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Prostanoid EP_1- and TP-receptors involved in the contraction of human pulmonary veins

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1 To characterize the prostanoid receptors (TP, FP, EP_1 and/or EP_3) involved in the vasoconstriction of human pulmonary veins, isolated venous preparations were challenged with different prostanoid-receptor agonists in the absence or presence of selective antagonists.

2 The stable thromboxane A_2 mimetic, U46619, was a potent constrictor agonist on human pulmonary veins ($pEC_{50} = 8.60 \pm 0.11$ and $E_{max} = 4.61 \pm 0.46$ g; $n = 15$). The affinity values for two selective TP-antagonists (BAY u3405 and GR32191B) versus U46619 were BAY u3405: $pA_2 = 8.94 \pm 0.23$ ($n = 3$) and GR32191B: apparent $pK_B = 8.25 \pm 0.34$ ($n = 3$), respectively. These results are consistent with the involvement of TP-receptor in the U46619 induced contractions.

3 The two EP_1-/EP_3- agonists (17-phenyl-PGE_2 and sulprostone) induced contraction of human pumonary veins ($pEC_{50} = 8.56 \pm 0.18$; $E_{max} = 0.56 \pm 0.24$ g; $n = 5$ and $pEC_{50} = 7.65 \pm 0.13$; $E_{max} = 1.10 \pm 0.12$ g; $n = 14$, respectively). The potency ranking for these agonists: 17-phenyl-PGE_2 > sulprostone suggests the involvement of an EP_1-receptor rather than EP_3. In addition, the contractions induced by sulprostone, 17-phenyl-PGE_2 and the IP-/EP_1- agonist (iloprost) were blocked by the DP-/EP_1-/EP_2-receptor antagonist (AH6809) as well as by the EP_1 antagonist (SC19220).

4 $PGF_{2\alpha}$ induced small contractions which were blocked by AH6809 while fluprostenol was ineffective. These results indicate that FP-receptors are not implicated in the contraction of human pulmonary veins.

5 These data suggest that the contractions induced by prostanoids involved TP- and EP_1-receptors in human pulmonary venous smooth muscle.

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Keywords: AH6809; BAY u3405; contraction; human pulmonary veins; GR32191B; iloprost; prostaglandin; prostanoid receptors; SC19220; sulprostone; U46619

Abbreviations: E_{max}: maximal contraction; K_B values: equilibrium dissociation constant for the antagonist; L-NOARG: N^G-nitro-L-arginine; NC: not calculable; PG: prostaglandin

Introduction

Prostanoids may contract or relax smooth muscle by activating different prostanoid receptors (Coleman *et al*., 1994). Results derived from isolated tissues showed that prostanoid activation of TP-, FP-, EP_1- or EP_3-receptors produced smooth muscle contraction by increasing Ca^{2+} or reducing cyclic AMP intracellular levels (Negishi *et al*., 1995). The preferential receptor for thromboxane A_2 (TP-receptor) has been extensively described in platelet aggregation and in smooth muscle contraction (Shen & Tai, 1998). In most of the human arteries, U46619 the TP selective agonist induced contraction (Maddox *et al*., 1985; Ohlstein *et al*., 1988; Uski *et al*., 1984; Baxter *et al*., 1995; Templeton *et al*., 1991), while studies on human veins have been rarely reported. However, the TP-receptor has been described in vasoconstriction of veins in the hand, the placenta and the leg (Arner *et al*., 1991; Boura *et al*., 1986; Mais *et al*., 1985). The involvement of FP-, EP_1- or EP_3-receptors in the contraction of numerous non-human smooth muscle preparations is frequently reported. The activation of FP-receptors by prostanoids induced contraction of cat iris sphincter, bovine ciliary muscle, rabbit uterus and ewe myometrium (Woodward *et al*., 1989; Krauss *et al*., 1997; Chen *et al*., 1998; Crankshaw & Gaspar, 1995). In mammals, activation of EP_3-receptor induced contraction of smooth muscles present in ileum, colon, myometrium and corpus luteum (Botella *et al*., 1993; 1995; Crankshaw & Gaspar, 1995; Sharif *et al*., 1998). The EP_1-receptor has been classically described using selective antagonist (SC19220) against PGE_2-induced contractions in preparations derived from either the guinea-pig trachea or gastrointestinal tract (Kennedy *et al*., 1982; Coleman & Kennedy, 1985). However, few studies characterizing FP-, EP_1- or EP_3- receptors involved in the control of human vascular tone have been reported. The FP-receptor has been described in different non-vascular human smooth muscle: urinary bladder, and myometrium (Palea *et al*., 1998; Senior *et al*., 1992). Furthermore, EP_1-receptors have not been associated with any human smooth muscle contraction and there is limited data on human pulmonary artery and myometrium contractions due to EP_3-receptor activation (Qian *et al*., 1994; Senior *et al*., 1993).

