In Vitro and in Vivo Pharmacological Characterization of 5-[(R)-2-(5,6-Diethyl-indan-2-ylamino)-1-hydroxy-ethyl]-8-hydroxy-1H-quinolin-2-one (Indacaterol), a Novel Inhaled \( \beta_2 \) Adrenoceptor Agonist with a 24-h Duration of Action


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ABSTRACT

Here, we describe the preclinical pharmacological profile of 5-[(R)-2-(5,6-diethyl-indan-2-ylamino)-1-hydroxy-ethyl]-8-hydroxy-1H-quinolin-2-one (indacaterol), a novel, chirally pure inhaled \( \beta_2 \) adrenoceptor agonist, in comparison with marketed drugs. Indacaterol is close to a full agonist at the human \( \beta_2 \) adrenoceptor (\( E_{\text{max}} \) of 73 ± 1% of the maximal effect of isoproterenol; pEC\(_{50}\) of 8.06 ± 0.02), whereas salmeterol displays only partial efficacy (38 ± 1%). The functional selectivity profile of indacaterol over human \( \alpha_1 \), \( \beta_3 \) and \( \beta_1 \) adrenoceptors is similar to that of formoterol, whereas its \( \beta_2 \) adrenoceptor selectivity profile is similar to that of formoterol and salbutamol. In isolated superfused guinea pig trachea, indacaterol has a fast onset of action (30 ± 4 min) similar to formoterol and salbutamol, and a long duration of action (529 ± 99 min) comparable with salmeterol. In the conscious guinea pig, when given intratracheally as a dry powder, indacaterol inhibits 5-hydroxytryptamine-induced bronchoconstriction for at least 24 h, whereas salmeterol, formoterol, and salbutamol have durations of action of 12, 4, and 2 h, respectively. When given via nebulization to anesthetized rhesus monkeys, all of the compounds dose-dependently inhibit methacholine-induced bronchoconstriction, although indacaterol produces the most prolonged bronchoprotective effect and induces the lowest increase in heart rate for a similar degree of antibronchoconstrictor activity. In conclusion, the preclinical profile of indacaterol suggests that this compound has a superior duration of action compatible with once-daily dosing in human, together with a fast onset of action and an improved cardiovascular safety profile over marketed inhaled \( \beta_2 \) adrenoceptor agonists.

Agents that act as agonists of the \( \beta_2 \) adrenoceptor are effective in the management of asthma and chronic obstructive pulmonary disease (COPD), primarily through their bronchodilatating properties. These drugs induce bronchodilatation by causing direct relaxation of airway smooth muscle through activation of adenylate cyclase, which in turn increases intracellular cAMP levels.

Salbutamol is an inhaled \( \beta_2 \) adrenoceptor agonist that provides rapid bronchodilatation and has been widely used over the past 30 years. However, its major drawback is its short duration of action (4–6 h), requiring the drug to be administered several times a day. Two longer acting inhaled \( \beta_2 \) adrenoceptor agonists, formoterol and salmeterol, are now available and are used in the management of asthma and COPD (Sutherland, 2004). These two drugs have a bronchodilating effect lasting for 12 h after a single inhalation and are therefore given twice daily. Despite the decrease in dosing frequency with the longer acting inhaled \( \beta_2 \) adrenoceptor agonists, patient compliance is still an issue (Ying et al., 1999). In addition, the recent launch of tiotropium bromide, a once-daily inhaled muscarinic antagonist for the treatment of COPD (Gross, 2004), and the development of once-daily inhaled corticosteroids for the treatment of asthma (Dent, 2002; Wardlaw et al., 2004), would suggest that a new inhaled \( \beta_2 \) adrenoceptor agonist with a duration of action compatible with once-daily administration is likely to become the future bronchodilator of choice, either on its own or when used with a once-daily muscarinic antagonist in COPD and when used with a once-daily inhaled corticosteroid for the treatment of asthma.

ABBRIDATIONS: COPD, chronic obstructive pulmonary disease; 5-HT, 5-hydroxytryptamine.
Systemic exposure to $\beta_2$ adrenoceptor agonists results in predictable adverse events such as tremor, palpitation, headache, muscle cramps, and hypokalemia. These adverse events are minimized when these drugs are given by inhalation; salbutamol, salmeterol, and formoterol are perceived to be safe at the recommended clinical doses. However, the therapeutic margin of these drugs is rather small, such that doubling the dose of, e.g., salmeterol and formoterol, can result in a significant increase in the incidence of side effects (Guan et al., 2000; Sovani et al., 2004). Furthermore, such undesirable side effects have been linked to reduced compliance to treatment (White and Sander, 1999). Therefore, a higher therapeutic margin would be desirable for the new generation of inhaled $\beta_2$ adrenoceptor agonists.

