Muscarinic receptor subtypes in airways

P.J. Barnes*

Cholinergic antagonists are now widely-used in the treatment of obstructive airways diseases, and may be the bronchodilators of choice in the treatment of chronic obstructive pulmonary disease (COPD), where cholinergic tone is the only reversible component. The recognition that there are multiple subtypes of muscarinic receptor in the lung has raised important questions about their role in the regulation of airway function, and creates the prospect of more selective therapy in the future [1].

Five distinct human muscarinic receptor genes have so far been identified [2], and four subtypes of muscarinic receptor (M1-M4) have now been recognized in the lung both pharmacologically and by use of specific complementary deoxyribonucleic acid (cDNA) probes [3-6]. There is now convincing evidence that these subtypes of muscarinic receptor may subserve different physiological roles in the airways, but their clinical relevance in the treatment of airway disease is not yet certain. Unravelling the role of muscarinic receptor subtypes will depend on the use of selective muscarinic antagonists and, in this issue of the journal Ućević et al. [7] report the effect of a selective M1-receptor antagonist on lung function of patients with COPD.

The vagus nerve releases acetylcholine, which activates muscarinic receptors on smooth muscle and submucosal gland cells, resulting in bronchoconstriction and mucous secretion, respectively [8]. Muscarinic receptors regulate the secretion of mucus from both submucosal glands and airway epithelial goblet cells [9]. Autoradiographic mapping studies, using [3H]quinuclidinyl benzilate, indicate that muscarinic receptors are predominantly localized to airway smooth muscle, vascular endothelium, submucosal gland cells and neuronal structures [10-12], although in some species, including humans, there is also localization to alveolar walls.

M1-receptors

Binding studies with lung homogenates indicate that there is a high proportion of pirenzepine-sensitive binding sites, presumed to be M1-receptors in several species, including humans and rabbits [13-15]. In human lung membrane, high affinity pirenzepine-sensitive sites make up approximately 70% of total binding, as confirmed by studies using [3H]pirenzepine as a radioligand [15]. Autoradiographic mapping studies indicate that these receptors are localized to the alveolar walls [11]. Other species, such as guinea-pig and ferret, do not appear to have these parenchymal muscarinic receptors [10, 11], but their significance is far from clear, as there is no evidence for cholinergic innervation of the lung periphery. These parenchymal muscarinic receptors have recently been confirmed as true M1-receptors, using specific cDNA probes. Northern blot analysis of human lung parenchyma shows a prominent band corresponding to M1-receptor messenger ribonucleic acid (mRNA) and in situ hybridization shows that M1-receptor mRNA is localized to alveolar walls [6].

M1-receptors are usually found in neuronal tissue, and there is evidence that M1-receptors are localized to parasym pathetic ganglia, and to sympathetic nerve terminals in airways [16]. In rabbit bronchi, low concentrations of pirenzepine inhibit ganglionic transmission, suggesting that M1-receptors may have a facilitating effect on transmission through airway ganglia [17]. M1-receptors may also be present in human airway cholinergic pathways. The effects of inhaled pirenzepine and the non-selective antagonist ipratropium bromide were compared on cholinergic reflex bronchoconstriction, triggered by the inhalation of the irritant gas, sulphur dioxide, in allergic volunteers [18]. A dose of inhaled pirenzepine was found, which did not inhibit bronchoconstriction due to inhaled methacholine, whereas ipratropium bromide blocked its bronchoconstrictor effect as expected. The same dose of pirenzepine, however, was as effective as ipratropium bromide in blocking the cholinergic reflex bronchoconstriction. Since pirenzine, in this dose, could not be acting directly on airway smooth muscle receptors, it might be acting on some peripheral part of the cholinergic pathway, which is most likely to be parasym pathetic ganglia in the airways.

The physiological role of the M1-receptors in ganglia is still not certain. Classically, ganglionic transmission is via nicotinic cholinergic receptors, which are blocked by hexamethonium. It is possible that excitatory M1-receptors are facilitatory to nicotinic receptors, and may be involved in "setting" the efficacy of ganglionic transmission. Activation of these receptors probably closes K+ channels, resulting in a slow depolarization of the ganglion cell [19]. Perhaps they might be involved in the chronic regulation of cholinergic tone, whereas nicotinic receptors (which act as "fast" receptors and open ion channels) are more important in rapid signalling, such as occurs during reflex activation of the cholinergic pathway. If so, then M1-antagonists, such as pirenzepine and telenzepine, might have a useful therapeutic role in...
asthma and COPD, since they may reduce vagal tone. Since increased vagal tone may play an important role in nocturnal exacerbations of asthma, then pirenzepine might prove to be efficacious in preventing nocturnal wheeze.