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The aim of the present study was to investigate not only the TP but also the FP-, EP_1- or EP_3-receptors associated with the contractions induced by prostanoids in human pulmonary veins.

Methods

Isolated preparations

Human lung tissues were obtained from patients (21 male and four female) who had undergone surgery for lung carcinoma. The mean age was 58 ± 2 years. Pulmonary venous preparations were removed, dissected free from adjoining connective tissue and lung parenchyma, placed in Tyrode's solution (concentration mM): NaCl 139.2, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.5; pH 7.4 and maintained at 4°C. All preparations were used within 1–12 h postsurgery. Vascular preparations with intact endothelium were cut as rings (3–6 mm internal diameter, 3–5 mm in length). The rings were then set up in 10 ml organ baths containing Tyrode's solution, gassed with 95% O_2/5% CO_2 and maintained at 37°C. An optimal load (1.5 g), which ensured maximal physiological responses to the agonists, was applied to each ring.

Changes in force were recorded by isometric force displacement transducers (Narco F-60) and physiographs (Linseis). Subsequently, preparations were allowed to equilibrate for 90 min with bath fluid changes taking place every 10 min.

Experimental protocol

After the equilibration period, the venous preparations were incubated 30 min with indomethacin (1.7 μM) and 15 min with N^G-nitro-L-arginine (L-NOARG; 0.1 mM). These agents were used to avoid any physiological effect induced by the release of endogenous prostanoids and nitric oxide. The preparations were then contracted with increasing concentrations of prostanoids or selective agonists applied in a cumulative fashion. In some experiments, during the incubation period, different antagonists: BAY u3405 (TP), GR32191B (TP), AH6809 (DP/EP_1/EP_2) or SC19220 (EP_1) were added with indomethacin and L-NOARG. These antagonist were used to determine either their affinity values or to illustrate one response through activation of a single receptor subtype for the agonists which may act on different receptor subtypes. The maximal contraction of each preparation with norepinephrine (10 μM) was obtained at the end of each experimental protocol.

Data analysis

The changes in force were measured from isometric recordings in grams (g). The contractions produced with the different agonists were expressed either as grams or as per cent of the contraction induced with norepinephrine. The maximal contraction (E_{max} value) produced with an agonist and the half-maximum effective concentration value (EC_{50} value) were interpolated from the individual concentration-effect curves. The pEC_{50} values were calculated as the negative log of EC_{50} values. When the pEC_{50} values obtained in the absence and presence of antagonist were significantly different and the tentative assumption was made that the Schild equation held in our experiments, then the apparent pK_B value was calculated as the negative log of the equilibrium dissociation constant for the antagonist (K_B value). The K_B value was determined using the Schild equation: $K_B = [B]/(DR-1)$, where [B] is the concentration of the antagonist and DR (dose ratio) is the ratio of EC_{50} values of agonist in the presence and absence of antagonist. In studies on veins with the contractile agonist U46619, different concentrations of BAY u3405 were used to determine the pA_2 value according to the method of Arunlakshana & Schild (1959). For each lung sample, Schild plot analysis was performed, the slope and pA_2 value were determined by least square fitting of a regression line to the points. All results are expressed as means $\pm$ s.e.mean and were derived from different lung samples (n). Statistical analysis was performed using Student's paired or unpaired t-test and Mann-Whitney rank sum test with a confidence level of 95%.

