Editorial

Muscarinic receptor subtypes: implications for lung disease

Introduction

Dale in 1914 first showed that acetylcholine produced two types of response, which were mimicked by nicotine and by muscarine, and that the muscarinic action was blocked by atropine.¹ Only very recently, however, have muscarinic receptors been subdivided. This has been made possible by the development of selective drugs, and muscarinic receptor subtypes have now been identified in several tissues.^{2,3} The evidence for muscarinic receptor subtypes was initially challenged, but advances in molecular biology have confirmed their existence and as many as five different receptor subtypes have now been cloned and expressed.⁴⁻⁷ The precise relation between these cloned receptor subtypes and receptors identified pharmacologically, however, is still not clear and the drugs that differentiate receptor subtypes lack a high degree of selectivity.

Muscarinic receptor classification

There is still some confusion over the classification and nomenclature of muscarinic receptors. At least three distinct receptor subtypes have been differentiated pharmacologically, both by radioligand binding assays and by functional studies.⁸

Pirenzepine was the first selective muscarinic antagonist to be recognised,⁹ and pirenzepine sensitive muscarinic receptors are termed M₁ receptors. Other muscarinic receptors were termed M₂ receptors, until it became clear that they were heterogeneous as new antagonists, such as AF-DX 116¹⁰ and methoctramine,^{11,12} were developed. M₂ receptors in atria were clearly different from those in smooth muscle and glands, which have now been termed M₃ receptors. M₃ receptors are selectively blocked by 4-diphenyl-acetoxy-*N*-methyl piperidine methiodide (4-DAMP) and hexahydrosiladifenidone. Further subdivision may be possible with the development of more selective drugs. The recognition of muscarinic

subtypes is more than a pharmacological curiosity as it is now clear that the subtypes may have different physiological roles, may be coupled to different transduction mechanisms, and may be differentially regulated.

Recent research has shown that different subtypes of muscarinic receptor are present in the airways of several species, including man¹³⁻¹⁵ (fig 1). These observations may have clinical importance, as the receptor subtypes will have different functions and the development of selective drugs may therefore be of clinical benefit in the treatment of airway disease.

Muscarinic receptors in airways

Cholinergic nerves are the predominant neural pathway in human airways^{16,17} and cholinergic bronchoconstriction may be important in airway obstruction. Cholinergic nerves pass down the vagus nerve to form synapses in parasympathetic ganglia within the airway wall, from which short postganglionic fibres pass to target cells, which include airway smooth muscle and submucosal glands. Acetylcholine is released from postganglionic nerve varicosities, leading to contraction of airway smooth muscle and mucus secretion, effects that are blocked by muscarinic antagonists such as atropine or ipratropium bromide. Autoradiographic mapping studies have shown that muscarinic receptors are localised to airway smooth muscle cells and to submucosal glands in the airways of animals¹⁸ and man.¹⁹ Muscarinic receptors are also localised to nerves and parasympathetic ganglia but they are not found on epithelium or bronchial vessels.

M₁ RECEPTORS

M₁ receptors with a high affinity for pirenzepine are generally found in the cerebral cortex and on autonomic ganglia.²⁰ Pirenzepine is used clinically to reduce gastric acid secretion and, although it was previously believed to act directly on acid secreting cells, it is now apparent that it acts predominantly on ganglia in the stomach to inhibit neurally mediated gastric secretion.²¹ As the innervation of the airways is derived embryologically from that of the gut,

Address for reprint requests: Professor P J Barnes, Department of Thoracic Medicine, National Heart and Lung Institute, London SW3 6LY.

