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Effect of indacaterol, a novel long-acting β_2-agonist, on isolated human bronchi

E. Naline*, A. Trifilieff#, R.A. Fairhurst#, C. Advenier* and M. Molimard¶

ABSTRACT: Indacaterol is a novel β_2-adrenoceptor agonist in development for the once-daily treatment of asthma and chronic obstructive pulmonary disease. The present study evaluated the relaxant effect of indacaterol on isolated human bronchi obtained from lungs of patients undergoing surgery for lung carcinoma.

Potency (-logEC50), maximal relaxant effect (Emax) 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, -logEC50 and Emax values were 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, respectively.

In contrast to salmeterol, indacaterol did not antagonise the isoprenaline response. Indacaterol's onset of action (7.8±0.7 min) was not significantly different from that of formoterol (5.8±0.7 min) or salbutamol (11.0±4.0 min), but it was significantly faster than that of salmeterol (19.4±4.3 min). EFS-induced contractions were inhibited with -logIC50 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 >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

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]. In fact, 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 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 onset of action [10, 11] and interaction with the β_2-receptor [10, 12, 13]. The β_2-receptor interaction is characterised in terms of the following factors: the ability to bind to the receptor and induce an intracellular 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 is a novel inhaled β_2-adrenoceptor agonist under clinical development for the treatment of asthma and COPD. In pre-clinical 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 compliance with treatment.

The present study aimed to evaluate the relaxant effect (potency and maximal efficacy) and the

AFFILIATIONS
*Research Unit EA220, Faculty of Medicine, Versailles University, Hospital Foch, Suresnes, and ¶Dept of Pharmacology, University Victor Segalen Bordeaux 2, Bordeaux, France.
#Novartis Institute for BioMedical Research, Horsham, UK.

CORRESPONDENCE
M. Molimard
Département de Pharmacologie
CHU de Bordeaux
Université Victor Segalen-INSERM U657
33076 Bordeaux cedex
France
Fax: 33 557574671
E-mail: mathieu.molimard@pharmaco.u-bordeaux2.fr

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onset and duration of action of indacaterol in comparison with marketed compounds (formoterol, salbutamol and salmeterol) on isolated human bronchial rings. The interaction of indacaterol, formoterol and salmeterol with isoprenaline-induced bronchial relaxation was also studied. Since isoprenaline is a SABA, this permitted the assessment of 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 males and eight females, mean age 61.5 ± 1.7 yrs) 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 $\geqslant$20 mm from the malignancy was supplied by the hospital pathologist and submerged in a physiological salt solution (PSS; Kreb's 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 4°C. After removal of adhering lung parenchyma and connective tissue, eight to 24 rings from each bronchus were prepared (4–5 mm length × 2–4 mm diameter) as previously described elsewhere [16]. The preparations were stored overnight at 4°C in PSS equilibrated with 5% CO_2 in O_2 and the experiments were performed the next day. Previous experience in the 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 present 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 37°C (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). 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 the equilibration period, the resting load was stable at 2–4 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 the present authors' laboratory and other studies [10, 16–18].

Potency and efficacy

A total of 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

A total of 93 rings obtained from seven 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 3×10^{-9} M) or indacaterol (10^{-7} M and 3×10^{-7} M), which induced ~20 and 35% inhibition of the carbachol-induced contraction. As a result of its partial agonistic activity, salmeterol (10^{-7} M and 3×10^{-7} M) did not induce >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}–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

A total of 32 rings obtained from four 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 observed for the compound [10, 17]. Equi-effective drug concentrations (salbutamol, salmeterol and indacaterol 3×10^{-8} M and formoterol 10^{-9} M) corresponding to 80% of the maximal relaxation observed for each compound, were used.