Compounds

U46619 (9,11-dideoxy-11α,9α-methanoepoxy $PGF_{2\alpha}$), PGE_2, $PGF_{2\alpha}$, fluprostenol ($\pm$) 16-m-trifluoromethylphenoxy tetranor $PGF_{2\alpha}$) and 17-phenyl-trinor-PGE_2, were purchased from Cayman Chemical Company, Ann Arbor, MI, U.S.A. Iloprost (5-[(E)-(1S,5S,6R,7R)-7-hydroxy-6-[(E)-(3S,4RS)-3-hydroxy-4-methyl-1-octen-6-inyl]bicyclo[3.3.0]-octan-3-ylidene] pentanoic acid) and sulprostone (N-(methylsulphonyl)-9-oxo-11α,15R-dihydroxy-16-phenoxy-17,18,19,20-tetranor-prosta-5Z, 13E-dien-1-amide) were a gift from Schering AG, Berlin, Germany. AH6809 (6-isopropoxy-9-oxaxanthene-2-carboxylic acid) and GR32191B ([1R-[1α(z),2β,3β,5α]]-(+)-7-[5[[(1,1′-biphenyl)-4-yl] methoxy]-3-hydroxy-2-(1-piperidinyl) cyclopentyl]-4-heptenoic acid, hydrochloride) were a gift from Glaxo Wellcome, U.K. BAY u3405 (3(R)-3-(4-fluorophenylsulphonamido)-1,2,3,4-tetrahydro-9-carbazole propanoic acid) was a gift from Bayer, Stokes Poges, U.K. SC19220 (8-chlorodibenz [b,f][1,4] oxazepine-10(11H)-carboxy-(2-acetyl)hydrazide) was a gift from Searle Research and Development, Skokie, IL, U.S.A. Norepinephrine, L-NOARG (N^G-nitro-L-arginine) and indomethacin were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Results

The cocktail of inhibitors (indomethacin, L-NOARG) and antagonists with which the venous preparations were incubated induced a small contraction on the resting tone of these preparations (0.07 ± 0.06 g; $n = 18$). At the end of the protocols, venous preparations were contracted with norepinephrine (10 μM: 3.10 ± 0.44 g; $n = 25$).

Concentration-dependent contractions of human pulmonary venous preparations produced by U46619 are shown Figure 1a and Table 1. U46619 was a potent constrictor with a maximal effect which was 50% greater than that induced by norepinephrine (10 μM). BAY u3405 (0.03; 0.1; 0.3 μM) caused a concentration-related rightward shift of the U46619 concentration-effect curves with a pA_2 of 8.94 ± 0.23 ($n = 3$) and a Schild plot slope of 1.07 ± 0.03, which was not significantly different from unity. These values

were derived from Schild plots (Figure 1b). In additional lung samples, BAY u3405 and GR32191B (1 μM) reduced the E_{max} and/or the pEC_{50} values obtained from U46619 concentration-effect curves (Table 1). In presence of BAY u3405 (1 μM) the U46619 concentration-effect curves were difficult to restore and no plateau was reached even at the maximal concentration available for U46619 (10 μM). When possible, the apparent pK_B values for each concentration of BAY u3405 and GR32191B were calculated and these values are presented Table 1.