$5\{[(R)-2\{5,6\text{-Diethyl-indan-2-ylamino\}-1\text{-hydroxy-ethyl\}}\}-8\text{-hydroxy-1H-quinolin-2-one (indacaterol) (Fig. 1), previously known as QAB149, is a novel, chirally pure inhaled $\beta_2$ adrenoceptor agonist that was discovered in a program to identify compounds with a duration of action compatible with once-daily dosing in human, combined with a fast onset of action and an increased therapeutic index compared with the available inhaled $\beta_2$ adrenoceptor agonists. Here, we report the preclinical in vitro and in vivo pharmacological characteristics of indacaterol and compare the properties of this compound to those of the marketed inhaled $\beta_2$ adrenoceptor agonists salbutamol, formoterol, and salmeterol (Fig. 1).

Materials and Methods

Materials. Indacaterol maleate or hydrochloride and formoterol fumarate were synthesized by the Department of Chemistry (Novartis, Horsham, UK). Salbutamol hemisulfate and isoprenaline were purchased from Sigma Chemical (Poole, Dorset, UK). Salmeterol was synthesized or isolated from clinical dosage forms by the Department of Chemistry (Novartis) or purchased from Tocris Cookson Inc. (Bristol, UK). The xinafoate salt form for salmeterol was isolated from a Hanks’ buffered saline solution containing 0.1% bovine serum albumin, pH 7.4 (assay buffer), in the presence of 100 μM guanosine 5’-triphosphate to ensure monophasic binding curves. For saturation binding experiments, up to 600 pM $^{[125]}$Iodo(-)-cyanopindolol was used for $\beta_1$ and $\beta_2$ and up to 1600 pM for $\beta_3$. Nonspecific binding was determined in the presence of 10 μM alprenolol. For competition binding, $^{[125]}$Iodo(-)-cyanopindolol was used at 10 pM for $\beta_1$ and $\beta_2$ and 120 pM for $\beta_3$. Membranes were incubated for 3 h at 22°C, filtered through Millipore GF/B filters [containing 0.5% (w/v) polyethyleneimine per well] using ice-cold wash buffer (75 mM Tris; pH 7.4).

Increases in intracellular cAMP levels were determined using AlphaScreen technology (Packard, Shelton, CT). Cells were harvested and transferred to 96-well OptiPlates (PerkinElmer Life and Analytical Sciences) at a concentration of $4 \times 10^4$ cells/well, before incubation with agonist or vehicle for 1 h at room temperature in Hanks’ buffered saline solution supplemented with 5 mM HEPES and 0.1% bovine serum albumin. Cells were lysed using AlphaScreen reagents, and after an additional 1 h, the plate was read on a Packard Fusion plate reader. The concentration of cAMP in the samples was calculated from a standard curve.

Animals. Guinea pigs were housed in plastic cages in air-conditioned rooms at 24°C in a 12-h light/dark cycle. Food and water were available ad libitum. All animals were acclimatized for a period of at least 7 days upon arrival before any experimental work began. The studies reported here conform to the UK Animals (Scientific Procedures) Act 1986.

A group of 17 male rhesus monkeys (Centre for Primatology, University of Strasbourg, Strasbourg, France) was used. All animals were kept in colonies in a specialized primate unit, and the interval

![Fig. 1. Chemical structure of indacaterol, formoterol, salmeterol, and salbutamol.](Image)
between experiments was a minimum of 21 days. All experiments were carried out with the approval of the Veterinary Authority of the City of Basel (Kantonales Veterinaeramt, Basel-Stadt, Switzerland).

**Isolated Guinea Pig Organ Preparation.** Male Dunkin-Hartley guinea pigs (350–700 g; Charles River, Margate, UK) were killed by exposure to an increasing concentration of CO2; their trachea and heart were removed and placed in ice-cold oxygenated (95% O2, 5% CO2) Krebs-Henseleit solution containing 2.8 mM glucose, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, and 1.2 mM Na2PO4.

The method used for the electrically stimulated tracheal strip is a modification of a previously published technique (Coleman and Nials, 1989). The trachea was cut into rings of three to five cartilage bands in width, which were subsequently opened into strips by cutting the cartilage opposite the smooth muscle band at 90° to each ring. Each strip was set up in a superfusion system and attached to an isometric force transducer (Fort 10; WPI, Stevenage, Hertfordshire, UK) under a resting tension of 1 g and superfused (1.2 ml/min) with oxygenated Krebs-Henseleit solution at 37°C containing 2.8 μM indoethacain. After a 1-h equilibration period, phasic contractile responses were induced by electrical stimulation with 10-s trains of square-wave pulses of 5-Hz frequency and 0.1 ms duration every 2 min. A supramaximal voltage was selected for each tissue within 10 to 15% of the maximal response following construction of a voltage-response curve from 8 to 16 V. After a 1-h equilibration period using the above-mentioned stimulation parameters, a baseline contractile response was determined over a 20-min period before the superfusion fluid was changed to Krebs-Henseleit solution containing compounds for 30 min. After this time, the superfusion fluid was changed back to compound-free Krebs-Henseleit solution for the remaining 11.5 h of the experiment.

