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PGE_2 receptor (EP_4) agonists: Potent dilators of human bronchi and future asthma therapy?

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ABSTRACT

Background: Asthma and chronic obstructive pulmonary disease are characterized by inappropriate constriction of the airway smooth muscle. In this context, the physiological response of the human airways to selective relaxant agonists like PGE_2 is highly relevant. The aim of this study was thus to characterize the PGE_2 receptor subtypes (EP_2 or EP_4) involved in the relaxation of human bronchial preparations.
Methods: Human bronchial preparations cut as rings were mounted in organ baths for isometric recording of tension and a pharmacological study was performed using selective EP_2 or EP_4 ligands.
Results: In the presence of a thromboxane TP receptor antagonist and indomethacin, PGE_2 induced the relaxation of human bronchi ($E_{max} = 86 \pm 04\%$ of papaverine response; pEC_{50} value $= 7.06 \pm 0.13$; $n = 6$). This bronchodilation was significantly blocked by a selective EP_4 receptor antagonist (GW627368X, 1 and 10 μmol/L) with a pK_B value of 6.38 ± 0.19 ($n = 5$). In addition, the selective EP_4 receptor agonists (ONO-AE1-329; L-902688), but not the selective EP_2 receptor agonist (ONO-AE1-259), induced potent relaxation of bronchial preparations pre-contracted with histamine or anti-IgE.
Conclusion: PGE_2 and EP_4 agonists induced potent relaxations of human bronchial preparations *via* EP_4 receptor. These observations suggest that EP_4 receptor agonists could constitute therapeutic agents to treat the increased airway resistance in asthma.

1. Introduction

Asthma is a chronic inflammatory disease of the airways characterized by reversible airflow obstruction and bronchoconstriction. Previous studies have shown that the use of beta-receptor agonists in asthma treatment has detrimental side effects. Beta-receptor agonists are associated with incident heart failure in patients treated with long lasting beta-agonists [1]. Researches are currently underway to identify new bronchodilators agents. Many *in vitro* studies using animal airways have reported relaxation induced by prostaglandin E_2 (PGE_2). The activation of EP_2 or EP_4 receptor subtypes by PGE_2 is responsible for these effects. In mice, guinea-pig and cat, the EP_2 receptor is involved [2,3] while in rat the EP_4 receptor has been implicated [4]. The initially characterized ligands: AH6809 (EP_2, DP, EP_1 receptor antagonist), AH23848B (TP, $EP_4 > EP_2$ receptor antagonist) and butaprost ($EP_2 > EP_4$ receptor agonist), used to discriminate between EP_2 and EP_4 receptor subtypes, had a poor selectivity [5]. PGE_2 in presence of a thromboxane TP receptor antagonist or butaprost are also potent relaxants of human bronchial preparations [3,6]. These pharmacological studies suggested the involvement of the EP_2 receptor in human bronchi. In the context of asthma treatment, two phase I clinical trials were conducted using inhalation of a selective EP_2 receptor agonist (AH13205) [7]. In these studies no bronchodilation was observed while AH13205 has been shown to produce airway irritancy in normal volunteers and coughing in mild asthmatics. In the last decade new selective (EP_2/EP_4) receptor agonists and antagonists have been developed [8,9]. For this reason, the aim of the present study was to clarify and determine which EP receptor subtype (EP_2/EP_4) is involved in human bronchodilation using these new compounds.

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2. Material and methods

2.1. Tissue preparations

All research programs involving the use of human tissue were approved and supported by the Ethics Committee of INSERM (the French National Institute for Health and Medical Research) and informed consent was obtained from each patient. Human lung tissue was obtained from patients (5 male and 5 female) who had undergone surgery for lung carcinoma. The mean age of the patients was 60 ± 4 years. Subsegmental (4th to 7th generation) bronchial preparations were used within 1–12 h post-surgery and cut as rings (2–4 mm internal diameter, 2–3 mm in length). The epithelium, in some human bronchial rings, was mechanically removed by inserting both smooth-edged arms of a dissecting forceps into the lumen of the vessel and gently rolling the moistened preparation between the surface of a forefinger and the forceps for 10 s without undue stretching.

Mice (C57BL/6, $n = 6$) tracheal preparations were used as a positive control to confirm the efficacy of the EP_2 receptor agonist (ONO-AE1-259) after pre-contractions induced by acetylcholine. Mice were handled in accordance with European Union directives (86/609/EEC) on the care and use of laboratory animals. The investigation was approved by the Animal Ethics Committee of INSERM and by the Local Animal Ethics Committee (No. B 7518 03).

Fresh human bronchial or mice tracheal preparations were set up in organ baths containing Tyrode's solution (concentration mmol/L): 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. An optimal load (1–2 g) was applied to each ring which ensured optimal physiological responses. Changes in force were recorded by isometric force displacement transducers (Narco F-60). Acquisition and processing of the physiological data (contraction/relaxation) was performed with the IOX software (EMKA Technologies, Paris, France).