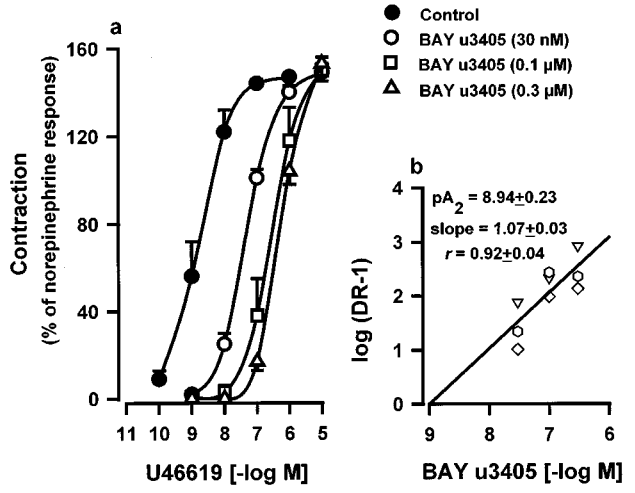

Figure 1 (a) Contraction of human isolated pulmonary veins induced by U46619. Responses were expressed as per cent of the norepinephrine (10 μM) contraction. Values are means$\pm$s.e.mean. (b) Schild-plot analysis of the antagonism by BAY u3405 of U46619-contractions in veins derived from three human lung samples (each lung sample is presented as a different symbol). Analysis was based on pEC_{50} values calculated in treated preparations which were significantly different from control values. The linear regression presented was performed using all data points. The calculated average of pA_2 values, slopes and regression coefficients performed with data derived from each lung sample are indicated.

Concentration-effect curves induced by sulprostone on human pulmonary venous preparations in the absence of antagonist were biphasic, the average curve failed to reach a plateau and was not sigmoïdal (Figure 2). The contraction obtained with the highest available concentration of sulprostone was 54$\pm$09% (n=5) of norepinephrine contraction (Figure 2). Sigmoïdal curves were obtained with sulprostone, in the presence of either BAY u3405 (1; 10 μM) or GR32191B (10 μM) and the E_{max} did not exceed 50% of the norepinephrine contraction (Figure 2 and Table 1).

In presence of BAY u3405 (1 μM), the contractions induced by sulprostone on venous preparations were inhibited by AH6809 (10; 30 or 100 μM; Figure 3a and Table 1). AH6809 (30, 100 μM) had a non-competitive behaviour on sulprostone responses, the antagonism was not reversible. Under the same conditions (presence of BAY u3405, 1 μM), SC19220 (100 μM) significantly inhibited the vasoconstrictions induced by sulprostone (Figure 3a). In presence of this latter treatment, the contraction obtained with the highest available concentration of sulprostone was 32$\pm$09% (n=3) of norepinephrine contraction, the derived estimations of pEC_{50} and apparent pK_B were ($<$6.97$\pm$0.41, $>$4.75$\pm$0.36), respectively. AH6809 (10 μM) significantly inhibited the vasoconstrictions induced by 17-phenyl-PGE_2 in the human pulmonary veins treated with BAY u3405, 1 μM, (Figure 3b and Table 1). In this latter protocol, the contraction obtained

Table 1 Effect of TP-, EP- and FP- receptor agonists or antagonists on human isolated pulmonary venous preparations

Treatment	*Concentration* (μM)	n	*Agonist* E_{max} (%)	*Agonist* pEC_{50}	*Apparent* pK_B
			U46619		
Tyrode		15	146$\pm$15	8.60$\pm$0.11	
BAY u3405	0.03	3	149$\pm$04	7.17$\pm$0.03*	8.93$\pm$0.25
BAY u3405	0.1	3	150$\pm$06	6.35$\pm$0.18*	9.24$\pm$0.14
BAY u3405	0.3	3	153$\pm$08	6.13$\pm$0.06*	8.99$\pm$0.24
BAY u3405	1	4	39$\pm$09*	NC	NC
GR32191B	1	3	133$\pm$06*	6.14$\pm$0.08*	8.25$\pm$0.34
			Sulprostone		
BAY u3405	1	14	41$\pm$04	7.65$\pm$0.13	
BAY u3405	10	6	37$\pm$05	7.29$\pm$0.05	
GR32191B	10	3	40$\pm$10	7.40$\pm$0.01	
AH6809+B	10	5	36$\pm$03	7.14$\pm$0.08*	5.52$\pm$0.31
AH6809+B	30	4	10$\pm$05*	NC	
AH6809+B	100	3	00$\pm$00*	NC	
			17-phenyl-PGE_2		
BAY u3405	1	5	17$\pm$02	8.56$\pm$0.18	
			$PGF_{2\alpha}$		
BAY u3405	1	5	22$\pm$04	7.61$\pm$0.37	
AH6809+B	10	5	01$\pm$03*	NC	
			Fluprostenol		
BAY u3405	1	5	08$\pm$03	NC	

