

The Preclinical Pharmacology of Indacaterol

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Abstract The preclinical pharmacological profile of indacaterol, a novel, chirally pure inhaled beta(2) adrenoceptor agonist, is described in this chapter. In various in vitro systems, indacaterol is close to a full agonist at the human β_2 -adrenoceptor with nanomolar potency. In isolated superfused human and guinea pig trachea, indacaterol has a fast onset of and a long duration of action. In the conscious guinea pig, when given intratracheally as a dry powder, indacaterol inhibits bronchoconstriction for at least 24 h and shows no tachyphylaxis when given for 5 consecutive days. When given via nebulization to anesthetized rhesus monkeys, indacaterol produces a prolonged bronchoprotective effect and induces a small increase in heart rate. In in vitro systems as well as a large cohort of COPD patients, no association could be demonstrated between β_2 -adrenoceptor polymorphisms and indacaterol response. In conclusion, the preclinical profile of indacaterol suggests that this compound has a duration of action compatible with once-daily dosing in human, together with a fast onset of action.

1 Introduction

Indacaterol has been developed to meet the current needs for a long-acting bronchodilator for the maintenance therapy of chronic obstructive pulmonary disease (COPD) [1]. Current bronchodilators for COPD include the β_2 -adrenoceptor agonist and the muscarinic receptor antagonist, both of them being delivered via the inhaled route. The available β_2 -adrenoceptor agonists are either short acting (salbutamol) and used as rescue medicine or compatible with twice-daily dosing

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(formoterol and salmeterol) and used as maintenance therapy. The muscarinic receptor antagonists consist of ipratropium, used as rescue medicine, and the more recently available once-daily drug, tiotropium, used for maintenance therapy. Up to recently, no once-daily β_2 -adrenoceptor agonists were available for the treatment of COPD. The indacaterol research program started in 1998 with the aim of delivering an inhaled β_2 -adrenoceptor agonist that combines high potency and intrinsic efficacy together with a duration of action compatible with once-daily dosing and a fast onset of action.

2 The β_2 -Adrenoceptor

β -Adrenoceptors have been subclassified into three different receptor subtypes called β_1 , β_2 , and β_3 . All three receptors belong to the seven transmembrane receptor family of G protein-coupled receptor and are coupled to the G_s type of G protein. Upon ligand receptor interaction, activation of the G_s protein leads to an increase of intracellular cyclic adenosine monophosphate (cAMP) via activation of adenylate cyclase [2]. In the case of the β_2 -adrenoceptor, one primary consequence of this increase in intracellular cAMP levels is the activation of protein kinase A (PKA), which causes airway smooth muscles to relax by a variety of complementary mechanisms, including activation of potassium channels leading to the efflux of potassium and dephosphorylation of the 20-kDa regulatory light chain of myosin II (MLC₂₀) (Fig. 1). In addition to this classical mechanism, recent evidence suggests that β_2 -adrenoceptor-induced airway smooth muscle relaxation could also be mediated via PKA-independent pathways such as the activation of protein kinase G, the tyrosine kinase Src, and the exchange protein activated by cAMP (Epac) [3].

3 Potency and Intrinsic Efficacy

Potency is pharmacologically defined as the concentration of an agonist needed to produce half of the maximal effect (efficacious concentration₅₀, EC₅₀). Intrinsic efficacy is a measure of how powerfully an agonist can activate a receptor. Compounds that bind to a receptor and are able to produce the maximum possible response in a given system are called full agonist. On the other hand, agonists that bind the receptor but are not able to fully activate the system are called partial agonists. As an example for the β_2 -adrenoceptor system, isoprenaline is a full agonist, whereas salbutamol is a partial agonist (Table 1). It is important to realize that the efficacy of an agonist is highly dependent on the system used (i.e., in a system where the receptors are highly expressed, a partial agonist can behave as a full agonist). Using genetically engineering cells expressing medium level of the

