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EFFECT OF INDACATEROL, A NOVEL LONG-ACTING BETA 2-AGONIST, ON HUMAN ISOLATED BRONCHI

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Short title: Indacaterol effect on isolated human bronchi

Abstract

Indacaterol is a novel β_2-adrenoceptor agonist in development for the once-daily treatment of asthma and COPD. This study evaluated the relaxant effect of indacaterol on isolated human bronchi obtained from lungs of patients undergoing surgery for lung carcinoma.

Potency (-logEC_{50}), intrinsic efficacy (E_{max}) and onset of action were determined at resting tone. Duration of action was determined against cholinergic neural contraction induced by electrical field stimulation (EFS).

At resting tone, -logEC_{50} and E_{max} values were, respectively, 8.82±0.41 and 77±5% for indacaterol, 9.84±0.22 and 94±1% for formoterol, 8.36±0.16 and 74±4% for salmeterol, and 8.43±0.22 and 84±4% for salbutamol.

In contrast to salmeterol, indacaterol did not antagonize the isoprenaline response. Indacaterol's onset of action (in minutes) (7.8±0.7) was not significantly different from that of formoterol (5.8±0.7) or salbutamol (11.0±0.4) but significantly faster than that of salmeterol (19.4±4.3). EFS-induced contractions were inhibited with –logIC_{50} values of 6.96±0.13 (indacaterol), 8.96±0.18 (formoterol), 7.18±0.34 (salmeterol) and 6.39±0.26 (salbutamol). Duration of action was over 12 h for indacaterol and salmeterol, and 35.3±8.8 and 14.6±3.7 min for formoterol and salbutamol, respectively.

In isolated human bronchi, indacaterol behaved as a long-acting β_2-adrenoceptor agonist with high intrinsic efficacy and fast onset of action.

Keywords: Airways smooth muscle; β_2-adrenoceptor agonists; formoterol; indacaterol; isolated human bronchus; salmeterol.

Abbrevations; COPD: Chronic obstructive pulmonary disease, EFS: Electrical field stimulation. LABAs: long-acting β_2-adrenoceptor agonists. SABAs: short-acting β_2-adrenoceptor agonists.

Introduction

The introduction of long-acting inhaled β_2-adrenoceptor agonists (LABAs; salmeterol and formoterol) represented an important advance in asthma therapy. Treatment with LABAs provides better control of symptoms and lung function than short-acting β_2-agonists (SABAs) [1,2] and, when combined with moderate doses of inhaled glucocorticosteroids, these drugs have been shown to improve symptoms and lung function more effectively than doubling the dose of inhaled corticosteroids [3–5]. They have also been shown to reduce the number of asthma exacerbations during a 12-month treatment period, although to a lesser extent than doubling the dose of inhaled corticosteroids [4]. LABAs are also used in the treatment of stable chronic obstructive pulmonary disease (COPD) according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines recommendations [6,7].

The clinical efficacy and duration of action of formoterol and salmeterol appear to be very similar [8,9]. There are, however, some differences between the two compounds, in terms of their onset of action [10,11] and their interaction with the β_2-receptor [10,12,13]. This β_2-receptor interaction is characterized in terms of: the ability to bind to the receptor and to induce an intra-cellular response; potency, which is related to the amount of drug required for a physiological response; and efficacy, which is related to the drug's ability to induce a maximum physiological effect [10,12,13].

Indacaterol (QAB149) is a novel inhaled β_2-adrenoceptor agonist under clinical development for the treatment of asthma and COPD. In preclinical studies in guinea-pigs, indacaterol demonstrated a longer duration of action and faster onset than salmeterol [14]. Clinical studies have indicated that indacaterol might be used as a once-daily compound [15], which would be of great interest regarding patient convenience and patient compliance with treatment.

The present study aimed to evaluate the relaxant effect (potency and maximal efficacy) and the onset and duration of action of indacaterol in comparison with marketed compounds (formoterol, salbutamol and salmeterol) on isolated human bronchial rings. We also studied the interaction of indacaterol, formoterol and salmeterol with isoprenaline-induced bronchial relaxation. Since isoprenaline is a SABA, this permitted us to examine the potential antagonism of these compounds with the use of SABA rescue medicine.