MUSCARINIC RECEPTOR SUBTYPES

Fig 1 Muscarinic receptor subtypes in airways. M_1 receptors are localised to parasympathetic ganglia, facilitating neurotransmission; M_2 receptors to cholinergic nerves, inhibiting acetylcholine (ACh) release (autoreceptors); and M_3 receptors to airway smooth muscle. From Barnes et al¹³; reproduced by courtesy of "Trends in Pharmacological Science."

excitatory M_1 receptors seem likely to exist also in airway ganglia. There appear to be considerable species differences in the location of muscarinic receptors in pulmonary nerves, however. Pirenzepine was shown to be very effective in blocking bronchoconstriction due to stimulation of the vagus nerve in both rabbits²² and dogs,²³ although the studies did not clearly show that pirenzepine was effective at doses that had little action on the direct bronchoconstrictor action of acetylcholine. In vitro, pirenzepine has a low potency against acetylcholine induced contraction of rabbit airways²² and for binding of the non-selective antagonist [³H]quinuclidinyl benzilate to bovine airways,^{24,25} suggesting that M_1 receptors are not present in airway smooth muscle. Similarly, pirenzepine is only weakly effective in inhibiting the phosphoinositide turnover stimulated by cholinergic agonist in bovine airway smooth muscle.²⁴

Pirenzepine is a bronchodilator when given intravenously to human subjects,^{26,27} although at the dose used it might be acting non-selectively and blocking smooth muscle muscarinic receptors. A recent study has shown that lower doses of intravenous pirenzepine, although having no effect on FEV₁, increase expiratory flow at low lung volumes, indicating perhaps a selective action on peripheral airways.²⁸

Recent evidence suggests that M_1 receptors may also be present on human airway cholinergic nerves. The effects of inhaled pirenzepine and ipratropium bromide on cholinergic reflex bronchoconstriction triggered by inhalation of sulphur dioxide, were compared in atopic subjects. A dose of inhaled pirenzepine was found that failed to inhibit significantly the bronchoconstriction due to inhaled methacholine (which acts directly on airway smooth muscle), whereas ipratropium bromide was able to block this response as expected. The same dose of pirenzepine, however, was as effective as ipratropium bromide in blocking bronchoconstriction induced by sulphur dioxide, and as it could not be acting directly on airway smooth muscle receptors it must be acting on some part of the cholinergic reflex pathway.²⁹ This is most likely to be the parasympathetic ganglia in the airways, which may be accessible to inhaled pirenzepine. In support of this possibility, pirenzepine has been shown to depress parasympathetic neurotransmission in rabbit bronchi in vitro.³⁰ Furthermore, human airway parasympathetic ganglia show a high density of muscarinic receptors in autoradiographic studies.¹⁹

The physiological role of M_1 receptors in autonomic ganglia is still not certain. Classically, ganglionic transmission is via nicotinic cholinergic receptors.

which are activated by nicotine and blocked by hexamethonium. Possibly excitatory M_1 receptors are facilitatory to nicotinic receptors and may play a part in "setting" the efficacy of ganglion transmission. Activation of these receptors probably closes potassium channels, resulting in a slow depolarisation of the ganglion cell.³¹ They might perhaps have a role in the long term regulation of cholinergic tone, whereas nicotinic receptors are more important in rapid signalling, such as occurs during reflex activation of the cholinergic pathway. If so, M_1 antagonists such as pirenzepine or telenzepine might have a useful therapeutic role in asthma as they may reduce vagal tone. As increased vagal tone may have an important role in nocturnal exacerbations of asthma,^{32,33} pirenzepine might prove to be efficacious in preventing nocturnal wheeze.

M_1 receptors have also been detected in the lungs by radioligand binding studies, but the results are unexpected. Binding of [3 H]quinuclidinyl benzilate to both rabbit and human peripheral lung membranes is displaced by pirenzepine with a shallow inhibitory curve, suggesting the presence of high and low affinity sites.³⁴⁻³⁶ The high affinity binding site has the characteristics of an M_1 receptor; the use of [3 H]pirenzepine, which selectively labels this receptor subtype, has provided confirmation. M_1 receptors make up more than half of the binding sites in the human lung. This cannot possibly be accounted for by receptors on parasympathetic ganglia or nerves, which would make up only a small fraction of the membranes. Recent autoradiographic mapping of muscarinic receptor subtypes in human airways has shown that M_1 receptors are present on submucosal glands and not on airway smooth muscle. They are also found on alveolar walls, which would account for the high density of M_1 receptors in peripheral lung.³⁷ The function of M_1 receptors in alveoli is not known.