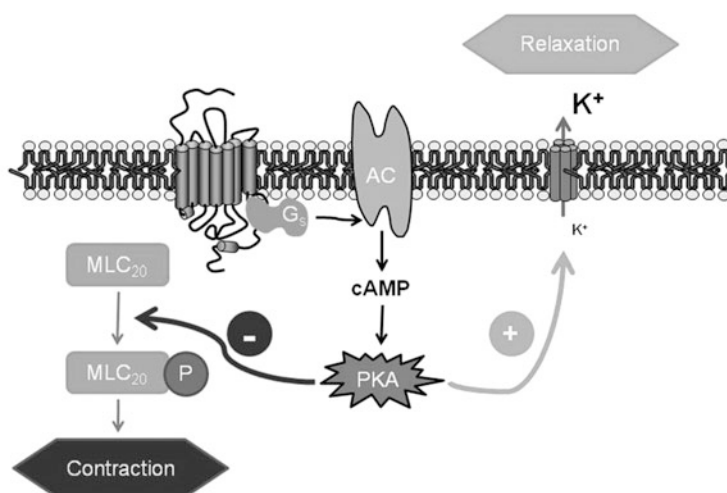


Fig. 1 Mechanism of action of the β_2 -adrenoceptor agonists. AC adenylyl cyclase, cAMP cyclic adenosine monophosphate, G_s G stimulatory protein, MLC20 20-kDa regulatory light chain of myosin II, P phosphate, PKA protein kinase A

β_2 -adrenoceptor, we have shown that salmeterol and salbutamol behave as partial agonists, inducing approximately 40 % of the maximal effect of isoprenaline, whereas the maximum effect of indacaterol and formoterol were 73 and 90 % of the maximal effect of isoprenaline, respectively (Table 1) [4]. Similar ranking of the intrinsic activities for the compounds have been observed in other cells such as human airway bronchial smooth muscle cells [5–7] and human lung mast cells [8].

Desensitization is a protective mechanism that prevents overstimulation of the receptor in the presence of an excess of the agonist. This phenomenon, also known as tachyphylaxis, reduces receptor activity and plays a role in signal duration, intensity, and quality. Desensitization is initiated by phosphorylation of the receptor that is followed by binding to β -arrestin. β -Arrestin serves to sterically inhibit G protein coupling, thereby terminating the G protein activation, and may also target the receptor for internalization [9].

Early studies investigating the desensitization of β_2 -adrenoceptor showed a relationship between agonist efficacy and desensitization, with partial agonists causing less phosphorylation and internalization than full agonists [10, 11]. This suggests that formoterol and indacaterol should elicit a greater degree of tachyphylaxis than salmeterol, but this has not been observed either in preclinical models or in the clinic.

The potential for indacaterol tachyphylaxis, in comparison with salmeterol and formoterol, was studied in the conscious guinea pig by comparing the bronchoprotective effect of the compounds following a single treatment or five-daily treatments. Results demonstrated that no tachyphylaxis was observed for indacaterol, formoterol, and salmeterol administered as dry powder formulations.

Table 1 Functional properties for the marketed inhaled β_2 -adrenoceptor agonists at the human adrenoceptors

					Selectivity ratio	
		β_1	β_2	β_3	β_1/β_2	β_3/β_2
Isoprenaline	Potency (EC ₅₀ , nM)	35	60	12	0.6	0.2
	Intrinsic efficacy (% isoprenaline)	99	98	99	–	–
Indacaterol	Potency (EC ₅₀ , nM)	251	8.7	190	29	22
	Intrinsic efficacy (% isoprenaline)	16	73	113	–	–
Formoterol	Potency (EC ₅₀ , nM)	110	2.6	27	42	10
	Intrinsic efficacy (% isoprenaline)	29	90	103	–	–
Salmeterol	Potency (EC ₅₀ , nM)	67	0.7	933	96	1,333
	Intrinsic efficacy (% isoprenaline)	–11	38	59	–	–
Salbutamol	Potency (EC ₅₀ , nM)	1,175	251	1,820	5	7
	Intrinsic efficacy (% isoprenaline)	–3	47	99	–	–

Data are from [4]

A significant improvement in protection against serotonin-induced bronchoconstriction was demonstrated after 5 days dosing of indacaterol and formoterol compared to a single treatment, but this was not demonstrated for salmeterol [4]. A similar pattern is observed in the clinic. Despite an initial loss of bronchoprotective efficacy with all ligands, their bronchodilator action is much more resilient to tolerance, demonstrating efficacy that is normally stable after the first few days (reviewed in [12]).