The maximal contraction (E_{max}) produced with an agonist was expressed as per cent of the contraction induced by norepinephrine (10 μM). See methods for definitions of pEC_{50} or apparent pK_B values. Values are means$\pm$s.e.mean and (n) indicates the number of lung samples used. NC=not calculable. *Values significantly different (Student t-test or Mann-Whitney rank sum test) from appropriate control (Tyrode or BAY u3405 (1 μM)). +B indicates a co-treatment with BAY u3405 (1 μM).

with the highest available concentration of 17-phenyl-PGE_2 was 15±05% ($n=4$) of norepinephrine contraction and the derived estimations of pEC_{50} and the apparent pK_B were ($<7.66\pm0.13$, $>5.88\pm0.20$), respectively. Venous preparations derived from three human lung samples, treated with BAY u3405 (1 μM), contracted when challenged with iloprost (Figure 4) this response was abolished in the presence of AH6809 (10 μM). In the presence of BAY u3405 (1 μM), the PGE_2 concentration-effect curves were quite variable, PGE_2 either had no effect, or induced contraction or relaxation of human pulmonary venous preparations (Figure 5). These contractions induced by PGE_2 were inhibited by AH6809 (10 μM, $n=3$; data not shown).

In human pulmonary veins pre-treated with BAY u3405 (1 μM), the concentration-effect curves produced with $PGF_{2\alpha}$ were small while fluprostenol failed to contract these tissues (Table 1). The $PGF_{2\alpha}$ induced curves were abolished in the presence of BAY u3405 (1 μM) and AH6809 (10 μM; Table 1).

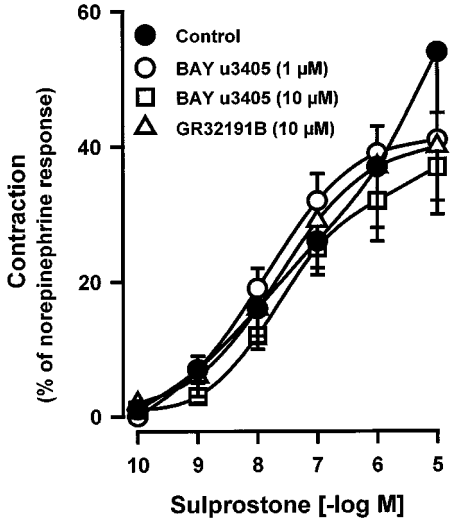

Figure 2 Effect of BAY u3405 (1; 10 μM) or GR32191B (10 μM) on the contraction of human isolated pulmonary veins induced by sulprostone. Control and treated preparations were incubated with indomethacin (1.7 μM) and L-NOARG (0.1 mM). Responses were expressed as per cent of the norepinephrine (10 μM) contraction. Values are means±s.e.mean, number of lung samples used are indicated in Table 1 and in the Results section.

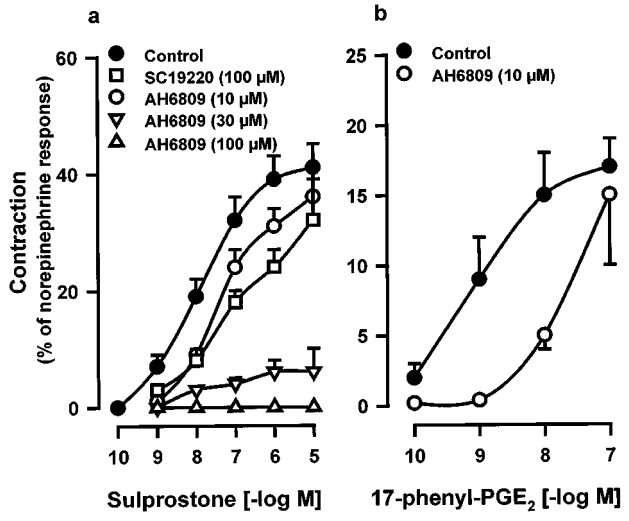

Figure 3 Effect of AH6809 (10; 30; 100 μM) or SC19220 (100 μM) on contraction of human isolated pulmonary veins induced by sulprostone (a) or 17-phenyl-PGE_2 (b). Control and treated preparations were incubated with indomethacin (1.7 μM), L-NOARG (0.1 mM) and BAY u3405 (1 μM). Responses were expressed as per cent of the norepinephrine (10 μM) contraction. Values are means±s.e.mean, number of lung samples used are indicated in Table 1 and in the Results section.

