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# Pharmacology and Therapeutics of Bronchodilators

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**Abstract**—Bronchodilators are central in the treatment of airways disorders. They are the mainstay of the current management of chronic obstructive pulmonary disease (COPD) and are critical in the symptomatic management of asthma, although controversies around the use of these drugs remain. Bron-

chodilators work through their direct relaxation effect on airway smooth muscle cells. At present, three major classes of bronchodilators,  $\beta_2$ -adrenoceptor (AR) agonists, muscarinic receptor antagonists, and xanthines are available and can be used individually or in combination. The use of the inhaled route is

currently preferred to minimize systemic effects. Fast- and short-acting agents are best used for rescue of symptoms, whereas long-acting agents are best used for maintenance therapy. It has proven difficult to discover novel classes of bronchodilator drugs, although potential new targets are emerging. Consequently, the logical approach has been to improve the existing bronchodilators, although several novel broncholytic classes are under development. An important step in simplifying asthma and COPD management and improving adherence with prescribed therapy is to reduce the dose frequency to the minimum necessary to maintain disease control. Therefore, the incorporation of once-daily dose administration is an important strategy to improve adherence. Several

once-daily  $\beta_2$ -AR agonists or ultra-long-acting  $\beta_2$ -AR agonists (LABAs), such as indacaterol, olodaterol, and vilanterol, are already in the market or under development for the treatment of COPD and asthma, but current recommendations suggest the use of LABAs only in combination with an inhaled corticosteroid. In addition, some new potentially long-acting antimuscarinic agents, such as glycopyrronium bromide (NVA-237), aclidinium bromide, and umeclidinium bromide (GSK573719), are under development, as well as combinations of several classes of long-acting bronchodilator drugs, in an attempt to simplify treatment regimens as much as possible. This review will describe the pharmacology and therapeutics of old, new, and emerging classes of bronchodilator.

## I. Introduction: The Physiological Rationale for Using Bronchodilators

A schematic description of the innervation of the airways is fundamental for understanding how bronchodila-

<sup>1</sup>Abbreviations: 95% CI, 95% confidence interval; ACh, acetylcholine; ALKS27, inhaled tropism; ANP, atrial natriuretic peptide; AR, adrenoceptor; ASM, airway smooth muscle; AWD 12-281, *N*-(3,5-dichloropyrid-4-yl)-(1-(4-fluorobenzyl)-5-hydroxy-indole-3-yl)glyoxylic acid amide; BI 1744 CL, olodaterol; BNP, brain natriuretic peptide; CHF 5407, 3-(((3-fluorophenyl)((3,4,5-trifluorophenyl)methyl)amino)carbonyl)oxy)-1-(2-oxo-2-(2-thienyl)ethyl)-1-azoniabicyclo(2.2.2)octane; CHF, chronic heart failure; COMT, catechol-*O*-methyltransferase; COPD, chronic obstructive pulmonary disease; ECG, electrocardiogram; EIA, exercise-induced asthma; EP, E-prostanoid; FDA, U.S. Food and Drug Administration; FEV<sub>1</sub>, forced expiratory volume in 1 s; FVC, forced vital capacity; GPCR, G protein-coupled receptor; GR, glucocorticoid receptor; GRC-3886, oglemilast; GRK, G-protein receptor kinase; GSK256066, 6-(((3-((dimethylamino)carbonyl)phenyl)sulfonyl)-8-methyl-4-((3-methyloxyphenyl)amino)-3-quinolinecarboxamide; GSK269962A, *N*-(3-[[2-(4-amino-1,2,5-oxadiazol-3-yl)-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-6-yl]oxy]phenyl)-4-[[2-(4-morpholinyl)ethyl]oxy]benzamide; GSK573719, umeclidinium bromide; GSK-642444, vilanterol; GSK961081, [1-[9-[[2-(2-hydroxy-2-(8-hydroxy-2-oxo-1*H*-quinolin-5-yl)ethyl]amino)nonyl]piperidin-4-yl] *N*-(2-phenylphenyl)carbamate; HA-1077, fasudil; HA-1100, hydroxyfasudil; HDAC, histone deacetylase; HFA, hydrofluoroalkane; IC, inspiratory capacity; ICI 118551, ( $\pm$ )-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol; ICS, inhaled corticosteroid; IK312532, [Arg<sup>15,20,21</sup>,Leu<sup>17</sup>]-VIP; IL, interleukin; IL, interleukin; iNANC, inhibitory NANC; LABA, long-acting  $\beta_2$ -AR agonists; LAMA, long-acting mAChRs antagonist; LAS-40464, formoterol plus aclidinium bromide; LAS100977: 5-(2-[[6-(2,2-difluoro-2-phenylethoxy)hexyl]amino]-1(*R*)-hydroxyethyl)-8-hydroxyquinolin-2(1*H*)-one; LV, left ventricular; MABA, mAChR antagonist- $\beta_2$ -AR agonist; mAChR, muscarinic acetylcholine receptor; MDI, metered-dose inhaler; MN-221, bedoradrine; NANC, nonadrenergic noncholinergic; NCX-950,  $\alpha'$ -[[1-(1-dimethylethyl)amino]methyl]-4-hydroxy-1,3-benzenedimethanol nitrate; NF- $\kappa$ B, nuclear factor of  $\kappa$  light polypeptide gene enhancer in B cells; NVA-237, glycopyrronium bromide; OR, odds ratio; P450, cytochrome P450; PD, pharmacodynamics; PDE, phosphodiesterase; PF-610355, *N*-(4'-hydroxybiphenyl-3-yl)methyl)-2-(3-(2-((2-hydroxy-2-(4-hydroxy-3-((methylsulfonyl)amino)phenyl)ethyl)amino)-2-methylpropyl)phenyl)acetamide; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; PK, pharmacokinetics; PKA, protein kinase A; PKC, protein kinase C; QAB149, indacaterol; QMF-149, mometasone; QVA149, indacaterol and glycopyrronium bromide; RhoK, Rho kinase; Ro 25-1392, Ac-His<sup>1</sup>[Glu<sup>8</sup>,OCH<sub>3</sub>-Tyr<sup>10</sup>,Lys<sup>12</sup>,Nle<sup>17</sup>,Ala<sup>19</sup>,Asp<sup>25</sup>,Leu<sup>26</sup>,Lys<sup>27,28</sup>]-VIP; Ro 25-1553, Ac-His<sup>1</sup>[Glu<sup>8</sup>,Lys<sup>12</sup>,Nle<sup>17</sup>,Ala<sup>19</sup>,Asp<sup>25</sup>,Leu<sup>26</sup>,Lys<sup>27,28</sup>,Gly<sup>29,30</sup>,Thr<sup>31</sup>]-NH<sub>2</sub> VIP; ROCK, Rho-associated protein kinase; RPL554, 9,10-dimethoxy-2-(2,4,6-trimethylphenylimino)-3-(*N*-carbamoyl-2-aminoethyl)-

tors work and why they have clinical utility. Airway tone is mainly controlled by the vagus nerve, and the parasympathetic nerves carried in the vagus nerve are tonically active, producing a stable, readily reversible baseline tone of the airway smooth muscle (ASM) (Kesler and Canning, 1999) (Fig. 1). Electrophysiological recordings from both preganglionic and postganglionic parasympathetic nerve fibers also confirm the existence of a persistent outflow of parasympathetic activity to the airways (Molfino et al., 1993). Preganglionic parasympathetic nerve fibers project to the airways via the vagus nerve. They form cholinergic synapses with postganglionic neurons via airway parasympathetic ganglia. Airway parasympathetic ganglia are associated mainly with the larger airways, but the subsequent postganglionic fibers innervate structures throughout the airway tree (Canning and Fischer, 2001). Postganglionic parasympathetic cholinergic and nonadrenergic noncholinergic (NANC) fibers innervate ASM, providing the dominant control of smooth muscle tone and thus airway caliber, as well as airway glands and microvasculature in the respiratory tract. There is no direct sympathetic innervation of ASM, although the airway vasculature does receive sympathetic innervation (Racké et al., 2006). There is evidence, however, for a sympathetic input to parasympathetic ganglia, and of course there are  $\beta_2$ -adrenoceptors (ARs) found on ASM (see below). The parasympathetic nervous system also has a role in reflex bronchoconstriction, induced by irritants such as histamine and SO<sub>2</sub> (Dixon et al., 1983).

Acetylcholine (ACh) is the "classic" neurotransmitter of the parasympathetic nervous system at both the level of ganglionic transmission and the neuroeffector junctions

3,4,6,7-tetrahydro-2*H*-pyrimidino(6,1- $\alpha$ )isoquinolin-4-one; RR, risk ratio; Rt50, the time taken for 50% recovery from the effects of EC<sub>50</sub> of agonist; RWS, rightward shift; SABA, short-acting  $\beta_2$ -AR agonist; SB-772070-B, 4-(7-[[[(3*S*)-3-amino-1-pyrrolidinyl]carbonyl]-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl]-1,2,5-oxadiazol-3-amine; sGaw, specific airway conductance; SNP, single nucleotide polymorphism; TM, transmembrane segment; TPI 1020, budesonide 21-[[4-(4-nitrooxymethyl)benzoate]; UCB-101333-3,6-(azepan-1-yl)-*N*,2-dicyclopropyl-5-methylpyrimidin-4-amine; VIP, vasoactive intestinal peptide; Y-27632, *N*-(4-pyridyl)-4-(1-aminoethyl)cyclohexanecarboxamide; Y-30141, 4-(1-aminoethyl)-*N*-(1*H*-pyrrolo(2,3-*b*)pyridin-4-yl)cyclohexanecarboxamide dihydrochloride.

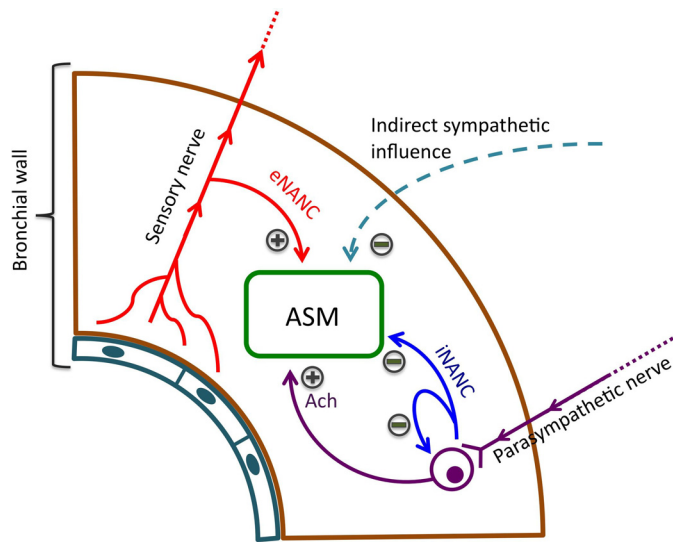


FIG. 1. Neural regulation of bronchial tone. eNANC, excitatory NANC; ACh, acetylcholine.

(Racké and Matthiesen, 2004; Racké et al., 2006). ACh acts via activation of muscarinic receptors (mAChRs) that belong to the large seven-transmembrane family of G protein-coupled receptors (GPCRs). Five different subtypes of mAChRs have been identified by molecular biological techniques ( $M_1$ – $M_5$ ), but so far, a sufficient pharmacological and functional characterization has been provided for only four of them ( $M_1$ – $M_4$ ). The coupling of mAChRs to their cellular effector systems is mediated via heterotrimeric G proteins. The different mAChRs couple differentially to the multiple G proteins. Thus, the odd-numbered mAChR subtypes ( $M_1$ ,  $M_3$ , and  $M_5$ ) couple preferentially to G proteins of the  $G_q$  family, whereas the even-numbered mAChRs ( $M_2$  and  $M_4$ ) prefer G proteins belonging to the  $G_{i/o}$  family (Racké and Matthiesen, 2004; Racké et al., 2006).

The mAChRs are expressed in almost every cell type of the airway and lung tissue, including airway and vascular smooth muscle, different glandular and surface epithelial cells, endothelial cells, and various inflammatory cells (Barnes, 1993). In humans,  $M_1$  mAChRs seem to be expressed particularly in peripheral lung tissue and in the alveolar wall, but they have not been detected in larger airways, where  $M_2$  and  $M_3$  mAChRs represent the major population of mAChRs. Under “physiological” conditions, the ASM contraction induced by ACh is mediated primarily via the  $M_3$  subtype.  $M_2$  mAChRs couple to adenylyl cyclase via  $G_i$  in an inhibitory manner. They functionally oppose the  $\beta$ -AR-mediated increase in cAMP, leading to attenuation of  $\beta$ -AR-induced relaxation of ASM (Racké and Matthiesen, 2004; Racké et al., 2006) and prevent activation of  $Ca^{2+}$ -dependent  $K^+$  ( $K_{Ca}$ ) channels.

Release of ACh from parasympathetic nerves is regulated predominantly by mAChRs in the ganglia and at pre- and postganglionic nerve endings. Airway ganglia express  $M_1$  mAChRs that inhibit the opening of  $K^+$  channels, resulting in depolarization of parasympathetic ganglion cells (Coulson and Fryer, 2003). Moreover, they express

prejunctional inhibitory  $M_2$  mAChRs that inhibit ACh release from parasympathetic nerves.  $M_2$  mAChRs have also been demonstrated functionally within the cholinergic ganglia, where they play a role in inhibiting the slow excitatory postsynaptic potential (Coulson and Fryer, 2003; Belmonte, 2005).

The  $M_3$  mAChRs are the predominant receptors mediating mucus secretion, whereas  $M_1$  mAChRs are postulated to have an accessory role in electrolyte and water balance (Belmonte, 2005).  $M_3$  mAChRs also mediate dilation of airway blood vessels, an action that has been demonstrated to be an endothelium-dependent mechanism (Belmonte, 2005). Moreover, they facilitate cigarette smoke extract-induced interleukin (IL)-8 secretion by human ASM cells via protein kinase C (PKC)-dependent activation of nuclear factor of  $\kappa$  light polypeptide gene enhancer in B cells (NF- $\kappa$ B), inhibitor  $\alpha$  and extracellular signal-regulated kinase 1/2 (Oenema et al., 2010).

Sympathetic nerve fibers emerge from the spinal cord and release ACh onto the sympathetic trunk located on either side of the spinal column. The postganglionic nerve fibers extend to the lung to release norepinephrine on effector targets similar to those of the parasympathetic nerves. In humans, sympathetic fibers innervate the submucosal mucus gland, blood vessels, and the parasympathetic ganglia, although sympathetic adrenergic innervation of human ASM is sparse and/or nonexistent (Richardson and Béland, 1976; Skoogh, 1986). Despite the lack of direct sympathetic innervation of ASM, ARs are present throughout the lung (Carstairs et al., 1985). Furthermore, sympathetic nerves are near the cholinergic parasympathetic nerve fibers, allowing for communication between the two systems, particularly at parasympathetic ganglia (Jones et al., 1980).

Functional studies have mostly suggested a limited role for sympathetic adrenergic nerves regulating airway function in normal or asthmatic human subjects, but relaxation and, under some conditions, contraction of ASM can be evoked by stimulation of sympathetic nerves. Such contraction and relaxation are attributed to norepinephrine release, which is capable of acting on  $\alpha$ - and  $\beta$ -ARs (van der Velden and Hulsmann, 1999). Hormonal catecholamines and perhaps adrenergic nerves may play a prominent role in regulating the airways in asthma under some conditions. Elevated sympathetic nerve activity has also been associated with COPD (Canning, 2006).

$\beta$ -ARs are present in high concentration in lung tissue, and autoradiographic mapping and in situ hybridization studies show that they are localized to several cell types (Barnes, 1995).  $\beta$ -ARs are subdivided into three types;  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$ . They are members of the seven-transmembrane spanning family of GPCRs related to bacteriorhodopsin and are composed of 413 amino acid residues. There is a 65 to 70% homology between  $\beta_1/\beta_3$ - and  $\beta_2$ -ARs. Binding studies show that approximately 70% of pulmonary  $\beta$ -ARs are of the  $\beta_2$ -AR subtype. These receptors are localized to ASM ( $3\text{--}4 \times 10^4$  per cell), epithelium, vascular



smooth muscle, and submucosal glands (Ruffin et al., 1982; Carstairs et al., 1985), whereas  $\beta_1$ -ARs in the lung are confined to glands and alveoli. There is a uniform distribution of  $\beta$ -ARs on the alveolar wall with a 2:1 ratio of  $\beta_1/\beta_2$ -ARs (Mak et al., 1996).  $\beta_2$ -AR density increases with increasing airway generation, and high levels are found in the alveolar region (Spina et al., 1989). Computed tomography scanning has confirmed that  $\beta_2$ -AR distribution is greater for small rather than large airways.  $\beta_2$ -ARs are also expressed on many proinflammatory and immune cells, including mast cells, macrophages, neutrophils, lymphocytes, eosinophils, epithelial and endothelial cells, and type I and type II alveolar cells.

GPCRs are dynamic proteins that switch between an inactive (R) state and an active (R\*) conformation that can engage G proteins (Fig. 2). Persistent activation of a GPCR is achieved through the binding of both an agonist and a G protein at opposite ends of the receptors relative to the lipid bilayer, where the combined binding interactions reduce the energy barriers to the formation of the R\* state (Sprang, 2011).  $\beta_2$ -ARs are coupled to  $G_s$ , where stimulation by a  $\beta_2$ -AR agonist activates adenylyl cyclase and increases cAMP levels. cAMP increases protein kinase A (PKA) activity, which phosphorylates downstream protein modulators (Giembycz and Raeburn, 1991). The overall activation of this signal transduction pathway can lead to the inhibition of phosphoinositol hydrolysis, a fall in intracellular  $Ca^{2+}$  levels, and the activation of large conductance  $K^+$  channels. The hyperpolarization of airway smooth muscle as a result of opening  $K^+$  channels can lead to relaxation of airway smooth muscle (Jones et al., 1990).  $\beta_2$ -AR stimulation produces airway relaxation, but prolonged  $\beta_2$ -AR activation leads to a decrease in receptor

responsiveness (i.e., desensitization) that differs depending on the cell type but is more readily demonstrable in inflammatory cells than ASM (Johnson, 1998).

$\beta_2$ -ARs can also couple to  $G_i$  proteins. Activation of this pathway needs phosphorylation of the receptor by PKA and is mediated by  $\beta\gamma$  subunits of the G protein. This mechanism may not only cause desensitization by uncoupling the  $\beta_2$ -AR from  $G_s$ , but also, by switching the coupling from  $G_s$  to  $G_i$ , represents a means of terminating the  $\beta_2$ -AR agonist/receptor signal and response (Johnson, 2001).

In addition,  $\beta_2$ -ARs can also be phosphorylated by members of a kinase family known as G protein receptor kinases (GRKs). GRK-mediated phosphorylation of  $\beta_2$ -AR serves to diminish receptor-G protein coupling and is specific for the agonist-occupied or spontaneously active form of the receptor, whereas PKA and other second-messenger kinases can phosphorylate receptors independently of their occupancy or activity status (Walker et al., 2011). GRK-mediated phosphorylation of residues within the receptor cytoplasmic tail triggers the translocation of  $\beta$ -arrestin-1 and  $\beta$ -arrestin-2 [proteins that were originally discovered to “arrest” G protein-mediated cell-signaling events (Benovic et al., 1987)] to the cell surface, where they interact with phosphorylated receptors, causing receptor uncoupling from stimulatory G protein and, consequently, reduction of cAMP signaling (Krupnick and Benovic, 1998). Furthermore, GRKs and  $\beta$ -arrestins are involved in an increasing number of interactions with nonreceptor proteins, broadening the variety of their cellular functions. Schematically, GRKs and  $\beta$ -arrestins orchestrate  $\beta_2$ -AR activities at three different levels: 1) silencing: the functional uncoupling of the receptor from its cognate G protein by a mechanism known as “homologous desensitization”; 2) trafficking: receptor internalization, “resensitization,” and/or degradation; and 3) signaling: the activation or inhibition of intracellular signaling pathways independently of heterotrimeric G proteins (Reiter and Lefkowitz, 2006). It has been found that  $\beta$ -arrestin-2 functions as a scaffold for  $\beta_2$ -AR activation of several mitogen-activated protein kinases including extracellular signal-regulated kinase 1, which is independent of G protein activation (Shenoy et al., 2006).

The  $\beta_2$ -AR protein regions, which are important for binding and coupling, have been identified (Johnson, 2001). The active receptor site, with which  $\beta_2$ -AR agonists must interact to exert their biological effects, is located approximately one third of the way (15 Å) into the receptor core. The residues of critical importance with respect to agonist binding to the active site are Asp113 (counted from the extracellular or N-terminal end) of the third domain; Ser204 and Ser207, which are both on the fifth domain; and Phe259 and Phe290, on the sixth domain. A model for the agonist binding site of the  $\beta_2$ -AR has emerged in which the ligand is bound within the hydrophobic core of the protein in the transmembrane helices and anchored by specific molecular interactions. The Asp residue binds to

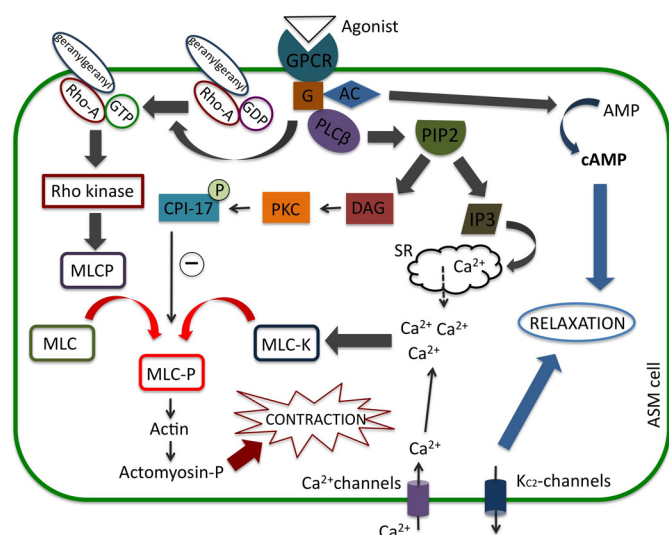


FIG. 2. Main intracellular pathways involved in the bronchomotor tone regulation. PLC $\beta$ , phospholipase C- $\beta$ ; PIP2, phosphatidylinositol 4,5-bisphosphate; IP3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; SR, sarcoplasmic reticulum; MLCK, myosin light chain kinase; MLC, myosin light chain; MLC-P, myosin light chain phosphorylation; CPI17, C kinase-potentiated phosphatase inhibitor; Rho-kinase, Rho-associated serine/threonine kinase; AC, adenylyl cyclase.

nitrogen whereas the two Ser residues interact with hydroxyl groups on the phenyl ring of the  $\beta_2$ -AR agonist molecule.

It has been proposed that the degree to which an agonist has an effect depends on whether it can fulfill two main criteria: ligand-induced conformational changes of Ser215<sup>5.46</sup> and Ser212<sup>5.43</sup> (numbers in superscript correspond to the Ballesteros-Weinstein numbering system for conserved GPCR residues) in the transmembrane segment (TM) 5 and contraction of the binding pocket (Warne et al., 2011). Rasmussen et al. (2011a) generated a camelid antibody fragment (known as a nanobody) targeted to the human  $\beta_2$ -AR, which was able to mimic G protein-like behavior and allowed the formation of a high-affinity agonist-bound R\*-state crystal structure. This structure revealed major changes in the cytoplasmic ends of TM5 and TM6, which are outwardly displaced, whereas TM3 and TM7 move inward. In the ligand-binding pocket, the largest change seen is an inward bulge of TM5 that is focused around Ser207<sup>5.46</sup>. In a companion publication, Rasmussen et al. (2011b) designed an irreversible agonist that efficiently formed crystals when bound to the low-affinity conformation of a human  $\beta_2$ -AR. The covalent agonist formed hydrogen bonding contacts with Ser203<sup>5.42</sup> and Ser207<sup>5.46</sup> on TM5 in the binding pocket, but the structure of the cytoplasmic domains was more similar to the inverse agonist-bound, R-state  $\beta_2$ -AR than to the nanobody-stabilized R\*-state structure. Therefore, agonist binding alone is not enough to stabilize the active conformation at the cytoplasmic surface.

Studies in human and animal models have suggested that stimulation of prejunctional  $\beta_2$ -ARs on parasympathetic ganglia inhibits cholinergic neurotransmission (Aizawa et al., 1991). Conversely, direct evidence of an excitatory  $\beta_2$ -AR in airway parasympathetic nerves has been identified (de Haas et al., 1998). Direct activation of the  $\beta_2$ -AR-coupled  $G_s$  protein by cholera toxin, which increases the activity of adenylyl cyclase, caused an increase in ACh release in epithelium-denuded trachea (Belvisi et al., 1996). Stimulation of the  $\beta_2$ -AR pathway thus seems to enhance cholinergic neurotransmission under certain circumstances. However, in ASM cells, stimulation of  $G_s$  protein directly opened large  $K_{Ca}$  channels (Kume et al., 1992), which has been found to decrease ACh release. Activation of  $K_{Ca}$  channels is thought to hyperpolarize the cell membrane, thus causing reductions in the concentration of intracellular  $Ca^{2+}$  and ACh release in prejunctional cholinergic nerves.

Autonomic neural control of ASM tone cannot be fully explained by the functions of the cholinergic and adrenergic nervous systems alone (Gu and Lee, 2006). For example, striking changes in ASM tone can be induced even in the presence of an anticholinergic agent (atropine) and a  $\beta$ -AR antagonist (propranolol). There is substantial evidence of contractile and relaxant NANC smooth muscle responses in mammalian airways (Shah et al., 1998; Said and Rattan, 2004). In fact, inhibitory NANC (iNANC) in-

ervation is considered the primary neural mechanism mediating ASM relaxation. iNANC relaxation is thought to be generated by a combined effect of vasoactive intestinal peptide (VIP), VIP structure-related peptides (e.g., peptide histidine methionine), and nitric oxide (NO) (Gu and Lee, 2006). Indeed, VIP, VIP-like peptides, and NO synthase have been identified in the parasympathetic ganglia and nerve fibers innervating ASM. Furthermore, endogenously released VIP-like and NO-like substances can attenuate ASM contraction induced by ACh. However, the precise anatomical pathways of iNANC innervation of human ASM are not clear. There is also a potent excitatory effect on the ASM involving the "efferent" functions of a specific subtype of bronchopulmonary sensory nerves containing tachykinins (e.g., substance P and neurokinin A) in guinea pig airways (Gu and Lee, 2006), although this is less evident in human airways (Spina et al., 1998). When these afferent endings are activated, the impulses trigger the release of tachykinins either locally or propagating antidromically to other peripheral branches via the axonal ramifications. These sensory neuropeptides can activate neurokinin-1 and -2 receptors located on the ASM membrane and produce intense and sustained bronchoconstriction.

## II. $\beta$ -Adrenergic Receptor Agonists

### A. A History of the Development of $\beta$ -Adrenergic Receptor Agonists: from Nonselective Adrenergic Receptor Agonists to $\beta_2$ -Adrenergic Receptor-Selective Drugs

In traditional Chinese medicine, the botanical ma huang (the plant *Ephedra equisetina*), from which the active material, an alkaloid identified as ephedrine, is extracted, was used for more than 2000 years for the short-term treatment of respiratory symptoms. Beginning at the turn of the last century, the nonselective  $\alpha$ -AR and  $\beta$ -AR agonist epinephrine was introduced into clinical practice and administered by the subcutaneous route for the treatment of acute asthma (Bullowa and Kaplan, 1903). Although highly efficacious, epinephrine was far from the ideal drug, because in addition to the desired therapeutic effect of bronchodilation, it caused hypertension and tachycardia, now known to be due to its effect on  $\alpha_1$ - and  $\beta_1$ -ARs in the cardiovascular system. Moreover, because of metabolic instability, it had a short duration of action and had to be given parenterally (Waldeck, 2002). In the 1940s, the nonselective  $\beta$ -AR agonist isoproterenol was introduced for the treatment of airway disease and became the standard-of-care bronchodilator, although its use was complicated by adverse effects that were due to activation of the  $\beta_1$ -AR in the heart (Waldeck, 2002), which elicits tachycardia and predisposes patients to cardiac dysrhythmias. A further disadvantage of isoproterenol is its short duration of action, which is due to its ready transportation into cells by the uptake process for catecholamines (Gryglewski and Vane, 1970) where, except in the gut, it is converted by

catechol-*O*-methyltransferase (COMT) to 3-*O*-methylisoprenaline (Blackwell et al., 1974). This limits the duration of effect, and the compound has a poor oral availability (Waldeck, 2002). Metaproterenol, a noncatechol resorcinol derivative of isoproterenol, was subsequently developed in the early 1960s. It was an effective bronchodilator when inhaled but also did not discriminate between  $\beta_1$ - and  $\beta_2$ -ARs and thus produced cardiac side effects. The modern era of selective  $\beta_2$ -AR agonists did not begin until the discovery of albuterol (called salbutamol in Europe) by Sir David Jack and colleagues working at Allen and Hanburys in the UK (now part of GlaxoSmithKline) (Jack, 1991).

All the clinically important  $\beta_2$ -AR agonists consist of a benzene ring with a chain of two carbon atoms and either an amine head group or a substituted amine head group. If a hydroxyl (OH) group is present at positions 3 or 4 on the benzene ring, the structure is a catechol nucleus and hence the agent is termed a catecholamine. If these OH groups are substituted or repositioned, the drug is generally less potent than the synthetic catecholamine isoproterenol, which has both strong  $\beta_1$ - and  $\beta_2$ -AR properties and is the more powerful bronchodilator (McFadden, 1981). However, this potential disadvantage may be outweighed by the relative resistance of substituted catecholamines to

metabolic degradation by COMT. The noncatecholamine  $\beta_2$ -AR agonists such as fenoterol, albuterol, and terbutaline differ in their substitutions in the amine group and benzene ring. These structural modifications, conferring resistance to metabolism by COMT, result in a longer half-life and also reduce their potency for  $\beta_1$ -ARs, making them relatively more selective for  $\beta_2$ -ARs (Fig. 3).