2.2. Study design

After a 90 min equilibration period, most of the human bronchial preparations were incubated for 30 min with a cyclooxygenase inhibitor (indomethacin; 1.7 μmol/L) to eliminate endogenous prostanoid production. When PGE_2 was used as the relaxant agonist, BAY u3405 (1 μmol/L) was added to avoid any physiological effects induced by the activation of the TP receptor [10]. In addition to this treatment, some rings were exposed to the selective EP_4 receptor antagonist, GW627368X (10 μmol/L) [9]. After incubation, the preparations were pre-contracted with histamine (50 μmol/L); when the response reached a plateau, increasing concentrations of EP receptor agonists (PGE_2 (EP_{1-4}), ONO-AE1-329 (EP_4; [8,11]), L-902688 (EP_4; [12]) or ONO-AE1-259 (EP_2; [8,11])) were applied in a cumulative fashion.

After the equilibration period, some human bronchial preparations were not incubated with indomethacin and L-902688 concentration-effect curves were performed after pre-contractions induced by histamine (50 μmol/L) or anti-IgE (1/100). This protocol is considered as an asthma/allergic model.

Maximal relaxation was obtained for each preparation with papaverine (0.1 mmol/L) at the end of the experiment.

2.3. Data analysis

Changes in airway smooth muscle tone induced by the different receptor agonists were expressed in grams (g) or normalised (%) with respect to the papaverine-induced maximal relaxation. The values presented are positive for the contractions and negative for the relaxations. Where possible, a four parameter logistic equation of the form: $E = (E_{max}[A]^{nH})/(EC_{50}^{nH} + [A]^{nH})$ was fitted to the data obtained from each organ bath protocol to provide estimates of the maximal effect (E_{max}) produced by the EP receptor ligands (A), the half-maximum effective concentration values (EC_{50}), as well as Hill slope (nH) parameters. The pEC_{50} values were calculated as the negative log of EC_{50} values. The equilibrium dissociation constant for the receptor antagonist (K_B) was calculated using the following equation: $K_B = [B]/(DR - 1)$, where [B] is the concentration of the receptor antagonist and DR (dose ratio) is the ratio of EC_{50} of receptor agonist in the presence and absence of receptor antagonist in paired preparations (derived from a same individual). The affinity of the receptor antagonist (pK_B) was calculated as the negative log of the K_B value.

All data are presented as means ± s.e. mean derived from (n) different patients or mice. Pharmacological values and statistical analysis (One or Two Way ANOVA followed by the Student–Newman–Keuls test with a confidence level of 95%) were obtained by using SigmaStat® (SPSS Inc. Chicago, USA).

2.4. Drugs

L-902688 was a gift from Merck (Kirkland, Canada). ONO-AE1-259 and ONO-AE1-329 were gifts from Ono Pharmaceutical Co. (Osaka, Japan). GW627368X was a gift from GlaxoSmithKline (Stevenage, UK). PGE_2 and BAY u3405 were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Sheep antiserum to human IgE (anti-IgE; ShAHu/IgE(Fc)) was obtained from Nordic Immunological Laboratories, (Tilberg, Netherlands). Histamine, acetylcholine and indomethacin were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

3. Results

The airway pre-contractions produced by the different receptor agonists (histamine, anti-IgE and acetylcholine) and maximal relaxations induced by papaverine are shown in Table 1. PGE_2 induced concentration-dependent relaxations of human bronchial preparations pre-contracted with histamine (Fig. 1; Table 2). These bronchial relaxations were significantly blocked by GW627368X (1 and 10 μmol/L) as these EP_4 receptor antagonist concentrations caused a rightward shift (5 and 15 fold) of the PGE_2 concentration-effect curve with a pK_B value of 6.38 ± 0.19 ($n = 5$). The selective EP_4 receptor agonists (ONO-AE1-329; L-902688) produced concentration-dependent relaxations of human bronchial preparations pre-contracted with histamine (Fig. 2; Table 2). L-902688 concentration-effect curves after histamine pre-contractions were significantly different when paired preparations ($n = 4$) were incubated with or without indomethacin (Fig. 3; Table 2). On the contrary, these relaxations produced by L-902688 stimulation, were not modified in absence of epithelium (Table 2).

Table 1
Airways pre-contraction induced by histamine, anti-IgE and acetylcholine and maximal relaxations induced by papaverine.

Stimulation	Human bronchial preparations	Mice tracheal preparations
Histamine (50 μmol/L)	1.38 ± 0.17 g ($n = 10$; 42 rings)	NP
anti-IgE (1/100)	1.54 ± 0.34 g ($n = 4$; 4 rings)	NP
Acetylcholine (10 μmol/L)	NP	0.55 ± 0.12 g ($n = 6$; 6 rings)
Papaverine (0.1 mmol/L)	−1.58 ± 0.14 g ($n = 10$; 46 rings)	−0.59 ± 0.11 g ($n = 6$; 6 rings)

Values represent means ± s.e. mean, NP (not performed).