Pirenzepine is a bronchodilator when given intravenously to human subjects [20], although, at the dose used, it might be acting non-selectively and blocking smooth muscle muscarinic receptors. Lower doses of intravenous pirenzepine, while having no effect on forced expiratory volume in one second (FEV₁), increase expired flow at low lung volumes, suggesting an effect on more peripheral airways [21]. The more potent and longer-lasting M₁-receptor antagonist, telenzepine, has been reported to cause bronchodilatation after a single 5 mg oral dose in patients with COPD [22], in contrast to the findings of a study reported in the present issue of the Journal [7]. Administration of telenzepine, in a dose of 3 mg daily for 5 days, had no effect on airway function in patients with COPD, although dryness of the mouth was observed in half of the patients, indicating that the dose was probably adequate [7].

However, M₁-receptors may be localized to structures other than airway ganglia. Receptor mapping studies also indicate the presence of M₁-receptors on submucosal glands in the larger airways in humans [11], although this has not been confirmed using M₁-receptor cDNA probes [6]. Functional studies of mucus secretion in human airways suggest that there are no functional M₁-receptors, since pirenzepine, at low and selective concentrations, has no inhibitory effect on secretion of mucus glycoproteins [23]. Whether M₁-receptors may be involved in some other secretory response of submucosal glands, or of goblet cells, remains to be determined.

**M₂-receptors**

Binding studies in lung membrane preparations indicate that the population of M₂-receptors is very low [15], although binding to airway smooth muscle indicates that there may be a sizeable proportion of M₂-receptors [24] and M₂-receptor protein has been identified immunologically in peripheral rabbit lung [25]. M₂-receptor mRNA has also been detected in cultured human airway smooth muscle cells, using Northern blot analysis [6]. M₂-receptors may play a very important physiological role in the regulation of cholinergic neurotransmission [16]. In several species, including guinea-pig, rat, dog cat and human, there is evidence for pre-junctional muscarinic receptors on post-ganglionic airway cholinergic nerves, which inhibit the release of acetylcholine and, therefore, function as feedback inhibitory receptors (reviewed in [16]). The pre-junctional receptors have the characteristics of M₂-receptors, and are selectively blocked by methoctramine [26].

The presence of these M₂-receptors has recently been confirmed by measurement of acetylcholine release in guinea-pig trachea [27]. In human airways, activation of pre-junctional M₂-receptors has a powerful inhibitory effect on cholinergic nerve-induced contraction of airway smooth muscle in vitro [28]. In non-asthmatic human subjects, inhalation of pilocarpine, which selectively stimulates the pre-junctional receptors, has an inhibitory effect on cholinergic reflex bronchoconstriction induced by SO₂, suggesting that these inhibitory receptors are present in vivo, and presumably serve to limit cholinergic bronchoconstriction [29]. In asthmatic patients, pilocarpine has no such inhibitory action, indicating that there might be some dysfunction of the autoreceptor, which would result in exaggerated cholinergic reflex bronchoconstriction [29]. Another study, using histamine challenge, also supports this conclusion [30]. A functional defect in muscarinic autoreceptors may also explain why β-blockers produce such marked bronchoconstriction in asthmatic patients, since any increase in cholinergic tone due to blockade of inhibitory β-receptors on cholinergic nerves would normally be switched off by M₂-receptors in the nerves, and a lack of such receptors may lead to increased acetylcholine release, resulting in exaggerated bronchoconstriction [1]. Support for this idea is provided by the protective effect of oxitropium bromide against propranolol induced bronchoconstriction in asthmatic patients [31].

The mechanisms by which M₂-autoreceptors on cholinergic nerves may become dysfunctional is not certain. It is possible that chronic inflammation in airways may lead to down-regulation of M₂-receptors, which may have an important functional effect, if the density of pre-junctional muscarinic receptors is relatively low. Recently, experimental studies have demonstrated that influenza virus and major basic protein from eosinophils may inactivate M₂- rather than M₁-receptors [32, 33]. This may account for an increase in cholinergic reflex bronchoconstriction during an exacerbation of asthma, either due to a virus infection or due to an allergen exposure.