A major limitation of the  $\beta_2$ -AR agonists in use during the 1960s and 1970s was their short duration of action, typically 4 to 6 h. Therefore, the next advance in the development of  $\beta_2$ -AR agonists was the development of the long-acting drugs salmeterol and formoterol, the duration of action of which is approximately 12 h, which made their use for maintenance treatment (e.g., for reducing nighttime symptoms) more appealing (Lötvall, 2002). A pure *R*-isomer of albuterol, levalbuterol (Berger, 2003), and the *R,R*-enantiomer of formoterol, arformoterol (Cazzola et al., 2010b), have been developed. It is claimed that they have a better safety profile than the racemic mixture because they do not have the *S*-enantiomer, which, at least for (*S*)-albuterol, is now known to have unwanted effects in the lung (Page and Morley, 1999). At present, several once-a-day ultra-long-acting  $\beta_2$ -AR agonists are in different stages of clinical development (Cazzola and Matera, 2009;

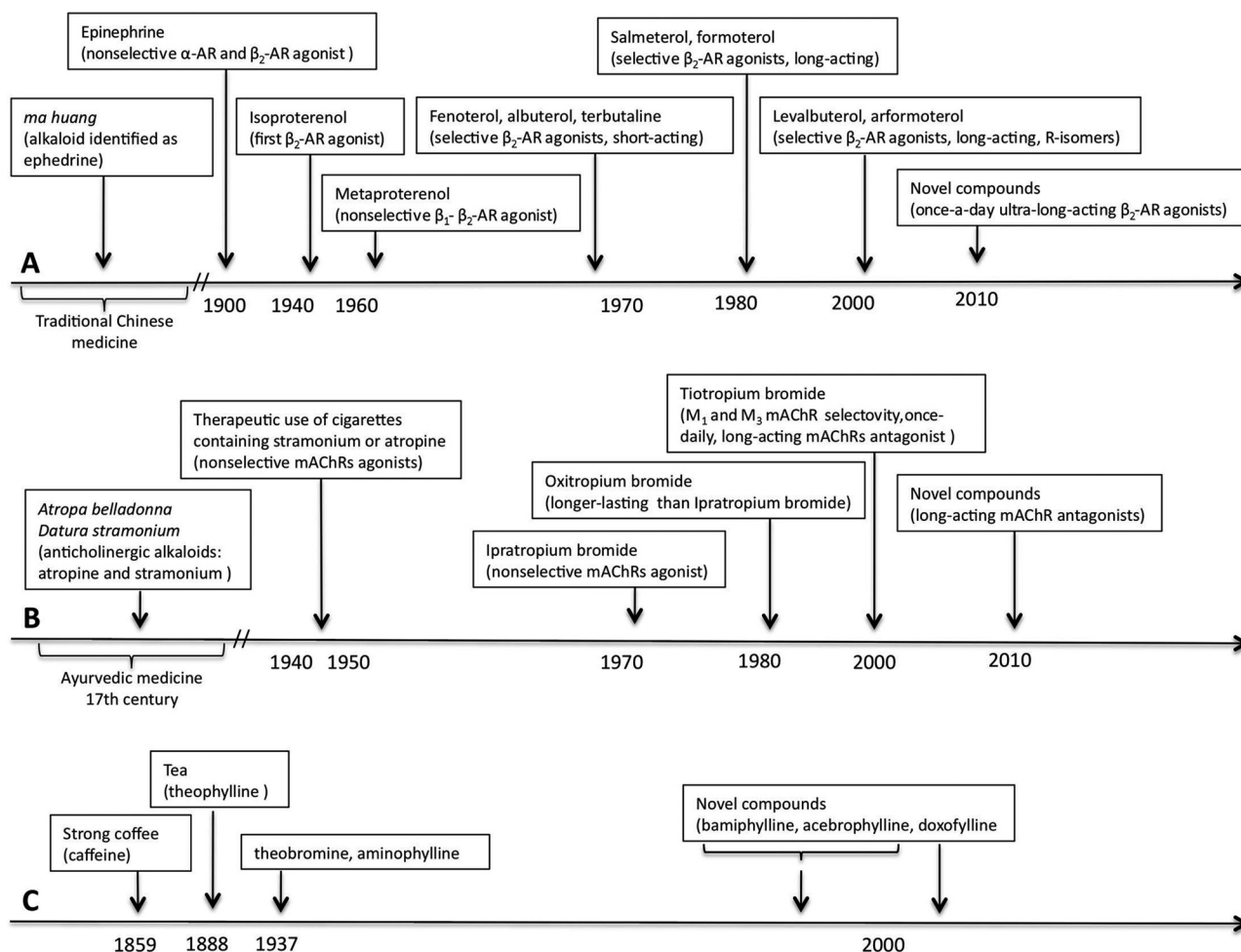


FIG. 3. Milestone development of  $\beta_2$ -AR agonists (A), antimuscarinics (B), and xanthines (C).



Cazzola et al., 2011) and will be discussed in more detail in section II.D.

### B. Short-Acting $\beta_2$ -Adrenergic Receptor Agonists

Short-acting  $\beta_2$ -AR agonists (SABAs) can be divided into two broad groups according to duration of action after inhalation of conventional doses: 1) the catecholamines isoprenaline and rimiterol, which have a very short action of 1 to 2 h; and 2) those conventionally described as short-acting, such as fenoterol, albuterol, and terbutaline, which are active for 3 to 6 h, although the action of fenoterol may be slightly longer (Beardshaw et al., 1974). In any case, the duration of action of all  $\beta_2$ -AR agonists increases with dose such that 1600  $\mu\text{g}$  of inhaled albuterol, for example, has a considerably longer action than 200  $\mu\text{g}$  (Corris et al., 1983).

1. *Albuterol.* Albuterol has negligible  $\alpha$ -AR activity at recommended clinical doses and demonstrates a substantial greater selectivity between  $\beta_2$ - and  $\beta_1$ -ARs than any other product previously available (Sears and Lötvall, 2005). In vitro tests that used guinea pig isolated atria for  $\beta_1$ -ARs and tracheal preparations for  $\beta_2$ -ARs, respectively, documented that isoproterenol has equal affinity for  $\beta_1$ - and  $\beta_2$ -ARs, orciprenaline is slightly more selective for  $\beta_1$ -ARs, whereas albuterol is more selective for  $\beta_2$ -ARs (O'Donnell, 1972). It is not surprising, therefore, that albuterol has approximately equivalent potency at relaxing human isolated bronchi in vitro and bronchodilator potency in subjects with asthma compared with epinephrine (Baldwin et al., 1994). However, the effects of albuterol and epinephrine on histamine-induced contraction in vitro are significantly different from their effects on histamine reactivity in vivo. Albuterol has no effect on the maximal response to histamine, whereas epinephrine reduces it by 54% in vitro. In contrast, albuterol is more potent in vivo (Baldwin et al., 1994). Other in vitro studies have also demonstrated that albuterol acts as a partial agonist at  $\beta_2$ -ARs compared with isoproterenol (O'Donnell and Wanstall, 1978). In human isolated bronchi, albuterol is a partial relaxant of ASM, whereas isoproterenol has greater efficacy (Goldie et al., 1986). Nonetheless, compared with isoproterenol, albuterol is at least as potent a bronchodilator, has a much longer duration of action, and is much less likely to influence blood pressure or heart rate. Whereas isoproterenol produces tachycardia that runs parallel to bronchodilation, albuterol causes the same maximum bronchodilation but with minimal cardiovascular responses. After inhalation of albuterol, maximum bronchodilation can be seen within 15 min of inhalation (Price and Clissold, 1989). However, albuterol binds only weakly to the receptor and quickly diffuses back into the microcirculation. This accounts for its short duration of action (4–6 h) (Sears and Lötvall, 2005). Nevertheless, because of its rapid onset of action, which is a clear clinical advantage for the reversal of bronchoconstriction, albuterol is usually consid-

ered the drug of choice as relief medication for symptoms of bronchospasm.

Although a dry powder inhaler and pressurized aerosol are effective means of delivery of albuterol to the lungs, even in patients with poor ventilation (Tukiainen and Terho, 1985), when the response to inhaled albuterol is reduced or absent, intravenous administration can be used as an alternative (Williams and Seaton, 1977).

Levalbuterol [(*R*)-albuterol], a single isomer of albuterol, is currently available as a nebulizer solution in the United States. Several studies have compared levalbuterol with racemic albuterol and suggest that use of the single isomer may reduce hospitalizations (Carl et al., 2003), have fewer adverse effects (Scott and Frazee, 2003), and provide similar bronchodilator effects at a reduced dose (Berger, 2003). However, other studies have questioned the improved safety profile of (*R*)-albuterol versus the racemic form (Ahrens and Weinberger, 2001; Lötvall et al., 2001).

2. *Fenoterol.* Fenoterol, the 4-hydroxyphenyl derivative of orciprenaline, is a resorcinol derivative with relative selectivity for  $\beta_2$ -ARs and negligible  $\alpha$ -AR-stimulating activity (Svedmyr, 1985). However, an in vitro comparison revealed that fenoterol was less selective for  $\beta_2$ -ARs than albuterol (O'Donnell, 1972). Nonetheless, in guinea pig trachea, the efficacy of fenoterol was found to be approximately twice that of albuterol, and in human isolated bronchi, it had greater efficacy than albuterol (Goldie et al., 1986). Moreover, on a microgram-equivalent basis, inhaled fenoterol exhibits greater systemic potency than albuterol at extrapulmonary  $\beta_2$ -ARs but has the same bronchodilator potency at airway  $\beta_2$ -ARs (Lipworth et al., 1995).

There is little difference in the time course of fenoterol, albuterol, and terbutaline, although there is some evidence that fenoterol might have a slightly longer duration of action (Beardshaw et al., 1974). It is noteworthy that fenoterol and albuterol administered as nebulizer solutions in patients with asthma have shown microgram equivalence (Newhouse et al., 1994).

The efficacy of fenoterol has been demonstrated in several trials with different methods of administration, but the recommended dosages have varied. Using administration by nebulized aerosol at doses of fenoterol of 200 to 800  $\mu\text{g}$ , Tweel (1971) reported maximal effect at 200  $\mu\text{g}$ . Watanabe et al. (1981) found that 500  $\mu\text{g}$  by nebulizer (of a range of 500–2500  $\mu\text{g}$ ) and 400  $\mu\text{g}$  from a metered-dose inhaler (MDI) were equally effective. Various recommendations have appeared concerning the optimal dose of fenoterol by MDI. De Troyer et al. (1978) compared doses of 400 and 1200  $\mu\text{g}$  and found no difference in change in airways resistance. Pennock et al. (1977) studied lower doses of fenoterol by MDI in adults with moderately severe asthma. Doses administered were 100, 200, and 400  $\mu\text{g}$ . It was found that 100  $\mu\text{g}$  was as effective as the two higher doses. In contrast, Conrad et al. (1986) found that 200  $\mu\text{g}$  was required to produce a maximal response.



**3. Terbutaline.** Terbutaline is a synthetic sympathomimetic amine. It possesses a tertiary butyl group on the terminal nitrogen of the side chain that gives it greater  $\beta_2$ -AR specificity than isoetharine or metaproterenol. The drug has the same 3,5 *meta*-dihydroxy groups as metaproterenol, making it a member of the resorcinol group and thereby increasing its duration of action to 4 to 6 h. Its structural formula differs from that of albuterol in that it has a dihydroxybenzene group at the  $\beta$ -carbon atom instead of a benzene ring, with *meta*-hydroxymethyl and *para*-hydroxyl groups. In anesthetized dogs, terbutaline was a relatively more potent activator of pulmonary and vascular  $\beta$ -ARs than of cardiac  $\beta$ -ARs (Wasserman and Levy, 1974). For reducing histamine-induced bronchospasm, terbutaline was one-sixth as potent as isoproterenol; for vascular responses, it was 1/30 to 1/20 as active as isoproterenol.

In subjects with asthma, terbutaline administered by aerosol inhalation in doses of 250 and 500  $\mu\text{g}$  yielded prompt bronchodilation above pretreatment levels that was maintained for 5 h (Formgren, 1970). Furthermore, inhalation of aerosols of 250  $\mu\text{g}$  of terbutaline and of 100  $\mu\text{g}$  of albuterol was followed by the same mean maximum increase in forced expiratory volume in 1 s ( $\text{FEV}_1$ ) in subjects with asthma (Freedman, 1972). After inhalation of these doses of each drug, there were rapid bronchodilator responses, the amplitudes of which were the same for 90 min. With these doses, bronchodilation was maintained at a higher level after terbutaline, the difference being statistically significant during the fourth hour.

When inhaled terbutaline (250  $\mu\text{g}$  per puff) and fenoterol (100  $\mu\text{g}$  per puff) were compared, equivalence of bronchodilator effects on a "puff per puff" basis was documented, but dose-response curves for heart rate showed a greater response with fenoterol, although the mean difference between the highest doses of fenoterol (1500  $\mu\text{g}$ ) and terbutaline (3750  $\mu\text{g}$ ) was only 10 beats/min (Gray et al., 1982).

Cumulative puffs of inhaled fenoterol (200  $\mu\text{g}$  per puff), albuterol (100  $\mu\text{g}$  per puff), and terbutaline (250  $\mu\text{g}$  per puff) were given up to a total of 26 puffs (Wong et al., 1990). This design was used to mirror the dose of drug delivered by each puff, as occurs in clinical practice. Conventional low doses (two puffs) of all three drugs produced equal bronchodilation and caused no systemic effects. Only at much higher doses did any separation of systemic effects between fenoterol and the other  $\beta_2$ -AR agonists emerge. The lowest doses of all three drugs produced  $\text{FEV}_1$  responses near the top of the dose-response curve, whereas systemic effects did not occur until after eight puffs.

Unfortunately, when given parenterally, terbutaline loses much of this selectivity, and cardiovascular effects similar to those of isoproterenol are observed. Compared with epinephrine, subcutaneous terbutaline can induce more bronchodilation for longer periods but with more side effects. Because the MDI formulation was not available originally, and terbutaline was considered by many clini-

cians to be superior to metaproterenol, the injectable ampule solution of 1 mg/ml was often used as a nebulizer solution. In any case, terbutaline by nebulization is at least as effective as epinephrine administered subcutaneously in the treatment of acute asthma (Uden et al., 1985). Terbutaline can also be administered orally as 5-mg terbutaline sustained-release tablets, twice daily.

Alternatively, it is possible to use bambuterol orally, which is an oral terbutaline prodrug with a prolonged duration of bronchodilator action (Olsson and Svensson, 1984) that allows once-daily administration. Bambuterol itself is devoid of sympathomimetic activity (Olsson and Svensson, 1984), but terbutaline is formed from bambuterol by hydrolysis, predominantly catalyzed by plasma cholinesterase (Tunek et al., 1988) and probably by cytochrome P450 (P450) enzymes (Svensson and Tunek, 1988). The slow metabolism of bambuterol to terbutaline contributes to the 24-h duration of action. The clinical efficacy of once-daily bambuterol seems to be equivalent to that of b.i.d. sustained-release terbutaline (Fugleholm et al., 1993). On the contrary, in patients with COPD, 20 mg of bambuterol once daily seems to be less effective than 50  $\mu\text{g}$  of salmeterol b.i.d. by inhalation (Cazzola et al., 1999a).

### C. Long-Acting $\beta_2$ -Adrenergic Receptor Agonists

Long-acting  $\beta_2$ -AR agonists (LABAs) such as salmeterol and formoterol provide 12-h bronchodilation (Lötvall, 2002). The duration of action of  $\beta_2$ -AR agonists in the human bronchus is in the following order: salmeterol  $\gg$  formoterol  $\geq$  albuterol  $\geq$  terbutaline  $>$  fenoterol.

Although formoterol and salmeterol are both potent and effective  $\beta_2$ -AR agonists, their different chemical structures confer markedly different pharmacological characteristics. It has been suggested that salmeterol specifically binds to the  $\beta_2$ -AR via the albuterol "head group," whereas a secondary exosite has been proposed in which the lipid tail binds to confer the long duration of action (Green et al., 1996). The exosite is an auxiliary binding site, a domain of highly hydrophobic amino acids within the fourth domain of the  $\beta_2$ -AR (Johnson, 1998). When the lipid tail is in association with the exosite, the salmeterol is prevented from dissociating from the  $\beta_2$ -AR, but the head can freely engage and disengage the active site by the Charnière (hinge) principle, the fulcrum being the oxygen atom in the side chain. The position of this oxygen atom is critical for the long duration of action (Johnson, 2001). The salmeterol molecule is  $>10,000$  times more lipophilic than albuterol. It partitions rapidly ( $<1$  min) into the cell membrane and then diffuses laterally to approach the active site of the  $\beta_2$ -AR through the membrane. The process is relatively slow ( $>30$  min) and accounts for the slow onset of action of salmeterol compared with albuterol (Johnson, 2001).

On the contrary, formoterol is not thought to be able to access the exosite and an alternative hypothesis for its long duration of action has been proposed whereby the lipophilic, basic nature of this drug allows formoterol to partition effectively into the lipid bilayers of ASM after inhala-

tion. This partitioning then permits an effective concentration of agonist to be present over time in the form of a depot within the ASM, from where formoterol progressively leaches out to interact with the active site of the  $\beta_2$ -AR, providing a prolonged duration of action. This is known as the diffusion microkinetic theory (Anderson et al., 1994). The size of the depot is determined by the concentration or dose of formoterol applied. In airway preparations, the onset of action of formoterol is somewhat delayed compared with albuterol, and the duration of relaxant activity, although longer, is concentration-dependent (Johnson, 2001). In any case, formoterol is somewhat less lipophilic than salmeterol and is believed to diffuse more rapidly through the lung tissues, reaching the site of action faster than salmeterol (Anderson, 1993).

**1. Formoterol.** Formoterol (eformoterol in the United Kingdom) is a phenylethanolamine derivative. It is a pure diastereomer of the *R,R*- and *S,S*-enantiomers of 2-hydroxy-5-[(1*R,S*)-1-hydroxy-2-[(1*R,S*)-2-(*p*-methoxyphenyl)-1-methylethylamino] formanilide prepared as the fumarate dihydrate salt. The two major structural features of the formoterol molecule are an *N*-aralkyl group to replace the *N*-alkyl group of phenylethanolamine, because this substitution greatly enhances  $\beta_2$ -AR selectivity, and a formamide moiety that confers strong hydrogen bonding with key amino acid residues in the  $\beta_2$ -AR active site and may be a major determinant of the intrinsic activity and very high affinity of formoterol at the  $\beta_2$ -AR (Anderson, 1993).

The pharmacology of formoterol was initially described by Ida (1976a,b). Formoterol was compared with isoproterenol, orciprenaline, trimetoquinol, and albuterol for its  $\beta$ -adrenergic activity and selectivity in vitro. On guinea pig trachea, the maximum relaxing responses to the five agonists were similar, but the order of potency was formoterol > trimetoquinol > isoproterenol  $\geq$  albuterol > orciprenaline. On atria, the maximum chronotropic and inotropic responses to isoproterenol were greater than those to formoterol, orciprenaline, and trimetoquinol, whereas albuterol caused the weakest cardiac stimulating effect. Formoterol, orciprenaline, trimetoquinol, and albuterol seemed to act as partial agonists on atria. Albuterol showed high selectivity for trachea, whereas orciprenaline and trimetoquinol were equipotent on trachea and atria. Isoproterenol was more potent on atria than on trachea, whereas formoterol had the highest bronchoselectivity among five agonists tested. The  $\beta$ -AR stimulant activity of formoterol was compared with those of isoproterenol, orciprenaline, trimetoquinol, and albuterol in conscious guinea pigs. The bronchodilator effect of formoterol was most potent among the five agonists after subcutaneous, oral, or aerosol administration and had the longest duration of action after oral or aerosol administration. Formoterol was similar to isoproterenol but more potent than trimetoquinol or orciprenaline and albuterol in increasing heart rate after administration by the subcutaneous route. However, the cardiostimulating effect of formoterol was

more potent than that of isoproterenol, trimetoquinol, or albuterol orally. The order of bronchoselectivity [the ratio between effective dose (ED)<sub>30</sub> heart beats per minute versus ED<sub>50</sub> for bronchodilator] in conscious guinea pigs was albuterol > formoterol = trimetoquinol > orciprenaline > isoproterenol; after either subcutaneous or oral administration, the order was formoterol = albuterol > trimetoquinol = isoproterenol.

In vitro studies using both animal and human ASM preparations have revealed that formoterol has a faster onset of action compared with salmeterol (Lindén et al., 1993; Naline et al., 1994). Moreover, a strongly contracted smooth muscle will relax to a greater extent if formoterol rather than salmeterol is added to the preparation (Lindén et al., 1993; Naline et al., 1994). In particular, Nials et al. (1994) documented that formoterol, like albuterol and salmeterol, relaxed isolated preparations of guinea pig trachea and human bronchus, and inhibited antigen-induced mediator release from human lung fragments in a concentration-dependent fashion. In each case, these actions were mediated through  $\beta_2$ -ARs, formoterol being 50- to 120-fold more potent than albuterol and 2- to 27-fold more potent than salmeterol. The duration of action of formoterol was longer than that of albuterol in all preparations but was markedly shorter than that of salmeterol, whose actions persisted for many hours despite continuous or extensive washing of the tissues. In conscious guinea pigs, inhaled formoterol, albuterol, and salmeterol each caused dose-related inhibition of histamine-induced bronchoconstriction. Formoterol was again more potent (10- to 20-fold) than either albuterol or salmeterol. However, although the actions of a threshold-effective dose of formoterol persisted for less than 3 h, somewhat longer than that observed with albuterol (< 1.5 h), an equivalent dose of salmeterol was active for at least 6 h.

An oral formulation of formoterol did not seem to offer a clear advantage to albuterol (Tasaka, 1986). In effect, formoterol has a long duration of action when given by inhalation but not when given orally (Löfdahl and Svedmyr, 1989). A dose-response comparison of formoterol and albuterol in patients with asthma suggested that formoterol is 5 to 15 times as potent as albuterol on the airways after inhalation and 50 times more potent after oral administration (Löfdahl and Svedmyr, 1989).

Formoterol has been shown to provide a rapid onset bronchodilating effect that occurs within minutes after inhalation. A number of studies have shown a comparable clinical effect of formoterol compared with the SABAs albuterol and terbutaline in stable patients with asthma (Lötvall, 2002). Moreover, the onset of effect of formoterol is comparable with that of albuterol. The onset of bronchodilation (defined as a 15% or greater increase from baseline in FEV<sub>1</sub>) has been demonstrated to be similar for 12  $\mu$ g of formoterol by Aerolizer (Novartis AG, Basel, Switzerland) and 180  $\mu$ g of albuterol by MDI. In patients with acute asthma, 36  $\mu$ g formoterol administered by Turbuhaler (AstraZeneca, Lund, Sweden) produced a rapid and clinically

relevant improvement in FEV<sub>1</sub> that was not statistically significantly different from that of 1600 µg of albuterol by MDI and spacer (Rubinfeld et al., 2006). Other studies have shown similar onset of bronchodilation with 12 and 24 µg of formoterol administered via Turbuhaler or MDI and 200 to 800 µg of albuterol given via MDI or dry powder inhaler in patients with COPD (Cazzola et al., 2001, 2002b). In patients with mild acute exacerbations of COPD, formoterol administered via the Turbuhaler induced a fast bronchodilation that was dose-dependent and not significantly different from that caused by albuterol (Cazzola et al., 2002a).

Formoterol protects against bronchial responsiveness, measured as methacholine responsiveness, in a dose-dependent manner and bronchoprotects for at least 12 h after inhalation (Ramsdale et al., 1991). In methacholine-induced bronchoconstriction, both formoterol and albuterol have a very fast onset of action, achieving prechallenge values of specific airway conductance (sGaw) within 3 min, albuterol being slightly faster than formoterol (van Noord et al., 1998).

In patients with COPD, formoterol (12–36 µg) induces a significant spirometric improvement over 12 h (Cazzola et al., 1995). A single high dose of formoterol (36 µg) is as effective as the same dose administered in a cumulative manner in patients with acute exacerbations of COPD (Cazzola et al., 2003).

**2. Salmeterol.** Salmeterol is a drug resulting from a specific research program designed to achieve prolonged duration of action by molecular modification of albuterol. The resulting 25-Å molecule consists of the saligenin head of albuterol that binds to the active site of the β<sub>2</sub>-AR, coupled to a long aliphatic side chain that profoundly increases the lipophilicity of the molecule.

At β<sub>2</sub>-ARs in ASM in vitro, salmeterol is more potent than isoprenaline and albuterol (Johnson et al., 1993). In bovine tracheal smooth muscle, a close temporal correlation between promotion of cAMP accumulation and tissue relaxation stimulated by salmeterol and albuterol was documented when both parameters were measured under identical conditions (Ellis et al., 1995).

The potency and duration of action of salmeterol, isoprenaline and a range of β<sub>2</sub>-AR agonists as relaxants of inherent tone in human superfused isolated bronchial smooth muscle was investigated (Nials et al., 1993). All of the β<sub>2</sub>-AR agonists caused concentration-related inhibition of inherent tone. The rank order of agonist potency was formoterol ≥ salmeterol ≥ clenbuterol > fenoterol = isoprenaline > terbutaline ≥ albuterol > quinprezaline. Rt50 [i.e., the time taken for 50% recovery from the effects of a median effective concentration required to induce a 50% effect (EC<sub>50</sub>) of agonist] differed considerably among the different β<sub>2</sub>-AR agonists. Most agonists were short-acting, having Rt50 values less than 13 min. Quinprezaline was of moderate duration, with an Rt50 value of ≥20 min. In contrast, salmeterol was extremely long-acting, with no sign of recovery within 4 h.

The onset of action of salmeterol on ASM is slower than that of other β<sub>2</sub>-AR agonists, such as albuterol and formoterol, but it seems to be inherently long-acting, in that its effects are independent of concentration as a result of exosite binding, whereas albuterol, fenoterol, and formoterol have shorter durations of action, but this can be prolonged by increasing the concentration of the β<sub>2</sub>-AR agonist applied to the tissue (Johnson et al., 1993).

Whereas isoprenaline, fenoterol, and formoterol are full agonists at β<sub>2</sub>-ARs, salmeterol and albuterol are partial agonists with ~60 and 85% of the efficacy of isoprenaline, respectively. In contrast to its effects on β<sub>2</sub>-ARs, at cardiac β<sub>1</sub>-ARs, salmeterol is >10,000-fold weaker than isoprenaline and has a very low efficacy (4%) (Johnson et al., 1993). Pharmacological studies using ASM cell preparations from animals and humans have shown that salmeterol has a smaller maximal effect compared with formoterol (lower pharmacological efficacy/lower intrinsic activity) (Lindén et al., 1993). Thus, strongly contracted smooth muscle will relax to a lesser extent with salmeterol compared with formoterol, showing that salmeterol is a partial agonist at the β<sub>2</sub>-AR site in relation to formoterol (Lindén et al., 1993). According to the basic principles for agonist/antagonist interaction, a partial agonist has to occupy more receptors than a full agonist to induce the same effect and behaves as an antagonist in the presence of an agonist with higher efficacy acting on the same receptor. In human precontracted bronchi, salmeterol behaves as an antagonist to all β<sub>2</sub>-AR agonists studied (i.e., isoprenaline, fenoterol, terbutaline, and albuterol) (Molimard et al., 1998). This antagonism seems to be more pronounced toward albuterol. For relaxations comparable with those obtained with salmeterol, formoterol had no antagonistic effect toward any of the β<sub>2</sub>-AR agonists tested (Molimard et al., 1998). A partial β<sub>2</sub>-AR agonist may attenuate the effects of a β<sub>2</sub>-AR agonist with greater efficacy, raising the possibility that pretreatment with salmeterol may affect the acute bronchodilatory effects of reliever medication (Källström et al., 1994). However, clinical data argue against such an effect in patients treated regularly with salmeterol (Cazzola et al., 1998b; Korosec et al., 1999), although some patients taking salmeterol regularly have been shown to have a higher risk of exacerbations (Guo et al., 2011).

In patients with asthma or COPD, the onset of bronchodilation with salmeterol is approximately 10 min, although maximal bronchodilation may not occur for hours (Tattersfield, 1993; Cazzola et al., 1994, 1995).

In subjects with asthma, 50 µg of salmeterol produced greater protection against histamine challenge than 200 µg of albuterol (Gongora et al., 1991), but high doses of formoterol protected more against methacholine-induced bronchoconstriction than salmeterol did (Palmqvist et al., 1999).

Using sGaw measurement in healthy subjects, it was demonstrated that 200 µg of salmeterol provides a significant increase in sGaw up to 24 h after a single dose (Pullerits et al., 2011). In patients with asthma or COPD,



the bronchodilation elicited by salmeterol lasts at least 12 h (van Noord et al., 1996; Cazzola et al., 1994, 1995). It is noteworthy that in patients with mild bronchial asthma, 50  $\mu$ g of salmeterol seems to have a duration of action up to 24 h (Rabe et al., 1993).

#### D. Ultra-Long-Acting $\beta_2$ -Adrenergic Receptor Agonists

A variety of  $\beta_2$ -AR agonists with longer half-lives are currently in development, with the hope of achieving once-daily dosing (Cazzola et al., 2005b, 2011; Matera and Cazzola, 2007; Cazzola and Matera, 2008, 2009). These agents include indacaterol (which received European regulatory approval in November 2009 and has already been launched in several countries worldwide), olodaterol, vilanterol, carmoterol, 5-(2-[[6-(2,2-difluoro-2-phenylethoxy)hexyl]amino]-1(*R*)-hydroxyethyl)-8-hydroxyquinolin-2(1*H*)-one (LAS100977), *N*-((4'-hydroxybiphenyl-3-yl)methyl)-2-(3-(2-((2-hydroxy-2-(4-hydroxy-3-((methylsulfonyl)amino)phenyl)ethyl)amino)-2-methylpropyl)phenyl)acetamide (PF-610355), and AZD-3199, the structure of which has not yet been disclosed but has been suggested by Norman (2009).

**1. Indacaterol.** Indacaterol, also known as QAB149, is a novel chirally pure inhaled ultra-LABA. Within a series of 8-hydroxyquinoline 2-aminoindan-derived  $\beta_2$ -agonists, the 5,6-diethyl substituted indan analog indacaterol was selected using lipophilicity as the basis for the design and rationalization of their onset and duration of action profiles, as assessed by a guinea pig tracheal-strip assay. In addition to lipophilicity, potency and intrinsic efficacy have also been shown to be contributing factors in regulating these in vitro time course profiles (Baur et al., 2010). Extensive preclinical studies involving indacaterol have been performed both in vitro and in vivo and have documented that it demonstrates a unique rapid onset of action and a bronchodilating effect that lasts for 24 h (Cazzola et al., 2010d).

Indacaterol seems to have a high intrinsic activity at human  $\beta_2$ -ARs in vitro. The mean maximum effect ( $E_{\max}$ ) for indacaterol was 73% of the maximum effect of isoprenaline, compared with 90, 38, and 47% for formoterol, salmeterol, and albuterol, respectively (Battram et al., 2006). Like formoterol, indacaterol is a very weak agonist at the  $\beta_1$ -AR (mean  $E_{\max}$  = 16% of the maximal effect of isoprenaline) but acts as a full agonist at the  $\beta_3$ -AR (mean  $E_{\max}$  = 113%) (Battram et al., 2006). Studies with isolated human bronchi and small-airway lung slices showed that indacaterol behaves as a high efficacy  $\beta_2$ -AR agonist, with an onset of action that is not significantly different from that of formoterol or albuterol but significantly faster than that of salmeterol, and a significantly longer duration of action than either formoterol or salmeterol (Naline et al., 2007; Sturton et al., 2008). In particular, a study that compared the properties of indacaterol with those of salmeterol, formoterol, and albuterol on small airways in precision-cut human lung slices contracted with carbachol (Sturton et al., 2008) confirmed that the onset of action is fast for albuterol, formoterol, and indacaterol, whereas it is signif-

icantly slower for salmeterol, and indicated that indacaterol and formoterol have a higher intrinsic efficacy than albuterol and salmeterol. It was also shown that indacaterol, in contrast to salmeterol, does not antagonize the bronchorelaxant effect of a SABA (Naline et al., 2007).