Material and Methods

Human bronchial tissue sampling

Lung tissue was obtained from 34 patients (26 men, 8 women, mean age 61.5 ± 1.7 years) who were undergoing surgery for lung carcinoma. None of the patients had a history of asthma. The use of human lung tissue for *in vitro* experiments was approved by the local ethics committee. After the resection of one or more lung lobes, a piece of macroscopically normal tissue at a distance of at least 20 mm from the malignancy was supplied by the hospital pathologist and submerged in a physiological salt solution (PSS, Krebs solution, composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.6, KH_2PO_4 1.1, $NaHCO_3$ 25.0, glucose 11.7) at 4ºC. After removal of adhering lung parenchyma and connective tissue, 8 to 24 rings from each bronchus were prepared (4–5 mm length x 2–4 mm diameter) as previously described [16]. Preparations were stored overnight at 4ºC in PSS equilibrated with 5% CO_2 in O_2 and the experiments were performed on the next day. Previous experience in this laboratory and other published data have demonstrated that overnight storage of tissue does not alter its reactivity [10, 16, 17].

Experimental procedures

Tissue preparation

The total number of rings used in the study was 411. Bronchial rings were suspended in parallel on tissue hooks in 10 ml organ baths containing PSS, gassed with 5% CO_2 in O_2 at 37ºC (pH 7.4). Each preparation was connected to a force displacement transducer (UF1 Pioden strain gauges, EMKA Technologies, Mitry Mory, France) and isometric tension changes were recorded (I.O.X. recorder system, EMKA Technologies, Mitry Mory, France). The preparations were suspended under an initial load of 3 g and equilibrated for 60–90 min with changes in bath PSS every 15–20 min before any pharmacological intervention occurred; at the end of equilibration period, the resting load was stable at between 2 and 4 g. In all experiments, human bronchi were first contracted maximally with acetylcholine (3 mM). The tissues were then washed and equilibrated for 60 min before beginning the experimental procedure. Under these conditions, responses were optimal and reproducible according to data from this and other laboratories [10, 16, 17, 18].

Potency and efficacy

110 rings obtained from 10 patients were used for the following experiments and only one concentration–response curve for one compound was recorded in each ring. Within a given set of experiments, the response for each patient was tested in duplicate. Concentration–response curves for indacaterol, formoterol, salmeterol and salbutamol were produced by cumulative addition of the compounds at intervals of 5–30 min to obtain a relaxation plateau on bronchi at resting tone and on bronchi pre-contracted with histamine (10 μM, representing 30% of 3 mM acetylcholine) or carbachol (1 μM, representing 40% of 3 mM acetylcholine). At the end of the experiment, theophylline 3 mM was added to the bath to determine the maximal relaxation.

Antagonism of isoprenaline-induced relaxation

93 rings obtained from 7 patients were used for the following experiments and only one concentration–response curve for isoprenaline was recorded in each ring. Within a given set of experiments, the response for each patient was tested in duplicate. Following the resting period, bronchial rings were contracted with 1 μM carbachol. After the contraction plateau was reached, bronchial rings were incubated for 30 min with PSS (control) or with equi-effective concentrations of formoterol (10^{-9} M and $3x10^{-9}$ M) or indacaterol (10^{-7} M and $3x10^{-7}$ M) that induced approximately 20 and 35% inhibition of the carbachol-induced contraction. As a result of its partial agonistic activity, salmeterol (10^{-7} M and $3x10^{-7}$ M) did not induce more than 20% inhibition of the carbachol-induced contraction. Thereafter, concentration–response curves to isoprenaline in the presence of β_2-adrenoceptor agonist drugs or PSS were recorded by applying increasing concentrations of isoprenaline (10^{-8} M to 10^{-5} M) at 8–15 min intervals. At the end of the experiment, theophylline (3 mM) was added to the bath to determine maximal relaxation.

Onset of action

32 rings obtained from 4 patients were used for the following experiments and only one compound and one concentration were studied in each ring. Within a given set of experiments, the response for each patient was tested in duplicate. The onset of action was measured on resting tone and was calculated as the time (min) to induce 50% of the maximal relaxation ($T_{1/2}$) observed for the compound [10, 17]. Equi-effective

drug concentrations, corresponding to 80% of the maximal relaxation observed for each compound, were used: salbutamol, salmeterol and indacaterol $3x10^{-8}$ M and formoterol 10^{-9} M.