Although the bronchoconstrictor responses to cholinergic agonists appear to involve the activation of M₂-receptors, leading to phosphoinositide hydrolysis, binding studies have indicated a high proportion of M₂-receptors in airway smooth muscle [24]. Receptor mapping studies indicate the presence of M₂-receptors in airway smooth muscle of more peripheral airways (at least in guinea-pig) [11], with a relatively low level of gene expression [6]. Recently, it has been established that these M₂-receptors, by inhibition of adenylyl cyclase, may have a functional role in counteracting the bronchodilator response to β-agonists due to activation of adenylyl cyclases, both in vitro [34], and in vivo [35]. It is not certain what the physiological role of these airway smooth muscle M₂-receptors might be, however, or whether their function may be alters in airway disease.

**M₃-receptors**

Binding studies in guinea-pig and human lung membranes indicate the presence of M₃-receptors [15]. Autoradiographic studies have demonstrated M₃-receptors in airway smooth muscle of large and small human airways [11], and this has been confirmed by in situ
In guinea-pig, M4 receptors are localized predominantly to smooth muscle of proximal airways [6], and a similar distribution (of total muscarinic receptors) is seen in ferret airways [36]. In the airways, smooth muscle muscarinic receptor activation results in rapid phosphoinositide hydrolysis [37–39], and the formation of inositol (1, 4, 5) trisphosphate [40], which releases calcium ions from intracellular stores.

M3 receptors are also localized to submucosal glands in human airways [11], and there is a high concentration of M3-receptor mRNA in these structures [6]. M2-selective antagonists potentiate inhibit mucus glycoprotein secretion from human airway in vitro suggesting that M2-receptors predominate [23]. M2-receptors are only weakly expressed on airway epithelial cells [11], in contrast to the strong in situ hybridization signal with an M2-receptor cDNA probe [6], indicating that there may be a very rapid turnover of receptors. A similar epithelial expression of M4-receptor mRNA is found in human nasal biopsies [41]. M3-receptors are also localized to endothelial cells of the bronchial circulation, and presumably mediate the vasodilator response to cholinergic stimulation of the proximal airways [42]. The vasodilator response to acetylcholine in pulmonary vessels is mediated via an M3-receptor on endothelial cells [43].

M2-receptors

In rabbit lung, there is evidence from binding studies for the existence of an M2-receptor, and this has been confirmed by the presence of M2-receptor mRNA on Northern blot analysis [44], and a preponderance of M2-receptor protein [25]. In situ hybridization has demonstrated that this M2-receptor mRNA is localized to alveolar walls, and vascular and airway smooth muscle (Mak J, Barnes PJ, unpublished observations). In human lung, Northern blot analysis has not revealed any evidence of either M2 or M4-receptor mRNA in situ hybridization has not yet revealed any evidence for expression of the genes for these receptor subtypes [6].

Clinical Relevance

The discovery of at least three muscarinic subtypes in human lung has important clinical implications, since it raises the possibility of more selective anticholinergic therapy in the future. Atropine, ipratropium bromide and oxitropium bromide are non-selective as anticholinergic drugs and, therefore, block pre-junctional (M3) and post-junctional (M2) receptors. Inhibition of the autacotransceptor means that more acetylcholine will be released during cholinergic nerve stimulation and this may overcome post-junctional blockade; thus, making these non-selective antagonists less efficient than a selective antagonist of M2-receptors. Direct evidence for this is the increase in acetylcholine release on nerve stimulation, which occurs in the presence of atropine [27, 45], and the fact that ipratropium bromide in low doses causes an increase in vagally-mediated bronchoconstriction [46]. Paradoxical bronchoconstriction has been reported with nebulized anticholinergic drugs, and this may be a contributory mechanism. A similar analogy exists with a-adrenoceptors, and the non-selective antagonist, phentolamine, by acting on a pre-junctional a-receptor, increases noradrenaline release and is, thus, far less effective in the treatment of high blood pressure than a selective a-receptor antagonist, such as prazosin, which acts only on the post-junctional receptor. Unfortunately, muscarinic drugs with the high selectivity for post-junctional receptors shown by prazosin are not yet available for clinical use. Selective anticholinergic drugs which block M2-receptors or M4- and M1-receptors may, therefore, have an advantage in the treatment of airways obstruction. It has been difficult to develop highly selective muscarinic receptor subtype antagonists, possibly because the binding site for acetylcholine is very similar between the different subtypes [47], although selective allosteric inhibitors have been discovered. A long-acting muscarinic antagonist, tiotropium bromide (Bu 679), which may have some receptor selectivity, looks promising [48].

References