It is noteworthy that no tachyphylaxis has been demonstrated for indacaterol, although significant improvement in protection against 5-hydroxytryptamine-induced bronchoconstriction has been documented after 5-day dosing of indacaterol and formoterol (compared with a single treatment), but not with salmeterol, at least in guinea pig airways (Battram et al., 2006). The fact that indacaterol behaves as a nearly full  $\beta_2$ -AR agonist could explain why indacaterol does not induce tachyphylaxis and also does not antagonize the bronchorelaxant effect of a SABA. Although low-efficacy agonists may cause less receptor desensitization at equal occupancy, they require more receptors to generate a subsequent response and so will be more sensitive to loss of functional receptors (Charlton, 2009). High-efficacy agonists, in contrast, may cause a greater loss of receptors, but are more tolerant to this, as they have "spare receptors," resulting in a loss in potency but not necessarily any loss of maximal effect and are therefore less sensitive to loss of receptors through desensitization (Charlton, 2009). Preclinical data also suggest that, for a given degree of bronchodilator activity, indacaterol has a greater cardiovascular safety margin than formoterol or salmeterol (Battram et al., 2006).

The faster onset of action and longer duration of action of indacaterol compared with some other  $\beta_2$ -AR agonists may be related to interactions with lipid bilayers (Lombardi et al., 2009). Indacaterol and salmeterol show no major, but several minor, differences in their steady-state and kinetic interactions with lipid membranes, and the sum of these small differences, including higher partitioning of indacaterol into the microenvironment of the receptor and its faster membrane permeation, is thought to contribute to its faster onset and longer duration of therapeutic action. A striking difference was observed between indacaterol and salmeterol on membrane fluidity. Although indacaterol did not alter membrane fluidity, salmeterol drastically increased membrane fluidity. This may affect the function of the  $\beta_2$ -AR, reducing the intrinsic efficacy of salmeterol (Lombardi et al., 2009). It has also been suggested that lipid rafts, which are areas of cell membranes in which  $\beta_2$ -ARs are held together in close contact with signaling molecules and effectors, and caveolae, which are a special type of lipid raft, being small (50–100 nm) invaginations of the plasma membrane in ASM, might play a role in the long duration of action of indacaterol (Lombardi et al., 2009). Indacaterol has a 2-fold higher affinity for raft microdomains compared with salmeterol, which might contribute to the difference in duration of action between these two drugs. It has also been suggested that the higher intrinsic efficacy of indacaterol offsets the high lipophilicity that is important for achieving the long duration of action (Rosethorne et al., 2010). In fact, in primary human bron-

chial smooth muscle cells, indacaterol displays a similar intrinsic activity to formoterol that, combined with comparable lipophilicity, translates to a faster rate of cAMP accumulation, which plays a key role in  $\beta_2$ -AR-induced smooth muscle relaxation in the airways.

In patients with COPD, comprehensive assessment of the dose response relationship of indacaterol provided a robust confirmation that 75  $\mu$ g is the minimum effective dose, and that 150 and 300  $\mu$ g provided optimal bronchodilation, particularly in patients with severe disease (Renard et al., 2011). Single doses (150 and 300  $\mu$ g) of indacaterol demonstrated a fast onset of action similar to that for albuterol and faster than that for salmeterol-fluticasone (Balint et al., 2010). Moreover, once-daily indacaterol (150  $\mu$ g) is at least as effective as tiotropium bromide, with a faster onset of action (within 5 min) on the first day of dosing (Vogelmeier et al., 2010) and indacaterol (300  $\mu$ g) treatment improves the ability of patients with COPD to exercise (O'Donnell et al., 2011). In addition, the improvements observed in resting and end-exercise inspiratory capacity (IC) indicate reductions in lung hyperinflation after 3 weeks of treatment with indacaterol.

The efficacy of indacaterol in the maintenance treatment of adults with COPD has been assessed in large, randomized, double-blind, parallel-group, placebo-controlled, multicenter phase III trials (Dahl et al., 2010; Donohue et al., 2010; Feldman et al., 2010; Buhl et al., 2011; Chapman et al., 2011; Korn et al., 2011; Kornmann et al., 2011; Laforce et al., 2011). Analysis of these trials (Moen, 2010) shows that 150 and/or 300  $\mu$ g of indacaterol once daily was more effective than tiotropium bromide, formoterol, or salmeterol for improving trough FEV<sub>1</sub> values versus placebo. COPD exacerbations were significantly reduced versus placebo for 150  $\mu$ g or 300  $\mu$ g of indacaterol once daily. In a 52-week study (Dahl et al., 2010), once-daily treatment with indacaterol prolonged the time to the first COPD exacerbation and was effective in reducing incidence and frequency of COPD exacerbations, with no significant difference between indacaterol and formoterol. Patients treated with indacaterol had a significantly higher percentage of days with no use of as-needed rescue albuterol than did placebo recipients in all large studies. Moreover, the percentages of days with no rescue medication were significantly ( $P < 0.05$ ) higher in the indacaterol groups than the active comparator groups in all studies. In general, indacaterol seemed to have greater effects on most COPD symptoms than tiotropium bromide, formoterol, or salmeterol, although differences between indacaterol and active comparators were not consistently statistically significant. Indacaterol also provided significant and clinically relevant better health-related quality of life. In all studies designed to investigate whether indacaterol has the same tolerability of LABAs already on the market, indacaterol was well tolerated at all doses and with a good overall safety profile (Cazzola et al., 2010d).

A meta-analysis of 15 placebo-controlled randomized clinical trials suggests that indacaterol monotherapy (150

$\mu$  and 300  $\mu$ g) is at least as good as formoterol/budesonide (9/320 and 9/160  $\mu$ g) and comparable with salmeterol/fluticasone (50/250 and 50/500  $\mu$ g) with respect to lung function (trough FEV<sub>1</sub>) (Cope et al., 2011). Indacaterol monotherapy (150 and 300  $\mu$ g) also provides comparable efficacy in terms of health status [St. George's Respiratory Questionnaire (SGRQ) total score] versus formoterol/budesonide (9/320 and 9/160  $\mu$ g) and salmeterol/fluticasone 50/500  $\mu$ g, as well as improvements in breathlessness (transition dyspnea index total score) similar to those provided by salmeterol/fluticasone (50/250 and 50/500  $\mu$ g) (Cope et al., 2011).

Several short-term studies have also explored the effect of indacaterol in patients with asthma (Cazzola et al., 2010e), although it is well known that the use of LABAs for asthma is contraindicated in patients of all ages without concomitant use of an asthma-controller medication such as an inhaled corticosteroid (ICS) (Chowdhury and Dal Pan, 2010). In particular, two large 7-day dose-finding trials examined the effect of 1) 100, 200, 300, 400, or 600  $\mu$ g of indacaterol once daily, delivered via single-dose dry powder inhaler (Kanniess et al., 2008a) and 2) 50, 100, 200, or 400  $\mu$ g of indacaterol delivered via a multiple-dose dry powder inhaler and 400  $\mu$ g delivered via single-dose dry powder inhaler once daily (LaForce et al., 2008) in patients with persistent asthma. Once-daily dosing with indacaterol provided sustained 24-h bronchodilation in patients with moderate to severe asthma, with a satisfactory overall safety profile. In the first study, mean FEV<sub>1</sub> for indacaterol doses of 200  $\mu$ g or more on day 7 was higher than placebo predose and at all postdose time points (Kanniess et al., 2008a). In the second study, all doses of indacaterol provided rapid-onset sustained bronchodilation over 24 h on once-daily dosing from day 1, with no loss of bronchodilator after 7 days of treatment, although 200  $\mu$ g of indacaterol seemed to be the optimum dose, offering the best efficacy/safety balance (LaForce et al., 2008). Given the concern about LABAs' association with serious exacerbations of asthma and asthma-related deaths, review of the safety data for indacaterol in patients with asthma suggested that the proposed doses might be higher than necessary. Compared with control groups, groups of patients with asthma who were treated with indacaterol (300 or 600  $\mu$ g) in addition to an ICS had a small numerical increase in serious asthma exacerbations and respiratory-related deaths (Chowdhury et al., 2011b).

**2. Olodaterol.** Olodaterol, previously known as BI 1744 CL, is a novel pure enantiomer, identified from a series of 6-hydroxy-4*H*-benzo[1,4]oxazin-3-ones, that is a potent agonist of the human  $\beta_2$ -AR with a high  $\beta_1/\beta_2$ -selectivity (Bouyssou et al., 2010b). In vitro, olodaterol showed a potent, nearly full agonist response at the human  $\beta_2$ -AR ( $EC_{50} = 0.1$  nM; intrinsic activity = 88% compared with isoprenaline) and, unlike formoterol and salmeterol, which act as full and partial agonist, respectively, at all  $\beta$ -ARs, olodaterol exhibited a significant selectivity profile (219- and 1622-fold against the human

$\beta_1$ - and  $\beta_3$ -ARs, respectively) (Bouyssou et al., 2010a). On isolated human bronchi, olodaterol concentration-dependently reversed the constriction induced by different stimuli, such as histamine, ACh, and electric field stimulation with an efficacy not statistically different from the full agonist formoterol under all conditions (Bouyssou et al., 2010a). Olodaterol and formoterol exhibited similar potencies and  $E_{\max}$  values at resting tone and in the presence of various contracting stimuli. Formoterol induced significant  $\beta_2$ -AR desensitization in vitro, whereas olodaterol preserved the  $\beta_2$ -AR signaling capacity, even after long-term preincubation (Naline et al., 2010). Studies with precision-cut lung slices obtained from rat lungs and human lung tissue showed that olodaterol is comparable with formoterol and displayed significantly increased relaxation after partial precontraction of human small airways in response to carbachol (Brown et al., 2011).

In vivo antagonistic effects of single doses of olodaterol and formoterol were measured against ACh challenges in anesthetized guinea pigs and dogs for up to 24 h by using the Respimat Soft Mist inhaler (Boehringer Ingelheim, Ingelheim, Germany), a propellant-free MDI. In both models, olodaterol provided bronchoprotection over 24 h. Formoterol applied at an equally effective dose did not retain efficacy over 24 h. In both models, olodaterol showed a rapid onset of action comparable with formoterol (Bouyssou et al., 2010a). It is noteworthy that olodaterol has a biphasic dissociation profile from human  $\beta_2$ -ARs, the slow component ( $\sim 30$ – $40\%$  of the total  $\beta_2$ -AR pool) showing a half-life of dissociation of more than 12 h, providing a rationale for its long duration of action (Schnapp et al., 2009).

Olodaterol associates moderately with lipid bilayers, but kinetic as well as equilibrium binding studies indicate the presence of a stable [ $^3\text{H}$ ]olodaterol/ $\beta_2$ -AR complex with a dissociation half-life of 17.8 h because of ternary complex formation. The tight binding of olodaterol to the human  $\beta_2$ -AR and stabilization of the ternary complex have been confirmed in functional experiments monitoring adenylyl cyclase activity after extensive washout (Casarosa et al., 2011). Taken together, binding, kinetic, and functional data support the existence of a stable complex with the  $\beta_2$ -AR that, with a dissociation half-life of  $>17$  h, might be a rationale for the 24-h duration of action of olodaterol. Initial studies achieved their objective by providing proof of concept of the 24-h bronchoprotective effect of olodaterol in patients with intermittent asthma (O'Byrne et al., 2009), and 24-h bronchodilation was sustained after 4 weeks of once-daily administration in patients with COPD (van Noord et al., 2011).

3. *Vilanterol*. Vilanterol, also known as GSK-642444, is the triphenylacetate salt of the 2,6-dichlorobenzyl analog of a series of novel  $\beta_2$ -AR agonists obtained by the incorporation of an oxygen atom at the homobenzylic position of the right-hand side phenyl ring of (*R*)-salmeterol (Procopiu et al., 2010). The triphenyl-

acetate salt was found to have suitable properties for inhaled administration.

Vilanterol is a potent, selective,  $\beta_2$ -AR agonist in human functional cellular assays. Vilanterol has a greater intrinsic efficacy than salmeterol and a greater potency than indacaterol and albuterol. In addition, it has been shown, using human recombinant  $\beta_{1/2/3}$ -AR cAMP assays, that it has significantly greater  $\beta_2$ -AR selectivity than formoterol, indacaterol, and albuterol (Barrett et al., 2010a; Procopiu et al., 2010). In isolated electrically stimulated guinea pig tracheal strips, vilanterol [negative logarithm of  $\text{EC}_{50}$  ( $\text{pEC}_{50}$ ) = 8.6] was equipotent with formoterol ( $\text{pEC}_{50}$  = 8.6), more potent than salmeterol ( $\text{pEC}_{50}$  = 6.7) or indacaterol ( $\text{pEC}_{50}$  = 7.0), and was shown to be antagonized in a competitive manner by propranolol (Ford et al., 2010a). Vilanterol, formoterol, and indacaterol had a more rapid onset of activity compared with salmeterol. On removal of vilanterol, the tissue showed no recovery after 1 h, suggesting a long duration of action in contrast with isoprenaline (rapid and full recovery) or formoterol (slow but continuous recovery) (Ford et al., 2010a). In human tissue precontracted with 0.1  $\mu\text{M}$  histamine, 1 nM vilanterol was shown to have a significantly faster onset of action (3.1 min) than 1 nM salmeterol (8.3 min) (Barrett et al., 2010b). Vilanterol had a significantly longer duration of action compared with salmeterol: vilanterol still demonstrated a significant bronchodilator effect at 22 h, but salmeterol did not.

Vilanterol inhibited histamine-induced bronchoconstriction when administered to conscious guinea pigs as a nebulized solution and was equipotent to salmeterol (Ford et al., 2010a). At equieffective doses the duration of action was similar to that of salmeterol (10 h) and longer than that of formoterol ( $<3$  h); however, unlike salmeterol, the duration of action of vilanterol increased in a dose-dependent manner. It is noteworthy that vilanterol is a metabolically labile LABA that undergoes conversion in human microsomes to metabolites with significantly lower  $\beta_2$ -AR activity and exhibits low systemic exposure in vivo after inhaled dosing (Ford et al., 2010b).

Vilanterol has been tested in patients with asthma or COPD. Single doses of inhaled vilanterol (25–100  $\mu\text{g}$ ) produced a rapid and prolonged bronchodilation over 24 h in patients with asthma, suggesting the potential for once-daily administration (Kempsford et al., 2010a). All doses were well tolerated with no clinically significant unwanted systemic effects. Vilanterol (25–100  $\mu\text{g}$ ) produced rapid bronchodilation in patients with COPD that was maintained over 24 h at all doses (Kempsford et al., 2010b). After dosing with vilanterol, there were no serious adverse events or withdrawals due to adverse events and no clinically significant laboratory, vital sign, 12-lead electrocardiogram (ECG), QTc, or Holter ECG abnormalities. Vilanterol was rapidly absorbed into plasma (median  $T_{\max}$  at 10 min), systemic exposure increasing in an approximately dose-proportional manner across the vilanterol dose range (25–100  $\mu\text{g}$ ). A 28-day study in patients  $\geq 12$



years with persistent asthma on maintenance ICS showed that once-daily vilanterol was well tolerated and resulted in a prolonged duration of action of at least 24 h at doses  $\geq 12.5 \mu\text{g}$  (Lötvall et al., 2010b). Another study in adults with persistent asthma documented that  $6.25 \mu\text{g}$  of vilanterol b.i.d. showed the greatest change in trough  $\text{FEV}_1$ ; however, similar changes in the weighted mean 24-h  $\text{FEV}_1$  with  $12.5 \mu\text{g}$  once-daily dosing suggests no advantage over a 24-h period of twice-daily over once-daily dosing for the same total daily dose (Sterling et al., 2011).

A 28-day dose-ranging (3, 6.25, 12.5, 25, or  $50 \mu\text{g}$ ) study of vilanterol in patients with COPD demonstrated statistically significant improvements in trough  $\text{FEV}_1$  for all doses compared with placebo (Hanania et al., 2012). Vilanterol significantly improved trough  $\text{FEV}_1$  in a dose-dependent manner. Clinically relevant treatment differences of  $\geq 130 \text{ ml}$  in trough and 0- to 24-h weighted mean  $\text{FEV}_1$  were observed with 25- and  $50\text{-}\mu\text{g}$  doses of vilanterol versus placebo. All doses of vilanterol were associated with a low incidence of treatment-related side effects.

**4. Carmoterol.** Carmoterol (CHF 4226, TA 2005), a pure *R,R*-isomer, is a noncatechol  $\beta_2$ -agonist with a *p*-methoxyphenyl group on the amine side chain and a 8-hydroxyl group on the carbostyryl aromatic ring (Kikkawa et al., 1991), possesses structural elements from both formoterol and procaterol, and binds very firmly to  $\beta_2$ -ARs (Voss et al., 1992), a property shared by some other agonists that, like carmoterol, are based on a carbostyryl skeleton (Standifer et al., 1989). In studies employing chimeric  $\beta_2$ -ARs, the methoxyphenyl group in carmoterol has been found to be critical to the  $\beta_2$ -AR selectivity of the molecule (Kikkawa et al., 1998).

Carmoterol has been demonstrated to be a highly potent and selective  $\beta_2$ -AR agonist [it has 53 times higher affinity for the  $\beta_2$ -ARs than for the  $\beta_1$ -AR (Voss, 1994), and is approximately 5 times more selective for the  $\beta_2$ -ARs present in the tracheal preparation than those mediating chronotropic response in the right atrium (Kikkawa et al., 1998)]. Moreover, it displays a fast onset and long duration of activity under both in vitro and in vivo experimental conditions (Kikkawa et al., 1991, 1994; Voss et al., 1992). It is noteworthy that the persistence of action of carmoterol at human recombinant  $\beta_2$ -ARs is similar to that of salmeterol, and longer than that of indacaterol, which is longer than that of formoterol (Summerhill et al., 2008). In a study that measured the rate of accumulation of cAMP in primary human bronchial smooth muscle cells and compared this with measures of intrinsic activity in the same systems, carmoterol displayed an intrinsic activity similar to that of formoterol, which, combined with comparable lipophilicity, translated to a fast rate of cAMP accumulation (Rosethorne et al., 2010).

Carmoterol is more potent than other LABAs in methacholine-precontracted guinea pig tracheal smooth muscle (Kikkawa et al., 1991; Voss et al., 1992; Voss, 1994). Carmoterol has a similar onset of action compared with albuterol and formoterol and a faster onset of action compared

with salmeterol. Furthermore, the duration of tracheal smooth muscle relaxation is longer for carmoterol compared with both formoterol and salmeterol (Voss, 1994).

In patients with asthma, the pharmacokinetics (PK) of carmoterol is proportional to the dose, and nonlinear accumulation of the drug after repeated dosing treatments is negligible (Cazzola et al., 2010e). It is noteworthy that using Modulite technology (Chiesi, Parma, Italy), which uses hydrofluoroalkane (HFA) 134a as a propellant, a lung deposition of carmoterol as high as 41% of the nominal dose can be reached (Haeussermann et al., 2006).

In patients with persistent asthma,  $2 \mu\text{g}$  of carmoterol administered once daily was as effective as formoterol  $12 \mu\text{g}$  b.i.d. (Kottakis et al., 2006). Safety and tolerability results were similar between carmoterol and formoterol (Nandeuil et al., 2006).

In COPD, a single  $4\text{-}\mu\text{g}$  dose, but not a 1- or  $2\text{-}\mu\text{g}$  dose, of carmoterol had an effect on 24-h trough  $\text{FEV}_1$  that was better than that of two  $50\text{-}\mu\text{g}$  doses of salmeterol given 12 h apart (Kanniess et al., 2008b). After a 2-week treatment period, once-daily doses of 2 and  $4 \mu\text{g}$  of carmoterol resulted in placebo-adjusted improvements compared with baseline in trough  $\text{FEV}_1$  of 94 and 112 ml, respectively, whereas  $50 \mu\text{g}$  salmeterol b.i.d. resulted in an increase of 78 ml (Make et al., 2008). There were no significant changes in ECG results, blood pressure, or serum potassium or glucose levels compared with salmeterol or placebo (Bateman et al., 2008a). No tolerance to the bronchodilatory effects of carmoterol or salmeterol was observed over the 2 weeks of treatment (Rossing et al., 2008).

**5. LAS100977.** LAS100977 is a novel once-daily LABA. Its in vitro pharmacological profile has been reported (Aparici et al., 2010). In radioligand displacement assays using human  $\beta_1$ -,  $\beta_2$ -, and  $\beta_3$ -ARs expressed in cell lines, LAS100977 showed the highest  $\beta_2$ -AR affinity (0.6 nM) compared with reference compounds. LAS100977 was 10 times more potent than salmeterol and similar to formoterol and indacaterol. The onset of action (10 min) of LAS100977 was faster than those of salmeterol and indacaterol (19 and 14 min, respectively) and slower than that of formoterol (6 min), whereas its duration of action (670 min) was longer than those of formoterol and salmeterol (77 and 230 min, respectively) and comparable with indacaterol (450 min). LAS100977 demonstrated higher  $\beta_2/\beta_1$ -AR selectivity than formoterol and indacaterol in both binding (64-fold) and tissue functional studies (10,750-fold). In anesthetized dogs, LAS100977 inhibited ACh-induced bronchoconstriction more potently and with a longer duration of action than salmeterol (Miralpeix et al., 2010). LAS100977 also had a higher therapeutic index than salmeterol, suggesting a reduced potential for cardiac side effects in man.

In healthy subjects, LAS100977 at doses of 5, 10, 25, and  $50 \mu\text{g}$  produced an increase in specific airway conductance (sGaw) at the 24-h postdose time point compared with the respective predose value in contrast to placebo (Timmer et al., 2010). In addition, airways resistance decreased for

LAS100977 at the 24-h after dosing time point compared with the predose value. The most frequent drug-related adverse events were palpitations and tremor, which were both of mild to moderate intensity. Two preliminary clinical studies have documented 24-h duration of action. In the first study, single different once-daily doses (5, 10, and 25  $\mu\text{g}$ ) of LAS100977 induced a significant increase in  $\text{FEV}_1$  compared with both placebo and salmeterol 50  $\mu\text{g}$  b.i.d., with a rapid onset of effect and improvements in lung function at 5 min after dosing in patients with mild to moderate asthma (Beier et al., 2010). Tachycardia and tremor were seen at the higher doses (Beier et al., 2010). In another study, LAS100977 also demonstrated sustained efficacy during multiple dose administrations with no significant increase in cardiovascular adverse events (Cazzola et al., 2010e).

**6. PF-610355.** PF-610355 is a member of a novel series of potent and selective sulfonamide-derived  $\beta_2$ -AR agonists (Glossop et al., 2010). The sulfonamide agonist headgroup confers high levels of intrinsic crystallinity that could relate to the acidic sulfonamide motif supporting a zwitterionic form in the solid state. Optimization of PK properties was achieved through targeted introduction of a phenolic moiety to support rapid phase II clearance, thereby minimizing systemic exposure after inhalation and reducing systemically mediated adverse events. It is intriguing that plasma PK of orally inhaled PF-610355 are consistent and exhibit a sustained plateau after single/multiple doses, but plasma exposure is reduced in patients with asthma compared with healthy volunteers (Li et al., 2009). In healthy subjects, the duration of action of PF-610355 450  $\mu\text{g}$  on airways determined by plethysmography was superior to 50  $\mu\text{g}$  salmeterol by 9.77 h indicating the potential for sustained pharmacological effect in the lung (Macintyre et al., 2009). A preliminary trial has documented that in patients with asthma, PF-610355 elicits a clear dose-response for peak and trough (32 h after dosing)  $\text{FEV}_1$ . At doses of 368, 736, and 1472  $\mu\text{g}$ , it produced higher peak  $\text{FEV}_1$  than 50  $\mu\text{g}$  of salmeterol (Ward et al., 2009).

**7. AZD3199.** AZD3199 is a novel selective ultra-LABA. It is a potent agonist (6 nM  $\text{EC}_{50}$ ) at the human  $\beta_2$ -ARs with an intrinsic activity of 0.8 relative to formoterol. AZD3199 displays a rapid onset of action in both guinea pig (22 min) and human (11 min) lung tissue, which is very similar to that determined for formoterol and significantly faster than salmeterol (>100 min) (Young et al., 2011a). Similar  $\beta_2$ -AR agonist activity has been demonstrated across multiple species, including guinea pig, rat, dog, mouse, and rabbit. AZD3199 is highly selective (>1500-fold) for the human  $\beta_2$ -AR over human  $\beta_1$  and  $\beta_3$ -ARs with no evidence of agonism (Young et al., 2011a). No activity has been observed at the channel coded by human ether-à-go-go-related gene at concentrations up to 26  $\mu\text{M}$ . High plasma protein binding (>90%) has been shown across multiple species (Young et al., 2011a). AZD3199 is also a potent

inhibitor of histamine-induced bronchoconstriction with an  $\text{ED}_{80}$  of 40  $\mu\text{g}/\text{kg}$  (Young et al., 2011b). When administered intratracheally at an  $\text{ED}_{80}$ , AZD3199 produces significant bronchoprotection up to 24 h after dosing, compared with equipotent doses of formoterol and salmeterol, which induce significant bronchoprotection up to a maximum of 12 h after dosing (Young et al., 2011b). AZD3199 also has the longest lung PK half-life. Inhalation exposure of nebulized AZD3199 at an approximate  $\text{ED}_{80}$  produces bronchoprotection lasting 24 h with no significant effects on blood potassium levels (Young et al., 2011b). An equipotent inhaled dose of formoterol induces bronchoprotection up to 8 h after dosing and significant decreases in blood potassium levels at 2 h after dosing. The reduced systemic effects for AZD3199 relative to formoterol are consistent with a lung to plasma concentration ratio of 308 for AZD3199 and 17 for formoterol at 2 h after dosing (Young et al., 2011b).

Three once-daily doses of AZD3199 (200, 400, and 800  $\mu\text{g}$ ) delivered via Turbuhaler were compared with b.i.d. formoterol (9  $\mu\text{g}$ ) and placebo over 4 weeks in patients with COPD (Kuna et al., 2011). For all three doses of AZD3199,  $\text{FEV}_1$  values gathered from 0 to 4 h and from 24 to 26 h were statistically significantly greater versus placebo after 4 weeks' treatment, but no dose response was seen. On the contrary, formoterol b.i.d. was not superior to placebo for  $\text{FEV}_1$  from 24 to 26 h. AZD3199 groups used less reliever medication than the placebo group. AZD3199 did not affect the acute bronchodilating effect of salbutamol. All AZD3199 groups showed a reduction in symptom scores, the 800- $\mu\text{g}$  dose reaching significance versus formoterol and placebo. AZD3199 was well tolerated with no safety concerns.

### E. Intravenous $\beta_2$ -Adrenergic Receptor Agonists

An interesting new option is the development of a  $\beta_2$ -AR agonist to be administered intravenously. Bedoradrine (MN-221) is a novel, highly selective  $\beta_2$ -adrenoceptor agonist under development for the treatment of acute exacerbation of asthma and COPD. Bedoradrine was calculated to be 832- and 126-fold more selective for  $\beta_2$ -ARs than for  $\beta_1$ - and  $\beta_3$ -ARs, respectively, which indicates that it is very highly selective for  $\beta_2$ -ARs in this assay system (Inoue et al., 2009). However, it was initially considered for the treatment of preterm labor because it is 1590-fold more specific for the uterus than for the trachea (Inoue et al., 2009).

The PK and pharmacodynamics (PD) of bedoradrine were investigated using data from a single intravenous dose study in patients with stable moderate to severe COPD (Sadler et al., 2010). Patients receiving doses of 600 and 1200  $\mu\text{g}$  showed responses superior to those of patients receiving 300  $\mu\text{g}$ . At 1200  $\mu\text{g}$ , the mean peak  $\text{FEV}_1$  increase was approximately 55% of maximal lending support to this dose.

Although both albuterol and bedoradrine independently induced an increase in heart rate, adverse effects on heart

rate were not observed upon combination in dogs. Other cardiovascular parameters (QTc and monophasic action potential) were not adversely affected in the albuterol + bedoradrine combination. These findings are consistent with some other data implicating bedoradrine as a partial agonist at  $\beta_1$ -ARs (Johnson et al., 2010).

A study that evaluated the safety and tolerability of bedoradrine at doses of 150 to 900  $\mu\text{g}$  via intravenous infusion in patients with mild to moderate stable asthma documented that it was safe and well tolerated, with dose-dependent improvements in FEV<sub>1</sub> (Matsuda et al., 2010). A preliminary small trial showed that bedoradrine added to standard therapy for severe acute asthma exacerbations was safe and provided additional clinical benefit (Nowak et al., 2010).

In a small group of patients with COPD, bedoradrine, at doses of 300, 600, or 1200  $\mu\text{g}$  i.v., seemed to improve lung function at all dose levels and reached statistical significance at both 600 and 1200  $\mu\text{g}$  compared with placebo (Pearle et al., 2010). FEV<sub>1</sub> (L) increased compared with baseline by an average of 21.5% ( $P = 0.0025$ ) for the 1200- $\mu\text{g}$  dose, 16.2% ( $P = 0.02$ ) for the 600- $\mu\text{g}$  dose, and 9.2% ( $P = \text{NS}$ ) for the 300- $\mu\text{g}$  dose compared with a decrease of 4.0% for placebo. Bedoradrine was generally well tolerated by all patients.

#### *F. Side Effects of $\beta_2$ -Adrenergic Receptor Agonists*

Because of the widespread distribution of  $\beta_2$ -ARs, a number of undesired responses result when  $\beta_2$ -AR agonists are absorbed into the systemic circulation. Therefore, side effects of  $\beta_2$ -AR agonists are greatest when the drugs are administered orally or parenterally (Svedmyr and Löfdahl, 1996), but there is some risk of cardiac actions even with inhaled formulations (Cazzola et al., 1998c, 2005a).

Increased heart rate and palpitations are less common with the selective  $\beta_2$ -AR agonists than with nonselective  $\beta_1$ - and  $\beta_2$ -AR agonists, although even stimulation of  $\beta_2$ -ARs can result in vasodilation and reflex tachycardia. Furthermore, some of the  $\beta$ -ARs in the atria and ventricles are  $\beta_2$  in type, and thus direct stimulation of the heart results from the use of even selective  $\beta_2$ -AR agonists (Cazzola et al., 1998c, 2005a). Consequently, they should be used with caution in patients with hyperthyroidism or cardiovascular disease (arrhythmias, hypertension, QT-interval prolongation) (Cazzola et al., 1998c, 2005a).