Electrical field stimulation

Experiments were performed as described previously [19]. 176 rings obtained from 13 patients were used for the following experiments and only one compound and one concentration were studied in each ring. Each organ bath was fitted with two platinum plate electrodes (1 cm^2) placed alongside the tissue (10 mm apart) for transmural electrical field stimulation (EFS) (biphasic pulse width 1 ms, constant current of 320 mA for 10 s at 5 Hz). A first control response was established for all bronchi preparations by adding 3 mM acetylcholine to obtain a plateau of maximal contraction. After washing, bronchi were allowed to equilibrate for 60 min with a change of the PSS every 15 min. For all the subsequent duration of the experiment, 1 µM montelukast and 1 µM indomethacin were present in the buffer to avoid the influence of indirect effects of leukotrienes and prostaglandins, respectively, on the neuronal responses.

After tension had returned to the baseline tone, the preparation was stimulated every 10 min at 5 Hz, pulse width 1 ms and 320 mA current for 10 s using a stimulator (EMKA Technologies, Mitry Mory, France) where the voltage output was adjusted to give a constant current and biphasic rectangular pulse of alternating polarity. These contractions represent 20–50% of the maximal contraction induced by 3 mM acetylcholine. Compounds or vehicle were then added to the bath for 1 h and washed out before the beginning of a second train of stimulations every 20 min for 12 h (Figure 1).

To determine their respective potency in preventing EFS-induced contraction ($-\log IC_{50}$), the following drugs were tested at different concentrations: indacaterol (10^{-8} M to 10^{-5} M), formoterol ($3x10^{-10}$ M to 10^{-6} M), salbutamol (10^{-6} M to 10^{-4} M), salmeterol (10^{-9} M to 10^{-5} M). For the measurement of the duration of action, drug concentrations that induced 50% of maximal inhibition of EFS-induced contraction were used: salbutamol 10^{-6} M, formoterol 10^{-9} M, salmeterol and indacaterol 10^{-7} M.

Expression and analysis of data

The maximal relaxant effect (E_{max}) of each β_2-adrenoceptor agonist was expressed as a percentage of the relaxation induced by 3 mM theophylline. Potency was determined by the EC_{50} value (concentration producing 50% of the maximal effect of a given compound). The onset of action was calculated as the time (in min) from compound administration to the attainment of half its maximal relaxation.

The concentration of each β_2-adrenoceptor agonist that induced a diminution of EFS response equal to 50% of the first control response (IC_{50}) was determined. The duration of action was then calculated as the time taken for response to 50% recovery from maximum inhibition.

Statistical significance, $p<0.05$, was assessed using analysis of variance and Student's t-test for paired or unpaired data. Data are presented as means ± s.e.m.

Drugs

Indacaterol maleate and formoterol fumarate were synthesized by the Department of Chemistry (Novartis Horsham Research Centre, Horsham, UK). Salmeterol xinafoate was either synthesized or isolated from clinical dosage forms by the Department of Chemistry (Novartis Horsham Research Centre, Horsham, UK) or purchased from Tocris Cookson Ltd (Bristol, UK). Acetylcholine HCl, indomethacin, carbachol (carbamylcholine chloride), salbutamol hemisulfate, theophylline and histamine were purchased from Sigma (Saint Quentin Fallavier, France), MK-476 (montelukast) from Merck (Paris, France), and isoprenaline HCl from Laboratoires Winthrop (Paris, France). Salmeterol and formoterol were dissolved in distilled water in the presence of HCl and DMSO (3%). Indacaterol was dissolved in distilled water in the presence of acetic acid (2%) and ethanol (20%). Indomethacin and montelukast were dissolved in pure ethanol and then diluted in PSS. Stock solutions (1 mM) were kept at –20°C until use.

Results

Potency and Efficacy

On basal tone preparations, all compounds relaxed the bronchi with the following order of potency: formoterol > indacaterol > salbutamol ≥ salmeterol. The order of maximal efficacy was: formoterol > salbutamol > indacaterol ≥ salmeterol (table 1, figure 2A).

