Prostanoid receptors involved in the relaxation of human pulmonary vessels

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1 To characterize the prostanoid receptors on human pulmonary smooth muscle involved in vasodilatations, isolated arteries and veins were contracted with norepinephrine (10 μ M) and vessels were subsequently challenged with different prostanoid-receptor agonists in the absence or presence of selective antagonists.

2 Prostaglandin D₂ (PGD₂) and the selective DP-receptor agonist, BW245C, induced relaxations in the contracted human pulmonary venous preparations. The pD₂ values were: 6.88 ± 0.11 ($n=17$) and 7.31 ± 0.12 ($n=5$), respectively. The relaxant responses induced by PGD₂ were reduced by the selective DP-receptor antagonist, BWA868C, and the estimated pA₂ value was 7.84 ± 0.16 ($n=4$). PGD₂ and BW245C did not relax contracted human pulmonary arteries.

3 The selective IP-receptor agonists, iloprost and cicaprost, both induced relaxations in the contracted human vascular preparations. The pD₂ values for iloprost were: 7.84 ± 0.08 ($n=6$) and 8.25 ± 0.06 ($n=4$) and for cicaprost: 8.06 ± 0.12 ($n=5$) and 8.11 ± 0.09 ($n=5$) in arteries and veins respectively.

4 Prostaglandin E₂ (PGE₂) and the EP₂/EP₃-receptor agonist, misoprostol, partially relaxed the contracted venous preparations and the pD₂ values were: 8.10 ± 0.15 ($n=15$) and 6.24 ± 0.33 ($n=3$), respectively. These relaxations suggest the presence of an EP receptor in the human pulmonary veins. The contracted human pulmonary arteries did not relax when challenged with PGE₂.

5 In human pulmonary venous preparations, the PGE₂-induced relaxations were neither modified by treatment with TP/EP₄-receptor antagonist, AH23848B (10 and 30 μ M, $n=6$), nor by the DP/EP₁/EP₂-receptor antagonist, AH6809 (3 μ M, $n=6$).

6 These data suggest that the relaxation induced by prostanoids involved DP-, IP-receptors and to a lesser extent an EP-receptor on human pulmonary venous smooth muscle. In contrast, only the IP-receptor is involved in the prostanoid induced relaxations on human pulmonary arterial smooth muscle.

Keywords: Human pulmonary vessels; relaxation; prostanoid receptors; prostaglandin; cicaprost; iloprost; AH6809; AH23848B; BW245C; BWA868C

Abbreviations: E_{max}, maximal relaxation; K_B value, 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.*, 1994b). Results derived from isolated tissue show that the activation of TP-, FP-, EP₁- or EP₃-receptor subtypes produce smooth muscle contraction by increasing Ca²⁺ or reducing cyclic AMP intracellular levels (Negishi *et al.*, 1995). TP- and EP₃-receptors have already been implicated in the vasoconstriction of human pulmonary vessels (Maddox *et al.*, 1985; Lumley *et al.*, 1989; Norel *et al.*, 1991; Quian *et al.*, 1994). When prostanoids activate the EP₂-, EP₄-, DP- or IP-receptor subtypes, present on smooth muscle, they induce relaxation by elevating intracellular cyclic AMP levels (Negishi *et al.*, 1995). Receptors present on the endothelial layer have only a small effect on the vasorelaxant response induced by the prostanoids. In fact, the relaxant responses provoked by these mediators were slightly or not modified after removal of endothelium in human isolated hand veins or pulmonary arteries (Arner *et al.*, 1994; Jones *et al.*, 1997).

In whole human lung samples, using the techniques of molecular biology, the prostanoid receptors associated with smooth muscle relaxation have been demonstrated, namely, the EP₂- (Regan *et al.*, 1994), EP₄- (An *et al.*, 1993; Bastien *et al.*, 1994) and IP-receptor (Nakagawa *et al.*, 1994; Boie *et al.*, 1994). However, the DP-receptor (Boie *et al.*, 1995) has not been detected. In human pulmonary vessels and specifically in pulmonary veins, the prostanoid receptors implicated in smooth muscle relaxation have not been determined. The aim of the present study was to characterize the prostanoid receptors present on human pulmonary vascular smooth muscle which are associated with vasodilatation.