Administration of  $\beta_2$ -AR agonists to patients with airways obstruction often results in a transient decrease in partial pressure of oxygen in arterial blood (PaO<sub>2</sub>) despite concomitant bronchodilation. This transient decrease in PaO<sub>2</sub> has been attributed to the pulmonary vasodilator action of these agents (Knudson and Constantine, 1967) as a result of the activation of  $\beta_2$ -ARs that are present in pulmonary blood vessels (Conner and Reid, 1984), increasing blood flow to poorly ventilated lung regions and thus increasing ventilation-perfusion (VA/Q) inequality, a shunt-like effect, at least in patients with asthma (Wagner et al., 1978). Hypoxemia induced by  $\beta_2$ -AR agonists is

usually mild (Cazzola et al., 2006; Santus et al., 2007). Intriguingly, there is evidence that, in more vulnerable patients with severe COPD undergoing exacerbations, nebulized albuterol does not aggravate pulmonary gas exchange. In contrast, there is a small deterioration in the gas exchange response to albuterol in the same patients whose baseline lung function has improved as a result of increased perfusion of low-V/Q-ratio regions, while these patients are in convalescence (Polverino et al., 2007).

$\beta_2$ -AR agonists should also be used with caution in patients with diabetes because of the risk of ketoacidosis.  $\beta_2$ -AR stimulation in the liver induces glycogenolysis and therefore raises blood sugar levels (Philipson, 2002). Hypokalemia is also a risk because of stimulation in skeletal muscle of the Na<sup>+</sup>,K<sup>+</sup>-ATPase-driven pump coupled to  $\beta_2$ -ARs. This increases the ability of the Na<sup>+</sup>,K<sup>+</sup> pump to push Na<sup>+</sup> out of the cell and facilitate intracellular accumulation of K<sup>+</sup>, thereby lowering plasma levels. An increase in blood flow to the skeletal muscles through  $\beta_2$ -AR-mediated vasodilation could also contribute to the increase in skeletal muscle K<sup>+</sup> levels (Tesfamariam et al., 1998). Numerous studies have shown a dose-related fall in serum K<sup>+</sup> level with increasing doses of  $\beta_2$ -AR agonist (Scheinin et al., 1987), although there is some evidence that this effect shows tolerance and that serum K<sup>+</sup> levels after regular treatment are not reduced (Canepa-Anson et al., 1987). Such hypokalemia may precipitate arrhythmias; hence, the use of  $\beta_2$ -AR agonists is linked to an increased incidence of tachyarrhythmias (Du Plooy et al., 1994).

A further side effect of selective  $\beta_2$ -AR agonists is a fine tremor of skeletal muscle, particularly of the hands, which can be inhibited in experimental animals by the selective  $\beta_2$ -AR antagonist ( $\pm$ )-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol (ICI 118551) (Tesfamariam et al., 1998). It is greatest after oral or parenteral routes of administration (Ahrens, 1990). An early explanation of the tremor was that  $\beta_2$ -AR stimulation shortens the active state of skeletal muscle, which leads to incomplete fusion and reduced tension of tetanic contractions (Waldeck, 1976). Normally, tetanic contractions involved in maintaining steady muscle tone are fused. This property, together with  $\beta_2$ -AR-mediated enhancement of skeletal muscle spindle discharge, could explain the development of muscle tremor often experienced by patients using  $\beta_2$ -AR agonists. More recently, the tremor has been correlated closely with the hypokalemia, and it was suggested that the tremor was a direct result of the raised intracellular K<sup>+</sup> levels in skeletal muscle (Tesfamariam et al., 1998). However, tolerance to the tremorogenic effects of  $\beta_2$ -AR agonists occurs with their long-term use (Ahrens, 1990).

$\beta_2$ -AR agonists can induce the mobilization of triglycerides resulting in elevated blood levels of fatty acids and glycerol (Smith and Kendall, 1984), although it is the  $\beta_1$ -AR that is responsible for mediating this effect. Albu-



terol, terbutaline, and fenoterol can induce mild appetite suppression, headache, nausea, and sleep disturbances (Miller and Rice, 1980; Pratt, 1982). This is consistent with their ability to cross the blood-brain barrier, leading to CNS levels approximately 5% of those seen in plasma (Caccia and Fong 1984). Use of indacaterol can also elicit cough in some users, although when cough does occur, it is mild in severity, transient in nature and duration, and declines with the duration of treatment (Cazzola et al., 2010d).

### III. Muscarinic Acetylcholine Receptor Antagonists

#### A. Brief History of Development

Inhaled mAChR antagonists have been used as treatments for respiratory diseases for centuries. The smoking of plant alkaloids was recommended as a therapy for asthma in the literature of Ayurvedic medicine as early as the 17th century (Gandevia, 1975; Jackson, 2010). *Atropa belladonna* and *Datura stramonium* are rich in anticholinergic alkaloids such as atropine and stramonium (Miraldi et al., 2001). These plants can be dried and smoked as a mechanism to deliver these agents to the airways. The practice was introduced to Great Britain in the early 19th century by travelers from India who had observed its beneficial effects. It was soon adopted widely, and for the next century and a half, stramonium and belladonna in the form of burning powders, cigarettes, and cigars were used throughout Europe and North America in the inhalation treatment of asthma. Surveys and clinical studies conducted during the 1940s and 1950s continued occasionally to emphasize the therapeutic value of cigarettes containing either stramonium or atropine.

Unfortunately, atropine, which is a tertiary ammonium compound, is well absorbed into the systemic circulation and penetrates the blood-brain barrier. As a result, it has multiple systemic side effects that limit its clinical usefulness. For this reason, it slowly lost popularity. However, a renewed interest in anticholinergic drugs as therapy for respiratory diseases has been sparked by the development of safe yet effective quaternary anticholinergic compounds (Fig. 3). Chemical modifications of the atropine molecule, in particular by making its nitrogen atom pentavalent, have yielded a number of synthetic congeners that are very poorly absorbed from mucosae and cross the blood-brain barrier with difficulty, but in other respects they have pharmacological properties similar to those of atropine (Cazzola et al., 1998a; Gross, 2006). The quaternary structure of these anticholinergics prevents a substantial absorption from mucosal surfaces and penetration of the blood-brain barrier. When given by inhalation, these agents are as effective as atropine at improving lung function but longer acting and much less prone to side effects (Gross, 2006).

#### B. Short-Acting Muscarinic Acetylcholine Receptor Antagonists

As well as atropine methonitrate, ipratropium bromide and oxitropium bromide are effective short-acting quaternary anticholinergic drugs that have been used in the treatment of respiratory diseases. Their duration of action is approximately 6 to 8 h, but compared with SABAs, they have a slower onset of action, although probably a longer duration of action (Cazzola et al., 1998c; Gross, 2006).

1. *Atropine Methonitrate*. Atropine methonitrate, a quaternary ammonium congener of atropine, seems to be a more potent bronchodilator agent than its parent compound, atropine sulfate (Altounyan, 1964; Allen and Campbell, 1980). This may be due to the poor absorption of atropine methonitrate from the respiratory epithelium. Maximum bronchodilation seems to be achieved by nebulization of 1 to 1.5 mg of the drug; its peak effect is seen at 40 to 60 min and lasts for up to 6 h (Pierce et al., 1979). In patients with asthma, atropine methonitrate produces a peak bronchodilator effect similar to that of albuterol, both administered in doses that produced close to maximum bronchodilation for the drug concerned, but the effect of atropine is more prolonged, the response being significantly greater at 4 and 6 h than with albuterol (Pierce et al., 1979).

2. *Ipratropium Bromide*. Ipratropium bromide is a topically active quaternary derivative of *N*-isopropyl noratropine. Unlike atropine, it has low lipid solubility and does not pass the blood-brain barrier. Ipratropium bromide is poorly absorbed by the oral and nasal mucosa, and the swallowed drug is poorly absorbed from the gastrointestinal tract (Simons, 1987). Ipratropium bromide was introduced in 1974, and this drug made it possible to provide local anticholinergic effects to the lung while avoiding the systemic side effects of atropine as a result of its poor absorption. It is a nonselective antagonist of  $M_1$ ,  $M_2$ , and  $M_3$  mAChRs. Serum ipratropium bromide concentrations are extremely low after inhalation of the drug, peak serum concentrations being achieved at approximately 3 h after administration. The elimination half-life is 3.2 to 3.8 h, and ipratropium metabolites have little or no anticholinergic activity (Simons, 1987).

Ipratropium bromide starts to act within 15 to 30 min, but maximal bronchodilation may take up to 90 min in patients with COPD (Scullion, 2007). The duration of action is approximately 6 h so in comparison with  $\beta_2$ -AR agonists, its broncholytic effect is slower in onset and probably longer in duration (Scullion, 2007). On the basis of the duration of action, ipratropium bromide is given four times daily, and the maximum number of doses (40  $\mu$ g) per day should not exceed 12.

Ipratropium bromide produces effects similar to those of atropine when given parenterally and may actually be more potent on a per-weight basis. However, inhaled ipratropium bromide has proved to be safer than atropine,

showing little or no evidence of adverse hemodynamic or ocular effects, or detrimental effects on salivation, micturition, or arterial blood gases (Anderson, 1986). When administered via inhalation at therapeutic doses of 20 to 40  $\mu\text{g}$ , ipratropium bromide is somewhat less effective than SABAs in patients with asthma (Cazzola et al., 1998a) and less effective than LABAs in patients with COPD (Matera et al., 1995).

**3. Oxitropium Bromide.** Oxitropium bromide is another quaternary anticholinergic compound but is based on the scopolamine molecule instead of atropine. The peak bronchodilation of oxitropium bromide may take 60 to 90 min, and its duration is 5 to 8 h (Restrepo, 2007). Its bronchodilator effect is similar to that of ipratropium bromide, but oxitropium bromide is longer-lasting (Restrepo, 2007). Oxitropium bromide is considered to have twice the strength of ipratropium bromide per dose (Restrepo, 2007). In patients with severe COPD, the FEV<sub>1</sub> and forced vital capacity (FVC) plateau attained at a total cumulative dose of 600  $\mu\text{g}$  of oxitropium bromide (six puffs at 100  $\mu\text{g}$ /puff) was slightly higher than the plateau achieved with 280  $\mu\text{g}$  of ipratropium bromide (14 puffs at 20  $\mu\text{g}$ /puff) (Ikeda et al., 1995). This indicates a slight clinical advantage of oxitropium bromide, but at high doses that also cause tachycardia. A review of studies by Hardy et al. (1992) concluded that there was little evidence of this longer duration of action using equivalent doses and on the basis of the duration of action, oxitropium bromide is usually given three times daily.

Skorodin et al. (1986) found that 100- to 400- $\mu\text{g}$  doses of oxitropium bromide were better than 150  $\mu\text{g}$  of isoproterenol. Frith et al. (1986) showed that the effect of 200  $\mu\text{g}$  of oxitropium bromide was less rapid than that of 400  $\mu\text{g}$  of fenoterol, but its duration of action was greater than that of fenoterol. In patients with COPD, 50  $\mu\text{g}$  of salmeterol elicited a greater peak bronchodilation and longer duration of action than 200  $\mu\text{g}$  of oxitropium bromide, but 400  $\mu\text{g}$  of oxitropium bromide was similar to 50  $\mu\text{g}$  of salmeterol in terms of mean peak response and duration of action (Cazzola et al., 1998d).

### C. Long-Acting Muscarinic Acetylcholine Receptor Antagonists

**1. Tiotropium Bromide.** Tiotropium bromide is a once-daily, long-acting mAChR antagonist (LAMA) with high potency and kinetic selectivity at the mAChRs (Barnes, 2000; Littner et al., 2000). It displays a 6- to 20-fold higher affinity for mAChRs than does ipratropium bromide (Restrepo, 2007). Although tiotropium bromide binds to all three mAChRs, it dissociates much faster from the M<sub>2</sub> mAChRs, which results in a more selective antagonist action for M<sub>1</sub> and M<sub>3</sub> mAChR subtypes (Restrepo, 2007; Scullion, 2007). Its prolonged pharmacologic activity is the result of its slow dissociation from M<sub>1</sub> and M<sub>3</sub> mAChRs. The half-life of the tiotropium bromide M<sub>3</sub> mAChR complex is approximately

35 h, compared with 0.3 h for ipratropium bromide (Restrepo, 2007). The mechanism allowing for the long residency of tiotropium bromide at M<sub>3</sub> mAChRs is not completely known. However, the presence of a reactive epoxide on the tropane ring portion of tiotropium bromide could create a covalent interaction with key amino acids on M<sub>3</sub> mAChRs but not with other mAChRs, leading to the reported “kinetic” selectivity for M<sub>3</sub> mAChRs (Belmonte, 2005). The increased duration of binding at the M<sub>3</sub> mAChRs results in prolonged improvement in lung function, allowing a once-daily dose compared with the three to four doses per day previously necessary with ipratropium bromide (Barnes, 2000; Littner et al., 2000). Tiotropium bromide is rapidly absorbed into the circulation with a peak plasma concentration within 5 min followed by a rapid fall within an hour to a steady state and a terminal half-life of 5 to 6 days that is independent of dose. Calculations are that this concentration would occupy <5% of mAChRs and thus help explain the low incidence of side effects (Barnes, 2000; Littner et al., 2000). The peak onset of bronchodilation with tiotropium bromide occurs between 1 to 3 h with improvements in FEV<sub>1</sub> for more than 24 h (Barnes, 2000). In one PD study, it was discovered that 90% of the improvement in FEV<sub>1</sub> was achieved within 24 h of the first inhalation of tiotropium bromide, whereas ongoing improvements in forced vital capacity were measured beyond the 1 week of treatment (van Noord et al., 2002).

Tiotropium bromide has been studied mainly in the dry powder formulation (Handihaler; Boehringer Ingelheim, Ingelheim, Germany) in patients with COPD, with clinical trials lasting from 1 week to 4 years. More recent trials of tiotropium bromide have evaluated its efficacy and safety in a new micronebulizer device (Respimat). A recent meta-analysis has documented the benefits of tiotropium bromide in COPD management and provided reassurance regarding its safety (Yohannes et al., 2011). Trials that were  $\geq 12$  weeks showed that tiotropium bromide improved health-related quality of life (measured with SGRQ) compared with placebo and ipratropium bromide. Tiotropium bromide also improved dyspnea (measured with the transition dyspnea index) compared with placebo and ipratropium bromide and decreased the likelihood of an exacerbation and related hospitalizations, but not serious adverse events, compared with placebo. A recent 1-year, randomized, double-blind, double-dummy, parallel-group trial has documented that tiotropium bromide is more effective than salmeterol in preventing exacerbations (Vogelmeier et al., 2011). Moreover, the large UPLIFT (Understanding Potential Long-Term Impacts on Function with Tiotropium) trial, in which tiotropium bromide was compared with placebo in 5993 patients followed for 4 years, has provided supportive evidence for the safety and efficacy of tiotropium bromide in patients with COPD already receiving treatment with LABAs and ICSs (Tashkin et al., 2008).

# *D. Novel Long-Acting Muscarinic Acetylcholine Receptor Antagonists*

The growing evidence of the importance of tiotropium bromide in the maintenance treatment of COPD has spurred further research to identify new long-acting mAChR antagonists that could be as successful as tiotropium bromide (Cazzola and Matera, 2009). Several novel inhaled LAMAs are currently being developed, including glycopyrronium bromide (NVA-237), aclidinium bromide, umeclidinium bromide (GSK573719), TD-4208, 3-(((3-fluorophenyl)((3,4,5-trifluorophenyl)methyl)amino)carbonyloxy)-1-(2-oxo-2-(2-thienyl)ethyl)-1-azoniabicyclo(2.2.2)octane (CHF 5407), QAT370 (structure not disclosed), BEA-2180BR (structure not disclosed), trospium, dexpirronium, and PF-4522971 (structure not disclosed).

We still do not know whether these new mAChR antagonists are really as long-lasting as tiotropium bromide or whether they have a longer duration of action simply as a consequence of increasing the dose to overcome their shorter duration of action, possibly through a mechanism implying the generation of a drug depot in the lungs. Recently, however, it has been documented that with the same total daily dose of a new mAChR antagonist, a twice-daily regimen provides higher bronchodilation at trough than the once-daily regimen (Wu et al., 2011). For the maximum bronchodilation, there is a small difference between the two regimens, with the once-daily regimen being slightly better than the twice-daily regimen. For the overall bronchodilation response, as quantified by the FEV<sub>1</sub> response 24-h area under the curve, the difference between the two regimens becomes even smaller.

**1. Glycopyrronium Bromide.** Glycopyrronium bromide, also known as NVA237, is an mAChR antagonist that has a dissociation half-life from the human M<sub>3</sub> mAChR that is significantly shorter than tiotropium bromide or aclidinium bromide (Casarosa et al., 2009). In line with this finding, in isolated human bronchi, glycopyrronium bromide elicits a duration of action intermediate between that produced by tiotropium bromide and that of ipratropium bromide (Villetti et al., 2006). Clinical studies have shown that glycopyrronium bromide has a fast onset of effect that is sustained over 24 h (Verkindre et al., 2010; Fogarty et al., 2011), although another recent study with nebulized glycopyrronium bromide shows that this was probably shorter acting, at least at doses below 50  $\mu$ g (Singh et al., 2011a). This finding suggests that receptor binding kinetics are only one part of the pharmacology of this drug and can therefore not reliably predict the duration of action of a compound administered to the lung in vivo (Fogarty et al., 2011). The safety and efficacy of glycopyrronium bromide have been documented in patients with moderate-to-severe COPD. Glycopyrronium bromide is well tolerated at doses of up to 100  $\mu$ g in this patient population (Verkindre et al., 2010). A 26-week double-blind treatment with 50  $\mu$ g of glycopyrronium

bromide once daily administered via a low-resistance single-dose dry powder inhaler (Breezhaler; Novartis, Basel, Switzerland) induced clinically meaningful bronchodilation that was rapid in onset and maintained for 24 h throughout the study (D'Urzo et al., 2011). Moreover, it provided a significant and clinically relevant improvement in dyspnea at 26 weeks versus placebo, which was accompanied by a significant improvement in health-related quality of life and reduced rescue medication use. It is noteworthy that 50  $\mu$ g of glycopyrronium bromide once daily produced immediate and significant improvement in exercise endurance from day 1, accompanied by sustained and significant improvements in IC at isotime and meaningful improvements in trough FEV<sub>1</sub> in patients with COPD (Beeh et al., 2011). Improvement in endurance time increased over the study period, suggesting that mechanisms beyond improved lung function play a role in enhanced exercise tolerance.

**2. Aclidinium Bromide.** Preclinical studies have demonstrated that aclidinium bromide exhibits M<sub>3</sub>/M<sub>2</sub> mAChR kinetic selectivity (Gavalda et al., 2009). This mAChR antagonist dissociates from human M<sub>3</sub> mAChRs at a rate that was similar to that of ipratropium bromide and 2.6 times faster than that of tiotropium bromide. On the contrary, it dissociates from the same receptors slightly faster than tiotropium bromide (Gavalda et al., 2009) and, in line with these findings, is equivalent to ipratropium bromide for speed of onset but with a longer duration. However, in human isolated bronchi, aclidinium bromide has a faster onset and shorter duration of action than tiotropium bromide (Cazzola, 2009). Aclidinium bromide has the advantage of rapid hydrolytic inactivation once absorbed into the plasma, thereby enhancing its safety profile (Jansat et al., 2009).

In phase II studies, aclidinium bromide, at doses of 100 to 900  $\mu$ g, provided significant improvements in FEV<sub>1</sub> from 15 min after dosing (Joos et al., 2010). In another study, 200  $\mu$ g of aclidinium bromide provided effective bronchodilation in patients with COPD, similar to that seen with tiotropium bromide, with significant improvements compared with placebo observed from 10 min after dosing (Vestbo et al., 2010). However, data from two phase III studies, ACCLAIM/COPD (Aclidinium Clinical Trial Assessing Efficacy and Safety in Moderate to Severe COPD) I and II, indicated that treatment with 200  $\mu$ g of aclidinium bromide once-daily is safe and improves lung function and symptomatic endpoints in patients with moderate or severe COPD, but the clinical relevance of the observed improvements in trough FEV<sub>1</sub> has been questioned, because one trial was unable to document a significant impact on health-related quality of life at the end of the trial and the other was unable to document a significant change in delaying the time to disease exacerbation (Jones et al., 2011c). Nonetheless, in a phase II placebo- and active comparator-controlled study, 400  $\mu$ g of aclidinium bromide b.i.d. provided bronchodilation over 24 h that was statistically superior and clinically meaningful



compared with placebo, and comparable with 18  $\mu\text{g}$  of tiotropium bromide once daily, but with significant differences in favor of aclidinium bromide observed in the average nighttime period (Fuhr et al., 2012). ATTAIN (Aclidinium to Treat Airway Obstruction in COPD Patients), a 24-week study that assessed the long term bronchodilator efficacy and safety of 200 and 400  $\mu\text{g}$  of inhaled aclidinium, both administered b.i.d., compared with placebo in 828 patients with moderate to severe COPD, documented that both doses produced statistically significant increases from baseline in morning predose (trough)  $\text{FEV}_1$  versus placebo at week 24 (Jones et al., 2011a). All secondary endpoints (peak  $\text{FEV}_1$ , the percentage of patients achieving a clinically meaningful reduction in breathlessness and the percentage of patients with improved health status) demonstrated statistically significant differences versus placebo for both doses. In a 12-week, double-blind study, 200 and 400  $\mu\text{g}$  of aclidinium bromide b.i.d. significantly reduced night-time/early morning symptoms and daily rescue medication use in patients with COPD (Kerwin et al., 2011). Moreover, it significantly improved quality of life and reduced dyspnea for patients with moderate to severe COPD (Gelb et al., 2011). Aclidinium bromide has recently been approved for the treatment of COPD in the European Union.

**3. Other Muscarinic Acetylcholine Receptor Antagonists under Development.** Several other mAChR antagonists are also under development. Unfortunately, the available public information on these bronchodilators is still limited.

Umeclidinium bromide, previously known as GSK573719, is a novel high-affinity specific mAChR antagonist (Cazzola and Matera, 2009). It is a potent agent that demonstrates slow functional reversibility at cloned human  $\text{M}_3$  mAChRs and at endogenous mAChR in isolated human bronchus (Lainé et al., 2011). Superfusion of human bronchial strips with carbachol showed that umeclidinium bromide was slowly reversible in a concentration-dependent manner (1–100 nM). Time to 50% restoration of contraction at 10 nM was >600 min (versus 413 min with 10 nM tiotropium bromide). In conscious guinea pigs, umeclidinium bromide dose-dependently blocked ACh-induced bronchoconstriction with long duration of action; a 2.5- $\mu\text{g}$  intratracheal dose elicited >50% bronchoprotection for >24 h (Lainé et al., 2011). Umeclidinium bromide is rapidly absorbed ( $t_{\text{max}}$ , 5–15 min); 1 to 2% of the total dose is excreted unchanged in the urine. Accumulation (day 7:day 1) is low, 1.5 $\times$  to 1.9 $\times$  based on plasma data (1.8–2.4 $\times$ , urine data) (Kelleher et al., 2011). Single 250- to 1000- $\mu\text{g}$  doses of umeclidinium bromide are well tolerated and are associated with clinically relevant improvements in lung function in patients with COPD (Decramer et al., 2011; Mehta et al., 2011b). The 1000- $\mu\text{g}$  dose evokes larger increases in heart rate (0–4 h) than the 250- $\mu\text{g}$  dose versus placebo, but 24-h Holter monitoring shows no dose-related effects over 24 h, and in any case, the treatment effects are small (Kelleher et al., 2011). In healthy adults, steady state with

repeated doses of umeclidinium bromide inhalation powder is achieved after 6 to 8 days of dosing, but there is large data variability (Mehta et al., 2011a).

CHF 5407 shows subnanomolar affinities for human  $\text{M}_1$ ,  $\text{M}_2$ , and  $\text{M}_3$  mAChRs and dissociates very slowly from  $\text{M}_3$  mAChRs (a large part of the receptor complex remaining undissociated at 32 h from radioligand washout) (Villetti et al., 2010). This behavior contributes to the effects of CHF 5407 in human isolated ASMs' lasting for several hours, despite the fact that the antagonist had been washed out from the organ bath, and may explain the residual anti-bronchoconstrictor effect exerted by CHF 5407 up to 24 to 48 h from its administration. It must also be mentioned that, in sharp contrast to tiotropium bromide, CHF 5407 dissociates quickly and completely from human  $\text{M}_2$  mAChRs but is as slow as tiotropium bromide in dissociating from  $\text{M}_3$  mAChRs.

Trospium has been used in several indications and been delivered by a number of different routes and is currently used as a urinary antispasmodic drug. Inhaled trospium (ALKS27) is able to induce a fast onset of bronchodilation (within 15 min after administration) with an effect that lasts 24 h. No significant differences in  $\text{FEV}_1$  were observed in trospium doses of 100 or 400  $\mu\text{g}$ , demonstrating that the lower dose was maximally effective (Oleson et al., 2010).

PF-4522971 is another novel  $\text{M}_3$  mAChR antagonist designed for inhaled delivery. In human isolated bronchi, PF-4522971 inhibited electric field stimulation response with an  $\text{EC}_{50}$  of 18.0 (5.8–56.1), being  $\sim 36$  times less potent than tiotropium bromide and  $\sim 10$  times less potent than ipratropium bromide. At just maximal concentrations, PF-4522971 and tiotropium bromide had a duration of action of >16 h (Patel et al., 2010b).

### *E. Side Effects of Muscarinic Acetylcholine Receptor Antagonists*

All of the currently approved inhaled mAChR antagonists have a very wide therapeutic margin and are very well tolerated, in part because they are very poorly absorbed after inhalation. However, if any of these agents makes inadvertent contact with the eye, they can cause papillary dilation and blurred vision (Kizer et al., 1999), a true risk in patients with glaucoma (Mulpeter et al., 1992). Angle-closure glaucoma precipitated by ipratropium bromide after both nebulizer and pressurized aerosol treatment (Malani et al., 1982) and by tiotropium bromide (Okusz et al., 2007) has been reported. Angle closure glaucoma could be attributed to a possible angle crowding as a result of pupillary dilation caused by the inhibitory effect of mAChR antagonists on the parasympathetic nervous system. This effect on the eye is particularly important in the case of tiotropium bromide, because its prolonged duration of action may precipitate acute glaucoma.

In older men, who may have prostatic hyperplasia, mAChR antagonists should be used with caution because they can cause urinary retention (Pras et al., 1991). A

nested case-control study of patients with COPD aged 66 years or older documented that men who just initiated a regimen of inhaled mAChR antagonists were at increased risk for acute urinary retention compared with nonusers (Stephenson et al., 2011). In men with evidence of benign prostatic hyperplasia, the risk was further increased. Men using both short- and long-acting inhaled mAChR antagonists had a significantly higher risk of acute urinary retention compared with monotherapy users or nonusers. Patients with moderate-to-severe renal impairment (creatinine clearance of  $\leq 50$  ml/min) under treatment with tiotropium bromide should be closely monitored, because tiotropium bromide is predominantly excreted by the kidneys through active secretion (Keam and Keating, 2004). A bad taste and dryness of the mouth has been reported by 20 to 30% of patients, with a frequency in tiotropium bromide that may be slightly greater than that observed with ipratropium bromide (Gross, 2004).

Paradoxical bronchoconstriction to ipratropium bromide has been reported in humans (Mann et al., 1984). It is possible that this results from blockade of prejunctional  $M_2$  mAChRs on airway cholinergic nerves, which normally inhibit ACh release; when the drug is given by nebulizer, however, it is largely explained by the hypotonicity of the nebulizer solution and by antibacterial additives, such as benzalkonium chloride. Paradoxical bronchoconstriction, which may also occur with other mAChR antagonists, warrants withdrawal of the drug from the patient.

Concerns have been raised about possible associations of mAChR antagonists with cardiovascular morbidity and mortality (Lee et al., 2008; Singh et al., 2008b). However, the results of the UPLIFT trial and a robust and extensive analysis of more than 19,000 patients participating in placebo-controlled clinical trials with tiotropium bromide indicate that there is not a real increased risk for death or cardiovascular morbidity during treatment with this inhaled anticholinergic agent in patients with COPD (Tashkin et al., 2008; Celli et al., 2010). Nonetheless, the results of a recent systematic review and meta-analysis indicate that tiotropium bromide mist inhaler is associated with a statistically significant increased risk of mortality, possibly because this new formulation and device delivers the drug more effectively to the lungs and leads to greater absorption into the systemic circulation (Singh et al., 2011b).

## IV. Xanthines

### A. History of Development

One of the earliest reports of the efficacy of methylxanthines in asthma was published in the *Edinburgh Medical Journal* in 1859, where Henry Hyde Salter, himself an asthmatic, described his experience that “one of the commonest and best reputed remedies of asthma... is strong coffee” (Salter, 1859). The first analysis of a xanthine derivative extracted from tea leaves was accomplished by Kossel (1888), who was able to extract not only caffeine but also another xanthine derivative, a dimethylxanthine. The

name theophylline was then applied to a compound that has two methyl groups (1–3 dimethylxanthine) (Mazza, 1982; Schultze-Werninghaus and Meier-Sydow, 1982) (Fig. 3). Until 1930, xanthine derivatives were used in clinical practice only because of their diuretic and cardiotonic properties, even though the original studies, which clearly demonstrated the antispasmodic action of theophylline on smooth muscle of bronchi and its superiority in the relief of attacks of asthma, were carried out independently by Macht and Ting (1921) in the United States and Hirsch (1922) in Germany. Years later, a combination of theophylline and ethylenediamine (aminophylline) was used intravenously as an effective bronchodilator in acute asthma (Herrmann et al., 1937). Since then several other xanthines have been synthesized and are used clinically in various parts of the world.

### B. Theophylline

Theophylline is a methylxanthine similar in structure to the common dietary xanthines caffeine and theobromine. It directly relaxes human ASM *in vitro*, but it is a relatively weak bronchodilator with an effective concentration giving 50% response of  $1.5 \times 10^{-4}$  M *in vitro*, which equates to a plasma concentration of 67 mg/l, assuming 60% protein binding (Guillot et al., 1984). However, the therapeutic range of plasma concentrations has been established to be 10 to 20 mg/l because of unacceptable side effects above plasma concentrations of 20 mg/l (Barnes, 2003), although there is also evidence that maintaining levels of theophylline as low as 5 mg/l provides good anti-inflammatory activity (Sullivan et al., 1994). The dose of theophylline required to give therapeutic concentrations varies among patients, largely because of differences in clearance of the drug. However, theophylline is an effective bronchoprotective agent at therapeutic levels (Magnussen et al., 1987) and is also able to inhibit airway hyperresponsiveness (Ward et al., 1993; Page et al., 1998). Theophylline has been used for the treatment of asthma for many years. Prolonged treatment with theophylline was associated with a significant change in the sensitivity and slope of the methacholine dose-response curve that was qualitatively similar to that observed after regular treatment with corticosteroids, albeit to a lesser extent (Page et al., 1998).

Theophylline is rapidly and completely absorbed, but there are large interindividual variations in clearance because of differences in hepatic metabolism. It is eliminated by the hepatic cytochrome P450 system (85–90%) and urinary excretion (10–15%). Theophylline is predominantly metabolized by the CYP1A2 enzyme, whereas at higher plasma concentrations, CYP2E1 is also involved (Zhang and Kaminsky, 1995). Diet, concomitant cardiac or liver disease, tobacco use, and several medications affecting the cytochrome P450 system can modify the half-life of theophylline (Boswell-Smith et al., 2006). Theophylline is approximately 40% protein bound in plasma, the remaining portion circulating throughout the body. Disease states that

may affect protein binding, such as end-stage liver disease, renal dysfunction, and hypoalbuminemia, may result in a higher concentration of unbound drug.