The left atrium was dissected, mounted between two parallel electrodes (Radnoti Glass, Monovia, CA) in 25-ml organ baths containing oxygenated Krebs-Henseleit solution at 32°C, and attached to an isometric force transducer (Fort 10; WPI) under a resting tension of 1 g. Atria were electrically stimulated with square-wave pulses of 3-Hz frequency and 2.5 ms duration. The voltage selected for each atrium was that of threshold plus 30% (threshold is defined as the voltage required to induce minimal changes in cardiac force). After a 1-h equilibration period, the viability of each preparation was determined by an increase in force of contraction upon the addition of 100 nM isoprenaline to the bath. After washout of isoprenaline and recovery to a stable baseline, cumulative concentration-response curves were constructed to compounds.

Isoprenaline was dissolved in distilled water containing 100 μM ascorbic acid. All other compounds were dissolved in dimethyl sulfoxide, and working solutions were made up in Krebs-Henseleit solution. For both preparations, tension changes were monitored and recorded with Buxco preamplifiers and Buxsis XA software (Buxco Europe Limited, Winchester, UK).

**In Vivo Duration of Action and Tachyphylaxis Studies in the Guinea Pig.** Male Dunkin-Hartley guinea pigs (450–500 g; supplied by Harlan, Horst, The Netherlands) were placed in a plethysmograph chamber (Buxco, Wilmington, NC) and left to aclimatize for 5 min. Bias flow (air removed from chamber) was set at 2.5 l/min, the nebulizer air flow was set at 8 l/min, and the trickle flow (the flow through nebulizer chamber) was set at 1.2 l/min. Four milliliters of nebulant was placed in the nebulizer. There were four chambers attached to each nebulizer. The guinea pigs were then challenged with aerosolized saline (0.9% NaCl) for 1 min using an ultrasonic nebulizer (deVilbiss, Wollaston, West Midlands, UK) to obtain the airway baseline reading. This was followed by aerosolized 5-hydroxytryptamine (5-HT) (175 μg/ml for 1 min) to determine the guinea pig’s sensitivity to the spasmogen (pretreatment control). Each 1-min challenge was followed by a 15-min lung function measurement to determine Penh (area under the curve). The dose of 5-HT was determined from a dose-response curve in a previous study (data not shown) to give a reproducible submaximal bronchoconstrictive response. The pressure waveforms generated by respiration of the guinea pigs were analyzed by Buxco software, and enhanced pause (Penh) was calculated on a breath-by-breath basis as described previously (Chong et al., 1998). The following day animals were anesthetized with a short-acting anesthetic (4% halothane in oxygen/nitrous oxide) for 4 min and were dosed with compounds or vehicle (lactose) by dry-powder intratracheal instillation. Before use, the compounds (indacaterol maleate, formoterol fumarate, salbutamol hemisulfate, and salmeterol xinafate) were blended with lactose, using a bore milling technique. Each intratracheal dose was 5 mg of compound blend or lactose. The animals were then rechallenged with saline and 5-HT (175 μg/ml for 1 min) 2, 4, 6, 9, 12, 18, and 24 h after treatment.

Once the dose-response and duration of action of each compound had been determined, the tachyphylaxis study was performed. In a series of experiments, male Dunkin-Hartley guinea pigs (500–550 g) were challenged with aerosolized saline followed by aerosolized 5-HT; Penh was recorded as described above (predose control). From days 1 to 5, the same animals were anesthetized and dosed daily with compounds or vehicle as described above. The animals were challenged with saline and 5-HT 2 h after treatment on days 1 and 5. The number of repeated treatments was based on clinical studies demonstrating that tolerance to the bronchoconstrictive effect of salmeterol was apparent after two doses and persisted up to the seventh dose (Kalra et al., 1996).

**Bronchoprotection and Cardiovascular Side Effects in the Anesthetized Rhesus Monkey.** All experimental details have been described previously (Fozard and Buescher, 2001). In brief, cardiovascular and respiratory parameters were measured in anesthetized male rhesus monkeys at rest and after exposure to a submaximal dose of methacholine given by aerosol. Forty minutes later, aerosolized salmeterol β2-adrenoceptor agonist or vehicle was administered for 10 min, and airway resistance changes (induced by aerosolized methacholine), cardiovascular parameters, and serum potassium concentrations were measured at regular intervals for up to 285 min thereafter. The administered dose was calculated for each animal, and the mean dose for each group was determined. Salbutamol hemisulfate and formoterol fumarate were dissolved in 0.9% NaCl (saline). Indacaterol hydrochloride was dissolved in saline containing 0.03% ethanol. Salmeterol free-base was suspended in saline containing 1 mg/ml gum arabic. In vehicle-treated animals (n = 32), heart rate, serum potassium concentrations, and bronchoconstrictor responses to methacholine were not significantly affected.

**Data Analysis.** K_{50} values for [125I]iodo-(-)-cyanopindolol and ligand IC_{50} values were calculated with a nonlinear curve fit in Prism version 4.02 (GraphPad Software Inc., San Diego, CA). Ligand IC_{50} values were transformed to K{50} values (Cheng and Prusoff, 1973). The cAMP concentration-response data were fitted to a sigmoidal curve (slope not fixed) in Prism.