Electrical field stimulation

Experiments were performed as previously described elsewhere [19]. A total of 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

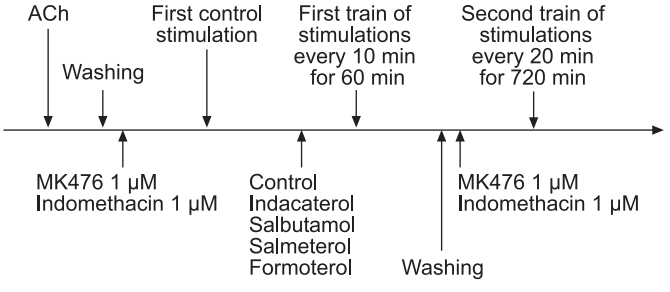

FIGURE 1. Schematic representation of the protocol to measure duration of action of β_2-agonists on electrical field stimulation-induced contraction. ACh: acetylcholine; MK476: montelukast.

TABLE 1 Potency (-logEC50) and intrinsic efficacy (Emax) of β_2-adrenoceptor agonists on isolated human bronchi at resting tone or after pre-contraction with 10 μM histamine or 1 μM carbachol

	At resting tone		Pre-contraction with 10 μM histamine		Pre-contraction with 1 μM carbachol	
	Subjects n	Mean±SEM	Subjects n	Mean±SEM	Subjects n	Mean±SEM
-logEC50						
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
Emax %						
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***

**: $p<0.01$ *versus* resting tone; ***: $p<0.001$ *versus* resting tone.

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 the subsequent duration of the experiment, 1 μM montelukast and 1 μM indomethacin were present in the buffer to avoid the influence on the neuronal responses of indirect effects of leukotrienes and prostaglandins, respectively.

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) 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 (fig. 1).

To determine the respective potency in preventing EFS-induced contraction (-logIC50), the following drugs were tested at different concentrations: indacaterol (10^{-8}–10^{-5} M), formoterol (3×10^{-10}–10^{-6} M), salbutamol (10^{-6}–10^{-4} M) and salmeterol (10^{-9}–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, *i.e.* salbutamol 10^{-6} M, formoterol 10^{-9} M and salmeterol and indacaterol 10^{-7} M.

Expression and analysis of data

The maximal relaxant effect (Emax) of each β_2-adrenoceptor agonist was expressed as a percentage of the relaxation induced by 3 mM theophylline. Potency was determined by the value of concentration producing 50% of the maximal effect of a given compound (EC50). The onset of action was calculated as the time (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 (IC50) was determined. The duration of action was then calculated as the time taken for response to 50% recovery from maximum inhibition.

A statistical significance of $p<0.05$ was assessed using ANOVA and a t-test for paired or unpaired data. Data are presented as mean±SEM.

Drugs

Indacaterol maleate and formoterol fumarate were synthesised by the Dept of Chemistry (Novartis Horsham Research Centre, Horsham, UK). Salmeterol xinafoate was either synthesised or isolated from clinical dosage forms by the Dept of Chemistry (Novartis Horsham Research Centre) or purchased from Tocris Cookson Ltd (Bristol, UK). Acetylcholine HCl, indomethacin, carbachol (carbamylcholine chloride), salbutamol hemisulphate, theophylline and histamine were purchased from Sigma (Saint Quentin Fallavier, France), montelukast (MK476) 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 MK476 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, fig. 2a).

When compared with the effect on basal tone, the potency and efficacy of all compounds were not statistically different when