It has been observed that FEV<sub>1</sub>, FVC, and peak expiratory flow rate changed only slightly (approximately 13%) over the range of doses that induced steady-state serum theophylline concentrations of 5 to 10, 10 to 15, and 15 to 20 mg/l, respectively (Chrystyn et al., 1988). Theophylline can also act as a respiratory stimulant. Moreover, theophylline is able to improve diaphragmatic contractility and has anti-inflammatory properties. There is, in fact, good evidence for inhibitory effects of theophylline on airway inflammation in patients with asthma (Sullivan et al., 1994) and COPD, and these effects are seen at plasma concentrations below 10 mg/l (Kobayashi et al., 2004).

Theophylline has been widely used in the treatment of patients with asthma and COPD. Many studies have examined its efficacy in these patients, but results are conflicting and difficult to interpret because of differences in patient populations, duration of therapy, and measurements of outcome. Apparently, the withdrawal of theophylline can worsen lung function, clinical status, exercise performance, and ratings of dyspnea (Kirsten et al., 1993). Studies comparing theophylline with  $\beta_2$ -AR agonists found similar improvements in spirometry and in various measures of patients' functional state and well-being (Cazzola et al., 1999b). However, very few studies have compared theophylline with inhaled mAChR antagonists.

Despite its extensive use in the treatment of respiratory disease, the precise molecular action(s) of theophylline have not been fully elucidated. Its efficacy in the treatment of patients with COPD or asthma has traditionally been attributed to nonselective phosphodiesterase (PDE) inhibition (Nicholson and Shahid, 1994), resulting in an increase in cAMP by inhibition of PDE3 and PDE4 and an increase in cGMP by inhibition of PDE5. However, it has been demonstrated that theophylline administered at therapeutic plasma levels (10 mg/l) exerts only a very modest nonselective inhibition of PDEs, which might not be clinically relevant (Barnes, 2003); at the lower doses now recommended, which have been shown to have significant anti-inflammatory activity, there is little evidence that theophylline inhibits any of the known PDE enzyme families (Boswell-Smith et al., 2006). Thus a number of other potential mechanisms of action of theophylline have been proposed, including adenosine receptor antagonism (Yasui et al., 2000) and increasing histone deacetylase 2 (HDAC2) activity (Ito et al., 2002). Intriguingly, theophylline restores HDAC2 activity to normal in macrophages obtained from patients with COPD and reverses corticosteroid resistance (Cosio et al., 2004). This can explain why mice exposed to cigarette smoke develop corticosteroid-resistant inflammation that is reversed by low doses of oral theophylline (Fox et al., 2007; To et al., 2010). The molecular mechanism of action of theophylline in restoring HDAC2 seems to be via selective inhibition of phosphoinositide 3-kinase  $\delta$ , which is activated by oxidative stress in pa-

tients with COPD and in smokers with asthma (Marwick et al., 2010; To et al., 2010). Although the side-effect profile of xanthines may relate to adenosine receptor antagonism (particularly A<sub>1</sub> receptor), it is unlikely that the therapeutic benefit of xanthines occurs via this mechanism because enprophylline has been shown to be as effective as theophylline in the treatment of patients with asthma, yet this drug lacks adenosine receptor antagonism (Persson and Pauwels, 1991).

### C. Aminophylline

Aminophylline is the ethylenediamine salt of theophylline with higher solubility at a neutral pH. In vivo intravenous aminophylline has an acute bronchodilator effect in patients with asthma that is most likely to be due to a relaxant effect on ASM (Mitenko and Ogilvie, 1973). However, it should not be given to a patient taking oral theophylline until the serum theophylline level has been measured. In any case, there is no evidence to support the use of intravenous aminophylline in acute severe asthma in adults (Hart, 2000). In critically ill children with severe acute asthma, aminophylline can confer early benefits on airway function and oxygenation, sustained for 24 h for oxygenation but not for airway function, and reduces the risk of endotracheal intubation (Yung and South, 1998). However, side effects such as nausea and vomiting are common, and the benefit/risk ratio is unknown. Aminophylline is also widely used for the treatment of acute exacerbations of COPD despite a lack of evidence to support this practice (Brochard et al., 1995). It remains possible that aminophylline confers a small but clinically significant benefit when added to standard therapy. However, Duffy et al. (2005) were unable to find evidence for any clinically important additional effect of aminophylline treatment when used with high-dose nebulized bronchodilators and oral corticosteroids in patients with nonacidotic COPD exacerbations. Aminophylline also increases diaphragmatic contractility and reverses diaphragm fatigue (Aubier et al., 1981).

### E. Novel Xanthine Drugs

The positive clinical effects of theophylline in airway disease, combined with its advantageous oral bioavailability, has spurred the development of other xanthines, such as bamiphylline, acebrophylline, and doxofylline, for the treatment of respiratory disease, with the anticipation that such drugs would have greater efficacy than theophylline but with an improved side-effect profile.

The structural novelty in doxofylline arises because of the unique methylene 1,3-dioxolone substitution at the N7 position of the xanthine ring. It has greatly decreased affinity toward adenosine A<sub>1</sub> and A<sub>2</sub> receptors compared with theophylline, which may contribute to the better safety profile, because in a number of studies, doxofylline has been shown to have better efficacy and fewer side effects than theophylline (Page, 2010). Moreover, unlike



theophylline, doxofylline does not interfere with calcium influx into cells or antagonize the action of calcium-channel blockers (Page, 2010). As a consequence, the effective therapeutic dose of doxofylline has fewer cardiostimulant effects than theophylline, such that doxofylline does not significantly increase the cardiac frequency or have arrhythmogenic effects (Page, 2010). Recent data also suggest that doxofylline does not directly inhibit any of the known HDAC enzymes and has much reduced ability to antagonize adenosine receptors or inhibit any known PDE enzyme (van Mastbergen et al., 2011). However, despite its superior safety efficacy, the potential of doxofylline has not been fully exploited.

A patented once-daily doxofylline tablet has been developed. This new sustained release formulation contains 650 mg of doxofylline and was shown to be bioequivalent to the standard recommended dose of 400 mg b.i.d. (Page, 2010). This novel formulation also shows lower peak plasma concentrations, thus widening the therapeutic window further; it is anticipated that this once-daily administration of 650 mg of doxofylline to patients with asthma or COPD will provide increased compliance and dosage convenience (Page, 2010).

#### *F. Side Effects of Xanthines*

Theophylline has a narrow therapeutic index; serum levels slightly outside of the target range may lead to serious toxicity or lack of efficacy. Long-term theophylline overmedication generally results from its sometimes unpredictable elimination kinetics [particularly in those at the extremes of age (i.e., very young and very old)], erratic use by patients, and administration to patients with underlying hepatic or cardiac disease.

Serum theophylline levels can be used to predict which patients are at risk for toxicity. However, life-threatening events are not predictable from very high serum theophylline levels, making it difficult to identify patients in whom aggressive interventional therapy is warranted before toxicity arises (Aitken and Martin, 1987). That theophylline concentrations are not predictive of toxic effects in patients with long-term overmedication has been recognized for many years (Shannon, 1999). This unusual feature of long-term overmedication can be explained by alterations in theophylline PK. For example, the volume of distribution of theophylline is 0.4 to 0.7 l/kg. Long-term overexposure to the drug would increase tissue concentrations, resulting in a greater body burden of drug relative to serum concentration. The lack of association between theophylline serum concentrations and adverse clinical effects in patients with long-term overmedication complicates the identification of high-risk patients who might benefit from early hemodialysis or hemoperfusion. Chronologic age has been increasingly shown to have greater predictive value in identifying patients at high risk, with those older than 60 to 65 years most likely to develop major toxic effects.

The most commonly reported adverse effects associated with theophylline include anorexia, nausea, headache, and sleep disturbance. Theophylline was also found to worsen symptoms of gastroesophageal reflux disease and to cause cardiac arrhythmias. Given that most of these adverse effects can be avoided at lower plasma levels, therapeutic drug level monitoring plays a pivotal role. However, levels within the therapeutic range should not preclude dosage decreases when a theophylline-related adverse event is suspected (Paul et al., 2010).

Several significant drug interactions can affect both the initial dose and subsequent dosing of theophylline. Because theophylline is metabolized by cytochrome P450 1A2 and to a lesser extent 3A3 and 2E1, medications that are inducers or inhibitors of these enzymes can affect the metabolism and elimination of theophylline. Concurrent administration of phenytoin and phenobarbitone increases activity of P450, resulting in increased metabolic breakdown, so that higher doses may be required. In addition, medications that are substrates of the same P450 enzymes, such as quinolone antibiotics (ciprofloxacin, but not ofloxacin), allopurinol, cimetidine (but not ranitidine), serotonin uptake inhibitors (fluvoxamine), and the 5-lipoxygenase inhibitor zileuton, which interferes with CYP1A2 function, will compete with theophylline for metabolism and potentially elevate theophylline levels (Aronson, 2006).

Older adults have a higher risk of toxicity when taking theophylline because of concomitant diseases, especially cardiovascular disease, reduced drug clearance, and polypharmacy, with consequent potential drug-drug interactions. It has been suggested that theophylline should be considered as potentially inappropriate for the older patients unless its plasma levels can be closely monitored, because it has a narrow therapeutic index and because plasma levels of theophylline are sensitive to reduced clearance, underlying diseases, and drug interactions (De Smet et al., 2007).

Some of the side effects of theophylline (central stimulation, gastric secretion, diuresis, and arrhythmias) may be due to adenosine receptor antagonism (predominantly A<sub>1</sub> receptor antagonism), but the most common side effects of theophylline are nausea, gastrointestinal symptoms, and headaches, which may be due to inhibition of certain PDEs (e.g., PDE4 in the vomiting center) (Howell et al., 1990). It is therefore of interest that doxofylline, which lacks significant A<sub>1</sub> receptor antagonism and PDE inhibitory activity, has a much improved side-effect profile compared with theophylline (Page, 2010; van Mastbergen et al., 2012). When theophylline is used as a bronchodilator at doses that give plasma concentrations of 10 to 20 mg/l, side effects due to PDE inhibition and adenosine antagonism are relatively common and often lead to discontinuation of therapy. However, a considerable body of evidence now suggests that theophylline works as a bronchodilator and anti-inflammatory agent at 5 to 10 mg/l concentration and that the use of low doses of theophylline that give plasma

concentrations of 5 to 10 mg/l largely avoids side effects, thus makes it unnecessary to monitor plasma concentrations (unless checking for compliance) (Sullivan et al., 1994; Barnes, 2005).

## V. Novel Classes of Bronchodilators

Novel classes of bronchodilators have proved difficult to develop, but there is still a continued interest in generating new classes of bronchodilators that act via emerging targets, particularly given the recent concerns over the long-term safety of  $\beta_2$ -AR agonists (Kuehn, 2010) and mAChR antagonists (Lee et al., 2008; Singh et al., 2008b).

### A. Selective Phosphodiesterase Inhibitors

Theophylline, long considered a nonselective PDE inhibitor, is known to be effective in the treatment of respiratory diseases; as described in section IV.B, the wider use of this drug is limited by its narrow therapeutic window (Boswell-Smith et al., 2006; Diamant and Spina, 2011). There has therefore been a longstanding interest in finding more selective PDE inhibitors, and this has become more plausible with the identification over the past decade of 11 different isoenzymes (PDE1–11) within the PDE superfamily that catalyze the breakdown of the signaling molecules cAMP and/or cGMP. Each isoenzyme has different tissue subdivision and different properties, enabling targeted therapy with potentially fewer (systemic) side effects (Boswell-Smith et al., 2006; Diamant and Spina, 2011).

In particular, in contrast to nonselective PDE inhibition, there is considerable interest in selectively targeting the PDE4 isoenzyme because it is the predominant isoenzyme in the majority of inflammatory cells, including neutrophils, which are implicated in the pathogenesis of COPD. PDE4 is also present in ASM, but selective PDE4 inhibitors have not shown acute bronchodilator activity in a variety of clinical trials (Boswell-Smith et al., 2006; Calverley et al., 2009). However, the selective PDE4 inhibitors are often considered members of a new class of potential bronchodilators, which is misleading, and they should be classified as novel anti-inflammatory drugs. Nonetheless, we will consider the evidence that PDE4 selective inhibitors can influence ASM function.

Theophylline is a weak and nonselective inhibitor of PDEs with less than 10% inhibition of PDE4, in comparison, for example, with roflumilast, the first PDE4 inhibitor to be approved for clinical use, which achieves 30 to 60% inhibition of PDE4 subtypes at clinically effective doses (Boswell-Smith et al., 2006). However, although PDE4 is one of the enzymes found in human ASM and a number of PDE4 inhibitors have been shown to improve lung function in patients with COPD after long-term administration (for review, see Boswell-Smith et al., 2006), no PDE4 inhibitor to date has shown any direct bronchodilator effect in the clinic. It is therefore thought that the impact of PDE inhibitors on lung function after long-term dosing clinically is secondary to the well described anti-inflammatory ef-

fects of these agents (for review, see Boswell-Smith et al., 2006). Certainly in vitro studies using isolated human bronchial tissues demonstrate that various PDE4-inhibitors are poor functional antagonists of spasmogen-induced bronchoconstriction and produce only modest relaxation of ASM directly (Diamant and Spina, 2011).

A number of selective PDE4 inhibitors have been investigated clinically for their effects on lung function. Cilomilast failed to induce an acute bronchodilator effect (Groottendorst et al., 2003) and when administered for 6 weeks in a dose-finding study in patients with moderate to severe COPD, although it significantly increased baseline FEV<sub>1</sub> by 11% by the end of the study (Compton et al., 2001). Several clinical trials with roflumilast have documented that this PDE4 inhibitor caused a small improvement in pre- and postbronchodilator FEV<sub>1</sub> (48 and 55 ml, respectively) in patients with more severe COPD, especially in those with a chronic bronchitis phenotype, those with recent exacerbations, and those requiring frequent rescue inhaler use (Calverley et al., 2009). These small changes in FEV<sub>1</sub> are not thought to be because of direct bronchodilation and occurred only after regular treatment. It is possible, as suggested by Celli (2006), that orally active PDE4 inhibitors such as cilomilast and roflumilast could reach the distal airways because of their systemic distribution, where decreasing inflammation around the small airways could result in more important changes in resting lung volumes than in absolute FEV<sub>1</sub>. Another possible explanation for the improvements in lung function could be partly attributed to a modulation of sensory nerve function by PDE4 inhibitors. A consequence of reduced sensory input to the central nervous system would lead to a decrease in parasympathetic outflow to the airways and a reduction in contraction of ASM and submucosal gland secretion, both leading to improvements in airflow (Spina et al., 1995). In any case, it has been demonstrated that PDE4 inhibitors can relax inherent tone in isolated human bronchial muscle (Schmidt et al., 2000) and that the PDE4D variant 5 is the key physiological regulator of  $\beta_2$ -AR-induced cAMP turnover within human ASM (Billington et al., 2008).

Several other oral PDE4 inhibitors, such as oglemilast (GRC-3886), MEM1414 (structure not disclosed), and tetomilast are under development (Matera et al., 2011b), although to date, the therapeutic usefulness of oral PDE4 inhibitors is limited by their side effects, particularly effects on the gastrointestinal system (Boswell-Smith et al., 2006; Diamant and Spina, 2011).

As an alternative to oral administration, a number of groups are now investigating the topical application of PDE4 inhibitors as a possible way to improve their efficacy in the treatment of inflammatory airway diseases while minimizing side effects. 6-((3-((Dimethylamino)carbonyl)phenyl)sulfonyl)-8-methyl-4-((3-methoxyphenyl)amino)-3-quinolinecarboxamide (GSK256066), SCH900182 (structure not disclosed) (Chapman et al., 2010), and 1-[[5-(1(S)-aminoethyl)-2-[8-methoxy-2-(trifluoromethyl)-5-quinolinyl]-4-oxazolyl]carbonyl]-4(R)-[(cyclopropylcarbonyl)amino]-L-

proline, ethyl ester xinafoate salt are examples of inhaled PDE4 inhibitors under development. GSK256066 has shown modest therapeutic potential in patients with asthma (Singh et al., 2010), but to date there are no studies in the literature with this drug in patients with COPD. However, failures in clinical trials have been reported for three other inhaled PDE4 inhibitors: tofomilast (Danto et al., 2007), *N*-(3,5-dichloropyrid-4-yl)-(1-(4-fluorobenzyl)-5-hydroxy-indole-3-yl)glyoxylic acid amide (AWD 12-281) (Gutke et al., 2005), and UK500001 (structure not disclosed) (Vestbo et al., 2009).

As discussed above, it is clear that PDE4 inhibitors have minimal direct effects of clinical relevance on ASM. In contrast, it has long been appreciated that PDE3 is the prevalent isoenzyme in the ASM that leads to smooth muscle relaxation. Indeed, a number of PDE3 inhibitors have been evaluated clinically for their effects on bronchodilation and have been demonstrated to have some activity (Leeman et al., 1987; Fujimura et al., 1997; Myou et al., 2003). However, PDE3 is also an important isoenzyme in cardiac and vascular tissue, and so PDE3 inhibitors may have detrimental effects on the cardiovascular system (Torphy, 1998). Nonetheless, it is clear that a safe mixed PDE3/4 inhibitor would provide both bronchodilator and anti-inflammatory activity in one molecule, something that is very attractive. Indeed, attempts have previously been made to clinically evaluate inhaled mixed PDE3/4 inhibitors such as zardaverine (Brunnée et al., 1992), but at a dose that acutely improved lung function in patients with asthma, the swallowed portion of the inhaled medicine led to unwanted gastrointestinal side effects. However, a nonemetic mixed PDE3/4 inhibitor, 9,10-dimethoxy-2-(2,4,6-trimethylphenylimino)-3-(*N*-carbamoyl-2-aminoethyl)-3,4,6,7-tetrahydro-2*H*-pyrimidino(6,1- $\alpha$ )isoquinolin-4-one (RPL554), has been demonstrated (Boswell-Smith et al., 2006) that is both a bronchodilator and anti-inflammatory. This drug has already successfully undergone a number of phase 2 clinical trials in patients with either COPD or asthma (<http://www.veronapharma.com>). There are also some recent pilot data showing that PDE5 inhibitors such as sildenafil can have bronchodilator activities (Sousa et al., 2011).

### B. $K^+$ Channel Openers

In ASM cells,  $K^+$  channels, such as large-conductance, voltage-dependent  $Ca^{2+}$ -activated  $K^+$  channels ( $K_{Ca}$ ) or the ATP-dependent  $K^+$  potassium channels ( $K_{ATP}$ ), play an important role in modulating contractile activity. Activation of these channels will cause cell hyperpolarization that should oppose  $Ca^{2+}$  entry through voltage-dependent  $Ca^{2+}$  channels, leading to smooth muscle relaxation. Consequently,  $K^+$  channel modulators may be of value in the treatment of chronic airway disorders. However,  $K_{ATP}$  openers, although effective in relaxing human airways in vitro, are not effective in treating asthma or COPD because they are more potent as vasodilators, which limits the dose that can be administered safely (Nardi et al., 2008).

Emerging attention can instead be seen in the patent literature for  $K_{Ca}$  channel openers.  $K_{Ca}$  channels regulate smooth muscle responses to both contractile agonists that elevate intracellular calcium and relaxant agonists that elevate cAMP (Jaggar et al., 1998). Freshly isolated human ASM cells express the large conductance  $K_{Ca1.1}$  channel. In addition to  $K_{Ca1.1}$ , two other types of  $K_{Ca}$  channel occur in human cells: the low conductance  $SK_{Ca}$  family ( $K_{Ca2.1-2.3}$ ) and the intermediate conductance channel  $K_{Ca3.1}$ . Preclinical data support this interest, although clinical proof of concept has not yet been obtained because of the lack of potent and selective  $K_{Ca}$  channel openers. In vascular smooth muscle cells, the large conductance channel ( $K_{Ca1.1}$ ), leads to membrane hyperpolarization and the inhibition of voltage-gated L-type  $Ca^{2+}$  channels. This, in turn, reduces the amount of  $Ca^{2+}$  influx and thereby decreases smooth muscle cell tone (Perez-Zoghbi et al., 2009). However, the observations that voltage-gated  $Ca^{2+}$ -channel inhibitors have no effect on agonist-induced  $Ca^{2+}$  signaling in ASM and are ineffective bronchodilators and that the deletion of  $K_{Ca1.1}$  channels in transgenic mice reduced airway contractility all suggest that the contribution of membrane potential to regulation of  $[Ca^{2+}]_i$  in ASM cells is minimal (Perez-Zoghbi et al., 2009). Consequently, the role of  $K_{Ca1.1}$  in ASM cell contraction is still controversial. In any case, it remains to be determined whether an oral  $K_{Ca}$  channel activator could selectively interact with ASM without causing adverse effects. There is documentation that  $K_{Ca3.1}$  is expressed by both normal and asthmatic human ASM (Shepherd et al., 2007), and it has been suggested that  $K_{Ca3.1}$  mediates important biological effects in the ASM of subjects with asthma and its blockade might at least partially prevent ASM remodelling (Bradding and Wulff, 2009). The only  $K^+$  channel modulating agent under clinical development in an airway indication is senicapoc, an orally active  $K_{Ca3.1}$  blocker that inhibits the late airway response and the development of bronchial hyper-responsiveness after allergen challenge in a sheep model of asthma (Bradding and Wulff, 2009).

### C. Vasoactive Intestinal Peptide Analogs

VIP, one of the major peptide transmitters in the central and peripheral nervous systems, is abundantly present in the normal human lung, and VIP-immunoreactive nerves are found in the smooth muscle layer and glands of the airway and within the walls of pulmonary and bronchial vessels (Onoue et al., 2007). The effects of VIP are mediated through interaction with two common G protein-coupled receptors named VPAC1 and VPAC2 (Laburthe and Couvineau, 2002). The bronchodilatory effect of VIP in human bronchi is almost 100 times more potent than adrenergic dilation induced by isoproterenol (Wu et al., 2011). Unfortunately, clinical application of VIP has been limited for a number of reasons, including its short plasma half-life and problems with administration, because intravenous VIP will lead to profound bronchodilation but also vasodilation; after inhalation to reduce systemic exposure, VIP is



subject to degeneration by proteases present in the lung lining fluid (Wu et al., 2011). As a consequence several peptidase-resistant VIP analogs, including Ac-His<sup>1</sup>[Glu<sup>8</sup>,OCH<sub>3</sub>-Tyr<sup>10</sup>,Lys<sup>12</sup>,Nle<sup>17</sup>,Ala<sup>19</sup>,Asp<sup>25</sup>,Leu<sup>26</sup>,Lys<sup>27,28</sup>]-VIP (Ro 25-1392), Ac-His<sup>1</sup>[Glu<sup>8</sup>,Lys<sup>12</sup>,Nle<sup>17</sup>,Ala<sup>19</sup>,Asp<sup>25</sup>,Leu<sup>26</sup>,Lys<sup>27,28</sup>,Gly<sup>29,30</sup>,Thr<sup>31</sup>]-NH<sub>2</sub> VIP (Ro 25-1553), and [Arg<sup>15,20,21</sup>,Leu<sup>17</sup>]-VIP (IK312532), have been developed. Ro 25-1553 is 24 to 89 times more potent than VIP as a relaxant of the guinea pig trachea, and pulmonary responses evoked by histamine, leukotriene D<sub>4</sub>, platelet-activating factor, and ACh are inhibited dose dependently by intratracheally instilled Ro 25-1553 (O'Donnell et al., 1994). However, in patients with asthma, it elicits a short-lasting bronchodilatory effect (Wu et al., 2011). IK312532 exhibits longer-lasting bronchodilation and suppression of the antigen-evoked infiltration of granulocytes in the bronchiolar mucosa compared with VIP, possibly because of higher stability against enzymatic digestion (Ohmori et al., 2004).

#### D. Rho Kinase Inhibitors

There is now substantial evidence that Rho kinase (RhoK) is involved in bronchoconstriction (Fernandes et al., 2007). RhoK can modulate smooth muscle contraction by multiple mechanisms. Agonists can activate RhoK via Ca<sup>2+</sup>-independent pathways involving G protein-coupled membrane receptors, subsequent activation of Rho family guanine nucleotide exchange factors and monomeric G protein RhoA, and eventual RhoK activation (Somlyo and Somlyo, 2003). RhoA, which is a member of the Rho (Ras-homologous) subfamily of the Ras superfamily, has been identified as the main upstream activator of RhoK. However, several studies, including some in airways, have shown that the RhoK pathway may also be activated by Ca<sup>2+</sup>-dependent mechanisms (Ito et al., 2001; Hirota et al., 2007). The contractile response to cholinergic stimulation of airways has been shown to depend partly on agonist-induced RhoK activation, which contributes to the early phase of the contractile response. RhoK modulates airway responsiveness via myosin light-chain phosphatase inhibition, without modifying the Ca<sup>2+</sup> signal. RhoK is activated via a Ca<sup>2+</sup>-dependent pathway, but independently of Ca<sup>2+</sup>-calmodulin-dependent protein kinase II and Ca<sup>2+</sup>-activated Cl<sup>-</sup> currents (Mbikou et al., 2011). Hence, the implication of RhoK signaling pathways in ASM is not fully determined, although it seems to be important.

Considering that Rho/RhoK signaling is thought to be involved in various processes that contribute to chronic airway diseases, the use of RhoK inhibitors in asthma and COPD therapy clearly holds promise. Inhibition of RhoK reduces contractile responses induced by spasmogens. Indeed, the RhoK inhibitor *N*-(4-pyridyl)-4-(1-aminoethyl)cyclohexanecarboxamide (Y-27632) has been shown to relax human isolated bronchial preparations (Gosens et al., 2004). Several analogs of Y-27632 exist that have similarly high inhibitory constants for RhoK and similar smooth muscle relaxant properties. Of those, 4-(1-aminoethyl)-*N*-(1*H*-pyrrolo(2,3-*b*)pyridin-4-yl)cyclohexanecarboxamide

dihydrochloride (Y-30141) and Y-30694 (structure not disclosed) (Schaafsma et al., 2008) may be particularly worth mentioning, because their selectivity profiles with respect to PKC and myosin light chain kinase are only slightly dissimilar from that of Y-27632 (Gosens et al., 2006). Fasudil (HA-1077) has affinity for RhoK similar to that of Y-27632. A metabolite of fasudil, hydroxyfasudil (HA-1100) also relaxes smooth muscle with a potency similar to the parent compound. It is, however, far more potent (*K<sub>i</sub>* for RhoK = 1.6 nM), and its selectivity profile (with respect to PKA, PKC, and myosin light chain kinase) is even better than that of Y-27632 (Schaafsma et al., 2008). Two amino-furazan-based inhibitors, *N*-(3-([2-(4-amino-1,2,5-oxadiazol-3-yl)-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-6-yl]oxy)phenyl)-4-([2-(4-morpholinyl)ethyl]-oxy)benzamide (GSK269962A) and 4-(7-([(3*S*)-3-amino-1-pyrrolidinyl]carbonyl)-1-ethyl-1*H*-imidazo[4,5-*c*]pyridin-2-yl)-1,2,5-oxadiazol-3-amine (SB-7720770-B), have been identified as a novel class of potent inhibitors of ROCK activity. IC<sub>50</sub>-values of GSK269962A and SB-7720770-B toward Rho-associated protein kinase 1 (ROCK-1) are 1.6 and 5.6 nM, respectively, and toward ROCK-2, 6 and 4 nM, respectively (Doe et al., 2007). Compared with Y-27632 and fasudil, both compounds were far more potent in inhibiting ROCK-1. In addition to ROCK-1 and -2 inhibition, SB-7720770-B potentially inhibits several other kinases (e.g., mitogen- and stress-activated protein kinase 1 and ribosomal S6 kinase 1) as well, whereas GSK269962A is at least >30-fold more selective for ROCK compared with the protein kinases tested.

#### E. Brain Natriuretic Peptide and Analogs

It is well known that the guanylyl cyclase/cGMP second messenger system has a parallel role to the adenylyl cyclase/cAMP system in ASM, regulating its contractile and proliferative functions. Therefore, it is not surprising that agents activating this signaling pathway bronchodilate in vivo and relax ASM in vitro (Hamad et al., 1997). Particulate guanylyl cyclases act as plasma membrane receptors for natriuretic and related peptides. Several membrane forms of the enzyme have been identified up to now. Some of them serve as receptors for the natriuretic peptides, a family of peptides that includes atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide, three peptides known to play important roles in renal and cardiovascular physiology. There is evidence that ANP relaxes human ASM in vitro (Angus et al., 1994b). Moreover, in humans, exogenous ANP reverses airway hyper-responsiveness when given intravenously or by inhalation in high doses, although it seems to be less effective when administered by inhalation (Angus et al., 1993, 1994a), and has also been shown to modify bronchial responsiveness to inhaled histamine (Angus et al., 1995) and nebulized water (Mcalpine et al., 1992). We have documented that BNP, which is able to induce a time- and concentration-dependent increase of cGMP levels in human ASM cells (Hamad et al., 2003), induces a weak but intriguing relaxant effect on human ASM (Matera et al.,

2009) through the activation of the natriuretic peptide receptor-A (Matera et al., 2011a). The incubation of human ASM with BNP can elicit a significant reduction of cholinergic and histaminergic responses (Matera et al., 2011a). We speculate that the direct activation of the natriuretic peptide receptor-A might influence bronchial tone, inducing bronchodilation, and certainly it has been documented that human recombinant BNP (nesiritide) is a potent bronchodilator in patients with asthma (Akerman et al., 2006).

#### F. Nitric Oxide Donors

Exogenous NO has the ability to exert bronchodilatory effects in patients with bronchial asthma (Högman et al., 1993), and NO has been used in the treatment of preterm children to improve lung capacity (Barrington and Finer, 2007). The augmented availability of NO in the lungs may represent a plausible approach for the treatment of asthma and COPD. Nitrates, NO, and NO donors relax ASM *in vitro* and in guinea pigs and humans (Ward et al., 1995), and inhaled NO exerts bronchodilatory effects against methacholine-induced bronchoconstriction *in vivo* (Kacmarek et al., 1996). Studies in bronchial and tracheal smooth muscle have shown that a major target of NO is the enzyme soluble guanylyl cyclase (Ellis, 1997), although NO is also a very active agonist at inducing vascular smooth muscle relaxation. There is therefore a need for suitable NO donors that have minimal effects on the vasculature.

NO donors can produce nitrites ( $\text{NO}_2^-$ ) and nitrates ( $\text{NO}_3^-$ ) and mediate their relaxing effects through the cGMP-mediated pathway. The main target of cGMP is the family of cGMP-dependent protein kinases. Activation of these kinases and subsequent phosphorylation of various proteins constitute a cascade of reactions that cause the reduction of cytosolic  $\text{Ca}^{2+}$  concentration, leading to relaxation (McDaniel et al., 1992). However, not all studies support the role of soluble guanylyl cyclase/cGMP pathway in mediating the relaxant effect of NO, and cGMP-independent pathways are increasingly being recognized (Redington, 2006). The controversial results of these studies may be due to the different species and NO donors used.