In the guinea pig isolated tracheal strip preparation, the onset of action for each concentration of compound was taken as the time from the start of compound superfusion until maximal inhibition of contraction to electrical stimulation was observed. The duration of action was defined as the time taken from the end of compound superfusion to 50% recovery from maximal inhibition. The percentage maximal inhibition at each concentration of a given compound was used to construct a concentration-response curve to calculate potency in Prism. For the isolated atria preparation, increases in force of contraction to each concentration of agonist were expressed as the percentage of increase from baseline contraction.

For the in vivo guinea pig experiments, percentage of inhibition was calculated for each compound-treated animal versus vehicle- and time-matched animals. For the duration of action studies, analysis of variance was calculated followed by a one-sided Dunnett’s multiple comparison test versus the time-matched vehicle control. Significance was determined as p < 0.05. For the tachyphylaxis studies, differences between a single treatment (day 1) and daily treatment for 5 days (day 5) were determined using the Mann-Whitney rank sum test.
For the rhesus monkey experiments, percentage of inhibition of bronchoconstriction or percentage of increase in heart rate was calculated for each compound-treated animal related to vehicle- and time-matched animals; statistical significance ($p < 0.05$) was determined using a Student’s $t$ test for unpaired observations.

## Results

### In Vitro Binding and Functional Activity at the Human Adrenoceptors

The receptor density on the three stably transfected clones and the dissociation constant of $[^{125}I]$iodo-($\pm$)-cyanopindolol were measured using saturation experiments. $B_{\text{max}}$ values (in femtomoles per milligram of protein) of $334 \pm 51$, $356 \pm 22$, and $435 \pm 40$ and $K_d$ values (in picomolar) of $33 \pm 5$, $20 \pm 5$, and $301 \pm 35$ were found for the $\beta_1$ ($n = 4$), $\beta_2$ ($n = 4$), and $\beta_3$ ($n = 5$) adrenoceptors, respectively.

The binding affinity of the $\beta_2$ adrenoceptor agonists for the $\beta_2$ adrenoceptor, as calculated by their dissociation constants, was as follows: salmeterol $>$ formoterol $>$ indacaterol $>$ salbutamol (Table 1). All of the compounds bound to the other two adrenoceptors with various levels of selectivity. Salmeterol was the most selective compound followed by formoterol, indacaterol, and salbutamol (Table 1).

The functional potency and intrinsic efficacy of the compounds was studied by measuring cAMP production in cells stably transfected with human $\beta_2$ adrenoceptors. The order of functional potency for the compounds was the same as their binding affinity for the $\beta_2$ adrenoceptor (Table 2). However, a clear difference was observed in their intrinsic activity at the $\beta_2$ adrenoceptor. Salmeterol and salbutamol induced approximately 40% of the maximal effect of isoprenaline, whereas the maximum effect of indacaterol and formoterol was 73 and 90%, respectively, of the maximal response to isoprenaline (Fig. 2; Table 2). Salmeterol and salbutamol had no agonistic activity at the $\beta_1$ adrenoceptor, whereas formoterol and indacaterol were very weak $\beta_1$ agonists. With the exception of salmeterol, all of the compounds were full agonists at the $\beta_3$ adrenoceptor; $\beta_3$ versus $\beta_2$ selectivity was similar for indacaterol, formoterol, and salbutamol; and salmeterol had the best $\beta_2/\beta_3$ selectivity profile (Fig. 2; Table 2).

### In Vitro Functional Activity in the Isolated Guinea Pig Organs

In the isolated guinea pig trachea (a $\beta_2$ adrenoceptor-containing preparation), all of the compounds inhibited electrically induced contraction in a concentration-dependent manner. Formoterol was the most potent compound, with a pEC$_{50}$ value of 9.13 $\pm$ 0.12. Indacaterol (pEC$_{50}$ = 8.23 $\pm$ 0.13) had similar potency to salmeterol (pEC$_{50}$ = 8.56 $\pm$ 0.16); salbutamol was of lower potency (pEC$_{50}$ = 7.82 $\pm$ 0.32) (Fig. 3). The onset and duration of action of each of the compounds were derived using the concentration closest to the pEC$_{50}$ values calculated from the concentration-response curves: 1 nM for formoterol ($n = 4$), 3 nM for indacaterol ($n = 5$) and salmeterol ($n = 5$), and 10 nM for salbutamol ($n = 5$). The onset was fast for indacaterol (30 $\pm$ 4 min), formoterol (32 $\pm$ 1 min), and salbutamol (28 $\pm$ 3 min), whereas it was much slower for salmeterol (169 $\pm$ 32 min). The durations of action of indacaterol (529 $\pm$ 99 min) and salmeterol (475 $\pm$ 130 min) were greater than they were for formoterol (158 $\pm$ 30 min) or salbutamol (22 $\pm$ 9 min) (Fig. 4). The inhibitory effect of the four $\beta_2$ adrenoceptor agonists was reversed by the $\beta$ adrenoceptor antagonists propranolol (0.1 $\mu$M) and sotalol (10 $\mu$M) (data not shown).