When compared with the effect on basal tone, the potency and efficacy of all compounds were not statistically different when histamine was used as a contractile agent (table 1, figure 2B). On the other hand, carbachol pre-contraction decreased the potency of indacaterol ($p<0.01$), formoterol ($p<0.01$) and salbutamol ($p<0.01$). However, a decrease in the maximal relaxant effect was only observed for formoterol ($p<0.01$), salmeterol ($p<0.001$) and salbutamol ($p<0.01$) (table 1, figure 2C).

Antagonism of isoprenaline-induced relaxation

On preparations pre-contracted with 1 µM carbachol, and at concentrations inducing approximately 20 and 35% inhibition, formoterol and indacaterol did not affect the potency of isoprenaline-induced bronchi relaxation. In contrast, in the presence of salmeterol, at a concentration inhibiting the contraction by about 20%, a statistically significant decrease in isoprenaline potency was observed (table 2 and figure 3).

Onset of action on human bronchi at resting tone

Using concentrations that induced about 80% of the maximal relaxation at resting tone, the onset of action of indacaterol ($3x10^{-8}$ M) (7.8 ± 0.7 min, n = 4) was not significantly different from that of formoterol (10^{-9} M) (5.8 ± 0.7 min, n = 4) and salbutamol ($3x10^{-8}$ M) (11.0 ± 4.0 min, n = 4) but was significantly faster than that of salmeterol ($3x10^{-8}$ M) (19.4 ± 4.3 min, n = 4; $p<0.05$).

Protection against cholinergic neural bronchoconstriction

Electrical field stimulation (EFS)-induced contractions were inhibited in a concentration-dependent manner by all compounds with the following order of potency: formoterol > salmeterol > indacaterol > salbutamol (figure 4 & table 3). The duration of protection against cholinergic neural bronchoconstriction, determined at doses corresponding to their respective IC_{50} values, was more than 12 h for indacaterol and

salmeterol, 35 min for formoterol and 15 min for salbutamol (table 3). At high doses, indacaterol, formoterol and salbutamol induced close to full inhibition of the EFS-induced contraction. At the highest dose tested (10^{-5} M), the effect of indacaterol lasted up to 12 h, whereas formoterol (10^{-6} M) and salbutamol (10^{-4} M) showed a 20% reduction of the inhibition of the cholinergic response at 5 and 1.5 h, respectively. Salmeterol (10^{-5} M) induced a maximal inhibition of the cholinergic response of 80%, followed by a 20% reduction of the inhibition of the cholinergic response at 4 h.

Discussion

The present study describes for the first time the pharmacological profile of indacaterol on isolated human bronchi. In this system, indacaterol behaved as a nearly full β_2-adrenoceptor agonist that has no antagonistic effect on isoprenaline-induced relaxation. In addition, indacaterol had an onset of action similar to that of salbutamol and a duration of action in excess of 12 hours.

Under our experimental conditions, indacaterol at resting tone or in bronchi contracted with histamine was about 10 times less potent than formoterol but about three times more potent than salbutamol and salmeterol. When bronchi were precontracted with carbachol, the compounds' potency was moderately (formoterol, 1 log unit) or strongly reduced (salbutamol, 1.98 log unit and indacaterol, 2.61 log unit). Salmeterol's potency was not affected when compared with the resting tone condition. This decrease in potency observed in carbachol-treated bronchi could be related to the fact that carbachol induced a greater contraction when compared with histamine (40% versus 30% of the maximal effect seen with acetylcholine, respectively). However, we find it difficult to believe that such a minimal difference in the strength of the contraction could explain such a dramatic effect on the potency of the compounds studied. A more likely explanation resides in a functional antagonism between the β_2-adrenoceptor and the muscarinic receptor, probably due to β_2-adrenoceptor or Gs protein phosphorylation by protein kinase C [20, 21], or to the inhibition of adenylate cyclase by the muscarinic M_2 receptor-mediated Gi protein [22].