M_2 RECEPTORS

Muscarinic receptors in the atria, which mediate tachycardia, are of the M_2 subtype and are selectively blocked by gallamine, AF-DX 116, and methoctramine.¹⁰⁻¹² Methoctramine is the most selective of the muscarinic antagonists currently available and it clearly distinguishes M_2 ("atrial type") receptors from those in smooth muscle.¹² M_2 receptors are selectively activated by pilocarpine and blocked by gallamine.

M_2 receptors have now been described in airways; they are localised to the cholinergic nerves themselves and function as feedback inhibitory receptors or autoreceptors. Muscarinic receptors that inhibit the release of acetylcholine from cholinergic nerves are well recognised in the gut,³⁸ so it is not surprising to

find similar receptors in airway cholinergic nerves. These autoreceptors are inhibited by gallamine and are therefore classified as M_2 receptors, thus differing from the muscarinic receptor subtypes on airway smooth muscle, which are classified as M_3 receptors.^{39,40} M_2 receptors appear to be located before the junctions on postganglionic parasympathetic nerves,⁴¹ where they have a powerful inhibitory effect on acetylcholine release. Muscarinic autoreceptors have now been identified in guinea pig,^{39,42} cat,⁴⁰ and dog airways,⁴³ but they are lacking in the rat and rabbit.³⁹ Recently, similar feedback inhibitory receptors have been localised to postganglionic cholinergic nerves in human airways *in vitro*.⁴²

Pilocarpine, a selective agonist of M_2 receptors, inhibits cholinergic nerve induced contraction of human bronchi elicited by electrical field stimulation, while not affecting contraction induced directly by acetylcholine. This suppressive effect is blocked in a non-competitive manner by gallamine, confirming that it is mediated by an M_2 receptor, which is presumably localised to postganglionic cholinergic nerves.⁴² The suppressive effect of these autoreceptors is substantial, with complete inhibition of cholinergic nerve activity with higher doses of pilocarpine. Autoradiographic studies have shown muscarinic receptors on human airway cholinergic nerves.^{19,37} In binding studies of human lung homogenates no appreciable population of M_2 receptors has been identified,³⁶ although cholinergic nerves must make up only a trivial proportion of lung membranes and so their contribution to a lung homogenate would not be likely to be detected.

In normal subjects pilocarpine, which selectively stimulates prejunctional receptors (but has some direct effect on airway smooth muscle receptors and so causes bronchoconstriction), had an inhibitory effect on cholinergic reflex bronchoconstriction induced by sulphur dioxide, whereas a similar degree of bronchoconstriction with histamine did not affect the bronchoconstriction induced by sulphur dioxide.⁴⁴ This suggests that these inhibitory receptors may be present *in vivo*, presumably serving to limit cholinergic bronchoconstriction. In asthmatic patients pilocarpine had no such inhibitory action, indicating that there might be some dysfunction of the autoreceptor; this might result in exaggerated cholinergic reflex bronchoconstriction.⁴⁴ The reason why muscarinic autoreceptors may be dysfunctional in asthma is not certain. Possibly inflammatory changes in the airways lead to a reduction in muscarinic receptor number or coupling. This would have little or no effect on airway smooth muscle muscarinic receptors, which are very much in excess, but there may not be as many "spare" prejunctional receptors and they would therefore be more susceptible to damage.

M₃ RECEPTORS

The muscarinic receptors on airway smooth muscle are sensitive to 4-DAMP and hexahydrosiladifenidol and are therefore classified as M₃ receptors. Binding studies in guinea pig lung membranes indicate a preponderance of M₃ receptors, whereas in human lung M₃ receptors make up less than half of the receptors (the remainder being M₁).³⁶ Autoradiographic mapping studies have shown that M₃ receptors are localised to airway smooth muscle and to sub-mucosal glands.³⁷ Muscarinic receptors in airway smooth muscle are linked to phosphoinositide breakdown²⁴ and the formation of inositol-1,4,5-triphosphate,⁴⁵ which releases calcium ions from intracellular stores.