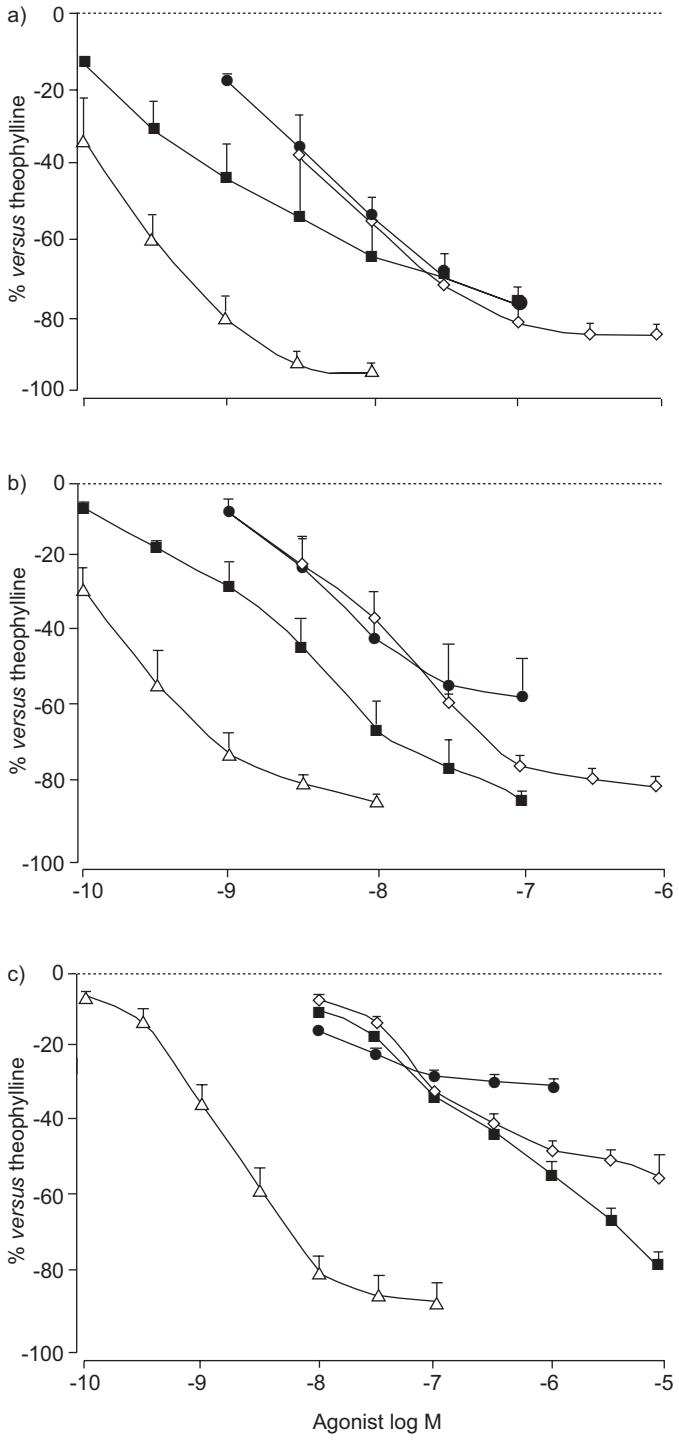

FIGURE 2. Effect of β_2-agonists on the isolated human bronchus a) at resting tone and on bronchi pre-contracted with b) 10 μM histamine (30% acetylcholine (ACh) maximum (max)) or c) with 1 μM carbachol (40% ACh max). Data are shown as % of theophylline-induced relaxation and are expressed as mean + SEM from three to seven patients (table 1). ■: Indacaterol; △: formoterol; ●: salmeterol; ◇: salbutamol.

histamine was used as a contractile agent (table 1, fig. 2b). Conversely, 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, fig. 2c).

TABLE 2 Isoprenaline potency (-logEC50) in the absence or presence of β_2-adrenoceptor agonists on the isolated human bronchus

Drug concentration	Subjects n	-logEC50
PSS	6	7.48±0.08
PSS + indacaterol		
10^{-7} M	6	7.20±0.16
3×10^{-7} M	6	7.81±0.15
PSS	5	7.48±0.07
PSS + formoterol		
10^{-9} M	5	7.59±0.16
3×10^{-9} M	5	7.05±0.23
PSS	6	7.49±0.16
PSS + salmeterol		
10^{-7} M	6	5.58±0.35*
3×10^{-7} M	6	5.24±0.44*

Data are expressed as mean±SEM. PSS: physiological salt solution. *: $p<0.05$ *versus* control.

Antagonism of isoprenaline-induced relaxation

On preparations pre-contracted with 1 μM carbachol and at concentrations inducing ~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 ~20%, a statistically significant decrease in isoprenaline potency was observed (table 2, fig. 3).