Methods

Isolated preparations

Human lung tissues were obtained from patients (43 male and 3 female) who had undergone surgery for lung carcinoma. The mean age was 62 ± 1 years. Pulmonary arterial and venous

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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, CaCl₂ 1.8, MgCl₂ 0.49, NaHCO₃ 11.9, NaH₂PO₄ 0.4 and glucose 5.5; pH 7.4 and maintained at 4°C. All tissues were used within 1–12 h postsurgery. Vascular preparations 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% O₂/5% CO₂ and maintained at 37°C. An optimal load (1.5 g), which ensured maximal physiological responses to the agonists, was applied to each ring.

Sprague-Dawley rat tracheal preparations were used to confirm the efficacy of the antagonist AH23848B. These tissues were set up as rings under an initial load of 2 g.

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 vascular preparations were incubated 30 min with BAY u3405 (1 µM), 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 activation of the TP-receptor or by the release of endogenous prostanoids and nitric oxide. The preparations were then contracted with norepinephrine (10 µM), when the contraction reached a plateau, increasing concentrations of prostanoids or selective agonists were applied in a cumulative fashion. In some experiments different antagonists: AH23848B (10 or 30 µM), AH6809 (3 µM) or BWA868C (0.1–30 µM) were added to the tissue bath for 30 min before the norepinephrine-induced contraction to determine their affinity values. The maximal relaxation of each preparation with papaverine (0.1 mM) was obtained at the end of the experimental protocols.

In experiments using rat tissues, subsequent to the equilibration period, four rat tracheal rings were incubated (30 min) with BAY u3405 (1 µM), indomethacin (1.7 µM), atropine (1 µM) and (last 15 min) with L-NOARG (0.1 mM). Concomitantly, two of these rings were also incubated (30 min) with AH23848B (30 µM). Preparations were then contracted with carbachol (10 µM), when a plateau was reached, cumulative concentration-effect curves were induced with PGE₂.

Data analysis

The changes in force were measured from isometric recordings and expressed in grams (g). The relaxations produced with the different agonists were expressed as per cent of the relaxation induced with papaverine. The maximal relaxation ($E_{\max}$ value) produced with the highest concentration of an agonist and the half-maximum effective concentration value (EC_{50} value) were interpolated from the individual concentration-effect curves. The pD_2 values were calculated as the negative log of EC_{50} values. When the pD_2 values obtained in the presence and absence of antagonist were significantly different, the equilibrium dissociation constant for the antagonist (K_B value) was calculated. The following equation was used: $K_B = [B]/(DR - 1)$, where $[B]$ is the concentration of the antagonist and DR (dose ratio) is the ratio of EC_{50} of agonist in the presence and absence of antagonist. The pK_B values were calculated as the negative log of K_B values. In the relaxation study with

PGD₂ on the veins, different concentrations of BWA868C 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 was 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 ANOVA with a confidence level of 95%.

Compounds

PGE₂, PGE₁, PGD₂ and misoprostol (($\pm$)-11, 16-dihydroxy-16-methyl-9-oxoprost-13-en-1-oic acid methyl ester) 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-ynyl]bicyclo[3.3.0]octan-3-ylidene]pentanoic acid) and cicaprost ([2-[hexahydro-5-hydroxy-4-(3-hydroxy-4-methyl-1,6-nonadynyl)-2-(1H)-pentalenylidene]ethoxy] acetic acid) were a gift from Schering AG, Berlin, Germany. AH6809 (6-isopropoxy-9-oxaxanthene-2-carboxylic acid) and AH23848B ([1 α (z),2 β ,5 α)-(±)-7-[5-[[1,1'-biphenyl]-4-yl]methoxy]-2-(4-morpholinyl)-3-oxo-cyclopentyl]-4-heptenoic acid) were a gift from Glaxo Wellcome, U.K. BAY u3405 (3(R)-3-(4-fluorophenylsulphonamido)-1,2,3,4-tetrahydro-9-carbazole propanoic acid) and butaprost were a gift from Bayer, Stokes Poges, U.K. BW245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl) hydantoin) and BWA868C (3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino) hydantoin) were a gift from Wellcome Research Laboratories, Beckenham, U.K. Norepinephrine, atropine sulphate, carbamylcholine chloride (carbachol), L-NOARG (N^G-nitro-L-arginine) and indomethacin were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Papaverine was obtained from Meram Laboratories (77020 Melun, France).