The main prototypes of NO donors traditionally used, such as sodium nitroprusside and nitroglycerine, have several well known adverse effects, such as rapid tachycardia, high toxicity, and rapid induction of tolerance. Likewise, sydnonimines, another well known class of NO donor drugs, have a characteristically low therapeutic index (because of cyanide toxicity) (Scatena et al., 2010).

At present, a number of groups are designing and synthesizing various chemical compounds capable of modulating NO metabolism for therapeutic purposes that also possess an improved therapeutic index (Scatena et al., 2010). Specifically, various new classes of NO donors are under intense pharmacological investigation (such as *S*-nitrosothiols, diazeniumdiolates, furoxans, and zeolites), each characterized by a particular PK and PD profile. The most important obstacle in the field of new NO donor drugs

seems to be carefully targeting NO release to lungs at an optimal concentration to achieve a beneficial action and to limit possible adverse effects, particularly on the cardiovascular system.

$\alpha'$ -[[[1,1-Dimethylethylamino)methyl]-4-hydroxy-1,3-benzenedimethanol nitrate (NCX-950), a NO-releasing albuterol, elicits potent relaxant and anti-inflammatory activities compared with albuterol (Lagente et al., 2004). These effects seem to be associated mainly with the stimulation of  $\beta_2$ -AR. However, it has been suggested that the release of NO from the NO moiety may be involved in the effects of NCX-950 because the effects of NCX-950, at equimolar doses, are slightly but significantly superior to that of albuterol alone (Lagente et al., 2004).

Budesonide 21-[(4'-nitrooxymethyl)benzoate] (TPI 1020, formerly NCX 1020 or NO-budesonide) is a novel compound, currently in development for the treatment of chronic respiratory disorders, with a dual mechanism of action involving corticosteroid activity (derived from its metabolite budesonide) and donation of NO. TPI 1020 has been shown to be superior to budesonide in an animal model of COPD, inhibiting airway hyper-responsiveness and reducing neutrophil infiltration into lungs (Nevin and Broadley, 2004). It has been shown that the NO component of TPI 1020 potentiates the bronchodilator activity of albuterol, and their combination lasted longer than either drug administered individually (Turner et al., 2010).

Another new NO donor TERPY (ruthenium complex  $[\text{Ru}(\text{terpy})(\text{bdq})\text{NO}^+]^{3+}$ ) has been shown to be more effective in reducing cytosolic  $\text{Ca}^{2+}$  concentration and inducing relaxation in rat trachea greater than that of the sodium nitroprusside alone (Castro et al., 2011). In contrast to sodium nitroprusside, the NO released from the ruthenium complex induces ASM relaxation by cGMP-independent mechanisms and seems to involve  $\text{Ca}^{2+}$  and  $\text{K}^+$  fluxes across the membrane, leading to decreased cytosolic  $\text{Ca}^{2+}$  concentrations.

#### G. E-Prostanoid Receptor 4 Agonists

Inhaled prostaglandin  $\text{E}_2$  ( $\text{PGE}_2$ ) has been shown to be a bronchodilator in subjects with asthma (Melillo et al., 1994) or COPD (Kawakami et al., 1973). However,  $\text{PGE}_2$  itself has the potential to cause the adverse effects of cough and retrosternal burning when inhaled by humans, particularly in subjects with asthma (Choudry et al., 1989). Moreover,  $\text{PGE}_2$  also stimulates other prostaglandin-like receptors having inhibitory and stimulatory activity on the intrinsic tone of the airway smooth muscle.  $\text{PGE}_2$  acts predominantly via specific E-prostanoid (EP) receptors. Whereas it has been established that  $\text{PGE}_2$ -induced sensory nerve activation and cough are mediated via the  $\text{EP}_3$  receptor (Maher et al., 2009), the  $\text{EP}_4$  receptor seems to be the predominant receptor responsible for  $\text{PGE}_2$ -induced relaxation of human ASM *in vitro* (Buckley et al., 2011). Stimulation of the  $\text{EP}_4$  receptor with  $\text{PGE}_2$  leads to a directly stimulation of the adenylyl cyclase via the  $\text{G}\alpha_s$  subunit, which converts ATP to cAMP. Thus, highly potent

EP<sub>4</sub> subtype-selective receptor agonists have been suggested to have therapeutic potential without side effects (Buckley et al., 2011).

### H. Bitter Taste Receptor Agonists

Bitter taste receptors work as chemoreceptors that interact with taste stimuli to initiate an afferent signal to the brain, where it becomes taste perception. Stimulation of the taste 2 receptors is responsible for the bitter taste (Behrens et al., 2007). These receptors were recently found on airway smooth muscle (Deshpande et al., 2010). When activated, they caused relaxation through a calcium-dependent mechanism. Agonists to these receptors may make up a new class of useful direct bronchodilators for treating obstructive lung disease, but because they are members of the G protein-coupled receptor superfamily, they may undergo desensitization (Robinett et al., 2011). Saccharin, chloroquine, denatonium, aristocholic acid, strychnine, quinine, colchicine, and yohimbine were used in the preclinical studies. Aristocholic acid, strychnine, and yohimbine are probably too toxic to be considered for human use. Quinine was selected for use in the mouse model of asthma, possibly because it was expected to be effective with minimal toxicity (Deshpande et al., 2010). In the low doses of quinine used in the treatment of muscle cramps, the incidence of serious adverse effects is not higher than that for the placebo (El-Tawil et al., 2010). Thus, perhaps it is worth considering developing an inhalation preparation of quinine for testing in humans with asthma (Doggrell, 2011).

## VI. Combination Therapy

For patients with COPD whose symptoms are not sufficiently controlled by maintenance monotherapy, combining bronchodilators of different classes seems a convenient way of delivering treatment and obtaining better results (Cazzola and Matera, 2006). In fact, it seems reasonable to postulate that targeting bronchoconstriction through two distinct mechanisms should maximize the bronchodilator response and help to overcome inter- and inpatient variability in bronchomotor tone associated with airway obstruction (Cazzola and Matera, 2006). Moreover, combining two or more classes of molecules allows the use of lower doses to achieve the same efficacy while decreasing adverse effects (Donohue, 2005). Combination therapy with a LABA and an ICS is considered an important approach for treating patients suffering from asthma and patients with severe COPD who have frequent exacerbations (Chung et al., 2009). In addition, combination therapy with a LAMA and an ICS seems to be intriguing, although the clinical effects of such combination are largely unknown.

### A. Pharmacologic Rationale for Combination Therapy

1. *Combining  $\beta_2$ -Adrenergic Receptor Agonists and Muscarinic Acetylcholine Receptor Antagonists.* Airway tone is regulated by both the parasympathetic and

sympathetic nervous systems. The complete nature of interactions between the two physiological systems is not yet fully understood, but there is enough evidence to suggest that combining  $\beta_2$ -AR agonists and mAChR antagonists is pharmacologically reasonable for two reasons. First, the addition of a  $\beta_2$ -AR agonist decreases the release of ACh through the modulation of cholinergic neurotransmission by presynaptic  $\beta_2$ -ARs and thereby amplifies the bronchial smooth muscle relaxation induced by the mAChR antagonist. Second, the addition of a mAChR antagonist can reduce bronchoconstrictor effects of ACh, the release of which has been modified by the  $\beta_2$ -AR agonist, and thereby amplify the bronchodilation elicited by the  $\beta_2$ -AR agonist through the direct stimulation of smooth muscle  $\beta_2$ -ARs (Cazzola and Molimard, 2010). Another possibility is the fact that the mAChR antagonist and not the  $\beta_2$ -AR agonist can suppress mucus/fluid secretions; hence, surface tension changes that would normally collapse the airways are less likely to occur (Cazzola et al., 2011).

2. *Combining  $\beta_2$ -Adrenergic Receptor Agonists and Inhaled Corticosteroids.* Inflammation plays a major role in the pathology of asthma and has an important role in COPD. ICS therapy forms the basis for treatment of asthma of all severities, improving asthma control and lung function and preventing exacerbations of disease. Use of ICS has also been increasingly established in the treatment of COPD, particularly in symptomatic patients, who experience useful gains in quality of life (likely from an improvement in symptoms such as breathlessness and in reduction in exacerbations) and an attenuation of the yearly rate of deterioration in lung function. The addition of LABA therapy with ICS has been suggested to improve the efficacy of ICS effects (Chung et al., 2009), and a number of molecular interactions between corticosteroids and  $\beta_2$ -ARs have been described (Adcock et al., 2002; Cazzola and Dahl, 2004). ICSs freely diffuse from the circulation across cell membranes into cells, where they activate the glucocorticoid receptor (GR). On ligand binding, the GR is activated, dissociates from chaperone proteins, and translocates to the nucleus, where it can bind as a dimer to specific DNA sequences (glucocorticoid response elements) upstream of the start site of transcription. The activated GCS-GR also binds to the  $\beta_2$ -AR gene, leading to an increase in the number of  $\beta_2$ -ARs in the cell membrane. On the other side, LABA stimulates  $\beta_2$ -ARs, leading to the priming of the GR and increasing translocation of the receptor into the nucleus of the cell. The overall response is increased anti-inflammatory activity from a given ICS dose. The primed GR may also have enhanced actions against other transcription factors, such as NF- $\kappa$ B. Besides, LABAs may inhibit NF- $\kappa$ B by inducing an increase in inhibitor  $\alpha$  levels. The combination of ICSs and LABA potentiates inhibition of CXCL8 (IL-8) and CCL11 (eotaxin) release from human ASM cells and their proliferation and has additive effects on granulo-



cyte-macrophage colony-stimulating factor release from epithelial cells.

3. *Combining Muscarinic Acetylcholine Receptor Antagonists and Inhaled Corticosteroids.* Experimental evidence has also suggested an influence of corticosteroids on mAChRs, signifying the potential of a LAMA/ICS combination for the treatment of asthma and COPD. In dogs, long-term treatment with methylprednisolone leads to a decreased expression of both  $M_2$  and  $M_3$  mAChRs in ASM (Emala et al., 1997). In guinea pigs, dexamethasone decreases airway responsiveness to vagal stimulation via two mechanisms: increased  $M_2$  mAChR function and increased degradation of ACh by cholinesterases (Jacoby et al., 2001). Studies in cultured airway parasympathetic neurons show that  $M_2$  mAChR mRNA expression is increased by dexamethasone and that this is associated with increased  $M_2$  mAChR protein and increased  $M_2$  mAChR function (Jacoby et al., 2001). Increased  $M_2$  mAChR function results in decreased ACh release. At the same time, the release ACh is degraded more rapidly through an increased cholinesterases activity (Jacoby et al., 2001).

### *B. Combination Therapy of $\beta_2$ -Adrenergic Receptor Agonists with Muscarinic Acetylcholine Receptor Antagonists*

Early studies questioned the value of combination therapy with ipratropium bromide and albuterol (Easton et al., 1986). However, several large trials found that concomitant administration of the two drugs by MDI (COMBIVENT Inhalation Aerosol Study Group, 1994) or nebulization (COMBIVENT Inhalation Aerosol Study Group, 1997) had additive bronchodilator effects.

The introduction of LABAs gave physicians additional therapeutic options for COPD. Results from two short-term studies that evaluated small COPD-patient cohorts suggested no substantial additive effect when combining salmeterol or formoterol with ipratropium bromide 40  $\mu$ g (Matera et al., 1996; Sichletidis et al., 1999). In contrast to these earlier studies, van Noord et al. (2000) demonstrated that a 12-week treatment with 50  $\mu$ g of salmeterol b.i.d. plus 40  $\mu$ g of ipratropium bromide four times daily was more effective than the salmeterol alone in improving FEV<sub>1</sub> and specific airway conductance. Subsequently, D'Urzo et al. (2001) demonstrated that adding formoterol (12  $\mu$ g b.i.d.) to ipratropium bromide (40  $\mu$ g four times a day) was more effective than the addition of albuterol (200  $\mu$ g four times a day) in patients with COPD who required combined bronchodilator therapy.

Addition of LABAs to tiotropium bromide has also been studied in short-term trials ( $\leq 6$  weeks) (Cazzola et al., 2004a,b; van Noord et al., 2005, 2006). Taken together, these studies indicate that once-daily formoterol/tiotropium bromide and salmeterol/tiotropium bromide combinations provide significant additive effects in patients with moderate to severe COPD and are well tolerated. A bronchodilator-mediated symptom benefit of the once-daily

combination is also reflected in the significant decrease in albuterol use as rescue therapy and symptoms of dyspnea. However, additional and more consistent improvements during the day and night were provided when the LABA was dosed twice daily.

Several other studies examined the regular treatment with LABA plus tiotropium bromide combination. In a 12-week study comparing 12  $\mu$ g of formoterol b.i.d. plus 18  $\mu$ g of tiotropium bromide once daily with tiotropium bromide monotherapy, the combination significantly increased the FEV<sub>1</sub> area under the curve at 0 to 4 h compared with tiotropium bromide at all time points (Tashkin et al., 2009). At the end of the study, increases from baseline in trough FEV<sub>1</sub> and FVC were significantly greater with the combination treatment than with tiotropium bromide alone. These outcomes were again found in a longer study lasting 6 months using similar treatment arms (Vogelmeier et al., 2008). This study also showed that the combination treatment arm had fewer exacerbations, although the difference between arms did not reach statistical significance. Finally, another study comparing salmeterol plus tiotropium bromide versus tiotropium bromide alone found that the combination improved quality of life scores, but not FEV<sub>1</sub>, and was unable to decrease the number of exacerbations over tiotropium bromide alone (Aaron et al., 2007). Overall, the outcomes of these studies demonstrate that there are a number of added benefits in using combinations over tiotropium bromide alone. LABA/LAMA combination seems to play an important role in maximizing bronchodilation, studies to date indicating that combining different classes of bronchodilators results in significantly greater improvements in lung function and other outcomes compared with individual drugs used alone and that these combinations are well tolerated in patients with moderate to severe COPD (Cazzola and Tashkin, 2009).

### *C. Novel Long-Acting $\beta_2$ -Adrenergic Receptor Agonists (Ultra-Long-Acting $\beta_2$ -Adrenergic Receptor Agonists) and Long-Acting Antimuscarinic Agent Combinations under Development*

It has been documented that adding indacaterol to tiotropium bromide is a beneficial strategy for patients with moderate to severe COPD, affording greater bronchodilation and lung deflation (as reflected by the increase in IC) (Mahler et al., 2011). The need for rescue medication was decreased after treatment with such combinations. The safety and tolerability profiles were similar in both treatment groups. It is not surprising, therefore, that current opinion is that it will be advantageous to develop inhalers containing combinations of two or more classes of once-daily long-acting bronchodilator drugs, in an attempt to simplify treatment regimens as much as possible (Cazzola and Matera, 2008, 2009; Cazzola et al., 2011). Nonetheless, some new combinations, such as formoterol plus glycopyrronium bromide and formoterol plus aclidinium

bromide (LAS-40464) are under development with an expected twice-daily dosing regimen (Matera et al., 2011a,b).

A range of once-daily LABA (ultra-LABAs) and LAMA fixed-dose combinations, including QVA149 (the combination of indacaterol and the LAMA glycopyrronium bromide), olodaterol plus tiotropium bromide, and vilanterol plus umeclidinium bromide, are in clinical development as fixed combinations (Cazzola and Matera, 2008, 2009; Cazzola et al., 2011). There is documentation that a 7-day treatment with QVA149 (300  $\mu$ g of indacaterol/50  $\mu$ g of glycopyrronium bromide) once daily via a single-dose dry powder inhaler is more effective than 300 and 600  $\mu$ g of indacaterol (van Noord et al., 2010). Moreover, QVA149 at the dosage of 600/100, 300/100, or 150/100  $\mu$ g has a safe cardiovascular profile, with no different 24-h mean heart rate at day 14 in QVA149 and placebo or indacaterol, and no clinically relevant differences in QTc intervals observed among treatment groups on days 1, 7, and 14 (Van de Maele et al., 2010). Addition of olodaterol enhanced the beneficial effect of the tiotropium bromide monotherapy on ACh-induced bronchoconstriction with a longer duration of action in anesthetized dogs (Bouyssou et al., 2010c). Moreover, the protective effect of olodaterol against histamine-induced bronchoconstriction in guinea pigs was augmented and prolonged in the presence of tiotropium bromide, which had no significant effect by itself (Meurs et al., 2011). Olodaterol and tiotropium bromide used in combination might control some proinflammatory events in COPD. In fact, they synergistically control TGF $\beta$ 1-mediated neutrophilic inflammation, at least in vitro (Profita et al., 2011). It has also been shown that olodaterol/tiotropium bromide (10/5  $\mu$ g) fixed-dose combination administered using the Respimat soft mist inhaler is more effective than 5  $\mu$ g of tiotropium bromide in patients with COPD, with superior bronchodilation over 24 h after 4 weeks of once-daily dosing (Maltais et al., 2010).

#### D. Bifunctional Muscarinic Acetylcholine Receptor Antagonists and $\beta_2$ -Adrenergic Receptor Agonists

An alternative strategy is the design and development of dual-acting agents that combine both mAChR antagonist and  $\beta_2$ -AR agonist pharmacology in a single molecule (Cazzola and Matera, 2009; Cazzola et al., 2011) by conjugating a  $\beta_2$ -AR agonist motif onto that of an  $M_3$  mAChR antagonist. This would create larger molecules with high lipophilicity that are likely to possess high metabolism and low oral absorption to minimize potential systemically driven side effects from the swallowed component after inhalation (Jones et al., 2011b). This approach may offer several advantages over combination therapy of two separate drug entities. These include the benefit of delivering a fixed ratio into every region of the lung, reducing the complexity of combination inhalers (Cazzola and Matera, 2009; Cazzola et al., 2011); a single PK profile; a uniform ratio of activities at the cellular level; and a simplified clinical development program (Steinfeld et al., 2011). These agents are known as dual-acting mAChR antagonist- $\beta_2$ -AR ago-

nist (MABA) bronchodilators. All MABA compounds disclosed so far have a  $M_3$  mAChR antagonist moiety and a  $\beta_2$ -AR agonist moiety connected by a linker, usually an aliphatic and linear linker with 7 to 10 atoms. Such compounds may feature the diphenylcarbamate moiety, a tiotropium-like moiety, or the tolterodine moiety. However, it must be mentioned that although the marketed inhaled mAChR antagonists ipratropium bromide and tiotropium bromide both contain quaternary ammonium groups, the incorporation of a quaternary ammonium pharmacophore into a bifunctional MABA constructs results in lower affinities for the  $M_3$  mAChR (Hughes et al., 2011).

TEI3252 (structure not disclosed) is a novel bifunctional bronchodilator that in experimental settings showed bronchoprotective activities against ACh- and histamine-induced contraction in a dose-dependent manner at the dose range of 1 to 5  $\mu$ g/kg (Sugiyama et al., 2010). The efficacy of TEI3252 was long-lasting compared with existing bronchodilators, such as tiotropium bromide and indacaterol. On the other hand, the inhibitory effect on salivation was not observed even at the dose up to 100  $\mu$ g/kg. This finding suggests that TEI3252 has a reduced side-effect profile.

Evaluation of THRX-200495 (structure not disclosed), a single bifunctional molecule that possesses both mAChR antagonist and  $\beta_2$ -AR agonist pharmacology, in a guinea pig model of bronchoconstriction revealed a matched mAChR antagonist and  $\beta_2$ -AR agonist potency [median dose that causes 50% inhibition ( $ID_{50}$ ) = 11.4 and 11.2  $\mu$ g/ml, respectively], with similar onset of action and potent dual pharmacology (MABA  $ID_{50}$  = 3.5  $\mu$ g/ml) lasting for >24 h (McNamara et al., 2009).

[1-[9[[2*R*]-2-Hydroxy-2-(8-hydroxy-2-oxo-1*H*-quinolin-5-yl)ethyl]amino]nonyl]piperidin-4-yl] *N*-(2-phenylphenyl) carbamate (GSK961081, formerly TD5959) is a further novel bifunctional molecule. It conferred potent 24-h bronchoprotection in guinea pigs through a dual mechanism involving antagonism of mAChRs and agonism of  $\beta_2$ -ARs. Dual pharmacology yielded bronchoprotection that was 2- to 5-fold more potent (MABA  $ID_{50}$  = 6.4 mg/ml) than either ipratropium bromide or albuterol alone (Pulido-Rios et al., 2009). In phase I randomized double-blind placebo-controlled single- and multiple-dose studies that enrolled healthy volunteers, GSK961081 was generally well tolerated and demonstrated evidence of bronchodilation over 24 h after a single dose and after seven consecutive daily doses; consequently, it has entered into phase II (Cazzola and Matera, 2009). In a phase II study, GSK961081 dosed at both 400 and 1200  $\mu$ g once daily showed bronchoprotection at day 14 that was at least equivalent to that of 50  $\mu$ g of salmeterol b.i.d. plus 18  $\mu$ g of tiotropium bromide once daily, as measured by changes in FEV<sub>1</sub> (Cazzola and Matera, 2009). Both the time to peak effect and maximum bronchodilation of GSK961081 were numerically better than salmeterol plus tiotropium bromide, although the study was not powered to compare the results to the salmeterol plus tiotropium bromide control. In patients with

COPD, after 14 days' dosing, 400 and 1200  $\mu\text{g}$  of GSK961081 induced a significant improvement in trough  $\text{FEV}_1$  (0.115 and 0.168 liters, respectively) similar to that elicited by salmeterol plus tiotropium bromide (0.103 liters) (Norris et al., 2011). There was no significant difference in maximum change from baseline heart rate or QTc after the final dose of any active treatment versus placebo. Adverse events were similar across all groups with the exception of tremor ( $n = 2$ , 1200- $\mu\text{g}$  dose) and dry mouth ( $n = 1$ , 1200- $\mu\text{g}$  dose), seen after GSK961081 only.

PF-3429281 (structure not disclosed) is another inhaled dual mAChR antagonist/ $\beta_2$ -AR agonist. In anesthetized guinea pigs, it caused a dose-related inhibition of ACh-induced bronchoconstriction (Philip et al., 2010). This was 26-fold weaker than tiotropium bromide and equipotent with salmeterol. Infusion of propranolol throughout the experiment blocked the effects of salmeterol on ACh-induced bronchoconstriction, whereas both tiotropium bromide and PF-3429281 were unaffected. Data generated in vitro using guinea pig isolated trachea suggest that the duration of action of the  $\beta_2$ -AR component is longer than the  $\text{M}_3$  mAChR component (Patel et al., 2010a). In an anesthetized dog model of bronchoconstriction, PF-3429281 had an potency equivalent to that of ipratropium bromide and a superior therapeutic index and duration of action compared with salmeterol (Wright et al., 2010).

PF-4348235 (structure not disclosed) is a novel and potent MABA (Patel et al., 2011). PF-4348235  $K_i$  at the  $\text{M}_3$  mAChR was 0.79 nM (0.52–1.21 nM). This was  $\sim 80$ -fold less potent than tiotropium bromide; however, both exhibited similar mAChR selectivity. PF-4348235 gave an apparent  $t_{1/2 \text{ off}}$  of 117.1 min (95% CI, 64.2–213.6 min) at the  $\text{M}_3$  mAChR. This was significantly longer than ipratropium bromide at  $t_{1/2 \text{ off}}$  of 11.8 min (95% CI, 10.2–13.8 min) and shorter than tiotropium bromide at  $t_{1/2 \text{ off}} > 1370$  min (95% CI, 1310–1420 min). PF-4348235 potency was 3.7 nM (95% CI, 2.2–6.2 nM) at the human  $\beta_2$ -AR, which was  $\sim 6$ -fold less potent than salmeterol. After washing, PF-4348235 gave a fold shift to the right (RWS) of 1.5, which was not significantly different from that of salmeterol (2.2 RWS) but was significantly smaller than that of formoterol (97.2 RWS). It demonstrated  $> 1000$ -fold selectivity over the human  $\beta_1$ -AR, similar to salmeterol and formoterol. PF-4348235 exhibited single and dual pharmacology in vivo using conscious guinea pigs with a potency and duration of action similar to salmeterol (Hulland et al., 2011).

#### *E. $\beta_2$ -Adrenergic Receptor Agonist or Long-Acting Antimuscarinic Agent/Phosphodiesterase Inhibitor Combinations*

There is evidence that (*R,R*)-glycopyrrolate can have beneficial interactions with the PDE4 inhibitor roflumilast in inhibiting inflammatory mediators (Pahl et al., 2006).

In patients with moderate to severe COPD treated with salmeterol or tiotropium bromide, roflumilast improved lung function and some clinically relevant symptomatic outcomes (Fabbri et al., 2009). The improvement in pre-

and postbronchodilator  $\text{FEV}_1$  means that the beneficial effect of roflumilast on lung function is in addition to that achieved with standard bronchodilators, an effect that is probably not due primarily to smooth muscle relaxation but to other mechanisms. It has been suggested that the improvement in lung function might be associated with a reduction in numbers of sputum neutrophils and eosinophils in patients with COPD. Apparently, the addition of roflumilast to tiotropium bromide induces a larger improvement in prebronchodilator  $\text{FEV}_1$  than its addition to salmeterol, and this was not an unexpected finding considering the mechanism of actions of the two drugs (Cazzola et al., 2010c). However, it must also be mentioned that some trials documented that the addition of a LABA did not change the mean prebronchodilator  $\text{FEV}_1$  increase observed with roflumilast in monotherapy (Calverley et al., 2009).

#### *F. Combination Therapy of $\beta_2$ -Adrenergic Receptor Agonists with Inhaled Corticosteroids*

Combination therapy of ICS and inhaled LABA has become the “gold standard” therapy for asthma management. In adolescent and adult patients with asthma with suboptimal control on low-dose ICS monotherapy, the combination of LABA and ICS is modestly more effective in reducing the risk of exacerbations requiring oral corticosteroids than a higher dose of ICS. Combination therapy also led to modestly greater improvement in lung function, symptoms, and use of rescue  $\beta_2$ -AR agonists and to fewer withdrawals as a result of poor asthma control than with a higher dose of ICSs (Ducharme et al., 2010a). In asthmatic adults who are symptomatic on low to high doses of ICS monotherapy, the addition of a LABA at licensed doses reduces the rate of exacerbations requiring oral steroids, improves lung function and symptoms, and modestly decreases use of rescue short-acting  $\beta_2$ -AR agonists (Ducharme et al., 2010b). In children, the effects of this treatment option are much more uncertain (Ducharme et al., 2010b). LABAs should not be used as monotherapy in patients with asthma, but rather only with an ICS; however, because of issues in using two inhalers for two different drugs, combination inhalers are now considered standard (Sears, 2011).

The LABA/ICS combination is now increasingly recommended in patients with COPD because, in general, the addition of LABA to ICS provides additional benefits (Cazzola and Hanania, 2006; Hanania, 2008). Several large-scale studies in patients with moderate to severe COPD have demonstrated that treatment with salmeterol/fluticasone propionate and formoterol/budesonide leads to significantly greater improvements in lung function, exacerbations, health status, and breathlessness compared with placebo or monotherapy with the component drugs, although combination treatments may differ with regard to specific outcomes. Compared with placebo, combination therapy leads to a significant reduction of a quarter in exacerbation rates (Nannini et al., 2007c). Compared with



mono component ICS therapy, LABA/ICS combination significantly reduces morbidity and mortality in COPD (Nannini et al., 2007a). Moreover, combination therapy is more effective than LABAs in reducing exacerbation rates, although the evidence for the effects on hospitalizations is mixed (Nannini et al., 2007b). There is a significant reduction in all-cause mortality with the addition of data from the Toward a Revolution in COPD Health (TORCH) study, although in the TORCH study regular treatment with salmeterol/fluticasone propionate narrowly missed demonstrating a statistically significant benefit on the reduction in all-cause mortality over 3 years (17.5% reduction in risk,  $P = 0.052$ ) (Calverley et al., 2007). In addition, in the TORCH study, the LABA/ICS combination reduced the rate of decline in FEV<sub>1</sub> in patients with moderate to severe COPD by 16 ml/year compared with placebo. However, this improvement was also observed in the LABA-only and ICS-only groups (Celli et al., 2008). Moreover, the superiority of combination inhalers should be viewed against the increased risk of side effects, particularly pneumonia in patients with COPD (Nannini et al., 2007c).

*1. Novel Combinations of Long-Acting  $\beta_2$ -Adrenergic Receptor Agonists and Inhaled Corticosteroids under Development.* There is a strong interest in developing a once-daily combination therapy, again in an attempt to simplify treatment, but also to overcome the loss of patent protection. The awareness that new once-daily ICSs, such as ciclesonide, fluticasone furoate, and mometasone furoate, are in development has further supported the development of new ultra-LABAs that can be used on a once-a-day basis with these ICS (Cazzola and Matera, 2009; Cazzola et al., 2011), although a combination of formoterol and mometasone furoate administered on a twice-daily basis has been developed and successfully tested in patients with asthma (Maspero et al., 2010).

A new inhaled therapy combines indacaterol with mometasone (QMF-149) and another indacaterol with QAE-397 (structure not disclosed), a novel corticosteroid in phase II development for the treatment of asthma (Cazzola and Matera, 2009). In particular, two trials have investigated the safety and tolerability of QMF-149. The first was designed to evaluate the bronchodilatory efficacy of QMF-149 delivered via a multiple-dose dry powder inhaler (Twisthaler) in adult patients with persistent asthma using open-label salmeterol/fluticasone (50/250  $\mu\text{g}$  b.i.d.) as an active control (Cazzola and Matera, 2009), whereas the second one investigated the safety and tolerability of 14-day treatment with QMF-149 500/800  $\mu\text{g}$  in patients with mild to moderate asthma (Cazzola and Matera, 2009). The results of these trials have not yet been released.

A next-generation, once-daily combination consisting of vilanterol and fluticasone furoate is another combination inhaler under development. Administration of vilanterol and fluticasone furoate in combination is not associated with an increase in systemic exposure or systemic PD effects compared with administration of either compound

alone (Kempford et al., 2011). A small study that enrolled 60 patients with COPD (Global Initiative for Chronic Obstructive Lung Disease stage II–III) documented that this combination had greater improvements than placebo in trough FEV<sub>1</sub> after a 4-week treatment with a good safety and tolerability profile (Lötvald et al., 2010a).

A positive interaction of carmoterol with budesonide in the control of bronchoconstriction induced by acetaldehyde in guinea pigs has also been documented (Rossoni et al., 2005). Intriguingly, carmoterol/budesonide was 2-fold more effective than the formoterol/budesonide combination, which suggests that carmoterol/budesonide, by optimizing each other's beneficial pharmacological potential, may represent a new fixed combination in asthma. In effect, in patients with mild or moderate asthma, the systemic exposure to 2  $\mu\text{g}$  of carmoterol and 400  $\mu\text{g}$  of budesonide was not increased with the combination compared with each component administered alone (Poli et al., 2009). However, a prolonged bronchodilation was observed with carmoterol/budesonide combination (Poli et al., 2009). The fixed combination carmoterol/budesonide formulated as HFA 134a pressurized MDI (Chiesi Modulite HFA technology) administered once a day to patients with moderate or severe persistent asthma maintained the bronchodilator effect over 24 h and was as effective as twice-daily formoterol/budesonide Turbuhaler (Woodcock et al., 2009).

