cameron & Baird, 1984a; Kilpatrick & Thorburn, 1990). There have also been reports of myocardial infarction (Fliers *et al.*, 1991) and of several deaths associated with the use of sulprostone, usually administered in combination with the progesterone antagonist, mifepristone (Editorial, 1991). If pulmonary hypertension were a causative factor in these incidents, then the balance between EP_2 dilator and EP_3 constrictor actions in the pulmonary circulation may have been weighted towards the latter. In terms of the receptor selectivity of the prostanoid, sulprostone does not have EP_2 dilator activity. Misoprostol does however, and in combination with the progesterone antagonist, mifepristone, is being strongly recommended for termination of early pregnancy (Peyron *et al.*, 1993). In relation to the relative sensitivity of the contractile/relaxant systems in the lung vessels, it would be of great interest to measure this during pregnancy. How-

ever, from both ethical and logistical standpoints, this is unlikely to be possible.

It is also possible that sulprostone has other cardiovascular actions which could accentuate the harmful effects of its direct pulmonary vasoconstrictor action. Thus in preliminary experiments performed by us in the anaesthetized cat, sulprostone ($0.1-1\ \mu g\ kg^{-1}$) induced a sustained rise in arterial blood pressure. An increase in cardiac output secondary to systemic venoconstriction appears a likely mechanism, in view of our observation that sulprostone at concentrations of 10 nM and above contracted circular smooth muscle of the abdominal vena cava of the cat.

Finally it must be borne in mind that EP_3-receptor agonists may have two interactive and potentially dangerous actions in man, the pulmonary vasoconstrictor action reported here and the ability to potentiate platelet aggregation investigated previously by us (Matthews & Jones, 1993).

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