It therefore appears that the early *in vitro* studies did not accurately predict the tendency for tachyphylaxis. This is likely because these studies were designed to match receptor occupancy for each of the agonists, regardless of their efficacy. This is important, because low-efficacy agonists must bind to a greater proportion of receptors to elicit a given pharmacological response than higher-efficacy agonists, which often have “spare receptors” [12]. It is therefore more appropriate to compare concentrations based on their magnitude of pharmacological response, analogous to the way clinical dose is chosen. When the tachyphylaxis induced by a series of β_2 -adrenoceptor agonists was compared at equi-effective concentrations, it was found that after 24 h exposure, all of the agonists desensitized the response to a subsequent formoterol challenge to the same degree, irrespective of their intrinsic efficacies [6]. In an effort to simulate the *in vivo* clearance of the drugs, the authors also examined a “pulse” protocol where drugs were washed away after 1 h. Under these conditions, it appeared that the lower-efficacy ligands caused more desensitization, with indacaterol inducing much less tachyphylaxis than salmeterol. This is presumably because the higher-efficacy ligands have a greater “receptor reserve” than lower-efficacy agonists, so they not only require a lower occupancy to generate an equivalent response but also are less sensitive to loss of receptors than low-efficacy ligands (discussed in [12]).

Importantly, these studies demonstrate that using *in vitro* systems to assess the tendency for agonists to cause desensitization in the clinic is highly dependent on the experimental design and of poor predictive value.

4 Receptor Selectivity

β -Adrenoceptors were originally classically identified as the cardiac (β_1), airway smooth muscle (β_2), and adipose tissue (β_3) receptors. However, it is now recognized that they are widely distributed within the human body. Because the targeted receptor for a β_2 -adrenoceptor bronchodilator is the airway smooth muscle and to avoid side effects due to systemic activation of the receptor, the inhaled route is preferred. However, despite the use of the inhaled route and probably because the lung is one of the most vascularized organ, systemic exposure, albeit in low amount, of the compound is inevitable. When entering the circulation, an inhaled β_2 -adrenoceptor agonist would induce a number of undesired responses such as tremor, a direct consequence of activation of the receptor on skeletal muscle [13]; metabolic responses such as hyperglycemia, hypokalemia, and hypomagnesemia [14]; and cardiac effects [15]. Although tremor and the metabolic effects are entirely mediated by the β_2 -adrenoceptor, the cardiac effects are mediated by both the β_1 - and β_2 -adrenoceptors [16]. In addition, it has been reported that activity at the β_1 -adrenoceptor might be responsible for some of the cardiovascular side effects often observed with terbutaline, a β_2 -adrenoceptor agonist [17]. It is therefore thought that a highly selective β_2 -adrenoceptor would have a better cardiac side effect profile when compared to an agonist that has activity at the β_1 -adrenoceptor. At the human adrenoceptors, the marketed long-acting inhaled β_2 -adrenoceptor agonists have different degree of functional selectivity at the β_2 -adrenoceptor when compared with the β_1 -adrenoceptor. As such, salmeterol has no or very weak functional activity on the β_1 -adrenoceptor, whereas formoterol is a weak partial β_1 -adrenoceptor agonist with a selectivity ratio of 42. Indacaterol also behaves as a weak partial β_1 -adrenoceptor agonist with a selectivity ratio of 29 (Table 1). However, despite this lower β_1/β_2 selectivity ratio for indacaterol when compared to salmeterol and formoterol, 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 [4]. This observation has been confirmed in a single-dose clinical study, where a supramaximal dose of indacaterol (1,000 μg) had a better safety profile when compared to a supramaximal dose of salmeterol (250 μg) [18]. All these data suggest that, at least for the cardiac side effects observed with the inhaled β_2 -adrenoceptor, the β_1/β_2 degree of functional selectivity does not play a major role. Indeed, direct activation of the β_2 -adrenoceptor in the human atrium and ventricle caused an increase in contractile force [16]. In addition, tachycardia may result from dilatation of peripheral vasculature, partially mediated by the β_2 -adrenoceptor, resulting in reflex sympathetic nervous system stimulation, thereby increasing inotropic and chronotropic effects [19]. Regarding the activity of the marketed β_2 -adrenoceptor agonists at the β_3 -adrenoceptor, salmeterol is a partial agonist with a selectivity ratio of more than 1,000, whereas formoterol and indacaterol are full agonists with selectivity ratios of 10 and 22, respectively (Table 1). In contrast to the β_1 - and β_2 -adrenoceptors, the β_3 -adrenoceptor has only been recently characterized [20], and