In the isolated guinea pig left atrium (a $\beta_1$ adrenoceptor-containing preparation), all of the compounds induced a concentration-dependent negative inotropic effect (Fig. 3). The maximal efficacy observed at the highest concentration tested (1 $\mu$M) in percentage increase from baseline was 163 $\pm$ 28, 75 $\pm$ 25, 54 $\pm$ 13, and 46 $\pm$ 4% for formoterol, indacaterol, salbutamol, and isoprenaline, respectively.

### Table 1

Dissociation constants for the $\beta_2$ adrenoceptor agonists at the human adrenoceptors

Data are expressed as mean $\pm$ S.E.M. of the number of experiment indicated in parentheses.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>$\mu_1$</th>
<th>$\mu_2$</th>
<th>$\mu_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indacaterol</td>
<td>6.21 $\pm$ 0.12 (5)</td>
<td>7.36 $\pm$ 0.06 (5)</td>
<td>5.48 $\pm$ 0.14 (4)</td>
</tr>
<tr>
<td>Formoterol</td>
<td>6.12 $\pm$ 0.09 (5)</td>
<td>7.84 $\pm$ 0.05 (3)</td>
<td>5.49 $\pm$ 0.08 (3)</td>
</tr>
<tr>
<td>Salmeterol</td>
<td>6.11 $\pm$ 0.09 (5)</td>
<td>9.19 $\pm$ 0.12 (4)</td>
<td>5.58 $\pm$ 0.05 (4)</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>5.39 $\pm$ 0.06 (5)</td>
<td>6.12 $\pm$ 0.09 (5)</td>
<td>4.62 $\pm$ 0.09 (3)</td>
</tr>
</tbody>
</table>

### Table 2

Functional properties for the $\beta_2$ adrenoceptor agonists at the human $\beta_2$ adrenoceptors

Data are expressed as mean $\pm$ S.E.M. of $n$ different experiments.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>$n$</th>
<th>pEC$_{50}$</th>
<th>$E_{\text{max} %}$ isoprenaline</th>
<th>$n$</th>
<th>pEC$_{50}$</th>
<th>$E_{\text{max} %}$ isoprenaline</th>
<th>$n$</th>
<th>pEC$_{50}$</th>
<th>$E_{\text{max} %}$ isoprenaline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoprenaline</td>
<td>12</td>
<td>7.46 $\pm$ 0.05</td>
<td>99 $\pm$ 2</td>
<td>10</td>
<td>7.22 $\pm$ 0.01</td>
<td>98 $\pm$ 1</td>
<td>8</td>
<td>7.91 $\pm$ 0.07</td>
<td>99 $\pm$ 2</td>
</tr>
<tr>
<td>Indacaterol</td>
<td>5</td>
<td>6.60 $\pm$ 0.24</td>
<td>16 $\pm$ 2</td>
<td>5</td>
<td>8.06 $\pm$ 0.02</td>
<td>73 $\pm$ 1</td>
<td>4</td>
<td>6.72 $\pm$ 0.13</td>
<td>113 $\pm$ 7</td>
</tr>
<tr>
<td>Formoterol</td>
<td>5</td>
<td>6.96 $\pm$ 0.12</td>
<td>29 $\pm$ 2</td>
<td>5</td>
<td>8.58 $\pm$ 0.02</td>
<td>90 $\pm$ 1</td>
<td>4</td>
<td>7.56 $\pm$ 0.14</td>
<td>103.0 $\pm$ 4.7</td>
</tr>
<tr>
<td>Salmeterol</td>
<td>5</td>
<td>7.17 $\pm$ 0.85</td>
<td>-11 $\pm$ 2</td>
<td>5</td>
<td>9.15 $\pm$ 0.05</td>
<td>38 $\pm$ 1</td>
<td>4</td>
<td>6.03 $\pm$ 0.05</td>
<td>59 $\pm$ 2</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>5</td>
<td>5.93 $\pm$ 0.44</td>
<td>-3 $\pm$ 1</td>
<td>5</td>
<td>6.60 $\pm$ 0.02</td>
<td>47 $\pm$ 1</td>
<td>4</td>
<td>5.74 $\pm$ 0.09</td>
<td>99 $\pm$ 6</td>
</tr>
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</table>

**Fig. 2.** Effect of the $\beta_2$ adrenoceptor agonists on intracellular cAMP levels at the human $\beta_1$ (middle), $\beta_2$ (left), and $\beta_3$ (right) adrenoceptors. Data are presented as percentage of maximal isoprenaline-induced cAMP increase. Data are shown as mean $\pm$ S.E.M. of the number of experiments indicated in Table 2.
and salmeterol, respectively. However, at their respective pEC\textsubscript{50} values in the tracheal preparation, the inotropic effect was 17 ± 3, 4 ± 1, 9 ± 1, and 10 ± 2% for formoterol, indacaterol, salbutamol, and salmeterol, respectively.