#### *G. Combination Therapy of Long-Acting Muscarinic Agonists with Inhaled Corticosteroids*

Very few studies published to date have been designed specifically to evaluate the effect of ICS and tiotropium bromide combinations on clinical outcomes, and this is an area that warrants future study. A retrospective analysis of the pooled results of two similar 6-month, randomized, double-blind, double-dummy, placebo-controlled parallel group studies of inhaled tiotropium bromide (18  $\mu\text{g}$  once daily) and salmeterol (50  $\mu\text{g}$  b.i.d.) in the treatment of patients with COPD demonstrated superior spirometric responses with tiotropium bromide plus ICS compared with salmeterol plus ICS (Hodder et al., 2007). In addition, clinically important endpoints such as dyspnea, health status, and frequency of exacerbations were superior with tiotropium bromide plus ICS compared with salmeterol plus ICS (Hodder et al., 2007).

Treatment with tiotropium bromide and budesonide in patients with COPD has led to significant improvements in the quality of life status according to SGRQ (Um et al., 2007), measuring both dyspnea and lung function, and a reduction of the number of exacerbations over 6 months of treatment in patients with COPD and chronic asthma (Choi et al., 2007). It is noteworthy that the effects of tiotropium bromide added to ICSs have also been explored in patients with asthma, demonstrating noninferiority to the concomitant treatment with LABAs and ICSs (Lee et al., 2008). These results suggest the possibility of LAMA as an alternative to LABA for the treatment of asthma.

### H. Other Possible Combination Therapies

A number of studies have reported that when theophylline and  $\beta_2$ -AR agonists are administered together (Cazzola et al., 2000; ZuWallack et al., 2001), some added clinical benefit is gained compared with the drugs administered alone. However, it has been questioned whether this has been due to the use of a suboptimal dose of inhaled  $\beta_2$ -AR agonist in the studies. In any case, it has been shown that regular theophylline treatment neither prevents nor worsens the development of tolerance to the bronchoprotective effect of salmeterol (Cheung et al., 1998).

The addition of theophylline to ICSs therapy is clinically beneficial in patients with asthma or COPD. It has been shown that combined treatment with low-dose inhaled budesonide plus low-dose theophylline yields similar improvement in lung function tests compared with patients receiving high-dose budesonide alone, without the adrenal suppression associated with high dose-budesonide (Evans et al., 1997). Nonetheless, a recent meta-analysis of randomized clinical trials has suggested that the addition of theophylline elicits a greater increase in lung function than increasing the dose of ICS, but only in short-duration treatment (Wang et al., 2011). However, these results should be interpreted with caution because of the small sample of included studies and the possible presence of a publication bias (Wang et al., 2011).

Theophylline has been shown to activate HDAC, resulting in a marked potentiation of the anti-inflammatory effects of ICSs (Ito et al., 2002). In patients with COPD, the combination of theophylline and ICS is more effective in reducing airway inflammation than either drug alone (Ford et al., 2010c). This is now leading to therapeutic trials in COPD with low doses of theophylline. Low-dose theophylline also improves asthma control in asthmatic patients who smoke and show no response to ICS alone (Spears et al., 2009).

### I. Triple Combination Therapies

There is limited documented clinical evidence for the use of triple therapy in COPD, but studies published to date indicate that ICS/LABA in combination with tiotropium bromide improves lung function, COPD symptoms, and health status and reduces the risk of hospitalizations compared with tiotropium bromide alone in patients with moderate to severe COPD (Aaron et al., 2007; Cazzola et al., 2007; Singh et al., 2008a; Welte et al., 2009). Intriguingly, a proof-of-concept study has recently shown that the addition of once-daily tiotropium bromide to a high-dose ICS plus a LABA significantly improves lung function over 24 h in patients with inadequately controlled severe, persistent asthma (Kerstjens et al., 2011). In any case, Bouyssou et al. (2011) has shown that olodaterol in combination with tiotropium bromide and ciclesonide elicits potent synergistic bronchoprotective activity in the guinea pig. This effect is significantly higher than the summarized values of the

respective monotherapies and supports the concept of the triple therapy.

Clearly, a triple inhaler would be advantageous from an adherence point of view because of the convenience of having three inhalation products in one inhaler. This improved patient compliance and adherence to the therapy regimen will certainly affect the management of such a chronic condition but will also reduce the repeating error that arises from the incorrect use of two or more inhaler devices. However, there is no hard evidence to date that this approach is useful or even necessary (Salama et al., 2011).

It is likely that the development of once-daily dual-action ultra LABA + LAMA combination products may serve as a basis for improved "triple-therapy" combinations through coformulation with novel anti-inflammatory compounds such as inhaled PDE4 inhibitors that could deliver three complementary therapeutic effects for patients with COPD (Cazzola and Matera, 2006); in addition, the use of mixed PDE3/4 inhibitors such as RPL554 in combination with an  $M_3$  mAChR antagonist may prove beneficial. In any case, the development of once-daily dual-action ultra LABA plus LAMA combination products may serve also as a basis for improved triple-therapy combinations through coformulation with novel ICSs. The potential for these therapeutic strategies to be administered once daily simplifies patient treatment regimens and therefore increases the likelihood of compliance with therapy.

The only inhalation triple formulation commercially available is an HFA pressurized MDI suspension that contains tiotropium bromide monohydrate equivalent to bromide 9  $\mu$ g of tiotropium, 6  $\mu$ g of formoterol, and 20  $\mu$ g of ciclesonide. The three drugs are suspended in HFA 227, with apparently no other additives (Salama et al., 2011).

A combination of indacaterol, glycopyrronium bromide, and mometasone seems to be a real possibility (Fitzgerald and Fox, 2007). A combination of vilanterol with umeclidinium bromide and fluticasone furoate is another valid option. Furthermore, LAS-40369 (structure not disclosed), an association of acridinium bromide and formoterol plus an undisclosed ICS under development (Matera et al., 2011b), is another combination under development. A combination that features the inhaled PDE4 inhibitor tofimi-last with a LAMA (potentially tiotropium bromide) in addition to, potentially, a MABA seems to be less realistic (Fitzgerald and Fox, 2007). It is intriguing that a family of dual-action  $M_3$  mAChR antagonists/PDE4 inhibitors has recently been discovered (Provins et al., 2006; Provins et al., 2007). The pharmacological profile of 6-(azepan-1-yl)-N,2-dicyclopropyl-5-methylpyrimidin-4-amine (UCB-101333-3), a 4,6-diaminopyrimidine, is intriguing (Provins et al., 2007). The addition of an ultra-LABA to UCB-101333-3 could create an interesting combination for treating asthma or COPD (Cazzola and Matera, 2009).

## VII. Controversies Surrounding Bronchodilators

### A. $\beta_2$ -Adrenergic Receptor Agonists

1. *Tachyphylaxis and Tolerance.* Prolonged or repeated use of current  $\beta_2$ -AR agonist drugs leads to loss of their effects, a pervasive phenomenon termed tachyphylaxis, refractoriness, or desensitization (Sears, 2002). Repetitive  $\beta_2$ -AR agonist use may lead not only to reduced bronchoprotection but also to sensitization of excitation-contraction signaling pathways. Prolonged exposure of cultured ASM cells to  $\beta_2$ -AR agonists directly augments procontractile signaling pathways elicited by several compounds, including thrombin, bradykinin, and histamine (Yang et al., 2011). Such treatment did not increase surface receptor numbers or expression of G proteins and downstream effectors (phospholipase C $\beta$  and myosin light chain). In contrast,  $\beta_2$ -AR agonists decreased expression of regulator of G protein signaling 5 (an inhibitor of GPCR activity), knockdown of which in human ASM increased agonist-evoked intracellular calcium flux and myosin light chain phosphorylation, which are prerequisites for contraction.

In subjects with asthma, tolerance (which is commonly encountered when a subject's reaction to a specific concentration of a  $\beta_2$ -AR agonist is progressively reduced) seems to reach a plateau after 1 week of regular therapy, but  $\beta_2$ -AR agonist responsiveness recovers quickly with little tolerance 3 days after stopping therapy (Haney and Hancox, 2005). Tolerance is not overcome by administering higher doses of  $\beta_2$ -AR agonists and does not affect the response to ipratropium bromide (Haney and Hancox, 2007). Apparently, the degree of  $\beta_2$ -AR agonist tolerance increases with the degree of bronchoconstriction (Wraight et al., 2003). In COPD, a pretreatment with a conventional dose of formoterol or salmeterol does not preclude the possibility of inducing a further bronchodilation with albuterol (Cazzola et al., 1998c). Tolerance to nonbronchodilator effects of  $\beta_2$ -AR agonists may account for the increase in reactivity to indirect bronchoconstrictor challenges and explain why some studies have demonstrated enhanced bronchoconstriction in patients with asthma after regular  $\beta_2$ -AR agonist therapy (Cazzola and Matera, 2007).

2. *Morbidity and Mortality.* When SABAs are taken regularly, the outcome usually differs little from that seen with placebo (Drazen et al., 1996; Dennis et al., 2000) or is even worse (Sears et al., 1990), although these agents provide rapid relief and protect against bronchoconstrictor stimuli. Moreover, the regular use of  $\beta_2$ -AR agonists has been blamed for the increases in mortality and morbidity of asthma that coincided with their introduction into the market (Barnes and Chung, 1992).

Mechanistic studies showed that regular use of albuterol may enhance early and late asthmatic reactions to allergen (Cockcroft et al., 1995; Drazen et al., 1996) and the degree of bronchoconstriction resulting from standardized exercise challenge (Inman and O'Byrne, 1996). It has been

suggested that  $\beta_2$ -AR constitutive or agonist-activated signaling required for the development of these phenomena occurs via a  $\beta$ -arrestin-dependent pathway (Dickey et al., 2010).

It was also hypothesized that, by treating symptoms of asthma with  $\beta_2$ -AR agonists, inhibition of mast cell degranulation occurs swiftly, inhibiting the release of endogenous heparin as an anti-inflammatory agent, thus increasing inflammation, which may lead to the long-term appearance of excess repair tissue and the acceleration of the disease process (Page, 1993). Intriguingly, Lommatzsch et al. (2009) showed that unbalanced monotherapy with at least salmeterol in patients with mild asthma increases neurotrophin brain-derived neurotrophic factor production and storage and that increases in airway hyper-responsiveness are associated with this effect. There is increasing evidence that brain-derived neurotrophic factor may enhance airway hyper-responsiveness and eosinophilia in allergic airway inflammation (Nassenstein et al., 2003).

Several meta-analyses investigating the adverse event profile of LABAs have now been published. However, rather than providing a clear picture, these studies variously implicate or exonerate the safety of LABAs (Taylor, 2009). The salmeterol multicenter asthma research trial (SMART) found more asthma deaths (13 versus 3) and life-threatening asthma events (37 versus 22) in the salmeterol-treated patients with asthma, although it was documented that among African Americans, 5 times as many deaths and near-deaths from asthma occurred in those given salmeterol than in those given placebo. Among patients with asthma not using an ICS as a preventive (controller) medication, again more deaths and near-deaths from asthma occurred in those given salmeterol than in those given placebo (Nelson et al., 2006). In two Cochrane Collaboration studies, the odds ratio (OR) for nonfatal adverse events with formoterol compared with placebo was increased to 1.57 (95% CI, 1.05–2.37;  $n = 8032$ ) (Cates et al., 2008), and for salmeterol it was 1.14 (95% CI, 1.01–1.28;  $n = 59,864$ ) (Cates and Cates, 2008). This has led the U.S. Food and Drug Administration (FDA) to post black-box warnings on all medicines containing these LABAs and recommend that further research is needed into the nature and magnitude of risk with LABA treatment (Chowdhury and Dal Pan, 2010). Furthermore, the use of LABA monotherapy in the treatment of patients with asthma has been stopped by the FDA.

Although other large studies have failed to find any significant differences in mortality or morbidity (notably hospitalizations) in relation to LABA use (Jaeschke et al., 2008; Sears et al., 2009), in another meta-analysis conducted by the FDA (Levenson, 2008), it was concluded that any increased risk of asthma-related events was confined to patients taking salmeterol (and not formoterol) who were not simultaneously taking concomitant ICS. A recent study has examined the association between LABAs and severe asthma exacerbations in a large US Medicaid pop-



ulation (940,449 patients aged <40 years) of newly diagnosed asthma and pre-existing asthma cohorts, controlling for disease severity to the greatest extent possible (Guo et al., 2011). Compared with patients taking a SABA only, estimated severe asthma exacerbations hazard ratios were 0.63 (95% CI, 0.58–0.69) and 0.74 (95% CI, 0.70–0.79) for patients on a LABA without ICS, and 0.79 (95% CI, 0.77–0.81) and 0.90 (95% CI, 0.87–0.92) for those on a LABA/ICS single inhaler. Although hazard ratios were estimated to be similar for emergency department visits, LABA use was found to be positively associated with hospitalizations and intubations. Other key risk factors included being African American, having an alcohol/substance use disorder, being pregnant, and being obese.

### *B. Muscarinic Acetylcholine Receptor Antagonists*

As already mentioned, concerns have been raised about possible associations of mAChR antagonists with cardiovascular morbidity and mortality, and a body of evidence supports the possible existence of a link between the use of SAMAs and cardiovascular risk. Two pooled analyses of randomized controlled trials (Singh et al., 2008b; Worth et al., 2011) and a retrospective, nested case-control study (Lee et al., 2008) reported increased risks for cardiovascular events or mortality with inhaled mAChR antagonists use. The highest risk for mortality was among those with pre-existing arrhythmias.

A number of studies have investigated the influence of mAChR antagonists on the cardiovascular system. In humans, the mean maximal heart rate rose significantly for 0.03 mg/kg i.v. atropine (+46.2%) and for 0.03 mg/kg i.v. ipratropium bromide (+57.4%) (Libersa et al., 1988). The effects obtained with ipratropium bromide on the heart rate lasted nearly twice as long as those obtained with atropine ( $120 \pm 38.4$  and  $70 \pm 30$  min, respectively, for the pharmacological half-life). In 13 patients with sinus bradycardia, heart rate and hemodynamics were recorded before and 3, 30, 60, and 120 min after administration of 0.5 mg i.v. ipratropium bromide. Heart rate already started to rise during the injection of ipratropium bromide and increased by an average of 78% 3 min after administration compared with pretreatment control values (Kikis et al., 1982). Mean heart rate was still markedly increased by an average of 26% at 120 min after the injection of the drug. This effect of ipratropium bromide on heart rate was accompanied by an increase in cardiac index, whereas stroke volume decreased because of a decrease in ejection fraction and diastolic filling time.

Even so, acute inhalation of ipratropium bromide seemed to have no significant effect either on cardiac vagal tone or on heart rate, at least in young asthmatics (Lehrer et al., 1994). This is not surprising. In fact, although ipratropium bromide is structurally similar to atropine and has similar actions if given parenterally, it is a quaternary ammonium compound that has low oral bioactivity and few extrapulmonary effects, even after administration of very large doses.

The large UPLIFT trial has clarified the doubts generated by the reported retrospective analyses of using tiotropium bromide (Tashkin et al., 2008). In this study, cardiovascular adverse events were significantly less with tiotropium bromide (RR, 0.84; 95% CI, 0.73–0.98), although 74% of patients reported having received ICSs, 72% LABAs and 46% a fixed combination of the two (Tashkin et al., 2008). The RR of chronic heart failure (RR, 0.59; 95% CI, 0.37–0.96) and myocardial infarction (RR, 0.71; 95% CI, 0.52–0.99) was also significantly lower with tiotropium bromide compared with placebo, and those of angina (RR, 1.44; 95% CI, 0.91–2.24) and cardiac failure (RR, 1.25; 95% CI 0.84–1.87) were not significantly higher with tiotropium bromide compared with placebo. The RR of stroke in the tiotropium bromide versus placebo groups was not significantly different (RR, 0.95; 95% CI, 0.70–1.29).

Because patients receiving tiotropium bromide with the mist inhaler are, however, potentially exposed to higher concentrations and the powder and mist inhaler formulations are considered to be distinct products (US Food and Drug Administration, 2009), Singh et al. (2011b) systematically reviewed and performed a meta-analysis on data from randomized controlled trials on the risk of mortality associated with inhaled tiotropium bromide delivered by the mist inhaler compared with placebo in patients with COPD. This meta-analysis of 6522 subjects documented that tiotropium bromide administered by the mist inhaler was associated with a 52% increased risk of all-cause mortality compared with placebo. The annualized number needed to treat for mortality associated with tiotropium bromide was estimated at 124 (95% CI, 52–5682). This means that one excess death would be expected for every 124 patients treated with 5  $\mu$ g of tiotropium bromide for 1 year.

However, it is noteworthy that none of the examined studies had cardiovascular outcomes as the primary outcome, and the data reported are based on adverse event reporting. Thus cardiovascular parameters may not have been prospectively defined in a uniform fashion across the trials included in this meta-analysis and, consequently, there may have been important discrepancies between the trials in reporting of these outcomes (Cazzola et al., 2010a). Also of note is the fact that patients with a recent history of myocardial infarction, arrhythmia, or heart failure were excluded from the UPLIFT trial (Tashkin et al., 2008) (and possibly other studies); thus, it is not known whether such patients may be at an increased risk of any drug-related cardiovascular events in a real-world setting when using tiotropium bromide.

## **VIII. The Role of Bronchodilators in the Management of Asthma and Chronic Obstructive Pulmonary Disease**

Inhaled bronchodilators are the mainstay of the current management of COPD (O'Reilly et al., 2010) and are crit-

ical in the symptomatic management of asthma (National Heart, Lung, and Blood Institute National Asthma Education and Prevention Program, 2007; Bateman et al., 2008b).

According to the guidelines for the management of COPD published by the British National Institute for Health and Clinical Excellence (NICE) (O'Reilly et al., 2010), the treatment choice after initial SABA or SAMA bronchodilation for persistent breathlessness or exacerbations is determined by the level of post bronchodilator FEV<sub>1</sub>. If the FEV<sub>1</sub> is >50%, then the recommendation is to use a LAMA such as tiotropium bromide or a LABA such as salmeterol or formoterol. Treatment with LAMA plus LABA is recommended in people with COPD who remain symptomatic on treatment with a LABA, whereas the LABA plus LAMA combination is not recommended in those already taking a LAMA as sole maintenance therapy. If the FEV<sub>1</sub> is <50%, then the initial choice is between a LAMA or LABA/ICS combination. The evidence for LABA/ICS is strong in people with severe (FEV<sub>1</sub> <50%) or very severe (FEV<sub>1</sub> <30%) airflow obstruction and persistent symptoms, but the evidence is weaker (and the recommendation accordingly weaker as well) in those with only mild or moderate airflow obstruction. If ICS therapy is declined or not tolerated then treatment with a LAMA plus a LABA should be considered. In the presence of persistent symptoms or exacerbations, then triple therapy with ICS/LABA and LAMA is recommended, irrespective of their FEV<sub>1</sub>.

International guidelines on asthma management (National Heart, Lung, and Blood Institute National Asthma Education and Prevention Program, 2007; Bateman et al., 2008b) currently recommend rapid-onset inhaled  $\beta_2$ -AR agonists such as albuterol, terbutaline, or formoterol alone for symptom relief and for the pretreatment of exercise-induced bronchoconstriction in all patients with asthma. They should be used only on an as-needed basis at the lowest dose and frequency required. Increased use, especially daily use, is a warning of deterioration of asthma control and indicates the need to reassess treatment. Inhaled ipratropium bromide is a less effective reliever medication in asthma than rapid-acting inhaled  $\beta_2$ -AR agonists, and it is not recommended for the long-term management of asthma, except as an alternative bronchodilator for patients who experience tachycardia, arrhythmia, and tremor from rapid-acting  $\beta_2$ -AR agonists.

It has been suggested that there may be a  $\beta_2$ -AR-dependent signaling pathway that evokes airway hyper-responsiveness and thereby plays a critical role in the generation of the asthmatic state (Nguyen et al., 2009). Consequently, LABAs should never be used as monotherapy for asthma. They are most effective when combined with ICSs, and this combination therapy is the preferred treatment when a medium dose of ICS alone fails to achieve control of asthma. Obviously, a combination inhaler must always be used, because in many patients, the use of separate inhalers will inevitably

result in periods of LABA monotherapy because of poor compliance with ICSs in standard clinical practice (Beasley et al., 2010).

Nevertheless, the FDA has recently highlighted that the guidelines recommending long-term use of LABAs were partly influenced by studies showing a benefit from adding a LABA to an ICS for the long-term treatment of persistent asthma, but these studies have several limitations. For instance, the benefits shown were largely a measure of the  $\beta$ -agonist effect, such as improved airflow and reduced rescue use of SABAs. There are no studies showing that LABAs (alone or in conjunction with ICSs) increase survival or positively affect severe asthma exacerbations (those necessitating intubation or hospital-based care) (Chowdhury and Dal Pan, 2010). Because of their serious risks, the FDA has conducted a comprehensive review of the benefits and risks of using LABAs to treat asthma and reached the conclusion that use of the LABA must be stopped, if possible, once asthma control is achieved and the use of an asthma-controller medication, such as an ICS, then be maintained. Furthermore, LABA should not be used in patients whose asthma is adequately controlled with a low- or medium-dose ICS (Chowdhury and Dal Pan, 2010). LABAs should be reserved only for patients whose asthma cannot be adequately managed with asthma-controller medication such as an ICS (Chowdhury and Dal Pan, 2010). Furthermore, until additional data are available from large, randomized, controlled trials evaluating the safety of LABAs when administered with an ICS, the FDA believes that long-term use of LABAs should be limited to patients who require prolonged use of these drugs (Chowdhury and Dal Pan, 2010). It must be mentioned that on April 14, 2011, the FDA issued a requirement for all manufacturers of LABAs that are marketed for asthma in the United States to conduct large controlled phase 4 postmarketing safety studies to assess the safety of a regimen of LABAs plus ICSs compared with ICSs alone (Chowdhury et al., 2011a). Sustained-release theophylline has little effect as a first-line controller, but may provide clinical benefit, as add-on therapy in patients who do not achieve control on ICSs alone.

The use of LAMAs is not commented on in guidelines for the treatment of asthma. However, beneficial effects of tiotropium bromide maintenance dosing in patients with asthma have been reported in cohort studies and case reports (Iwamoto et al., 2008; Kapoor et al., 2009). Moreover, adding tiotropium bromide once daily as maintenance treatment in addition to at least high-dose ICSs combined with LABAs offers significant potential to improve airway patency in patients with severe persistent asthma who are still symptomatic and obstructed on maximal therapy (Kerstjens et al., 2011). Furthermore, the addition of salmeterol/tiotropium bromide facilitates the step down of ICS in severe patients (Fardon et al., 2007).

## IX. The Use of Bronchodilators in Special Populations

### A. Older People

Aging-related modifications in lung mechanics, in receptor populations, in nervous system control, etc., might be responsible for a different effectiveness of bronchodilators in older patients compared with younger subjects (Bellia et al., 2006). A modification of bronchodilator responses to  $\beta_2$ -AR agonists in older people has been suggested (Connolly et al., 1995). There is considerable evidence relating the reduction in  $\beta_2$ -AR affinity (or a reduced percentage of high-affinity receptors) with increasing age, possibly in association with receptor internalization in membrane-bound vesicles (Scarpace and Baresi, 1988). Abnormal postreceptor events may also be implicated in impaired  $\beta$ -adrenergic activity in older people.

In a study on bronchodilator responses to albuterol and ipratropium bromide, the FEV<sub>1</sub> response to the  $\beta_2$ -AR agonist declined with age; conversely, the response to ipratropium bromide was not related to age (Barros and Rees, 1990). The bronchodilator effects of albuterol after methacholine-induced bronchoconstriction was tested in a study carried out in healthy subjects (Connolly et al., 1995). This study showed that the older group (age range, 60–76 years) had a lower sensitivity to bronchodilator effects of albuterol, and this was interpreted by the authors as being due to an age-related decrease in airway  $\beta_2$ -AR responsiveness. However, a retrospective analysis, carried out in subjects with an FEV<sub>1</sub> reduced by at least 20%, documented that aging does not affect bronchodilator response to  $\beta$ -AR agonist after methacholine-induced bronchoconstriction (Parker, 2004). Furthermore, a study aimed at exploring the effect of age on bronchodilator responses in acute severe asthma concluded that age is not a predictor of response to  $\beta$ -AR agonists (Rodrigo and Rodrigo, 1997). However, in this study, the age groups were differentiated using the age of 35 years as cut-off level, which obviously was inadequate to allow inferences on older patients' (>65 years) response to the treatment.

$\beta_2$ -AR agonists may have significant side effects, especially on the cardiovascular system in older people. For this reason, oral  $\beta_2$ -AR agonists are normally avoided in older patients, and inhalation is the route of choice.  $\beta_2$ -AR agonist-induced side effects are dose-dependent and include an increase in myocardial oxygen consumption, changes in blood pressure, arrhythmias, hypokalemia (Haalboom et al., 1985), nausea, and tremor. Some of these side effects may be increased by concomitant use of other drugs, thus hypokalemia can be aggravated by concomitant treatments promoting potassium loss, including diuretics, ICSs, and theophylline.

Relatively little is known about possible age-dependent alterations in human mAChRs, although it has been postulated that during the aging process in humans, number and functional responsiveness of lung M<sub>2</sub> mAChR decrease. Under normal conditions, M<sub>2</sub> mAChRs limit the

release of ACh from parasympathetic nerves. Hence, loss of M<sub>2</sub> mAChRs results in increased ACh release and in increased vagal tone (Bellia et al., 2006). It has been suggested that older patients (>60 years) respond to a mAChR antagonist better than younger subjects who, conversely, benefit more from  $\beta_2$ -AR agonists (van Schayck et al., 1991). If this is true, older subjects may require increased doses of  $\beta_2$ -AR agonists, compared with mAChR antagonists, to obtain maximal bronchodilatory response. However, Kradjan et al. (1992) found no age-related differences in either the magnitude of response or in the time and dose required to reach peak effects after either albuterol or ipratropium bromide. This finding indicated that the patients were not less responsive to either drug compared with the young people. Nonetheless, a recent population-based, retrospective cohort study has shown that older adults initially prescribed LABAs for the management of moderate COPD seem to have lower mortality than those initially prescribed LAMA use (Gershon et al., 2011).

Older subjects are at greater risk of theophylline toxicity than younger patients, mainly because of a more frequent occurrence of concomitant diseases, such as liver dysfunction, cardiac failure, and fever, and for a more frequent use of polypharmacy, which may interfere with theophylline metabolism (Bellia et al., 2006). Therefore, when used in older patients, dosage adjustment of theophylline is mandatory because this drug has different PK in older people. It has been proposed that, in these subjects, serum concentrations of the drug should range between 8 and 12 mg/l (i.e., a lower level than the range proposed for the general population).

### B. Pediatric Populations

The doses of  $\beta_2$ -AR agonist delivered with the use of an MDI with spacer and face mask for infants and small children are not yet well defined. Moreover, the risk of administering high doses of drug to small children is still unknown. It has been suggested that very young children may require higher relative doses because of possible problems in nebulizer technique or differences in PK (higher clearance) (Penna et al., 1993).

A direct comparison between four doses of inhaled formoterol (4.5, 9, 18, and 36  $\mu$ g) with 50  $\mu$ g of salmeterol in children found that although both medications increased FEV<sub>1</sub>, the increase was significantly greater for doses of formoterol  $\geq 9$   $\mu$ g compared with salmeterol (Pohunek et al., 2004).

The European Respiratory Society task force, which has reviewed the evidence for pediatric medicines in respiratory disease, has advised that as-needed SABAs are the first choice for rescue treatment of acute asthma in children (Smyth et al., 2010). Regular, daily use of SABAs may cause deterioration of asthma and is not recommended. However, the scientific basis for this issue in children is limited. Levalbuterol may have some advantages over racemic albuterol, but Qureshi et al. (2005) documented that there was no difference in clinical improvement in children



with acute moderate to severe asthma exacerbations treated with either racemic albuterol or levalbuterol.

Single doses of LABAs provide at least 12 h of bronchodilation in asthmatic school-aged children. Bronchodilator and protective effects of single doses of LABA have been documented in children of preschool age and older. LABA for daily maintenance treatment in asthmatic children who remain symptomatic despite conventional doses of ICS is widely advocated and practiced but insufficiently supported by the literature and debated. In any case, the bronchodilator and bronchoprotective effects of LABAs in children become less during long-term use.

It must be mentioned that two Cochrane reviews have reported that the risk for serious adverse events with regular salmeterol or formoterol use compared with placebo is greater in children (Cates and Cates, 2008; Cates et al., 2009). A higher risk for serious adverse events associated with LABA therapy in children was also described by Rodrigo et al. (2009).

A small study that enrolled 48 steroid-naïve atopic asthmatic children, 7 to 11 years of age, demonstrated that add-on therapy with montelukast to low dosage of budesonide is more effective than the addition of LABA or doubling the dose of budesonide in controlling airway inflammation, measured as the fraction of nitric oxide in exhaled breath ( $FE_{NO}$ ), in asthmatic children (Miraglia del Giudice et al., 2007). Nonetheless, several meta-analyses of studies evaluating the safety and effectiveness of LABAs in adults and children with asthma published by the Cochrane Airways Group concluded that adding LABAs to ICSs reduces the requirement for rescue therapy, reduces exacerbations requiring systemic steroids, improves lung function compared with ICS alone, and is superior to adding an leukotriene receptor antagonist to ICS (Elkout et al., 2010).

Another Cochrane review (Plotnick and Ducharme, 2000) documented that a single dose of a mAChR antagonist is not effective for the treatment of mild and moderate exacerbations and is insufficient for the treatment of severe exacerbations. Nonetheless, adding multiple doses of a mAChR antagonist to a  $\beta_2$ -AR agonist seems safe, improves lung function, and would avoid hospital admission in 1 of 12 such treated patients. Although multiple doses should be preferred to single doses of mAChR antagonists, the available evidence supports their use only in school-aged children with severe asthma exacerbation. There is no conclusive evidence for using multiple doses of mAChR antagonists in children with mild or moderate exacerbations.

Children eliminate theophylline more rapidly on average than do adults and also show pronounced interindividual differences in the elimination of the drug (Ellis et al., 1976). Consequently, compared with adults, they tend to require relatively larger amounts of theophylline per day and the doses may have to be given at shorter intervals of time.

### C. Patients with Heart Failure

Bronchodilator use is a powerful independent predictor of worsening heart failure and increased mortality in a broad spectrum of patients with heart failure. Whether this relates to a toxic effect of bronchodilators, underlying pulmonary disease, or both is unclear (Hawkins et al., 2010).

In particular, it remains unknown whether the use of  $\beta_2$ -AR agonists leads to an increased risk of heart failure and/or it may affect the risk of hospitalization among patients with existing chronic heart failure (CHF) (Matera et al., 2010). When acutely administered,  $\beta_2$ -AR agonists are efficacious in improving cardiac performance in patients with CHF because they enhance cardiac and stroke volume indexes in a dose-dependent manner (Matera et al., 2010). For these reasons,  $\beta_2$ -AR agonists are often used for the short-term enhancement of heart contractility and support of the circulation.