selective ligands have only been recently made available. As a consequence, its physiological role is not entirely clear [21]; it is therefore difficult to assess whether systemic activation of the β_3 -adrenoceptor can induce undesirable side effects. However, the long-term clinical use of formoterol has not reveal potential β_3 -adrenoceptor-mediated side effect.

5 Onset of Action

The onset of action of an inhaled bronchodilator is related to how rapidly, after inhalation, the patient feels that the treatment is effective. As such, in the clinic, salbutamol, formoterol, and indacaterol have been shown to have a fast onset, with an effective bronchodilation that occurs within 2–3 min, whereas salmeterol is slower [22–24]. Although it can be argued that for an inhaled bronchodilator designed for chronic treatment of COPD a fast onset is not relevant, one can see at least two major advantages for a fast-acting bronchodilator when compared to a slow-acting drug: increased treatment adherence and improvement in the ability to perform morning activities. Very few data exist regarding adherence to inhaled medication in COPD [25]. From the available data, on average, about 50 % of patients do not adhere to their inhaled medication [26]. Although the reasons for this lack of adherence are numerous, the patient feeling that the drug is not beneficial for him and therefore doubt about personal need for medication is a major reason [27]. Therefore it is reasonable to assume that with a fast-acting inhaled bronchodilator, symptom relief will be experienced rapidly after inhalation, and thereby patients will feel that the treatment is effective and will continue using it. Impairment in performing early morning activities is particularly problematic for COPD patients [28, 29], and it was recently shown that the fixed dose combination of budesonide/formoterol (Symbicort[®], AstraZeneca), which has a more rapid onset of action than the fixed dose combination of salmeterol/fluticasone (Seretide[®], GlaxoSmithKline), had a greater improvement in the ability for COPD patients to perform morning activities [30].

Indacaterol onset of action has been compared with the marketed inhaled β_2 -adrenoceptor agonists in a number of experimental setups (Table 2). As such, in the electrically stimulated guinea pig tracheal preparation, indacaterol has a fast onset of action similar to that of salbutamol and formoterol. This is in contrast to the much slower onset observed with salmeterol [4]. Similarly, in the isolated human bronchus [31] or in the human small airways using the precision-cut lung slice model [32], indacaterol was characterized as a fast-acting compound with an equivalent onset as formoterol and salbutamol and faster than salmeterol.

Until recently, onset of action of inhaled β_2 -adrenoceptor agonists has been considered to be dependent upon the physicochemical properties of the ligands, in line with the plasmalemma diffusion microkinetic model [33]. In this model, a hydrophobic molecule such as salbutamol is considered to diffuse rapidly to the site of action and access the receptor directly from the aqueous environment, resulting

Table 2 Onset and duration of action for the marketed inhaled β_2 -adrenoceptor agonists in isolated tissues

	Guinea pig trachea		Human bronchus		Human small airways	
	Onset (min)	Duration (h)	Onset (min)	Duration (h)	Onset (min)	Duration (h)
Indacaterol	30	8.8	8	>12	3	>6
Formoterol	32	2.6	6	0.6	2	<2
Salmeterol	169	7.9	20	>12	7	>6
Salbutamol	28	0.4	11	0.25	2	<1

Data from [4, 31, 32]

in a fast onset of action. In contrast, lipophilic compounds such as salmeterol take longer to diffuse into tissues and may even access the receptor via the membrane compartment, resulting in a slower onset of action. This has been supported by observations that the onset of relaxation of guinea pig trachea gets longer in a series of homologous indacaterol analogues with increasing lipophilicity [34]. However, although lipophilicity undoubtedly influences the onset, it does not explain the clear differences in clinical onset of action between indacaterol and salmeterol, which have very similar lipophilicity. It has been suggested that the unique structure of salmeterol allows it to pack more effectively into lipid membranes, slowing onset of action [35], but this is not supported by studies that show salmeterol and indacaterol have very similar affinity for lipid bilayers, regardless of their structural differences [36]. This suggests that other factors play an important role in governing the clinical onset of action.