In terms of efficacy, which is related to the ability of drugs to activate the β_2-adrenoceptor, all four drugs seem to be full agonists on preparations at resting tone. In the presence of histamine, none of the compounds had a statistically significant reduction in their maximal effect. However, in the presence of carbachol, the efficacy of salbutamol and salmeterol was moderately to considerably reduced, whereas formoterol and indacaterol were marginally or not affected. This suggests that formoterol and indacaterol are nearly full agonists, whereas salbutamol and salmeterol are partial agonists, as previously demonstrated [10,23,24]. These differences in the intrinsic efficacy of the four drugs were confirmed by the inhibition by salmeterol in this and other studies [23,10,12] and by salbutamol [10,12], but not by formoterol and indacaterol, of the relaxation induced by isoprenaline. These results are in agreement

with receptor theories suggesting that a partial agonist has to occupy more receptors than a full agonist and thereby creates a situation in which functional receptor number becomes limiting in terms of obtaining a maximal response. As a consequence, a partial agonist behaves as an antagonist in the presence of an agonist with higher efficacy acting on the same receptor.

In isolated human bronchi, indacaterol has a fast onset of action, similar to that of salbutamol and formoterol. Although this could seem to be of secondary importance for maintenance therapy, such a profile could still be of interest. Indeed, a rapid improvement in breathlessness could increase a patient's confidence in their treatment, and subsequently increase treatment compliance. Furthermore, a fast onset of action could permit the use of indacaterol as rescue medicine.

The duration of action was evaluated as the protection against cholinergic neural bronchoconstriction induced by EFS and was shown to be markedly higher for indacaterol and salmeterol when compared with formoterol and salbutamol. Although, in our system, the duration of action for salmeterol is in line with its known clinical profile, the duration of action of formoterol does not reflect the clinical situation. This discrepancy has been reported previously in human bronchus [25, 26] and a number of theories have been put forward to explain this inconsistency. The most rational explanation for the observed duration of action of formoterol, in man, almost certainly lies within the high local concentrations achieved after inhalation and the interaction with the membrane lipid bilayer as a key component [27]. Our model of EFS-induced contraction does not allow a study longer than 12 h. Indeed, even under control conditions, we observed a 30–50% loss in bronchial cholinergic response after 12 h of EFS. As a result, the duration of action of indacaterol could not be differentiated from that of salmeterol. However, at the highest concentration tested, indacaterol induced almost 100% relaxation, with this level being maintained for up to 12 h, whereas salmeterol induced a maximal relaxation of 80%, and demonstrated a loss of about 20% of this maximal effect after 4 h of stimulation. This suggests that a longer duration of action can be achieved for indacaterol when compared with salmeterol. Indeed, in clinical studies, indacaterol has demonstrated a profile compatible with once-daily dosing [15].

In conclusion, in the isolated human bronchus indacaterol efficiently prevents the occurrence of bronchial contraction, has a fast onset of action and possesses a very long duration of action. Furthermore, in contrast to salmeterol, indacaterol does not

antagonize the effect of rescue medication. All these characteristics that are promising regarding the use of indacaterol as a very long-acting β_2-adrenoceptor agonist are now being tested in clinical studies [15].

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References

1 Kips JC, Pauwels RA. Long-acting inhaled beta(2)-agonist therapy in asthma. Am J Respir Crit Care Med 2001; 164: 923-932.

2 Sears MR, Lötvall J. Past, present and future--beta2-adrenoceptor agonists in asthma management. Respir Med 2005; 99: 152-170.

3 Greening AP, Ind PW, Northfield M, Shaw G. Added salmeterol versus higher-dose corticosteroid in asthma patients with symptoms on existing inhaled corticosteroid. Allen & Hanburys Limited UK Study Group. Lancet 1994; 344: 219-224.

4 Pauwels RA, Lofdahl CG, Postma DS, Tattersfield AE, O'Byrne P, Barnes PJ, Ullman A. Effect of inhaled formoterol and budesonide on exacerbations of asthma. Formoterol and Corticosteroids Establishing Therapy (FACET) International Study Group. N Engl J Med 1997; 337: 1405-1411.

5 Woolcock A, Lundback B, Ringdal N, Jacques LA. Comparison of addition of salmeterol to inhaled steroids with doubling of the dose of inhaled steroids. Am J Respir Crit Care Med 1996; 153: 1481-1488.

6 Cazzola M, Dahl R. Inhaled combination therapy with long-acting beta 2-agonists and corticosteroids in stable COPD. Chest 2004; 126: 220-237.

7 Pauwels RA, Buist AS, Ma P, Jenkins CR, Hurd SS; GOLD Scientific Committee. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: National Heart, Lung, and Blood Institute and World Health Organization Global Initiative for Chronic Obstructive Lung Disease (GOLD): executive summary. Respir Care 2001; 46: 798-825.