Results

Arterial and venous preparations were obtained from 24 and 40 different human lungs, respectively. The cocktail of inhibitors and antagonists (indomethacin, L-NOARG and BAY u3405) with which the vascular preparations were first incubated have a slight contractile effect on the basal tone of these preparations (artery: 0.39 ± 0.09 g and vein: 0.11 ± 0.04 g). Arterial and venous preparations were contracted with norepinephrine (10 µM: 1.7 ± 0.17 g and 2.21 ± 0.15 g, respectively). At the end of the protocols, preparations relaxed with papaverine (0.1 mM: 2.05 ± 0.18 g and 2.59 ± 0.18 g, respectively).

Concentration-dependent relaxations of human pulmonary venous preparations produced by PGD₂ and BW245C are shown in Figure 1a and Table 1. BWA868C (0.1–30 µM) caused a concentration-related rightward shift of the PGD₂ concentration-effect curves. A mean pA_2 value of 7.84 ± 0.16 ($n = 4$) and a mean slope of 0.67 ± 0.07 , which was significantly less than unity, were derived from Schild Plots (Figure 2). In additional lung samples pK_B values were calculated for BWA868C against PGD₂ or BW245C and are presented in Table 1. PGE₁ and the stable PGI₂ analogues (iloprost and cicaprost) produced concentration-dependent relaxations (Figure 3a and Table 2) in human pulmonary venous preparations. PGE₂ and misoprostol partially reversed the norepinephrine-induced contraction in the human pulmonary veins (Figure 4a and Table 1). The PGE₂-induced relaxations were not modified

Figure 1 Relaxation of human isolated pulmonary veins (a) and arteries (b) induced by PGD_2 or BW245C. Responses were expressed as per cent of the papaverine (0.1 mM) relaxation. Values are means $\pm$ s.e.mean and number of lung samples used are indicated in Table 1 and 3.

Table 1 Relaxation of human isolated pulmonary venous preparations: effect of DP- and EP-receptor agonists or antagonists

Treatment	Concentration (μM)	n	E_{max} (%)	Agonist	pD_2 value	pK_B value
Tyrode BWA868C	0.1 0.3 1 3	18	65 $\pm$ 05	PGD_2	6.89 $\pm$ 0.10	
		6	47 $\pm$ 11		6.10 $\pm$ 0.17*	7.23 $\pm$ 0.25
		5	51 $\pm$ 09		5.76 $\pm$ 0.27*	7.54 $\pm$ 0.14
		3	22 $\pm$ 03		5.33 $\pm$ 0.47*	7.37 $\pm$ 0.32
		4	42 $\pm$ 18		5.38 $\pm$ 0.30*	7.11 $\pm$ 0.16
Tyrode BWA868C	1	5	58 $\pm$ 07	BW245C	7.31 $\pm$ 0.12	
		3	69 $\pm$ 23		6.16 $\pm$ 0.06*	7.18 $\pm$ 0.12
Tyrode		3	31 $\pm$ 09	Misoprostol	6.24 $\pm$ 0.33	
Tyrode AH6809 AH23848B AH23848B	3 10 30	15	43 $\pm$ 04	PGE_2	8.10 $\pm$ 0.15	
		6	38 $\pm$ 08		8.24 $\pm$ 0.39	NC
		6	39 $\pm$ 08		7.38 $\pm$ 0.24	NC
		6	51 $\pm$ 13		7.46 $\pm$ 0.33	NC

The maximal responses (E_{max}) were expressed as per cent of the relaxation induced by papaverine (0.1 mM). Values are means $\pm$ s.e.mean, (n) indicates the number of lung samples used. NC: not calculable. *Values significantly different when results from control (Tyrode) and treated tissues were compared by ANOVA.