One factor that could influence the initial rate of receptor occupancy, and hence the initiation of downstream signalling, is the binding kinetics of the β_2 -adrenoceptor agonists. A recent study has measured the kinetics of a series of inhaled β_2 -adrenoceptor agonists and examined the effect this has on observed association rates [37]. It was found that the k_{off} value for salmeterol was 4.5-fold slower than that of indacaterol (Table 3), which at first appears to support this notion. However, at equi-effective concentrations, the rate of association to the receptor was equivalent, while at concentrations based on their relative clinical doses, salmeterol occupied the receptors more rapidly than the other agonists examined. This is likely due to the low efficacy of salmeterol, meaning it must be used at higher relative concentrations to occupy sufficient receptors to give a functional response. These higher relative concentrations of drug will speed the observed association rate. Interestingly, these simulations also suggested that the low efficacy of salmeterol means it needs to be dosed at higher relative levels in the clinic when compared to other inhaled β_2 -adrenoceptor agonists. Importantly, this study suggests that binding kinetics does not play a significant role in determining clinical onset of action.

A potential explanation for the differences in onset of action is variations in intrinsic efficacy between the molecules. A recent study compared the intrinsic efficacy of a series of β_2 -adrenoceptor agonists with the rate at which they stimulate cAMP production in primary human bronchial smooth muscle cells [7]. This study

Table 3 Affinity and kinetic binding parameters for agonists at the β_2 -adrenoceptor

Compound	$k_{\text{on}} \text{ M}^{-1} \text{ min}^{-1} (k_3)$	$k_{\text{off}} \text{ min}^{-1} (k_4)$	$K_d \text{ nM} (\text{p}K_d)$	$K_i \text{ nM} (\text{p}K_i)$
Isoprenaline	$2.47 \pm 1.39 \times 10^7$	3.06 ± 1.53	$132.9 \pm 24.2 (6.89)$	$218.3 \pm 81.7 (6.72)$
Salmeterol	$4.31 \pm 1.34 \times 10^9$	0.76 ± 0.06	$0.3 \pm 0.1 (9.70)$	$0.8 \pm 0.1 (9.18)$
Salbutamol	$2.05 \pm 1.03 \times 10^7$	4.06 ± 1.19	$249.1 \pm 85.8 (6.65)$	$295.0 \pm 42.1 (6.54)$
Formoterol	$2.15 \pm 0.45 \times 10^8$	3.29 ± 0.79	$15.9 \pm 3.6 (7.83)$	$56.3 \pm 14.6 (7.28)$
Indacaterol	$8.74 \pm 2.12 \times 10^7$	3.48 ± 0.42	$48.7 \pm 13.4 (7.37)$	$96.2 \pm 13.0 (7.04)$
Adrenaline	$3.15 \pm 0.61 \times 10^6$	5.12 ± 1.39	$1513.3 \pm 303.6 (5.85)$	$1547.4 \pm 182.8 (5.82)$
GSK444	$3.25 \pm 1.7 \times 10^8$	0.41 ± 0.04	$2.6 \pm 1.6 (8.79)$	$1.7 \pm 0.3 (8.80)$
Carmoterol	$8.66 \pm 0.46 \times 10^7$	0.46 ± 0.09	$5.4 \pm 1.2 (8.32)$	$7.9 \pm 1.6 (8.16)$

Data are expressed as mean \pm S.E.M. ($n \geq 3$) and are taken from [37]

showed a direct correlation of intrinsic efficacy with onset of cAMP signalling, with the highest-efficacy ligands having the faster onset of action. This supports the notion that strength of receptor activation determines the rate of second messenger signalling and suggests that the slower onset of action of salmeterol compared to indacaterol could be a result of its lower intrinsic efficacy.

