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Prostanoid receptors involved in the relaxation of human bronchial preparations

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1 Iloprost and cicaprost (IP-receptor agonists) induced relaxations in the histamine- (50 μM) contracted human bronchial preparations (pD_2 values, 6.63 ± 0.12 and 6.86 ± 0.08; E_{max} values, 90 ± 04 and 65 ± 08% of the papaverine response for iloprost ($n=6$) and cicaprost ($n=3$), respectively).

2 Prostaglandin E_2 (PGE_2) and misoprostol (EP-receptor agonist) relaxed the histamine-contracted human bronchial preparations (pD_2 values, 7.13 ± 0.07 and 6.33 ± 0.28; E_{max} values, 67 ± 04 and 57 ± 08% of the papaverine response for PGE_2 ($n=14$) and misoprostol ($n=4$), respectively). In addition, both relaxations were inhibited by AH6809 ($DP/EP_1/EP_2$-receptor antagonist; 3 μM; $n=5-6$).

3 The PGE_2-induced relaxations of human bronchial preparations were not modified by treatment with AH23848B (TP/EP_4-receptor antagonist; 30 μM; $n=4$).

4 The contracted human bronchial preparations were significantly relaxed by prostaglandin D_2 (PGD_2) or by BW245C a DP-receptor agonist. However, these responses did not exceed 40% of the relaxation induced by papaverine. In addition, the relaxations induced by PGD_2 were significantly inhibited by treatment with a DP-receptor antagonist BWA868C (0.1 μM; $n=3$).

5 These data suggest that the relaxation of human isolated bronchial preparations induced by prostanoids involved IP-, EP_2- and to a lesser extent DP-receptors but not EP_4-receptor.

Keywords: Human bronchial preparations; relaxation; prostanoid receptors; prostaglandin; misoprostol; cicaprost; AH6809; BW245C; BWA868C; AH23848B

Introduction

In asthmatic patients, pretreatment with oral prostaglandin E_1 (PGE_1) has been shown to prevent the bronchoconstriction to both inhaled histamine and methacholine (Manning *et al*., 1989). In addition, PGE_1 or prostaglandin E_2 (PGE_2) may attenuate allergen-induced early and late asthmatic response (Pavord *et al*., 1993; Pasargiklian *et al*., 1976). These clinical results suggest a role for the EP-receptors in the relaxation of the human airway smooth muscle tone. Most of the pharmacological studies performed in airways derived from animals have demonstrated that the EP-receptors are involved in the prostanoid-induced relaxations. The EP_2-receptor has been characterized in the cat trachea (Gardiner & Collier, 1980), while the EP_4-receptor has been detected in the rat trachea (Lydford & McKechnie, 1994). Prostacyclin (PGI_2) analogues are ineffective as airway muscle relaxants on isolated trachea derived from cat, guinea-pig (Dong *et al*., 1986) and rat (Lydford & McKechnie, 1994), suggesting no role for the IP-receptor in the relaxation of large airways. In contrast, these PGI_2 analogues relax human bronchial preparations (Haye-Legrand *et al*., 1987). However, the effect of inhaled PGI_2 does not alter airway calibre in normal or asthmatic subjects (Hardy *et al*., 1985; Bianco *et al*., 1979). Together, the results obtained in these studies suggest that the subtypes of prostanoid-receptors involved in the relaxation of airway smooth muscle vary between species. The DP-receptor and the EP-subtypes have not been systematically investigated in human airways. The aim of the present study was to characterize the different prostanoid receptors involved in relaxation of human bronchial preparations.