Discussion

The present report suggests the involvement of TP- and EP_1-receptors in the prostanoid induced contraction of human pulmonary venous preparations.

The contractions observed with U46619 (stable thromboxane A_2 mimetic) and their inhibition by the selective TP-antagonists (GR32191B, Lumley *et al*., 1989 and BAY u3405, McKenniff *et al*., 1991) suggest the presence of a TP-receptor in human pulmonary veins. These results have not previously been reported although the TP-receptor has been frequently described in human pulmonary arteries (Maddox *et al*., 1985; Sjoberg & Steen, 1989; Lumley *et al*., 1989; Norel *et al*., 1991; Ellis & Muller-Schweinitzer, 1991; Qian *et al*., 1994; Jino *et al*., 1996). Pharmacological studies as well as investigations using molecular biology have suggested heterogeneity among thromboxane receptors (Lumley *et al*., 1989; Tymkewycz *et al*., 1991; Furci *et al*., 1991; Pierce & Regan, 1998). Actually, the affinity values calculated in human pulmonary veins for GR32191B (8.25±0.34) and BAY u3405 (8.94±0.23) are comparable to those found in human pulmonary arteries: GR32191B (8.18–8.3; Lumley *et al*., 1989; Qian *et al*., 1994) and BAY u3405 (9.25; Norel *et al*., 1991), respectively. These data suggest that the TP-receptor present in both human pulmonary arteries and veins may be the same. In addition, the apparent pK_B values obtained with GR32191B (present study) were also in accordance with those derived from pharmacological studies performed on human umbilical

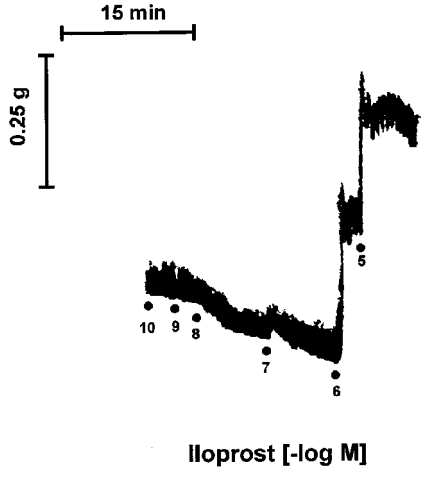

Figure 4 Representative tracing of the contraction induced by iloprost observed in isolated pulmonary veins derived from three human lung samples. Experiments were performed in presence of indomethacin (1.7 μM), L-NOARG (0.1 mM) and BAY u3405 (1 μM).

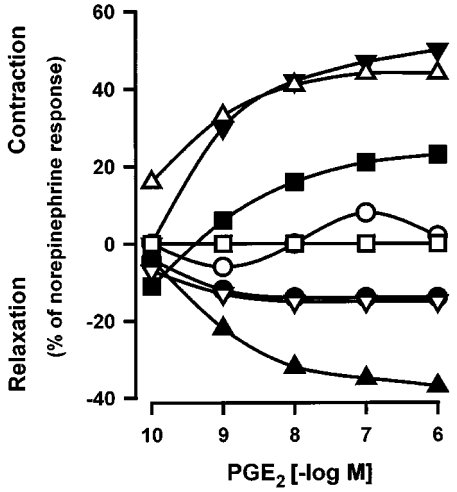

Figure 5 Variable effects induced by PGE_2 in human isolated pulmonary veins. Experiments were performed in presence of indomethacin (1.7 μM), L-NOARG (0.1 mM) and BAY u3405 (1 μM). Responses were expressed as per cent of the norepinephrine (10 μM) contraction. Each symbol indicates data obtained with one lung sample.

artery (8.04; Templeton *et al*., 1991), uterine artery (8.5; Baxter *et al*., 1995) or human platelet (8.2–8.8; Lumley *et al*., 1989).