Onset of action on human bronchi at resting tone

Using concentrations that induced ~80% of the maximal relaxation at resting tone, the onset of action of indacaterol (3×10^{-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 (3×10^{-8} M; 11.0±4.0 min, n=4) but was significantly faster than that of salmeterol (3×10^{-8} M; 19.4±4.3 min, n=4; $p<0.05$).

Protection against cholinergic neural bronchoconstriction

EFS-induced contractions were inhibited in a concentration-dependent manner by all compounds with the following order of potency: formoterol > salmeterol > indacaterol > salbutamol (fig. 4, table 3). The duration of protection against cholinergic neural bronchoconstriction determined at doses corresponding to their respective IC50 values was >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 ⩽12 h, whereas formoterol (10^{-6} M) and salbutamol (10^{-4} M) showed a 20% reduction of the

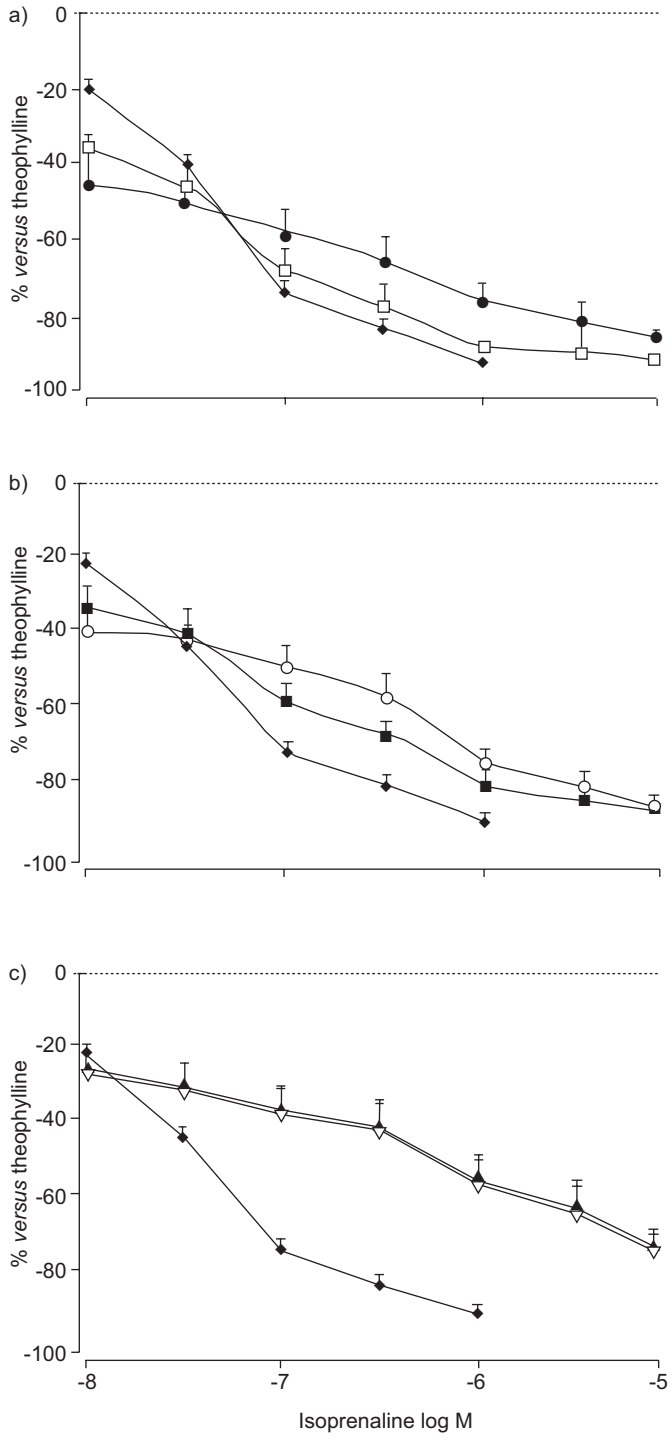

FIGURE 3. Effect of β_2-agonists on the isoprenaline-induced relaxation of the isolated human bronchus. Data are shown as % of theophylline-induced relaxation and expressed as mean+SEM from five to six patients (table 2). ♦: Physiological salt solution; □: indacaterol 10^{-7} M; ●: indacaterol 3×10^{-7} M; ■: formoterol 10^{-9} M; ○: formoterol 3×10^{-9} M; ▲: salmeterol 10^{-7} M; ▽: salmeterol 3×10^{-7} M.

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 the current system, indacaterol behaved as a nearly full β_2-adrenoceptor agonist that had 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 of >12 h.

Under the present experimental conditions, indacaterol at resting tone or in the bronchi contracted with histamine was about 10 times less potent than formoterol but about three times more potent than salbutamol and salmeterol. When the bronchi were pre-contracted with carbachol, the compounds' potencies were moderately (1 log unit for formoterol) or strongly reduced (1.98 log units for salbutamol and 2.61 log units for indacaterol). 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, it is 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 the 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 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 elsewhere [10, 23, 24]. These differences in the intrinsic efficacy of the four drugs were confirmed by the inhibition by salmeterol in the present and other studies [10, 12, 23], 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 treatment and, subsequently, increase treatment compliance. Furthermore, a fast onset of action could permit the use of indacaterol as rescue medicine.