in the presence of AH6809 (3 μM ; Figure 4a and Table 1) or AH23848B (10 or 30 μM ; Table 1). In paired tissues, comparison of pD_2 values obtained in presence of AH23848B were not statistically different from the control values (pD_2 values: 7.87 ± 0.17) obtained in venous preparations derived from the same human lung sample. However, this antagonist at 30 μM inhibited the relaxation curves induced by PGE_2 in the rat tracheal preparations (data not shown). Butaprost, a PGE_1 analogue, provoked concentration-dependent relaxations of human pulmonary venous preparations (pD_2 value: 5.00 ± 0.11 and E_{max} value: $62 \pm 15\%$ of papaverine response, $n = 4$; Figure 5a). However, AH6809 (3 μM) did not modify the butaprost-induced vasodilatations in this tissue (pD_2 value: $4.60 - 5.22$ and E_{max} value: $38 - 89\%$ of papaverine response,

$n = 2$; Figure 5a). BWA868C, AH6809 and AH23848B at the concentrations used had no effect on the basal tone of the human pulmonary veins.

The norepinephrine induced contractions in the human pulmonary arteries were reversed by PGE_1 , iloprost and cicaprost (Figure 3b and Table 2). Butaprost produced also concentrations-dependent relaxations in this tissue (pD_2 value: 4.40 ± 0.07 and E_{max} value: $59 \pm 17\%$ of papaverine response; $n = 3$; Figure 5b). In addition, the butaprost-induced responses were not altered when arterial preparations were pre-treated with AH6809 (3 μM ; pD_2 value: 4.57 ± 0.17 and E_{max} value: $37 \pm 15\%$ of papaverine response $n = 4$; Figure 5b). AH6809 (3 μM) did not modify the basal tone of the human pulmonary arteries. PGE_2 , PGD_2 and BW245C were without effect on the

Figure 2 Schild-plot analysis of the antagonism by BWA868C of relaxant concentration-effect curves to PGD_2 in veins derived from four human lung samples. Analysis was based on pD_2 values calculated in treated preparations which were significantly different from control values. The linear regression presented was performed by use of all data points. However, regressions ($r=0.95 \pm 0.02$) were also performed through data derived from each lung sample. The calculated average of pA_2 values and slopes are indicated in Results section.

Table 2 Relaxant effects of IP-receptor agonists on human isolated pulmonary vascular preparations

Agonist	n	Artery		n	Vein	
		$E_{\max}$ (%)	pD_2 value		$E_{\max}$ (%)	pD_2 value
Cicaprost	5	83 ± 02	8.06 ± 0.12	5	71 ± 07	8.11 ± 0.09
Iloprost	6	91 ± 03	7.84 ± 0.08	4	91 ± 03	8.25 ± 0.06
PGE_1	4	78 ± 04	6.34 ± 0.20	4	62 ± 14	7.86 ± 0.31

The maximal responses ($E_{\max}$) were expressed as per cent of the relaxation induced by papaverine (0.1 mM). Values are means $\pm$ s.e.mean and (n) indicates the number of lung samples used. The relaxation induced by PGE_1 or iloprost were performed in presence of AH6809 ($3 \mu\text{M}$).

norepinephrine-induced arterial contractions (Figures 1b, 4b and Table 3).

Discussion

IP-receptors appear to be the only prostanoid receptors implicated in the vasodilatation of the human pulmonary arteries. In contrast, the present report suggest the involvement of DP-, IP- and to a lesser extent EP-receptors in the relaxation of human pulmonary venous preparations. The subtype(s) of these latter receptors remain uncharacterized since the responses induced by PGE_2 were not modified by AH6809 and AH23848B.

The relaxations observed with iloprost and cicaprost, two IP-receptor selective agonists (Schrör *et al.*, 1981; Stürzebecher *et al.*, 1985), indicate the presence of IP-receptor in human pulmonary arterial preparations. These results are in agreement with the data previously reported by Haye-Legrand *et al.* (1987). In addition, results (present report) suggest that PGE_1

Figure 3 Relaxation of human isolated pulmonary veins (a) and arteries (b) induced by cicaprost or iloprost. Responses were expressed as per cent of the papaverine (0.1 mM) relaxation. Values are means $\pm$ s.e.mean and number of lung samples used are indicated in Table 2.