8 Rabe KF, Jorres R, Nowak D, Behr N, Magnussen H. Comparison of the effects of salmeterol and formoterol on airway tone and responsiveness over 24 hours in bronchial asthma. Am Rev Respir Dis 1993; 147: 1436-1441.

9 Van Noord JA, Smeets JJ, Raaijmakers JA, Bommer AM, Maesen FP. Salmeterol versus formoterol in patients with moderately severe asthma: onset and duration of action. Eur Respir J 1996; 9: 1684-1688.

10 Naline E, Zhang Y, Qian Y, Mairon N, Anderson GP, Grandordy B, Advenier C. Relaxant effects and durations of action of formoterol and salmeterol on the isolated human bronchus. Eur Respir J 1994; 7: 914-920.

11 Wegener T, Hedenstrom H, Melander B. Rapid onset of action of inhaled formoterol in asthmatic patients. Chest 1992; 102: 535-538.

12 Molimard M, Naline E, Zhang Y, Le Gros V, Begaud B, Advenier C. Long- and short-acting beta2 adrenoceptor agonists: interactions in human contracted bronchi. Eur Respir J 1998; 11: 583-588.

13 Hanania NA, Sharafkhaneh A, Barber R, Dickey BF. Beta-agonist intrinsic efficacy: measurement and clinical significance. Am J Respir Crit Care Med 2002; 165: 1353-1358.

14 Battram C, Charlton SJ, Cuenoud B, Dowling MR, Fairhurst RA, Farr D, Fozard JR, Leighton-Davies JR, Lewis CA, McEvoy L, Turner RJ, Trifilieff A. In-vitro and in-vivo pharmacological characterisation of indacaterol (5-[(R)-2-(5,6-Diethyl-indan-2-ylamino)-1-hydroxy-ethyl]-8-hydroxy-1H-quinolin-2-one), a novel inhaled beta-2 adrenoceptor agonist with a 24-hour duration of action. J Pharmacol Exp Ther 2006; 317:762-770

15 Beier J, Chanez P, Martinot J-B, Schreurs AJM, Tkáčová R, Bao W, Jack D, Higgins M, Safety, tolerability and efficacy of indacaterol, a novel once-daily β_2-agonist, in patients with COPD: a 28-day randomised, placebo controlled clinical trial. Pulm Pharm Ther 2006; in press.

16 Sarria B, Naline E, Cortijo J, Moreau J, Cerda JM, Morcillo EJ, Advenier C. Functional, biochemical and morphological studies on human bronchi after cryopreservation. Br J Pharmacol 1995; 116: 2569-2574.

17 Naline E, Qian Y, Advenier C, Raeburn D, Karlsson J-A. Effects of RP 73401, a novel, potent and selective phosphodiesterase type 4 inhibitor, on contractility of human, isolated bronchial muscle. Br J Pharmacol 1996; 118: 1939-1944.

18 Watson N, Magnussen H, Rabe KF. The relevance of resting tension to responsiveness and inherent tone of human bronchial smooth muscle. Br J Pharmacol 1998; 123: 694-700.

19 Takahashi T, Belvisi MG, Patel H, Ward JK, Tadjkarimi S, Yacoub MH, Barnes PJ. Effect of Ba 679 BR, a novel long-acting anticholinergic agent, on cholinergic neurotransmission in guinea pig and human airways. Am J Respir Crit Care Med 1994; 150: 1640-1645.

20 Van Amsterdam RG, Meurs H, Ten Berge RE, Veninga NC, Brouwer F, Zaagsma J. Role of phosphoinositide metabolism in human bronchial smooth

muscle contraction and in functional antagonism by beta-adrenoceptor agonists. Am Rev Respir Dis 1990; 142: 1124-1128.

21 Barnes PJ. Beta-adrenergic receptors and their regulation. Am J Respir Crit Care Med 1995; 152: 838-860.

22 Sarria B, Naline E, Zhang Y, Cortijo J, Molimard M, Moreau J, Therond P, Advenier C, Morcillo EJ. Muscarinic M2 receptors in acetylcholine-isoproterenol functional antagonism in human isolated bronchus. Am J Physiol Lung Cell Mol Physiol 2002; 283: L1125-L1132.