6 Duration of Action

The available inhaled β_2 -adrenoceptor agonists are either used as a rescue medication (salbutamol) or as maintenance therapy (formoterol and salmeterol). Salbutamol provides rapid bronchodilation; however, its major drawback is its short duration of action (4–6 h). In contrast, the longer-acting inhaled β_2 -adrenoceptor agonists, formoterol and salmeterol, are given twice a day [38, 39]. Despite the decrease in dosing frequency with the twice-daily inhaled β_2 -adrenoceptor agonists, patient compliance is still an issue [25]. In addition, the availability of tiotropium bromide, a once-daily inhaled muscarinic antagonist for the treatment of COPD [40], would suggest that a new inhaled β_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.

Indacaterol has been shown to have duration of action compatible with a once-daily dosing regimen in various in vitro preclinical models (Table 2). In the electrically stimulated human bronchus [31], the duration of action for both indacaterol and salmeterol was greater than 12 h compared to half an hour for formoterol and 15 min for salbutamol. A similar ranking for the duration of action was observed in the isolated electrically stimulated guinea pig trachea [4]. In isolated human small airways contracted with carbachol, indacaterol had a comparable duration of action to salmeterol (greater than 6 h), whereas salbutamol (less than 1 h) and formoterol (less than 2 h) had a shorter duration of action [32]. More recently, using a cell-based assay, it was demonstrated that indacaterol had a longer

persistence of action at the β_2 -adrenoceptor, when compared with salbutamol or formoterol [41].

The long duration of action for indacaterol was also demonstrated *in vivo*. In the conscious guinea pig using lactose-blended dry powder formulations and serotonin as a constrictor agent, the duration of action of indacaterol has been compared to that of formoterol, salmeterol, and salbutamol. Using equi-effective doses, the duration of action can be ranked in the following order: indacaterol (24 h) > salmeterol (12 h) > formoterol (4 h) > salbutamol (2 h) [4]. This long duration of action for indacaterol when compared to salbutamol and salmeterol has been recently confirmed using an unconscious guinea pig Einthoven model and histamine as a constrictor agent [42].

The mechanism of prolonged duration of action of inhaled β_2 -adrenoceptor agonists has been widely debated. One possible explanation is that the drugs exhibit slow dissociation from the receptor, a feature exhibited by the once-daily inhaled muscarinic receptor antagonist tiotropium [43]. Indeed, it has been suggested that long duration of the exploratory β_2 -adrenoceptor agonist carmoterol can be attributed to its slow dissociation from the receptor [44]. However, in a recent study examining the binding kinetics of a series of inhaled β_2 -adrenoceptor agonists in physiological buffers at 37 °C, it was discovered that although there were some small difference in off rate for the different agonists, even the agonist with the highest residency time would be fully dissociated from the receptor within 10 min ([37], Table 3). This suggests that prolonged receptor residency time alone is not sufficient to explain the duration of action of long-acting β_2 -adrenoceptor agonists.

A more widely accepted mechanism for the long duration of inhaled β_2 -adrenoceptor agonists action is the plasmalemma diffusion microkinetic model [33] that suggests the lipid membrane provides a “depot” for lipophilic ligands, maintaining high concentrations of drug in the local vicinity of the receptor even when the bulk of the compound has washed out of the lung. Hydrophilic ligands, in contrast, remain in the aqueous phase and are rapidly washed away once they have dissociated from the receptor. This hypothesis has been modeled for salmeterol and found to adequately describe both the long action and the ability of salmeterol to “reassert” its effect once an antagonist has been washed away [45]. An extension of the diffusion microkinetic model is that in addition to the drug moving between receptor and lipid membrane depot, ligands that have freshly dissociated from one receptor can immediately bind another receptor in the local vicinity. This has been termed “rebinding” and can provide even larger gains in pharmacodynamic duration, particularly when diffusion barriers are considered in an “unstirred” model [46].