Figure 4 Relaxations of human isolated pulmonary veins (a) and arteries (b) induced by PGE₂ or misoprostol. Some venous preparations were treated 30 min with AH6809 (3 μ M). Responses were expressed as per cent of the papaverine (0.1 mM) relaxation. Values are means $\pm$ s.e.mean and number of lung samples used are indicated in Table 1 and 3.

Figure 5 Relaxation of human isolated pulmonary veins (a) and arteries (b) produced by butaprost. Some preparations were treated 30 min with AH6809 (3 μ M). Responses were expressed as per cent of the papaverine (0.1 mM) relaxation. Values are means $\pm$ s.e.mean and number of lung samples used are indicated in text.

a preferential agonist for IP-receptors in both binding and physiological studies (Blair & McDermot, 1981; Corsini *et al.*, 1987; Adie *et al.*, 1992) relaxed human pulmonary arterial preparations. IP-receptors appear to be the only prostanoid

receptors involved in the prostanoid induced relaxations of human pulmonary arteries since EP- (except butaprost, see below) and DP-receptor agonists did not provoke vasodilatation.

Table 3 Relaxation of human isolated pulmonary arterial preparations: effect of EP- and DP-receptor agonists

Agonist	n	E_{max} (%)	pD_2 value
PGD ₂	5	04 ± 04	NC
BW245C	6	06 ± 06	NC
PGE ₂	5	00 ± 00	NC
Misoprostol	1	05	NC

The maximal responses (E_{max}) were expressed as per cent of the relaxation induced by paraverine (0.1 mM). Values are means ± s.e.mean and (*n*) indicates the number of lung samples used, NC, not calculable.

Butaprost, a PGE₁ analogue, was shown to be a selective agonist for EP₂-receptors (Gardiner, 1986). More recently, Matthews & Jones (1993) suggested that this compound may interact with IP-receptors in human platelets. These latter results were confirmed (present report) since butaprost but not PGE₂ or misoprostol provoked concentration-dependent relaxations in human pulmonary arteries. AH6809 was classically described as a DP/EP₁-antagonist (Coleman *et al.*, 1985; Eglen & Whiting, 1988; Keery & Lumley, 1988), but Woodward *et al.* (1995) have recently suggested that this compound has an antagonistic effect on a recombinant human EP₂-receptor. This latter finding was confirmed in physiological studies involving isolated tissues: human myometrium (Brown *et al.*, 1996) and human bronchial preparations (Norel *et al.*, 1999). The butaprost-induced relaxations in human pulmonary arterial preparations were resistant to treatment with AH6809, suggesting that EP₂-receptors are not implicated in this response.

The data obtained with PGD₂ and BW245C, two DP-receptor selective agonists, also suggested the presence of DP-receptors in the human pulmonary venous preparations. Narumiya & Toda (1985) as well as Eglen & Whiting (1989) showed that BW245C does not activate the EP₂-receptor in the guinea-pig trachea. In addition, Town *et al.* (1983) and Whittle *et al.* (1983) demonstrated that this agonist does not stimulate the IP-receptor present on the rat platelets. These results together with data (present report) suggest that relaxations induced by BW245C in the human pulmonary venous preparations may not be attributed to activation of the IP- or EP₂-receptors. Furthermore, in the human pulmonary venous preparations, the relaxations induced by PGD₂ were significantly inhibited in presence of the selective DP-receptor antagonist BWA868C (Giles *et al.*, 1989). This effect of BWA868C was not associated with an inhibitory effect on EP₂- or IP-receptors since Giles *et al.* (1989), Chen & Woodward (1992) and Bhattacharjee *et al.* (1993) have demonstrated that BWA868C does not block these receptors. These results suggest that prostanoid relaxation of pulmonary veins is *via* a heterogeneous group of receptors and is in contrast with observations in pulmonary arteries.