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Methods

Isolated preparations

Human lung tissues were obtained from patients (26 male and 2 female) who had undergone surgery for lung carcinoma. The mean age was 65 ± 2 years. Bronchial 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 tissues were used within 1–12 h postsurgery. Bronchial 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% O_2/5% CO_2 and maintained at 37°C. An optimal load (2 g) which ensured maximal physiological responses to the agonists used 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 bronchial preparations were incubated 30 min with BAY u3405 (1 μM), atropine (1 μM), indomethacin (1.7 μM) and 15 min with L-NOARG (0.1 mM). These agents were used to avoid any physiological effects induced by the activation of TP- or muscarinic receptors and by the release of endogenous prostanoids or nitric oxide. When PGE_1 or iloprost were used as the relaxant agonist, AH6809 (3 μM) was added to the previous drug combination (30 min) to avoid any physiological effects induced by the activation of EP_1-receptors. After incubation, the preparations were contracted with histamine (50 μM), when the response reached a plateau, increasing concentrations of prostanoid receptor agonists (PGE_2, PGE_1, PGD_2, cicaprost, iloprost, BW245C or misoprostol) were applied in a cumulative fashion. The maximal relaxation was obtained for each preparation with papaverine (0.1 mM) at the end of the experiment.

The same protocol was performed to determine the affinity values of prostanoid receptor antagonists (AH23848B, AH6809 or BWA868C) which were added simultaneously with the drug combination during 30 min before the histamine-induced contraction.

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 relaxations induced with papaverine. The E_{max} value was the maximal relaxation produced with the highest agonist-concentration used and EC_{50} value was the concentration which produced $E_{max}/2$. These values were interpolated from the individual agonist 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 an 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} values of agonist in the presence and absence of antagonist. The pK_B values were calculated as the negative log of the K_B values. All results were expressed as means $\pm$s.e.mean of data derived from n different lung samples. Statistical analysis was performed using ANOVA with a confidence level of 95% and taking into account the preparations derived from the same or different lung samples (covariate).

Compounds

PGE_2, PGE_1, PGD_2 and misoprostol ((±)-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-inyl]bicyclo[3.3.0]-octan-3-ylidene]pentanoic acid) and cicaprost ([-2-[hexahydro-5-hydroxy-4-(3-hydroxy-4-methyl-1,6-nonadinyl)-2-(1H)-pentalenylidene]ethoxy] acetic acid) were a gift from Schering AG, Berlin, Germany. AH6809 (6-isopropoxy-9-oxaxanthene-2-carboxylic acid) and AH23848B ([1α(z),2β,5α]-(±)-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) was 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. Histamine dihydrochloride, L-NOARG (N^G-nitro-L-arginine), indomethacin and atropine sulphate were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Papaverine was obtained from Meram Laboratories (77020 Melun, France).

Results

In each experiment, the human bronchial preparations contracted with histamine (50 μM: 2.37±0.16 g, n=28) and at the end of the protocols the preparations were relaxed with papaverine (0.1 mM: 2.90±0.18 g, n=28). The combination of inhibitors and antagonists (indomethacin, L-NOARG, BAY u3405 and atropine) with which the bronchial preparations were incubated, had no significant relaxant effect on the basal tone of these preparations (−0.06±0.06 g; n=28).

PGE_1 as well as two stable PGI_2 analogues, iloprost and cicaprost, produced concentration-dependent relaxations (Figure 1 and Table 1) in human bronchial preparations. PGE_2 and misoprostol also relaxed the histamine-contracted human bronchial preparations (Figure 2 and Table 2). Concentration-dependent relaxations produced by PGE_2 and misoprostol were significantly shifted in presence of AH6809 (3 μM; Figure 2). The pK_B value for this antagonist against PGE_2 is presented in Table 2. On the contrary, no significant displacement of the relaxation curves induced by PGE_2 was observed after an incubation with AH23848B (30 μM, Figure 3 and Table 2). In paired bronchial preparations, derived from the same lung sample, the pD_2 values obtained in presence of AH23848B were not statistically different from the control values (6.97±0.11, n=4).