Nonetheless, data from the Acute Decompensated Heart Failure National Registry Emergency Module (ADHERE-EM) (Singer et al., 2008) demonstrated that 14% of patients who present with dyspnea are treated for COPD when COPD is absent and acute decompensated heart failure is the cause of dyspnea. Inhaled  $\beta_2$ -AR agonist use in patients with heart failure who do not have COPD seems to be associated with worse outcome. Au et al. (2003) identified an increased risk of heart failure hospitalization among patients with left ventricular (LV) systolic dysfunction who received a  $\beta_2$ -AR agonist within 3 months of the hospitalization. This risk increased as the number of canisters used per month increased; the effect persisted after adjustment for other pulmonary therapies, including ipratropium bromide and corticosteroids. The mortality risk was also higher for patients treated with a  $\beta_2$ -AR agonist, but the effect was significant only in patients who received three or more bronchodilator canisters per month.

There is evidence that  $\beta$ -AR agonists, although useful acutely, lead to increases in mortality with long-term use (Felker and O'Connor, 2001). Although  $\beta$ -AR agonists can relieve symptoms and improve hemodynamic parameters, their long-term administration has been associated with increased mortality in all sufficiently powered clinical trials with a variety of drugs ranging from strong to weak agonists. Actually, some reports suggest the presence of an association between  $\beta_2$ -AR agonists and the risk of incident CHF. The Washington DC Dilated Cardiomyopathy Study compared 129 case subjects with newly diagnosed idiopathic dilated cardiomyopathy with 258 randomly dialled neighborhood control subjects (Coughlin et al., 1995). An association between idiopathic dilated cardiomyopathy and history of emphysema or chronic bronchitis (OR, 4.4), asthma (OR, 1.9), oral  $\beta$ -AR agonists (OR, 3.4), and  $\beta_2$ -AR agonist inhalers or nebulization (OR, 3.2) was demonstrated. A total of 20% of the case subjects had a reported history of  $\beta_2$ -AR agonist inhaler use compared with 6.7% of the control sub-

jects. On the contrary, the Ambulatory Care Quality Improvement Project (ACQUIP) (Au et al., 2004) found no association between the use of inhaled  $\beta_2$ -AR agonists and the risk of heart failure (1–2 canisters per month, OR, 1.3;  $\geq 3$  canisters per month, OR, 1.1). However, among the cohort with a history of CHF, there was a dose-response association between the number of inhaled  $\beta_2$ -AR agonists and the risk of hospitalization for CHF (1–2 canisters per month, adjusted OR, 1.8;  $\geq 3$  canisters per month, adjusted OR, 2.1). The increase in risk among patients with existing CHF was independent of history of COPD, corticosteroid use,  $\beta$ -AR blocker use, ACE inhibitor use, myocardial ischemia, and cardiovascular risk factors.

Whatever the case may be, the observation that  $\beta_2$ -AR agonists may exacerbate heart failure is supported by physiological observations (Matera et al., 2010).  $\beta_1$ -ARs are down-regulated and desensitized among patients with LV systolic dysfunction, and  $\beta_2$ -ARs, although desensitized, are found in normal numbers and represent a higher proportion of total  $\beta$ -ARs. Among patients with heart failure,  $\beta_2$ -AR agonists augment cardiac function, but, with regular exposure to  $\beta_2$ -AR agonists, myocardial  $\beta_2$ -ARs become desensitized and down-regulated. Moreover, long-term  $\beta_2$ -AR stimulation elevates  $G_i$  expression. The coupling of  $\beta_2$ -ARs to  $G_i$  proteins negatively regulates the  $G_s$ -mediated contractile response in the heart of many mammalian species. Intriguingly, the Ile<sup>164</sup> polymorphism of the  $\beta_2$ -AR, which leads to a dysfunctional receptor, has been demonstrated to be associated with decreased exercise tolerance and a 5-fold increased risk of death among patients with CHF. The possibility that long-term  $\beta$ -AR stimulation induces myocardial, but not systemic, elaboration of tumor necrosis factor- $\alpha$ , IL-1 $\beta$ , and IL-6 is another important finding. In fact, evidence suggests that proinflammatory cytokines are capable of modulating cardiovascular function by a variety of mechanisms, including promotion of LV remodelling, induction of contractile dysfunction, and uncoupling of myocardial  $\beta_2$ -ARs.

The possibility that  $\beta_2$ -AR agonists might exacerbate heart failure has led Ng et al. (2002) to explore whether long-term inhaled salmeterol therapy (100  $\mu$ g b.i.d.) improved pulmonary function without augmentation of neurohormonal systems or ventricular ectopy in subjects with symptomatic heart failure and LV ejection fraction  $<40\%$ . Salmeterol significantly increased mean rate-pressure product by 5%, and FEV<sub>1</sub> without producing measurable effects on neuroactivation or ventricular ectopy. The safe profile of salmeterol was also confirmed by the Drug and Safety Research Unit and the School of Medicine at Southampton (UK) (Jenne, 1998). Using the prescription-event monitoring technique, researchers from this Unit re-examined the safety issue for salmeterol and bambuterol, a long-acting oral agent and prodrug of terbutaline. There was an excess of nonfatal “cardiac failure” in the bambuterol group during the first month and a lower but increased incidence during the second to sixth months, whereas there was no excess in the salmeterol group. This

finding raised the question of why bambuterol and not salmeterol caused CHF. It is likely that this was linked to the route of administration (oral instead of inhaled) and the different dose (significantly higher with bambuterol), which induced a high systemic bioavailability and, consequently, higher risk of side effects.

Although elevated sympathetic activity is associated with an adverse prognosis, a high level of parasympathetic activation confers cardioprotection by several potential mechanisms (Olshansky et al., 2008). These parasympathetic actions on the heart are mediated not only by the direct consequences of cardiac mAChR stimulation but also by a multitude of indirect mechanisms. Parasympathetic withdrawal, in addition to the well known augmentation of sympathetic drive, is an integral component of the autonomic imbalance characteristic of CHF (Binkley et al., 1991). Nonetheless, there is documentation that, in the setting of heart failure and sympathetic activation, muscarinic receptor stimulation decreases cardiac norepinephrine spillover, an effect not observed in patients with preserved LV function. (Azevedo and Parker, 1999). A recent cohort study that examined the association between mAChR antagonists use and cardiovascular events in a cohort of 82,717 U.S. veterans with a new diagnosis of COPD between 1999 and 2002 found an increased risk of cardiovascular events, mainly heart failure, associated with the use of ipratropium bromide within the preceding 6 months, whereas the risk was not present in subjects who received anticholinergics more than 6 months prior (Ogale et al., 2010). However, the large UPLIFT trial documented that the relative risk of congestive heart failure (RR, 0.59; 95% CI, 0.37–0.96) was significantly lower with tiotropium bromide compared with placebo (Tashkin et al., 2008).

#### D. Polypharmacy

In clinical practice, the use of multiple medications or polypharmacy for patients with COPD or asthma is commonly observed, and the potential for adverse effects and drug interactions is therefore increased (McLean and Le Couteur, 2004). Patients at highest risk include older people and those with multiple comorbid medical conditions. Adverse reactions related to polypharmacy and comorbidity are frequent, mainly in older patients.

Combining thiazide and loop diuretics with  $\beta_2$ -AR agonists may enhance hypokalemia and risk ECG changes, especially if the dosage of  $\beta_2$ -AR agonists exceeds the recommended range (Lipworth et al., 1989; Newnham et al., 1991). Concomitant use of formoterol and other  $\beta_2$ -AR agonists with drugs that prolong the QTc interval may potentiate the risk of ventricular arrhythmias, including torsades de pointes (Schering Corp., 2010). The risk of QT prolongation may be increased by cardiovascular disease, hypokalemia, hypomagnesemia, bradycardia, or genetic predisposition (e.g., congenital QT prolongation). The use of monoamine oxidase inhibitors and tricyclic antidepressants may also potentiate the vascular system effects of

$\beta_2$ -AR agonists, even when the latter were initiated within 2 weeks of discontinuation. Concomitant administration of oral roflumilast and inhaled formoterol under steady-state conditions does not affect the PD or PK of either drug (de Mey et al., 2011). In particular, there is no evidence of a relevant PD interaction with regard to myocardial repolarization or cardiac function in general. Moreover, the concomitant administration of roflumilast and formoterol did not negatively influence the safety profile of either drug. Although cardioselective  $\beta$ -AR blockers have been designed to target  $\beta_1$ -ARs but avoid  $\beta_2$ -ARs in the lung and elsewhere, so called cardioselective  $\beta$ -AR blockers (such as atenolol and bisoprolol) are only relatively selective and exert significant  $\beta_2$ -AR antagonism at therapeutic doses, although to a lesser extent than nonselective  $\beta$ -AR blockers such as propranolol. Thus, it might be considered counterintuitive to prescribe both  $\beta$ -AR blockers and  $\beta$  agonists in the same patient, even when they are targeting different organs (Cazzola et al., 2002c; Matera et al., 2010). In fact, there is some risk that bronchospasm may occur in certain patients and that the bronchodilator response to inhaled  $\beta_2$ -AR agonists might be impaired with these agents. Nonetheless, a recent retrospective cohort study documented that the addition of a  $\beta$ -AR blocker had no deleterious impact when added to a regimen that included a LABA (Short et al., 2011). Coadministration of albuterol and digoxin has shown to decrease digoxin serum levels (Edner and Jogestr nd, 1989); therefore, close monitoring of the digoxin level in that context would be prudent.

As noted in section III.B, theophylline is metabolized by CYP1A2 and to a lesser extent by CYP3A3 and -2E1; medications that are inducers or inhibitors of these enzymes can affect the metabolism and elimination of theophylline. Consequently, a wide variety of drugs is known to alter the clearance of serum theophylline that may result in subtherapeutic effects or chronic intoxication (Aronson, 2006). Cardiac medications such as the  $\beta$ -AR blocker propranolol, the calcium channel blockers nifedipine and verapamil, and mexiletine reduce theophylline clearance by unknown mechanisms. Considering that increased theophylline concentrations can lead to an increased propensity for central and cardiac stimulation, it is prudent to avoid using a combination of theophylline and cardiac medications, especially antidysrhythmic drugs (Aronson, 2006). Combined therapy with theophylline and a  $\beta_2$ -AR agonist in young, otherwise healthy asthmatic subjects does not lead to an increase in the total number of ectopic beats but may increase the degree of complexity of ventricular premature beats (Coleman et al., 1986).

#### *E. Use of Bronchodilation in Athletes and the Abuse of Bronchodilators*

There is evidence that  $\beta_2$ -AR agonists can have an anabolic effect on muscle when given orally, but they are not ergogenic when given at the usual inhaled dose (McKenzie et al., 2002). These effects have been studied particularly for clenbuterol, but there is also proof that oral albuterol

can improve performance because it increases strength, endurance, and power, although side effects can be unpleasant (Fitch, 2006). The ergogenic effects are associated with an increase in skeletal muscle protein, largely because of the inhibition of protein degradation (Maltin et al., 1993). In addition to the increase in muscle mass,  $\beta_2$ -AR agonists also decrease body fat (Yang and McElligott, 1989), and for this reason, they are also defined as "repartitioning agents." The increase in muscle mass is associated with an increase in muscle force production (Dodd et al., 1996). The  $\beta_2$ -AR agonist-induced skeletal muscle hypertrophy is evident in fast- (type II) and slow- (type I) twitch muscles (Dodd et al., 1996), although in some muscles, a change in the proportion of fast- to slow-twitch fibers may occur (Burniston et al., 2007).

However, no effect has been reported in humans on oxygen uptake, lung function and performance with the use of therapeutic doses of inhaled bronchodilator in either patients with asthma or those without (Backer et al., 2007), but some studies have found that high doses of inhaled (van Baak et al., 2004) or systemic (Collomp et al., 2000)  $\beta_2$ -AR agonists have an ergogenic effect in patients without asthma. Although no effect on performance has been reported, increased lung function has been found in nonasthmatic elite athletes with inhaled  $\beta_2$ -AR agonists (Sue-Chu et al., 1999). Furthermore, some studies have noted a shorter period of breathlessness after exercise when healthy subjects have taken a  $\beta_2$ -AR agonist (Carlsen et al., 2001), indicating that the normal period with breathlessness after exercise is shortened when healthy subjects use a  $\beta_2$ -AR agonist.

Systemic use of  $\beta_2$ -AR agonists in an animal model increases weight and muscle mass. It is noteworthy that formoterol, and to a lesser extent salmeterol, are capable of producing skeletal muscle hypertrophy at microgram doses (Ryall et al., 2006). Nonetheless, inhaled formoterol neither improved endurance performance in healthy nonasthmatic athletes at hypobaric conditions equal to 2000 m above sea level (R iser et al., 2006) nor improved endurance performance in cold environments compared with placebo (T j rhom et al., 2007).

Inhaled  $\beta_2$ -AR agonists are the most effective bronchodilators for the relief of asthma symptoms and for pretreatment of exercise-induced asthma (EIA). However, daily use of SABAs may increase the severity of EIA (Hancox et al., 2002), daily administration of LABAs decreases the duration of their protection against EIA (Ramage et al., 1994), and the recovery from EIA after a bronchodilator is slower (Storms et al., 2004). Ideally, athletes should use  $\beta_2$ -AR agonists infrequently, but this may not be appropriate for those who train every day. Fortunately, EIA should be better controlled by use of other therapies. Such therapies are likely to target the production, release, and effects of the mediators of bronchoconstriction. Therefore,  $\beta_2$ -AR agonists should be reserved for occasional use and breakthrough symptoms (Fitch et al., 2008).



The management of the athlete with asthma should follow current national or international guidelines (e.g., Global Initiative for Asthma). Currently, there is no evidence that management of asthma in athletes should differ from that in nonathletes (Fitch et al., 2008). The International Olympic Commission (IOC) set up restrictions for the use of inhaled  $\beta_2$ -AR agonists in 1993 that have been modified repeatedly. The World Anti-Doping Agency (WADA) states that the use of  $\beta_2$ -AR agonists requires therapeutic use exemption and declared the following: "It is preferred to leave to the professional judgement of the physician the medical conditions under which these drugs are to be prescribed." The team manager and team doctor are also responsible when an athlete is caught in doping. A concentration of urinary albuterol  $\geq 1000$  ng/l is considered an adverse analytical finding regardless of the grant of any form of therapeutic use exemption (Carlsen et al., 2008), bearing in mind that, in any case, after inhalation of doses up to 800  $\mu$ g, urinary concentrations of albuterol are well below the limits used in doping control (Sporer et al., 2008).

There is evidence that mAChR antagonists may be effective against EIA in some patients, but not usually in the majority (Boulet et al., 1989). An additional protective bronchodilator effect may be obtained when ipratropium bromide is added to an inhaled  $\beta_2$ -AR agonist (Greenough et al., 1993). Freeman et al. (1992) reported that ipratropium bromide improved lung function both before and after exercise in asthmatic men compared with nonasthmatic men but had no effect on cardiorespiratory or cardiovascular parameters of performance after a step-wise cycle exercise test.

No ergogenic effects were attributable to theophylline therapy, which should therefore remain an acceptable means of management of athletes with asthma (Morton et al., 1989). However, a recent meta-analysis has documented that caffeine, which is a member of the methylxanthine group, significantly increases time to exhaustion compared with the placebo, and no dose-response relationship is evident for the effects of caffeine on time to exhaustion (Marshall, 2010).

#### F. Pregnancy

Witter et al. (2009) have suggested that the use of  $\beta_2$ -AR agonists increases the frequency of neurodevelopmental disorders in offspring, possibly resulting from  $\beta_2$ -AR agonist effects on the balance of sympathetic-parasympathetic tone, which may be particularly affected at specific developmental stages. However, based on the National Asthma Education and Prevention Program (NAEPP) review of six published studies that included a total of 6667 pregnant women, of whom 1929 had asthma and 1599 had taken  $\beta_2$ -AR agonists during pregnancy, data are reassuring regarding the safety of  $\beta_2$ -AR agonists used in pregnancy (National Heart, Lung, and Blood Institute National Asthma Education and Prevention Program Asthma and Pregnancy Working Group, 2005). Another large prospective study of 1828 women with asthma confirmed the lack

of relationship between the use of inhaled  $\beta$ -AR agonists and adverse maternal or fetal outcome (Schatz et al., 2004).

There are no solid data in the literature indicating that one specific  $\beta_2$ -AR agonist should be considered preferable. Nevertheless, albuterol, terbutaline, and metaproterenol are considered SABAs of first choice, because they have been used for decades without any reported significant side effect in humans (Liccardi et al., 2003). Oral/parenteral administration of  $\beta_2$ -AR agonists to asthmatic pregnant women is not recommended for some important causes, such as the lack of safety data in the first trimester, the potential inhibitory effect on the delivery, and the higher rate of side effects (tachycardia and/or tremors) compared with the inhaled route (Liccardi et al., 2003).

Only few data are available on the safety during pregnancy of salmeterol and formoterol. Animal studies with salmeterol administered parenterally are not reassuring (Physician's Desk Reference, 1998). Thorough fetal examinations revealed no significant effects of maternal treatment with salmeterol in the rat, although ossification of the second or fifth sternebrae, hyoid bone, and eleventh thoracic centrum was marginally retarded at the highest dose of 2 mg/kg per day (Owen et al., 2010). Dutch rabbit fetuses exposed in utero exhibited significant effects at oral doses of 1 mg/kg per day or more (Owen et al., 2010). The effects observed included premature opening of the eyelids, cleft palate, sternebral fusion, and limb and paw flexures and a reduction in the ossification rate of the frontal cranial bones. Increased incidences of minor skeletal variants were also noted. However, the experience with chemically similar drugs such as albuterol (animal but not human studies reporting adverse effects) suggests that animal studies cannot be entirely transposed to humans (American College of Obstetricians and Gynecologists, and American College of Allergy, Asthma and Immunology, 2000). A postmarketing surveillance study of formoterol in general practice in England showed no important side effect in 33 patients who took formoterol during pregnancy (Wilton and Shakir, 2002). Moreover, an epidemiologic study reported no adverse outcomes among 65 women who used salmeterol while pregnant (Mann et al., 1996). The use of LABAs as monotherapy was reported in one study that did not find any evidence of an effect on fetal growth in humans (Bracken et al., 2003).

Some studies in animals have shown that theophylline can cause birth defects when given in doses that are many times higher than the human dose (Shibata et al., 2000). Nevertheless, the NAEPP review of two case reports and six clinical studies that included a total of 57,163 pregnant women, of whom 3616 had asthma and 660 had taken theophylline, reassures regarding the safety of theophylline at recommended doses (to serum concentration of 5–12  $\mu$ g/ml) during pregnancy (National Heart, Lung, and Blood Institute National Asthma Education and Prevention Program Asthma and Pregnancy Working Group, 2005). Unfortunately, however, the ability to clear theophylline

ylline from the body may decrease later in pregnancy (Frederiksen et al., 1986); therefore, dosages of theophylline may need to be adjusted as a result of the changing PK as pregnancy progresses, and because theophylline readily crosses the placenta. Use of this medicine in pregnant women may cause unwanted effects, such as fast heartbeat, irritability, jitteriness, or vomiting, in the newborn infant if the amount of medicine in mother's blood were too high (Labovitz and Spector, 1982). In any case, theophylline may induce several side effects in pregnancy such as nausea/gastroesophageal reflux exacerbations, hypertension, delivery inhibition, etc. (Liccardi et al., 2003). No data concerning use of mAChR antagonists in pregnancy are available, although studies in rats, mice, and rabbits showed no birth defects when these medications were used (Liccardi et al., 2003).

## X. Pharmacogenetics of Airway Obstruction and the Future

Several genetic factors that affect the pharmacotherapeutic responses to bronchodilators have now been recognized. The  $\beta_2$ -AR is a G protein-coupled receptor encoded by the gene *ADRB2*, which is located on chromosome 5q31. Reihnsaus et al. (1993) described nine coding polymorphisms in *ADRB2*, four of which (G<sup>16</sup>R, Q<sup>27</sup>E, V<sup>34</sup>M, and T<sup>164</sup>I) create nonsynonymous changes in the amino acid sequence. Two nonsynonymous single-nucleotide polymorphisms (SNPs) at the +46 and +79 positions, encoding the amino acid changes of Gly for Arg at position 16 and Glu for Gln at position 27, respectively, have been investigated extensively. Both SNPs are common and have been found in all patients of all ethnicities surveyed thus far. They create common combination of polymorphisms (haplotypic) variations that produce three  $\beta_2$ -AR isoforms containing the amino acid pairs Gly<sup>16</sup>/Gln<sup>27</sup>, Gly<sup>16</sup>/Glu<sup>27</sup>, or Arg<sup>16</sup>/Gln<sup>27</sup>. The fourth haplotype encoding Arg<sup>16</sup>/Glu<sup>27</sup> is very rare. Under in vitro conditions, the four AR isoforms exhibit different responses to  $\beta$ -AR agonists. ARs containing Gly<sup>16</sup> and either Gln<sup>27</sup> or Glu<sup>27</sup> that were exposed to isoproterenol were shown to exhibit approximately 40% AR down-regulation, whereas ARs containing Arg<sup>16</sup>/Gln<sup>27</sup> exhibited approximately 26% down-regulation. ARs containing Arg<sup>16</sup>/Glu<sup>27</sup> did not exhibit down-regulation (Green et al., 1994). This last observation may explain why the Arg<sup>16</sup>/Glu<sup>27</sup> isoform is so rare.  $\beta_2$ -ARs that do not respond properly to endogenous catecholamines could potentially cause harmful effects in sympathetic pathway regulation and thus may be detrimental to long-term survival (Hawkins et al., 2008). Several of the haplotype frequencies differ in U.S. white and African American subjects, including haplotypes that are unique to one ethnicity. The Arg<sup>16</sup>/Arg<sup>16</sup> polymorphism is reported to occur in 20% of African American and 15% of white subjects (Rider and Craig, 2006).

Reduced clinical responses to single-dose administration of SABAs has been reported in children and adults ho-

mozygous for Gly<sup>16</sup> compared with those homozygous for Arg<sup>16</sup> (Martinez et al., 1997; Lima et al., 1999). Martinez et al. (1997) reported that in 269 children, those homozygous for Arg<sup>16</sup> were 5.3 times more likely to show a positive response to albuterol compared with children homozygous for Gly<sup>16</sup>. Children who were heterozygous Arg<sup>16</sup>/Gly<sup>16</sup> elicited an approximately 2.3-fold greater positive response to albuterol than children homozygous for Gly<sup>16</sup>, as might be expected from an intermediary genotype. However, when examining the influence of  $\beta_2$ -AR genotype on the response to long-term and repeated dosages of  $\beta_2$ -AR agonist therapy, the Arg/Arg genotype has been more closely associated with reduced responses. Adults homozygous for Arg<sup>16</sup> demonstrated impaired clinical response to regular use of SABAs (Israel et al., 2000, 2004). In particular, the  $\beta$ -Adrenergic Response by Genotype (BARGE) study concluded that long-term use of albuterol is affected by the G<sup>16</sup>R, and that regular albuterol therapy may not be appropriate for Arg<sup>16</sup> homozygous subjects (Israel et al., 2004). Higher exacerbation rates were reported in children homozygous for Arg<sup>16</sup> when treated with regular SABAs (Taylor et al., 2000) and regular LABAs (Basu et al., 2009). In another study, children homozygous for Gly<sup>16</sup> had a more rapid response to  $\beta_2$ -AR agonists during acute asthma exacerbations (Carroll et al., 2009).

Studies on position 27 polymorphisms have demonstrated that individuals homozygous for Glu<sup>27</sup> are relatively resistant to desensitization (Green et al., 1995). A single small study has documented no association between the polymorphism and desensitization to the bronchoprotective effect of regular SABA or LABA use (Lipworth et al., 1999). The polymorphism at position 27 had no influence in asthma exacerbation rates (Palmer et al., 2006) or on the clinical response to SABAs or LABAs (Martinez et al., 1997; Palmer et al., 2006). In 148 children with acute asthma exacerbation, children homozygous for Gly<sup>16</sup> were less responsive to inhaled SABAs than Glu<sup>27</sup> homozygous subjects (Martin et al., 2008).

Kim et al. (2009) genotyped a total of six SNPs in the *ADRB2* gene. In 389 subjects with emphysema and severe airflow obstruction from the National Emphysema Treatment Trial, two synonymous coding variants were associated with bronchodilator responsiveness. Individually, the codon 16 and 27 variants were not significant, but haplotypes of the two SNPs were marginally associated with one of the measures of bronchodilator responsiveness. Neither the two synonymous SNPs nor the codon 16 and 27 haplotype associations were associated with bronchodilator responsiveness in 949 individuals in 127 families from the Boston Early-Onset COPD Study (Palmer et al., 2003).

Whether altered responses to LABAs associated with *ADRB2* polymorphisms can be explained by the development of tolerance remains uncertain, because the Gly<sup>16</sup> genotype has been associated with agonist-induced bronchodilator subsensitivity in vitro (Green et al., 1995) and in vivo (Tan et al., 1997). A report on the responses to salmeterol found that Arg<sup>16</sup>/Arg<sup>16</sup> subjects had decreased re-

sponses to salmeterol in the presence or absence of concurrent ICSs (Wechsler et al., 2006), whereas in a retrospective analysis of six randomized placebo-controlled trials, the Arg<sup>16</sup> genotype was associated with subsensitivity in patients treated with regular LABAs (Lee et al., 2004). Studies in children reported an increased risk for asthma exacerbation in subjects homozygous for Arg<sup>16</sup> compared with those homozygous for Gly<sup>16</sup>, and the risk was greater in children receiving regular salmeterol (Palmer et al., 2006). However, evidence regarding the association between *ADRβ2* polymorphism and altered response to LABAs is inconsistent with other studies in adolescents and adults reporting no effect of polymorphisms on clinical responses to LABAs administered with ICS (Bleecker et al., 2006, 2007; Wechsler et al., 2009). A recent large, prospective study that examined *ADRβ2* polymorphisms and responses to LABA (salmeterol and salmeterol plus ICS) in the treatment of asthma confirmed the absence of a pharmacogenetic effect of *ADRβ2* polymorphisms (Bleecker et al., 2010). The discrepancy between studies may have arisen from differences in study design, outcome measures, ethnicity, or altered responses associated haplotypes rather than SNPs (Drysdale et al., 2000).

Recently, Panebra et al. (2010) have used the most common *ADRβ2* haplotypes (allele frequency of 0.05 or greater) found in a transfection-based system to ascertain expression and agonist-promoted down-regulation. They observed that the initial bronchodilator response from intermittent administration of  $\beta$ -AR agonist may be influenced by certain  $\beta_2$ -AR haplotypes (expression phenotypes), whereas other haplotypes may influence tachyphylaxis during the response to chronic therapy (down-regulation phenotypes). An ideal clinical outcome of high expression and less down-regulation was found for two haplotypes.

Intriguingly, a report by Umeda et al. (2008) has shown that the Arg/Arg homozygote in the 16 codon of *ADRβ2* affects bronchodilator response to tiotropium bromide in patients with COPD with significant effects on health-related quality of life. The mAChRs and ARs are G protein-coupled receptors that can activate or modulate similar signal transduction pathways (Proskocil and Fryer, 2005). Cross-talk between the two receptors existed (Cazzola and Molimard, 2010) and thus alterations in  $\beta_2$ -ARs also affected the signaling and function of other receptors that control airway contractility, such as mAChRs (McGraw et al., 2003). All these observations imply a possible influence of genetic polymorphisms in *ADRβ2* on the response to mAChR antagonists. Israel et al. (2004) also suggested that genetic polymorphisms in *ADRβ2* affect response to mAChR antagonists, but they reported that subjects with the Arg/Arg homozygote in the 16 codon of *ADRβ2* experienced greater increase in morning peak expiratory flow than subjects with other genotypes when albuterol was switched to ipratropium bromide as a primary rescue strategy. It has been suggested that R<sup>16</sup>G in *ADRβ2* should be regarded as a useful marker for discriminating tiotropium

bromide responders and nonresponders in severe persistent asthma (Park et al., 2009).

Polymorphic variation within M<sub>2</sub> and M<sub>3</sub> mAChRs could alter treatment responses to mAChR antagonists. In Maltese patients with asthma, two degenerate polymorphisms in the coding region (1197T/C, Thr-Thr and 976A/C, Arg-Arg) and a common SNP in the 3' noncoding region (1696T/A), in M<sub>2</sub> mAChR gene have been identified that are not relevant functionally (Fenech et al., 2001). However, no variation has been identified in the M<sub>3</sub> mAChR coding sequence. In the Japanese population, a degenerate polymorphism in M<sub>2</sub> mAChR coding region (1050A/G) and a degenerate M<sub>3</sub> mAChR substitution (261C/T) in M<sub>3</sub> mAChR coding region were documented (Yamamoto et al., 2002). A variable tandem repeat in the human M<sub>2</sub> mAChR gene promoter has been shown to influence gene transcription in cultured cells. It has been suggested that this variation may be contributory to the development of asthma symptoms and interindividual variability in response to mAChR antagonists (Fenech et al., 2001).

Studies of SNPs are probably limited because responses are mediated by numerous factors and interactions. Genome-wide association studies currently under way, which can evaluate the interactions between phenotypes and several genetic polymorphisms and their combinations, as well as other response elements, are more likely to provide clinically significant findings (Kelly, 2009). Given the discordant results, further work is required to fully evaluate the exact role of *ADRβ2* polymorphisms in the response to bronchodilators. Furthermore, it is likely that there are other genetic determinants of response to bronchodilator treatment. For example, Litonjua et al. (2008), assessing the effect of 844 SNPs in 111 candidate genes, identified the *ARG1* gene encoding arginase 1 as a predictor of acute response to albuterol. Haplotypes in the *ARG1* locus were associated with bronchodilator response to  $\beta_2$ -AR agonists in three independent asthma cohorts (Duan et al., 2011). In vitro studies with transfected cells revealed differential reporter gene expression by haplotype. Depending on ethnicity, the lower-responding haplotypes may represent approximately 25 to 50% of the population, which is sufficient to consider *ARG1* promoter haplotyping as part of a collection of pharmacogenetic tests to individualize asthma therapy. However, the treatment options for those with the unfavorable haplotypes are not known and require specific clinical trials.

Current pharmacogenetic studies with bronchodilators have been limited by a candidate gene approach, and the effects of gene-gene interactions on drug responses have been determined using multiple linear regression models. As the relative influence of multiple loci in multiple genes in multiple pathways are considered, the use of novel statistical analyses such as multifactor dimensionality reduction will be essential to identify novel multilocus associations that were not established a priori. In the era of personalized medicine to come, the identification of pharmacogenetic loci that predict response to a particular drug



may enable the targeted use of specific medications in populations most likely to benefit (Moore, 2011).

#### Authorship Contributions

Wrote or contributed to the writing of the manuscript: Cazzola, Page, Calzetta, and Matera.

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