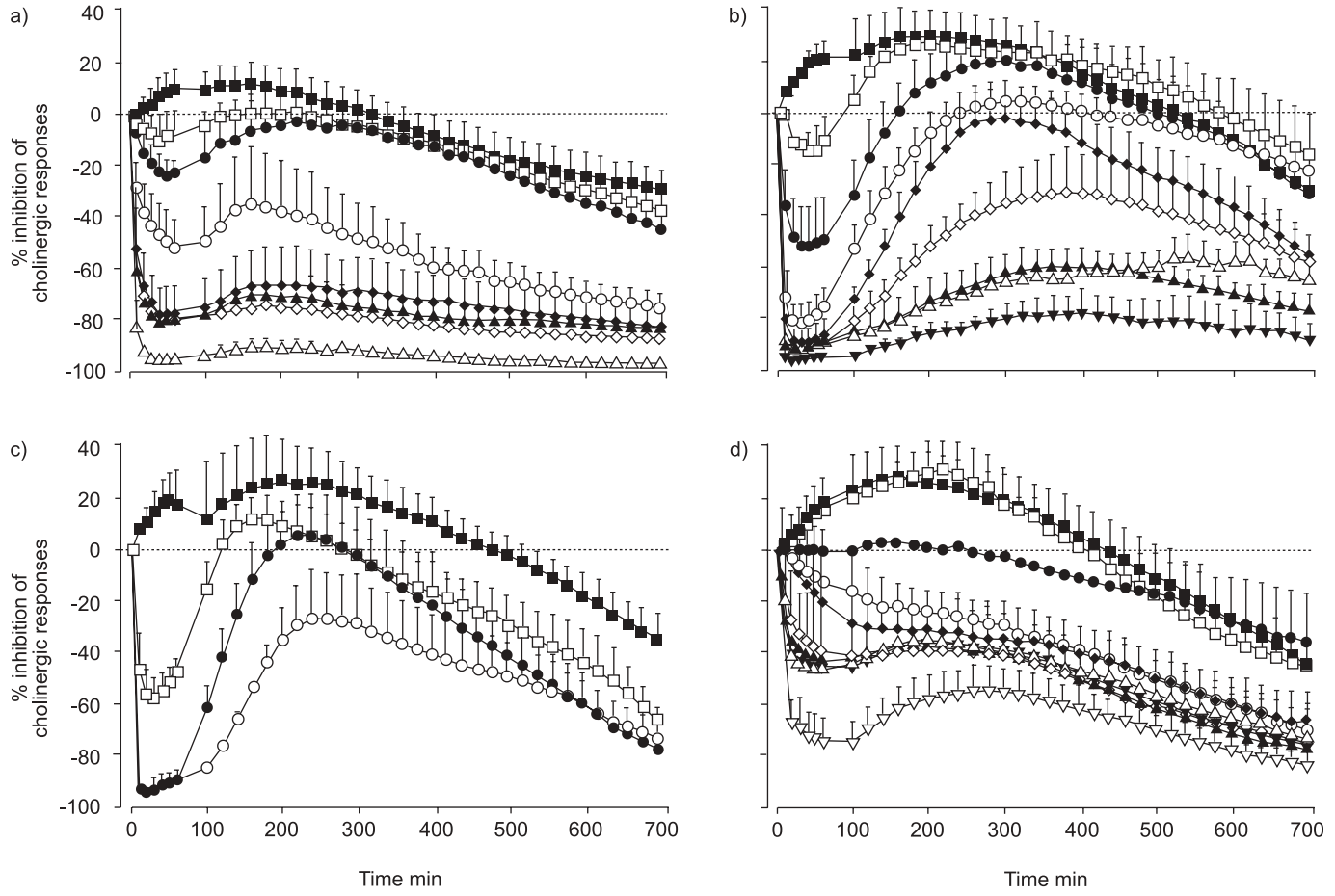

FIGURE 4. Effect of a) indacaterol (n=5; ■: stimulation control; □: 10^{-8} M ●: 3×10^{-8} M; ○: 10^{-7} M; ◆: 3×10^{-7} M; ◇: 10^{-6} M; ▲: 3×10^{-6} M; △: 10^{-5} M); b) formoterol (n=7; ■: stimulation control; □: 3×10^{-10} M; ●: 10^{-9} M; ○: 3×10^{-9} M; ◆: 10^{-8} M; ◇: 3×10^{-8} M; ▲: 10^{-7} M; △: 3×10^{-7} M; ▼: 10^{-6} M); c) salbutamol (n=3; ■: stimulation control; □: 10^{-6} M; ●: 10^{-5} M; ○: 10^{-4} M) and d) salmeterol (n=6; ■: stimulation control; □: 10^{-9} M; ●: 3×10^{-9} M; ○: 10^{-8} M; ◆: 3×10^{-8} M; ◇: 10^{-7} M; ▲: 3×10^{-7} M; △: 10^{-6} M; ▼: 3×10^{-6} M; ▽: 10^{-5} M) on electrical field stimulation (EFS)-induced contraction of isolated human bronchi. Data are shown as % of initial EFS contraction and expressed as mean + SEM.

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.

TABLE 3 Potency (-logIC50) and duration of action determined after electrical field stimulation

Drug	Subjects n	-logIC50	Duration of action	
			Concentration M	Time
Indacaterol	5	6.96±0.13#	1×10^{-7}	>12 h
Formoterol	4	8.96±0.18¶	1×10^{-9}	35.3±8.8 min
Salbutamol	3	6.39±0.26#	1×10^{-6}	14.6±3.7 min
Salmeterol	5	7.18±0.34+	1×10^{-7}	>12 h

Data are expressed as mean±SEM, unless otherwise stated. Duration of action was calculated as the time for response to reach 50% of the maximal relaxation. #: p<0.001 *versus* formoterol; ¶: p<0.01 *versus* salmeterol; +: p<0.01 *versus* formoterol.

Although in the present 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 previously reported 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 males almost certainly lies within the high local concentrations achieved after inhalation and the interaction with the membrane lipid bilayer as a key component [27]. The present model of EFS-induced contraction does not allow a study for >12 h. Indeed, even under control conditions, a 30–50% loss in bronchial cholinergic response was observed 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 ~100% relaxation, with this level being maintained for ⩽12 h, whereas salmeterol induced a maximal relaxation of 80% and demonstrated a loss of ~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 antagonise the effect of rescue medication. All these characteristics, which 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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