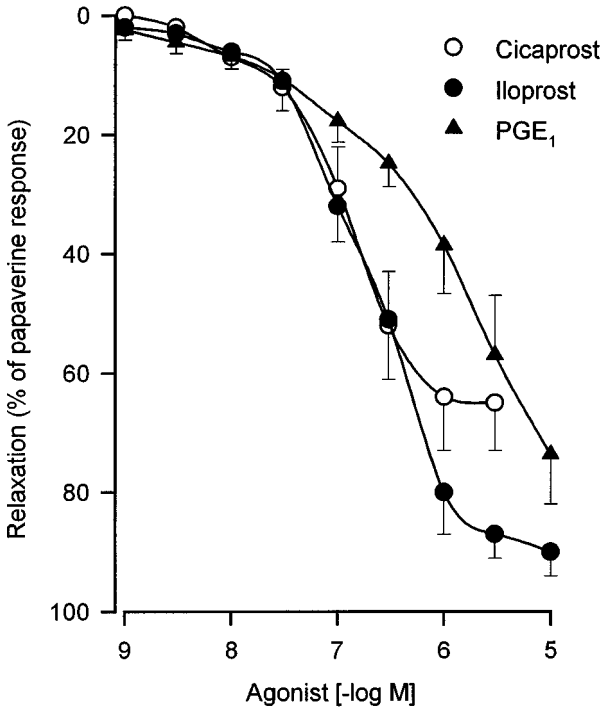

Figure 1 Relaxation of human isolated bronchial preparations induced by cicaprost, iloprost or PGE_1. Responses were expressed as per cent of the papaverine (0.1 mM) relaxation. Values are means±s.e.mean and the number of lung samples used are indicated in Table 1.

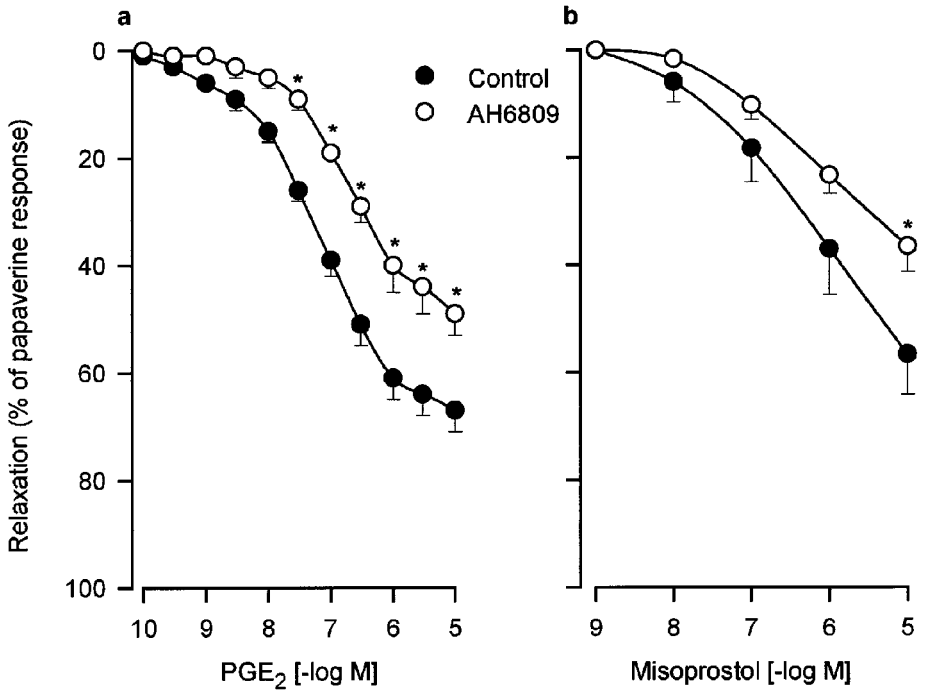

Figure 2 Relaxation of human isolated bronchial preparations induced by PGE_2 (a) or misoprostol (b). Some bronchial preparations were treated 30 min with AH6809 (3 μM). Responses were expressed as per cent of the papaverine (0.1 mM) relaxation. In each panel, values are means$\pm$s.e.mean and the number of lung samples used are indicated in Table 2. *Values significantly different when results from control (Tyrode) and treated tissues were compared.