**Dose Response and Duration of Action in the Conscious Guinea Pig.** Two hours after treatment with indacaterol, salmeterol, and salbutamol, a dose-dependent inhibition of 5-HT-induced bronchoconstriction was observed, with a maximal effect of 85 ± 7, 86 ± 6, and 91 ± 7% for indacaterol (6.7 μg/kg), salmeterol (66.7 μg/kg), and salbutamol (223.2 μg/kg), respectively (Fig. 5). In this experiment, no dose response was observed for formoterol, with all doses being fully effective; the maximal inhibition was 93 ± 3% for a dose of 0.7 μg/kg (Fig. 5). In a different experiment, formoterol pretreatment at lower doses of 0.00007 and 0.0007 μg/kg administered 2 h before 5-HT exposure inhibited bronchoconstriction in a dose-related manner by 15 ± 17 and 36 ± 20%, respectively (n = 8).

All of the compounds demonstrated increasing duration with increasing dose. Using the lowest dose required to produce approximately 80% inhibition of 5-HT-induced bronchoconstriction at the 2-h time point for each compound (6.7 μg/kg indacaterol, 85 ± 7%; 0.007 μg/kg formoterol, 83 ± 11%; 66.7 μg/kg salmeterol, 86 ± 6%; and 223.2 μg/kg salbutamol, 91 ± 7%), the duration of action could be ranked in the following order: indacaterol (24 h) > salmeterol (12 h) > formoterol (4 h) > salbutamol (2 h) (Fig. 5).

When the ability of the compounds to induce tachyphylaxis was evaluated, none of the compounds was subject to desensitization at any of the doses tested (Fig. 6). Indeed, for indacaterol and formoterol, the inhibitory effect of each dose after 5-day treatment compared with that of a single treatment was enhanced and reached significance for the indacaterol dose of 0.006 and 0.6 μg/kg and for the formoterol dose of 0.0006 μg/kg. Such a phenomenon was not observed for salmeterol (Fig. 6).

**Bronchoprotection and Cardiovascular Side Effects in the Anesthetized Rhesus Monkey.** Five minutes after the end of nebulization of the β\textsubscript{2} adrenoceptor agonists, all of the compounds dose-dependently inhibited methacholine-induced bronchoconstriction. At the highest dose tested, indacaterol (12.5 μg/kg), formoterol (1.2 μg/kg), and salbutamol (84 μg/kg) achieved a maximal bronchoprotection of 75 ± 11, 76 ± 4, and 102 ± 5%, respectively, whereas salmeterol was...
less effective, with maximal bronchoprotection of $47 \pm 15\%$ at a dose of 5.5 $\mu$g/kg (Fig. 7A). The calculated ED$_{50}$ values for indacaterol, formoterol, salmeterol, and salbutamol were $1.7 \pm 0.2$, $0.14 \pm 0.02$, $1.6 \pm 0.6$, and $25.7 \pm 1.7$ $\mu$g/kg, respectively. Immediately after application, all of the compounds induced an increase in heart rate with the following order of potency: formoterol $>$ salmeterol $>$ salbutamol $\approx$ indacaterol. Formoterol, salmeterol, and salbutamol gave greater increases in heart rate than indacaterol for an equivalent antibronchoconstrictor activity (Fig. 7A).

In a second set of experiments, the time course of bronchoprotection and changes in heart rate were studied for a single dose of each compound. We selected doses that induced approximately 80% bronchoprotection for indacaterol (12.5 $\mu$g/kg), formoterol (1.2 $\mu$g/kg), and salbutamol (27 $\mu$g/kg). Salmeterol was used at the dose that gave a just maximal bronchoprotective effect (5.5 $\mu$g/kg). Thus, at the 5-min time point, indacaterol, formoterol, and salbutamol had a similar bronchoprotective effect of approximately 70 to 80%, whereas salmeterol was only partially effective (bronchoprotective ef-
fect 47%). Salbutamol was short-acting in this model, with a bronchoprotective effect 95-min postdose not significantly different from control. Indacaterol had a significant bronchoprotective effect up to the last time point studied (275 min), whereas the bronchoprotective effects of salmeterol and formoterol only lasted up to 155 min (Fig. 7B). At the end of the treatment, all of the compounds induced an increase in heart rate. Salmeterol, formoterol, and salbutamol were of higher efficacy than indacaterol. This effect was short lasting for indacaterol, with the heart rate not significantly different from control values from 90 min onward. In contrast, the effect of the other three compounds on heart rate waned slowly and was still significant compared with control animals at the end of the experiment (275 min) (Fig. 7B). At these doses, none of the compounds had an effect on serum potassium, blood pressure, or respiratory rate (data not shown).

Discussion

In this article, we have described the preclinical pharmacological profile of indacaterol, a novel inhaled β₂ adrenoceptor agonist being developed for once-daily use for the treatment of asthma and COPD.