In human pulmonary veins, cicaprost and iloprost induced relaxations suggesting the presence of IP-receptors in this tissue. While the activation of the IP- or DP-receptors by their respective agonists produced a complete reversal of the norepinephrine contraction, PGE₂ and misoprostol, a selective agonist for the EP₂- and EP₃-receptors (Coleman *et al.*, 1988; Reeves *et al.*, 1988; Lydford & McKechnie, 1994), induced only a partial relaxation. In the human pulmonary artery, PGE₂ and misoprostol did not produce any relaxation suggesting that these agonists are ineffective on the IP-receptor. The inhibition of guinea-pig platelet aggregation is mediated by IP- and DP-receptors, Eglen & Whiting (1988) demonstrated that PGE₂ was ineffective in blocking this aggregation while misoprostol produced only a slight inhibition (pD_2 value = 4.71). These

results together with data (present report) suggest that the partial relaxation induced by PGE₂ or misoprostol in the human pulmonary venous preparations could not be attributed to the activation of IP- or DP-receptors. These reasons and the marked sensitivity of the venous preparations to misoprostol and PGE₂ suggest the presence of an EP-receptor.

EP₂-receptors do not seem to be involved in the human pulmonary venous relaxations as suggested by the two following observations. First, AH6809 did not modify the PGE₂- or the butaprost-induced responses; indicating that butaprost activated IP-receptors in venous preparations as in human pulmonary arteries. Actually, the sensitivity of human pulmonary veins (pD_2 value: 5) to this agonist was closer to the sensitivity of human pulmonary arteries (pD_2 value: 4.40) than to those of guinea-pig and cat trachea in which EP₂-receptors are present (pD_2 values: 6.30–6.35, respectively; Gardiner, 1986). Secondly, misoprostol was 70 fold less potent than PGE₂ to induce relaxation in venous preparations. This ratio is not in agreement with the activation of an EP₂-receptor since Wise & Jones (1994) have shown that misoprostol is 10 fold less potent than PGE₂ to induce inhibition of intracellular free calcium in rat neutrophils (IP- and EP₂-receptors).

Results (present report) were contradictory concerning the involvement of EP₄-receptors in human pulmonary venous relaxations. The presence of EP₄-receptors may be suggested by data obtained with the antagonist BWA868C. In fact, the pK_B and the pA_2 values (7.18–7.84) determined for BWA868C in human venous preparations were lower than the predicted values in preparations where this antagonist acts only on DP-receptor (pA_2 value = 9.26 in human washed platelets; Giles *et al.*, 1989). Furthermore, the mean slope value derived from Schild-plot analysis of the antagonism by BWA868C was significantly less than unity, suggesting that this antagonist interacts with a heterogeneous population of receptors. A similar *in vitro* result has been described in rabbit saphenous veins by Lydford *et al.* (1996), where BWA868C acts on DP- and EP₄-receptors. However, the presence of EP₄-receptors was not confirmed by results obtained with AH23848B, the TP/EP₄-antagonist (Coleman *et al.*, 1994a), which failed to inhibit the relaxation induced by PGE₂ in the human venous preparations. The efficacy of this antagonist was confirmed using rat isolated trachea which is a standard model for preparations containing EP₄-receptors (Lydford & McKechnie, 1994).

Finally, the EP-receptor subtype(s) implicated in the prostanoid induced relaxation of human pulmonary veins could not be characterized in the present study. Similarly, data obtained by Milne *et al.* (1995) in rabbit jugular veins did not differentiate between EP₄- and EP₂-receptors. In this latter study, while data derived from experiments using AH6809 and two EP₂-receptor agonists (AH13205 and butaprost) suggested the presence of EP₄-receptors, this was not confirmed when AH23848B was used as the antagonist. Similar observations were also reported by Meja *et al.* (1997) in human blood monocytes where AH6809 and AH23848B failed to antagonize the inhibitory response induced by PGE₂.

In conclusion, the DP- and IP-receptors are the dominant prostanoid receptors implicated in the relaxation produced by prostaglandins in human pulmonary venous smooth muscle and there is a small relaxant effect due to stimulation of EP-receptors. In the human pulmonary arterial smooth muscle only the IP-receptor is responsible for the prostanoid induced relaxations.

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