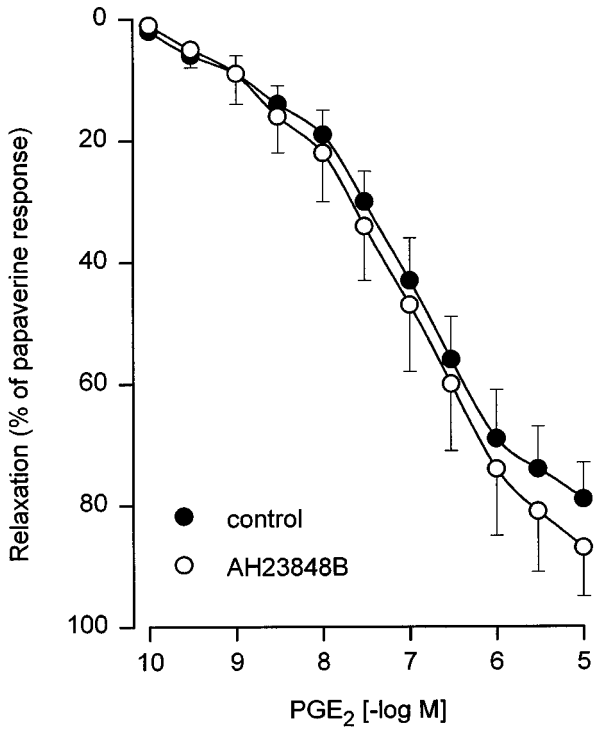

Figure 3 Relaxation of human isolated bronchial preparations induced by PGE_2 in absence or in presence of AH23848B (30 μM). Responses were expressed as per cent of the papaverine (0.1 mM) relaxation. Values are means$\pm$s.e.mean derived from four lung samples.

While PGE_2, misoprostol, PGE_1 and PGI_2 analogues totally reversed the histamine contraction, PGD_2 and BW245C induced only a partial reversal of this contraction. Concentration-dependent relaxations of human bronchial preparations produced by PGD_2 and BW245C are shown in Figure 4 and Table 2. In addition, BWA868C (0.1 μM) significantly reduced the relaxation induced by PGD_2 (Table 2).

AH6809, AH23848B and BWA868C at the concentrations used had no significant effect on the basal tone.

Table 1 Relaxant effects of IP-receptor agonists on the isolated human bronchial preparations

Agonist	n	E_{max} (%)	pD_2 *value*
Cicaprost	3	65$\pm$08	6.86$\pm$0.08
Iloprost	6	90$\pm$04	6.63$\pm$0.12
PGE_1	4	74$\pm$08	6.05$\pm$0.09

The maximal response (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. The relaxations induced by PGE_1 or iloprost were performed in presence of AH6809 (3 μM).

Discussion

These data suggest the involvement of IP- and EP_2-receptors and to a lesser extent DP-receptor in the relaxant response produced by prostanoids in human airways.

Gardiner & Collier (1980) and Lydford & McKechnie (1994) have demonstrated that in the guinea-pig and in the rat trachea, relaxations induced by the prostanoids are attributed to the activation of EP-receptors. Data (present report) are in contrast to the classical description of prostanoid receptors in the airways derived from animals. Actually, the relaxations induced by cicaprost (IP-receptor agonist; Stürzebecher *et al.*, 1985), indicate the presence of IP receptor in human bronchial preparations. Similar results were obtained with iloprost (EP_1/IP-receptor agonist; Schrör *et al.*, 1981; Sheldrick *et al.*, 1988) when the EP_1-receptors were blocked by AH6809 (DP/EP_1-

Table 2 Relaxations of isolated human bronchial preparations: effect of EP- and DP-receptor agonists or antagonists