Investigations regarding the binding activity of indacaterol to the human adrenoceptors reveal that this compound has similar binding affinity to the β₂ receptor and binding selectivity ratio over the other adrenoceptors as formoterol. Functional data indicate that indacaterol is a partial agonist with 2-fold higher intrinsic activity than salbutamol or salmeterol. It is accepted that a partial agonist can behave as an antagonist in the presence of an agonist with higher efficacy at the same receptor; in the case of the inhaled β₂ adrenoceptor agonists, such phenomenon could potentially result in an inhibitory action of rescue medicine (i.e., short-acting β₂ agonist such as salbutamol). Such an antagonistic effect has been reported for salmeterol, but not formoterol, a full agonist, in different in vitro systems, including the isolated human bronchi (Molimard et al., 1998). Therefore, based on the higher intrinsic activity of indacaterol compared with salmeterol, one would expect indacaterol to have somewhat lower antagonistic effects at the β₂ adrenoceptor. Indeed, in isolated human bronchi contracted with carbachol, salmeterol, but not indacaterol or formoterol, antagonize the isoprenaline response (Naline et al., 2005).

It is worth noting that the differences in intrinsic activities for the compounds observed at the β₂ adrenoceptor in the cell-based assays do not translate to the isolated superfused guinea pig tracheal preparation. This apparent discrepancy has been observed by other groups and is probably linked to the low magnitude of the contraction induced by the electrical field stimulation in the tracheal preparation (i.e., approximately 20 to 30% of the maximal response to carbachol). Indeed, it has been previously reported that both salmeterol and salbutamol behave as full agonists when tested against tracheal preparation at low tone (i.e., basal tone). If the tone is increased (i.e., precontracted with high concentrations of carbachol), then both salmeterol and salbutamol behave as partial agonists, whereas a full agonist, such as isoprenaline or formoterol, still has full efficacy (Lipworth and Grove, 1997).

Like formoterol, indacaterol is a low-partial agonist at the human β₂ adrenoceptor. It also has similar selectivity ratio to formoterol and salbutamol at the human β₂ adrenoceptor. It is always difficult to extrapolate the physiological activity of
a compound based only on its receptor pharmacology profile in vitro, and an in vivo evaluation is always more relevant. In this respect, regardless of the in vitro selectivity profile of indacaterol, we have demonstrated in the rhesus monkey that, for an equivalent degree of bronchoprotection, indacaterol has a better cardiac safety profile than formoterol, salmeterol, and salbutamol. In humans, it has been reported that activity at the $\beta_1$ adrenoceptor might be responsible for some of the cardiovascular side effects often observed with $\beta_2$ adrenoceptor agonists (Levine and Leenen, 1989). This suggests that $\beta_2$ selective compounds could provide an improved side effect profile. However, despite having the best selectivity profile for $\beta_2$ over $\beta_1$ adrenoceptors, salmeterol does have cardiovascular side effects in human, suggesting that the importance of $\beta_1/\beta_2$ selectivity is unclear in the clinic (Motomura et al., 1990). It is possible that these side effects could result from activating cardiac or systemic $\beta_2$ adrenoceptors. Indeed, tachycardia may result from dilatation of peripheral vasculature resulting in reflex sympathetic nervous system stimulation, thereby increasing inotropic and chronotropic effects. In addition, activation of $\beta_2$ adrenoceptors in both the left ventricle and the right atrium can directly increase heart rate. Therefore, even a “pure” $\beta_2$ agonist would be expected to have the potential for direct cardiovascular side effects (Motomura et al., 1990). In view of all of these potential mechanisms for generating cardiovascular side effects, it is important that the good overall cardiovascular side effect profile of indacaterol in the rhesus monkey has also been observed in clinical studies, both in asthma (Beeh et al., 2005; Chuchalin et al., 2005; Kanniess et al., 2005; Tarral et al., 2005) and COPD (Auibier et al., 2005; Beier et al., 2005). The physiological role of the $\beta_2$ adrenoceptor is still not firmly established, although it is thought to be involved in lipolysis (Philipson, 1990) and possibly in cardiac and vascular contractility (Gauthier et al., 2000). As a result, it is difficult to draw firm conclusions regarding the functional significance of the $\beta_3$ agonistic activity observed for all of the compounds in this study.

Although currently available inhaled $\beta_2$ adrenoceptor agonists are considered to be safe at their recommended therapeutic doses, they all exhibit a modest therapeutic window. To assess the therapeutic window for indacaterol, a comparison was made with the marketed $\beta_2$ adrenoceptor agonists in the rhesus monkey by evaluating the antibronchoconstrictive effect against aerosolized methacholine and the potential for systemic side effects as measured by increases in heart rate. Indacaterol, formoterol, and salbutamol are all able to inhibit methacholine-induced bronchoconstriction by more than 80%. In contrast, the maximal inhibition seen with salmeterol is 47%. The lower intrinsic activity of salmeterol against muscarinic agonist-induced airway contraction is a known phenomenon and has been described several times, notably in isolated human airways (Molimard et al., 1998). This has been attributed to a functional antagonism between muscarinic agonists and $\beta_2$ adrenoceptor agonists, probably because of the inhibition of adenylate cyclase by the muscarinic M2 receptor (Sarria et al., 2002). Regarding the increase in heart rate induced by the compounds, our results suggest that formoterol, salmeterol, and salbutamol would have a similar tendency to induce systemic side effects, whereas indacaterol would have a better therapeutic window. Clinical studies have shown that the side-effect profile of formoterol, salmeterol, and salbutamol are qualitatively similar (Bennett et al., 1994; Guhan et al., 2000; Rabe, 2001). Although a direct comparison of the clinical safety profile of indacaterol with the profiles of marketed inhaled $\beta_2$ adrenoceptor agonists is not yet available, indacaterol has demonstrated a good cardiovascular safety profile at a dose of up to 800 $\mu$g in patients with asthma (Tarral et al., 2005) or COPD (Auibier et al., 2005; Beier et al., 2005), whereas indacaterol doses as low as 200 $\mu$g once daily provide sustained 24-h bronchodilation (Beeh et al., 2005; Chuchalin et al., 2005; Kanniess et al., 2005).