23 Linden A, Bergendal A, Ullman A, Skoogh BE, Lofdahl CG. Salmeterol, formoterol, and salbutamol in the isolated guinea pig trachea: differences in maximum relaxant effect and potency but not in functional antagonism. Thorax 1993; 48: 547-553.

24 Rabe KF, Giembycz MA, Dent G, Perkins RS, Evans P, Barnes PJ. Salmeterol is a competitive antagonist at beta-adrenoceptors mediating inhibition of respiratory burst in guinea-pig eosinophils. Eur J Pharmacol 1993; 231: 305-308.

25 Anderson GP, Linden A, Rabe KF. Why are long-acting beta-adrenoceptor agonists long-acting? Eur Respir J 1994; 7: 569-578.

26 Nials AT, Coleman RA, Johnson M, Magnussen H, Rabe KF, Vardey CJ. Effects of beta-adrenoceptor agonists in human bronchial smooth muscle. Br J Pharmacol 1993; 110: 1112-1116

27 Nials AT, Ball DI, Butchers PR, Coleman RA, Humbles AA, Johnson M, Vardey CJ. Formoterol on airway smooth muscle and human lung mast cells: a comparison with salbutamol and salmeterol. Eur J Pharmacol 1994; 251: 127-135.

Table 1: Potency (–log EC_{50}) and maximal efficacy (E_{max}) of β_2-adrenoceptor agonists on isolated human bronchi at resting tone or after pre-contraction with 10 µM histamine or 1 µM carbachol

	n	At resting tone	n	Pre-contraction with 10 µM histamine	n	Pre-contraction with 1 µM carbachol
–log EC_{50}						
Indacaterol	5	8.82±0.41	4	8.51±0.24	4	6.58±0.28**
Formoterol	5	9.84±0.22	4	9.62±0.26	4	8.74±0.07**
Salbutamol	6	8.43±0.22	3	7.84±0.46	4	6.91±0.26**
Salmeterol	7	8.36±0.16	3	8.25±0.25	4	8.11±0.21
E_{max} (%)						
Indacaterol	5	77±5	4	84±7	4	77±4
Formoterol	5	94±1	4	86±4	4	84±2 **
Salbutamol	6	84±4	3	79±7	4	53±8**
Salmeterol	7	74±4	3	57±15	4	29±6***

Data are expressed as mean ± s.e.m. of n patients. **p<0.01, ***p<0.001 vs. resting tone.

Table 2: Isoprenaline potency (–log EC_{50}) in the absence or presence of β_2-adrenoceptor agonists on the isolated human bronchus

Drug (concentration)	n	–log EC_{50}
Control	6	7.48±0.08
+ Indacaterol (10^{-7}M)	5	7.20±0.16
+ Indacaterol ($3x10^{-7}$M)	6	7.81±0.15
Control	5	7.48±0.07
+ Formoterol (10^{-9}M)	5	7.59±0.16
+ Formoterol ($3x10^{-9}$M)	5	7.05±0.23
Control	6	7.49±0.16
+ Salmeterol (10^{-7}M)	6	5.58±0.35 *
+ Salmeterol ($3x10^{-7}$M)	6	5.24±0.44*

Data are expressed as mean ± s.e.m. of n patients.* $p<0.05$ vs. control

Table 3: Potency ($-\log IC_{50}$) and duration of action determined after electrical field stimulation

Drug	Potency		Duration of action		
	n	IC_{50} (nM)	n	concentration	Time
Indacaterol	5	6.96±0.13‡‡‡	5	($1x10^{-7}$ M)	>12 h
Formoterol	4	8.96±0.18†	4	($1x10^{-9}$ M)	35.3±8.8 min
Salbutamol	3	6.39±0.26‡‡‡	3	($1x10^{-6}$ M)	14.6±3.7 min
Salmeterol	5	7.18±0.34‡‡	5	($1x10^{-7}$ M)	>12 h

Duration of action was calculated as the time for response to reach 50% of the maximal relaxation. Data are expressed as mean ± s.e.m. of n patients.
$^{‡‡}p<0.01$, $^{‡‡‡}p<0.001$ vs. formoterol; $^{†}p<0.01$ vs. salmeterol.