Treatment	*Concentration*	n	*Agonist* E_{max} (%)	*Agonist* pD_2 *value*	pK_B *value*
			Misoprostol		
Tyrode		4	57±08	6.33±0.28	
AH6809	3 μM	5	36±05*	6.39±0.18	NC
			PGE_2		
Tyrode		14	67±04	7.13±0.07	
AH6809	3 μM	6	49±04*	6.68±0.08*	5.78
AH23848B	30 μM	4	87±08	6.96±0.27	NC
			PGD_2		
Tyrode		4	25±08	6.12±0.18	
BWA868C	0.1 μM	3	11±01*	NC	NC
			BW245C		
Tyrode		5	36±11	7.28±0.53	

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

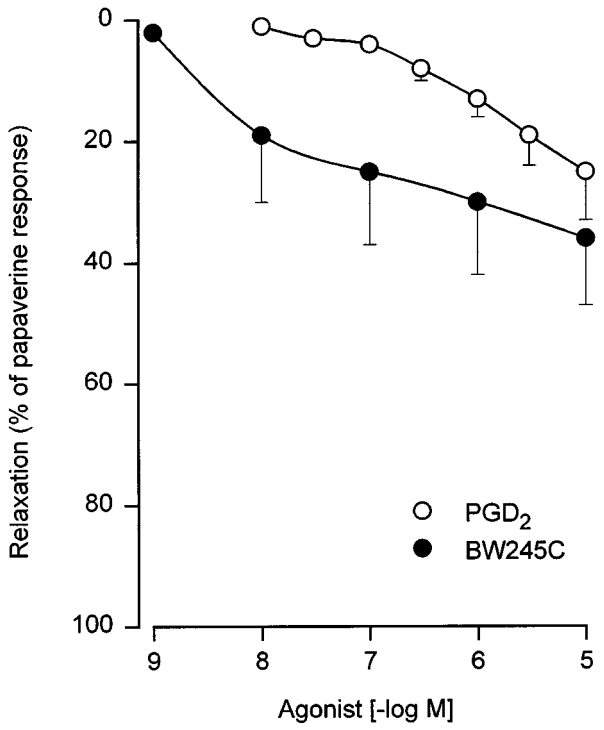

Figure 4 Relaxation of human isolated bronchial preparations induced by PGD_2 and BW245C. Responses were expressed as per cent of the papaverine (0.1 mM) relaxation. Values are means ±s.e.mean and the number of lung samples used are indicated in Table 2.

receptor antagonist; Coleman *et al*., 1985; Eglen & Whiting, 1988; Keery & Lumley, 1988). These results (present report) are in agreement with data obtained by Haye-Legrand *et al*. (1987) describing relaxations induced by iloprost, cicaprost (ZK 96480) and PGI_2 in the human isolated airways. These authors demonstrated that PGI_2, the natural agonist activating IP-receptor, induced quite variable relaxations of the human bronchial preparations. These variations may be due to the short half life of this prostaglandin. Blair & McDermot (1981), Corsini *et al*. (1987) and Adie *et al*. (1992) have shown that PGE_1, a more stable endogenous prostanoid, is a potent agonist for the IP-receptor in both binding and physiological studies. The effective relaxations of the human bronchi observed with PGE_1 (present report) suggest that, this prostaglandin, may be the preferential natural activator for IP-receptor in human airways *in vivo*.