In the guinea pig tracheal preparation, indacaterol has a fast onset of action similar that of salbutamol and formoterol. This is in contrast to the much slower onset observed with salmeterol. In the clinical situation, it is recognized that salbutamol and formoterol have fast onsets of action, whereas salmeterol has a slower onset (Wegener et al., 1992; van Noord et al., 1996). Therefore, the data generated in the tracheal preparation strongly suggest that, in a clinical setting, indacaterol will have a fast onset of action. This has been confirmed in a number of clinical studies (Beeh et al., 2005; Kanniess et al., 2005; Tarral et al., 2005). There are several potential advantages for a fast-acting inhaled $\beta_2$ adrenoceptor agonist. Because patients would experience rapid relief of symptoms, they will be confident that they have inhaled the drug properly; this could also reduce the risk of accidental over dosage through repeated dosing. In addition, a fast-acting compound has the potential to be used as rescue medication. Indeed, although formoterol and salmeterol are both approved as maintenance therapy for asthma, only formoterol has been approved as rescue medicine in the European Union (Lotvall, 2002).

Both in the in vitro preparation and in vivo in the guinea pig, the duration of action of salmeterol and salbutamol is in line with their known clinical profile. Although the duration of action of formoterol observed in both systems does not reflect the clinical situation, this discrepancy has been reported previously (Nials et al., 1993, 1994). A number of theories have been put forward to explain this inconsistency, and the most rational explanation for the observed duration of action of formoterol in human almost certainly lies within the high local concentrations achieved after inhalation and the interaction with the membrane lipid bilayer as a key component (Anderson et al., 1994). Our in vivo results would support this hypothesis, since an 18-h duration of action for this compound, in the guinea pig, is demonstrated when given at a higher dose. Although for all the compounds the relative potencies from the isolated tracheal preparation were carried over quantitatively to the in vivo guinea pig studies, this was not true for formoterol. Formoterol was approximately 10-fold more potent that the other compounds in the isolated trachea but approximately 1000-fold more potent in the in vivo guinea pig studies. This discrepancy is likely due to the physicochemical properties of the compounds and the route of delivery. In our early studies, when the compounds were given to the guinea pig by nebulization instead of dry powder, the relative potency of formoterol was much more in accord with the tissue data (i.e., approximately 10-fold more potent than indacaterol; data not shown). In line with this hypothesis, it should be noted that a similar 10-fold difference in the relative potency for formoterol over inda-
caterol and salmeterol was also observed in the rhesus monkey, where the compounds were given by nebulization.

In the case of indacaterol, the results obtained in both systems predict a duration of action in human of at least 24 h. Indeed, a range of studies have shown that, in patients with asthma (Beeh et al., 2005; Chuchalin et al., 2005; Kannies et al., 2005) or COPD (Aubier et al., 2005; Beier et al., 2005), indacaterol has a 24-h duration of action on once-daily dosing. Once-daily inhaled steroids (Dent, 2002; Wardlaw et al., 2004) and once-daily inhaled muscarinic antagonists (Gross, 2004) are now available; currently marketed long-acting indol-β2 adrenoceptor agonists have to be given twice a day. A once-daily β2 adrenoceptor agonist, such as indacaterol, has the potential to be given in combination with either a once-daily corticosteroid or a once-daily muscarinic antagonist or both. In addition to being more convenient to patients, this combination could also provide benefits in terms of improvements in lung function, exercise performance, and health status (Stoloff et al., 2002; Tennant et al., 2003).

Some clinical studies have noted a reduction in the bronchoprotective effect of β2 adrenoceptor agonists with regular treatment (Larj and Bleecker, 2002). The extent of desensitization is generally accepted to be dose-dependent for a given agonist and efficacy-dependent compared with other agonists. Since our studies demonstrate that indacaterol has a higher intrinsic efficacy than salmeterol, an assessment of the desensitization potential of this compound was made. The data from this study suggest that indacaterol, formoterol, and salmeterol do not induce any desensitization following 5-day treatment in the guinea pig. These results were further confirmed with those from clinical studies in patients with asthma (Chuchalin et al., 2005) or COPD (Beier et al., 2005), in which once-daily dosing with indacaterol provides 24-h bronchodilation with no loss of efficacy after 28-day therapy.

In the summary, the preclinical profile of indacaterol demonstrates that, as a single enantiomer, this compound combines a long duration of action, compatible with once-daily dosing in human, together with a fast onset of action and an improved cardiovascular safety profile.

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