The TP-receptor in human pulmonary veins was also activated by the high concentration (>1 μM) of sulprostone and the TP antagonists eliminated this response. Sulprostone is classically described as a selective agonist for EP_1- and EP_3- receptors (Coleman *et al*., 1987a, b; 1988; Reeves *et al*., 1988), however high concentrations (30 μM) have been reported to induce contractions of the human bronchial preparations and isolated uterine artery where TP-receptors are the only excitatory prostanoid-receptors (Coleman & Sheldrick, 1989; Baxter *et al*., 1995). The findings (present study) are in agreement with this previous observation, high concentrations of sulprostone may act on the TP-receptor and for this reason all the experiments involving this compound were carried out in the presence of BAY u3405.

In human pulmonary veins, sulprostone and 17-phenyl-PGE_2 (in presence of BAY u3405) induced smaller contractions than the response induced by U46619. The contractions of human pulmonary veins induced by sulprostone or 17-phenyl-PGE_2, suggest the involvement of EP_1- or EP_3-receptors. The sensitivities (pEC_{50} value) of the venous preparations to sulprostone and 17-phenyl-PGE_2 were comparable to those determined in standard functional assays for EP_1-receptor, namely, the contractions of guinea-pig fundus (Coleman *et al*., 1987a) and trachea (Lawrence *et al*., 1992). In the present study, the potency ranking for these agonist, 17-phenyl-PGE_2 > sulprostone (equi-effective molar ratio = 8 and 1, respectively), was similar to the one observed in the previous standard EP_1-tissues. In these later tissues or in binding studies with cloned EP_1-receptor (rat, Boie *et al*., 1997; mouse, Watabe *et al*., 1993; mouse, Kiriyama *et al*., 1997), 17-phenyl-PGE_2 was 1.5–4 fold more potent than sulprostone. A greater potency, 10 fold, was found for 17-phenyl-PGE_2 when compared with sulprostone to increase intracellular Ca^{2+} in rabbit cortical collecting duct *via* the activation of EP_1-receptor (Guan *et al*., 1998). In contrast, the rank order of potency for these agonists was reversed in studies where the effects were mediated by the activation of EP_3-receptors. Sulprostone was a more potent contractile agonist, 30–45 fold greater than 17-phenyl-PGE_2 either in human pulmonary artery (Qian *et al*., 1994) or when inhibiting the twitch contraction of guinea-pig vas deferens (Lawrence *et al*., 1992). These results (present study) support the presence of an EP_1-receptor associated with contraction in human pulmonary veins rather than an EP_3-receptor. In addition, iloprost, a selective agonist for IP and EP_1-receptors (Schrör *et al*., 1981; Dong & Jones, 1982; Dong *et al*., 1986) induced contraction of these preparations even though IP-receptors responsible for relaxation are present (Walch *et al*., 1999).

The effects of either the DP-/EP_1-/EP_2- receptor antagonist (AH6809; Coleman *et al*., 1985; Keery & Lumley, 1988; Woodward *et al*., 1995) or the EP_1-receptor antagonist (SC19220; Sanner, 1969; Kennedy *et al*., 1982; Coleman *et al*., 1987a, b) against the concentration-effect curves (present study) produced by both sulprostone and 17-phenyl-PGE_2 also suggest the involvement of the EP_1-receptor rather than EP_3. However, the affinity values calculated or estimated for AH6809 in preparations derived from human lung (apparent pK_B of 5.52 and >5.88) are lower than those obtained in similar physiological experiments performed in guinea-pig or dog EP_1-preparations. Lawrence *et al*. (1992) using sulprostone or 17-phenyl-PGE_2 as the contractile agonist, found the AH6809 affinity values ranking from 6.1–7.35 in either guinea-pig ileum or trachea. In other studies using PGE_2 with the EP_1-preparations (guinea-pig fundus, ileum and dog fundus), the affinity values for AH6809 ranked from 6.6–7.4 (Eglen & Whiting 1988; Coleman *et al*., 1985). Similarly, the estimated affinity value found for SC19220 in the present study (apparent pK_B > 4.75) was lower than the pA_2 of 5.6 calculated in experiments performed with the previous EP_1-preparations (Coleman *et al*., 1985; Coleman & Kennedy, 1985). The reason for this discrepancy remains to be established. However, when EP_1-receptors were assessed in either physiological or binding studies, the affinity values for AH6809 were always one order of magnitude greater than those for SC19220 (present study; Coleman *et al*., 1985; Boie *et al*., 1997; Funk *et al*., 1993). Since high concentrations of AH6809 (5–100 μM) or SC19220 (100–300 μM) did not block the EP_3 mediated effects in many of the EP_3 biological models (Table 2), the inhibitory effect of these antagonists in human pulmonary vein would suggest that the EP_3-receptor is not involved in contractions.