Kennedy *et al*. (1982) and Gardiner (1986) have demonstrated that the EP_2-receptor is involved in the relaxation of guinea-pig and cat tracheal preparations. These studies were based on the effects induced by butaprost, a PGE_1 analogue. In human bronchial preparations, butaprost induced concentration-dependent relaxations (Gardiner, 1986; Norel *et al*., 1991), these results suggest the presence of EP_2-receptor on human airways. Additional evidence consistent with the involvement of the EP_2-receptor in the relaxation of human bronchial preparations is suggested by the following observations. First, PGE_2 and misoprostol, two preferential agonists for the EP-receptors, were potent airway muscle relaxants. These relaxations cannot be attributed to the activation of the IP-receptor, since these agonists are totally ineffective on the IP-receptor as in human pulmonary arteries (Walch *et al*., 1999). Misoprostol is a preferential agonist for EP_2- and EP_3-receptors (Coleman *et al*., 1988; Reeves *et al*., 1988; Lydford & McKechnie, 1994). Wise & Jones (1994) showed that this agonist was 10 fold less potent than PGE_2 in producing an inhibition of intracellular free calcium in the rat neutrophils (EP_2- and IP-receptors). In contrast, Smith *et al*. (1994) demonstrated that misoprostol was 145 fold less potent than PGE_2 for inducing dilatation of the foetal rabbit ductus arteriosus (EP_4- and IP-receptors). In human bronchial preparations (present report), misoprostol was only 6 fold less potent than PGE_2 in provoking relaxations. Such a ratio is in agreement with the activation of an EP_2-receptor when the IP-receptor is present in the same preparation. Secondly, the TP/EP_4-receptor antagonist (AH23848B) failed to inhibit the relaxation induced by PGE_2, these data suggest that EP_4-receptor is probably not involved in the relaxations produced by either PGE_2 or misoprostol in human bronchial preparations. Finally, evidence in support of the presence of EP_2-receptor is derived from a new effect of AH6809 reported recently (Woodward *et al*., 1995; Brown *et al*., 1997) which indicates AH6809 as an EP_2 antagonist. The concentration-dependent relaxations induced by both EP agonists (PGE_2 or misoprostol) were significantly shifted in presence of this antagonist (present report) suggesting the presence of EP_2-receptor.

The human bronchial preparations relaxed to PGD_2 and BW245C (present report). These compounds have been

described as DP-receptor agonists. Actually, Narumiya & Toda (1985) and Eglen & Whiting (1989) have shown that BW245C is ineffective on the EP_2-receptor in the guinea-pig trachea. In a similar fashion, the relaxation induced by PGD_2 may not be attributed to the activation of IP-receptors since PGD_2 was totally ineffective on human pulmonary arteries (Walch *et al*., 1999). The involvement of DP-receptors in human bronchial relaxation, is suggested by the 15 fold greater potency of BW245C in comparison with PGD_2. This result is in agreement with those obtained by Narumiya & Toda (1985) and Giles *et al*. (1989) in human washed platelets. Furthermore, the relaxation induced by PGD_2 (present report) is significantly reduced in presence of BWA868C a DP-antagonist (Giles *et al*., 1989). This antagonist does not block the EP_2- or IP-receptors as demonstrated by Giles *et al*. (1989), Chen & Woodward (1992) and Bhattacherjee *et al*. (1993). Taken together, these results (present report) suggest that PGD_2 and BW245C induced relaxations by the activation of DP-receptors. However, these agonists produced relaxations which were less than 50% of the papaverine response. These results suggest a lower density of the DP-receptor or a less effective coupling of this receptor with adenylate cyclase when compared with EP_2- or IP-receptors in human airways.

The venous preparations exhibited a similar or a greater sensitivity to the prostanoid-receptor agonists (Walch *et al*., 1999) than the bronchial preparations (present report). A marked difference was observed with the prostacyclin analogues in bronchial versus pulmonary vascular preparations even though the E_{max} were the same. These results suggest that there is a difference at the receptorial level between the IP-receptor present in human pulmonary vessels and that in human airways. These data are in agreement with previous reports (Corsini *et al*., 1987; Armstrong *et al*., 1989; Merritt *et al*., 1991; Wise *et al*., 1995; Takechi *et al*., 1996) suggesting a heterogeneity of the IP-receptor in various tissues or cells. A comparison of the relaxation induced by PGE_2 in the airways (present report) and in the human pulmonary veins (Walch *et al*., 1999) demonstrates a difference in sensitivity and in maximal relaxations. These differences are consistent with the presence of two different subtypes of EP-receptor in these tissues. While an EP_2-receptor is involved in human bronchial preparations, the subtype of EP-receptor involved in venous preparations remains to be characterized.

In conclusion, the results (present report) suggest a major involvement of IP- and EP_2-receptors and a minor role for the DP-receptor in the bronchial relaxation induced by the prostanoids in the human lung.

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