These studies suggest that the duration of action of β_2 -adrenoceptor agonists should be directly related to their affinity for lipid membranes. The physicochemical properties governing this interaction with lipid membranes is described in detail elsewhere (see chapter “The Design of the Indacaterol Molecule”).

7 Interaction with Short-Acting β_2 -Adrenoceptor Agonists

Apart from their duration and onset of action, an interesting difference between the marketed long-acting β_2 -adrenoceptor agonists is their intrinsic efficacy. As discussed above, salmeterol is a partial agonist, formoterol an almost full agonist and indacaterol has intermediate efficacy (Table 1). Although when used as maintenance therapy, the clinical efficacy of these drugs is similar, the difference in their intrinsic efficacy may translate into meaningful clinical differences in condition of increased bronchial tone or overuse of short-acting β_2 -adrenoceptor relief medication. Indeed, in isolated human bronchi, we have shown that indacaterol, formoterol, and salmeterol have the same efficacy on preparations at resting tone. However, in bronchi precontracted with histamine or acetylcholine, the maximal effect induced by formoterol or indacaterol was not significantly modified, whereas the effect of salmeterol was moderately to considerably reduced [31]. In the same study, it was shown that preincubation of salmeterol but not indacaterol or formoterol decreased the potency of isoprenaline. These results are in agreement with the receptor theory suggesting that a partial agonist can behave as an antagonist in the presence of an agonist with higher efficacy acting on the same receptor. The lack of antagonism of isoprenaline-induced relaxation and the nearly full agonist behavior of indacaterol could be of particular interest when considering the use of rescue medication, where short-acting β_2 -adrenoceptor agonists are used on top of maintenance treatment with long-acting β_2 -adrenoceptor agonist. These results were confirmed *in vivo* using the conscious guinea pig model. In this experimental setup, pretreatment with indacaterol or formoterol did not blunt the effectiveness of the short-acting β_2 -adrenoceptor agonist salbutamol [47].

8 β_2 -Adrenoceptor Polymorphism and Agonist Efficacy

Several polymorphisms of the human β_2 -adrenoceptor have been described that can potentially modify the pharmacological properties of β_2 -adrenoceptor agonists. Of these, the two most frequent single-nucleotide polymorphisms result in the presence of either an arginine or a glycine at codon 16 and a glutamine or a glutamate at codon 27. In addition, there is a rare but potentially important single-nucleotide polymorphism that results in either a threonine or an isoleucine at codon 164 [48]. Although a number of clinical studies have been designed to assess the potential detrimental effects of these different polymorphisms, no consensus has been reached on the relationship between β_2 -adrenoceptor genetic variations and β_2 -adrenoceptor agonist response [48]. Nevertheless, since *in vitro* studies have reported significant effect of the genetic variations on the response to various β_2 -adrenoceptor agonists, it was important to assess the effect of indacaterol on the most common of these polymorphisms. In a study comparing the functional efficacy of indacaterol with formoterol and salmeterol on the most common

haplotypes, no marked genotype-dependent effects were observed for all compounds. Only for the rare single-nucleotide polymorphisms at codon 164, a reduced efficacy for all compounds was observed [5]. Recently, a large pharmacogenetic analysis testing for an association between common β_2 -adrenoceptor polymorphisms and indacaterol response in COPD patients was performed. A total of 648 indacaterol-treated patients were genotyped for the most commonly studied polymorphisms in the β_2 -adrenoceptor gene. Results showed little evidence for the association between β_2 -adrenoceptor variants and indacaterol response, suggesting that β_2 -adrenoceptor genetic variation is unlikely to have a major role in differential response to indacaterol treatment in COPD patients [49].

9 Conclusion

The preclinical profile of indacaterol shows that it is the first inhaled β_2 -adrenoceptor agonist that combines a fast onset of action together with a dosing regimen compatible with once-daily dosing. It has good potency and intermediate intrinsic efficacy at the β_2 -adrenoceptor. In addition, studies in animals or isolated tissues have shown that it has reduced potential for cardiovascular side effects and interaction with short-acting β_2 -adrenoceptor rescue medicine. All together, this favorable preclinical profile has led us to select this compound for further development in the treatment of COPD.

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