The variable effects induced by PGE_2 in the human pulmonary venous preparations in presence of the TP-antagonist may be explained by two opposing effects of this prostaglandin in these preparations. A contraction *via* the EP_1-receptor and a relaxation *via* another EP-receptor subtype as has been suggested by Walch *et al*. (1999). A similar paradoxical effect was observed in guinea-pig trachea as well as in human bronchial preparations where PGE_2 may act on EP_1- and/or TP- receptors to induce contraction while the activation of the EP_2-receptor provokes the relaxation (Gardiner, 1975; Coleman & Kennedy, 1980; McKenniff *et al*., 1988).

The results obtained in this report with the EP- agonists or antagonists suggest a role for EP_1- and not EP_3- receptor in

Table 2 EP_3-receptor biological models: Absence of antagonism of AH6809 and SC19220

Biological effect mediated via EP_3-receptor	*Tissue receiving agonist/antagonist*	*Inhibition*	*Reference*
Presynaptique inhibition of norepinephrine release	Guinea-pig vas deferens	no	Coleman *et al*., 1987b
	Rat vena cava	no	Molderings *et al*., 1992
	Mouse brain cortex	no	Exner & Schlicker, 1995
Inhibition of gastric secretion	Rat cerebral ventricle	no	Yokotani *et al*., 1996
	Rat gastric mucosa	no	Reeves *et al*., 1988
Smooth muscle contraction	Chick ileum	no	Coleman *et al*., 1987a,b
	Pig ileum	no	Botella *et al*., 1993
	Human pulmonary artery	no	Qian *et al*., 1994

The EP_3-receptor was activated by either PGE_2, enprostil, sulprostone or 17-phenyl-PGE_2.

the contraction of human pulmonary vein. The involvement of EP_1- or EP_3- receptors in the control of vascular tone has been principally investigated in the ocular vascular bed. The EP- agonists decrease the intraocular pressure in various animal models of glaucoma (Woodward *et al*., 1993; 1994; Bhattacherjee *et al*., 1999; Waterbury *et al*., 1990) and contract the pig retinal vessels (Abran *et al*., 1994). However, the EP_3-receptor is involved in vasoconstriction of guinea-pig aorta (Jones *et al*., 1998), rat renal afferent arteriole (Tang *et al*., 2000) and human pulmonary artery (Qian *et al*., 1994). Arner & Högestatt (1991) showed that iloprost contracted the human hand vein. One could associate this response with activation of an EP_1-receptor. The data (present study) demonstrated a similar response to that of the hand veins whereas human arterial preparations did not exhibit a contractile response to this agonist (Arner & Högestatt, 1991; Qian *et al*., 1994). Therefore, the presence of EP_1-receptors may be found only in human venous preparations.

The low potency of $PGF_{2\alpha}$ or fluprostenol, the selective agonist for FP-receptor, suggests that the FP-receptor is not involved in the contraction of human pulmonary veins. In addition, the small contractions induced by $PGF_{2\alpha}$ were inhibited by AH6809 suggesting that $PGF_{2\alpha}$ activates an EP_1-rather than FP- receptor.

In summary, the findings in the present study are consistent with the presence of TP- and EP_1- receptors mediating constriction of human pulmonary veins. These findings may be relevant to the pulmonary circulation. Sulprostone is used in obstetrics and gynaecology and one of the clinical side effects observed with this compound is pulmonary oedema (Stock *et al*., 1995; Levy *et al*., 1994; Puura *et al*., 1995). The present study and the work of Qian *et al*. (1994) suggest that this side effect may involve vasospasm of the whole pulmonary vasculature. Such EP-agonist may activate at the same time TP-, EP_3- receptors in the arteries and TP-, EP_1- receptors in the veins.

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