Figure legends

Figure 1: Schematic representation of the protocol to measure duration of action of β_2-agonists on EFS-induced contraction

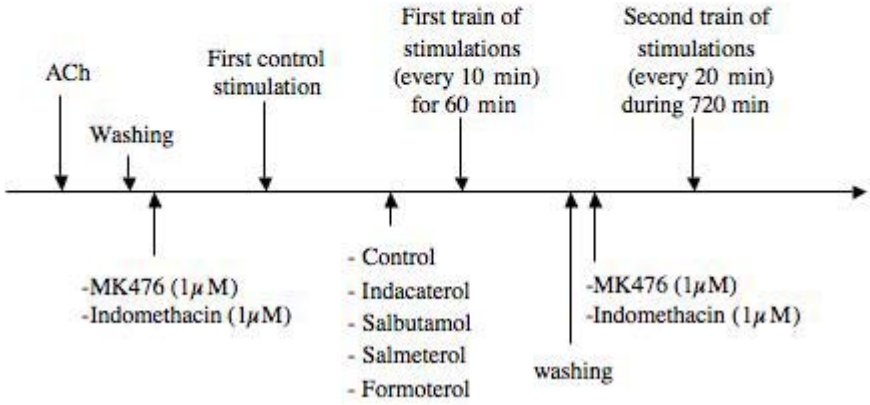

Figure 2: Effect of β_2-agonists on the isolated human bronchus at resting tone (A) and on bronchi pre-contracted with 10 µM histamine (B) or with 1 µM carbachol (C). Data are shown as percentage of theophylline-induced relaxation and expressed as mean ± s.e.m. from 3–7 patients (see table 1).

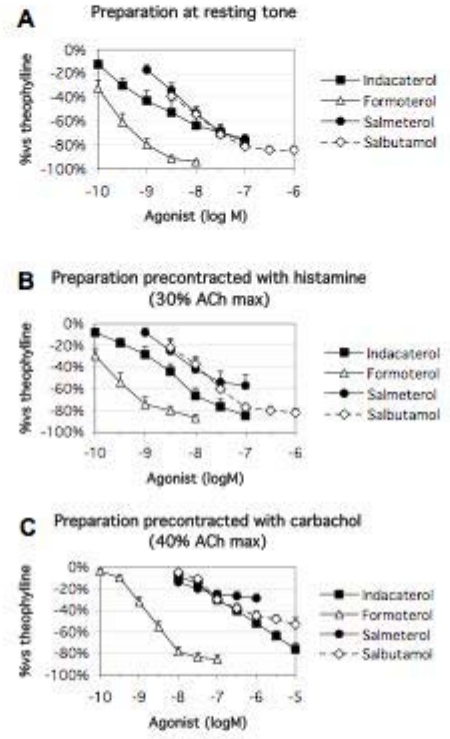

Figure 3: Effect of β_2-agonists on the isoprenaline-induced relaxation of the isolated human bronchus. Data are shown as percentage of theophylline-induced relaxation and expressed as mean ± s.e.m. from 5–6 patients (see table 2).

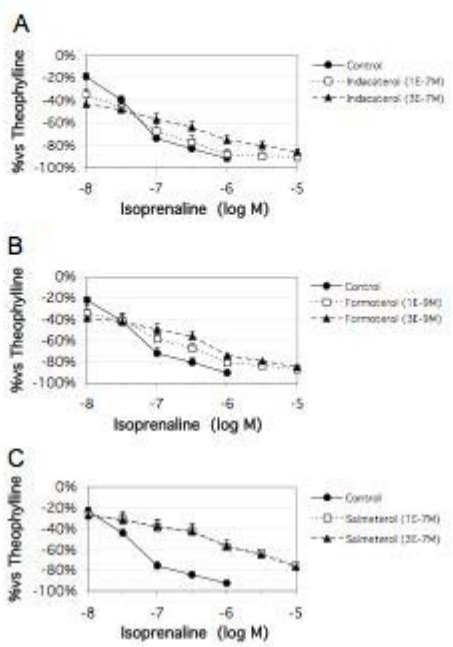

Figure 4: Effect of indacaterol (A), formoterol (B), salbutamol (C) and salmeterol (D) on EFS-induced contraction of isolated human bronchi. Data are shown as percentage of initial EFS contraction and expressed as mean ± s.e.m. from 5, 7, 3 and 6 patients for (A), (B), (C) and (D), respectively.