Autoradiographic studies suggest that submucosal glands have both M₁ and M₃ receptors, which are both presumed to stimulate mucus secretion. Functional studies with selective antagonists in isolated cat trachea suggest a response intermediate between M₁ and M₃ receptors, supporting the view that both receptor subtypes may play a part in airway mucus secretion.⁴⁶

Clinical relevance

The discovery of at least three muscarinic subtypes in the lung has important clinical implications, as it raises the possibility of more selective anticholinergic treatment in the future. Atropine, ipratropium bromide, and oxitropium bromide are non-selective anticholinergic drugs blocking prejunctional (M₂) and postjunctional (M₃) receptors with equal affinity. Inhibition of the autoreceptor means that more acetylcholine will be released during cholinergic nerve stimulation and this may overcome postjunctional blockade, thus making these non-selective antagonists less efficient than a selective antagonist of M₂ receptors. Alpha adrenoreceptors are analogous; the non-selective antagonist phentolamine, by acting on a prejunctional alpha₂ receptor, increases noradrenaline release and is thus far less effective in the treatment of hypertension than a selective alpha₁ antagonist such as prazosin, which acts only on the postjunctional receptor. Unfortunately, muscarinic drugs with the high selectivity of prazosin for postjunctional receptors are not yet available.

Blockade of muscarinic autoreceptors by anticholinergic drugs might account for paradoxical bronchoconstriction after a metered dose aerosol of ipratropium bromide in patients with chronic obstructive airways disease.⁴⁷ In guinea pigs ipratropium bromide in low doses potentiates vagally induced bronchoconstriction,⁴⁸ lending support to the idea that non-selective muscarinic antagonists might exacerbate bronchoconstriction by an action on prejunctional M₂

receptors. Such problems would not be expected from M₃ selective antagonists.

Beta blocker induced asthma

The worsening of asthma by beta blocking drugs remains a problem and deaths continue to be reported. Even beta blocker eye drops are capable of precipitating an asthma attack.⁴⁹ The cause has remained elusive, although cholinergic mechanisms may have a critical role as anticholinergic drugs may completely prevent the bronchoconstriction due to inhaled propranolol.⁵⁰ Muscarinic autoreceptors might help to explain how catastrophic bronchoconstriction may be precipitated by even a small dose of a beta blocking drug. Beta blocking drugs inhibit adrenergic bronchodilator tone, which in man is probably provided by circulating adrenaline.⁵¹ Beta₂ receptors that when activated inhibit the release of acetylcholine have now been found on cholinergic nerves of human bronchi,⁵² and when these are blocked an increase in tonic acetylcholine release would be expected. In normal individuals the acetylcholine would activate these autoreceptors to shut off any further acetylcholine release and thus no bronchoconstriction would occur. In asthmatic patients the same increase in acetylcholine would occur but, because of the apparent defect in muscarinic autoreceptors,⁴⁴ there would be no mechanism for switching off acetylcholine release. Furthermore, the asthmatic airway is hyperresponsive to acetylcholine, and thus even low doses of a beta blocker may precipitate a catastrophic bronchoconstrictor response (fig 2).

Summary and conclusions

Several subtypes of muscarinic receptor have now been identified and have been found recently in the airways of several species, including man, a discovery that may have important clinical implications. M₂ receptors may be found in parasympathetic ganglia, M₂ receptors on cholinergic nerves (autoreceptors) and M₃ receptors on airway smooth muscle and mucus secreting glands. It is suggested that a defect in M₂ receptor function may help to explain why asthma may be induced by beta blocking drugs. Further elucidation of the physiological role for these receptor subtypes will probably depend on the development of more selective antagonists. Drugs such as methoctramine, which have a high degree of selectivity for M₂ receptors, are promising tools for elucidating the role of muscarinic receptor subtypes,¹¹ but drugs with a higher selectivity for M₁ and M₃ receptors are likely to be most useful clinically in airway disease; in particular, M₃ blockers will not be associated with increased acetylcholine release. The recent availability

Fig 2 Possible mechanisms of beta blocker induced asthma implicating a dysfunction of muscarinic autoreceptors. Beta blockers inhibit the tonic inhibitory action of adrenaline on cholinergic nerve beta receptors and lead to increased acetylcholine (ACh) release. In normal individuals this is switched off by activation of M₂ autoreceptors. In asthmatic patients these receptors may be dysfunctional, so more acetylcholine is released. In addition, airway smooth muscle is hyperresponsive to acetylcholine in asthma, so substantial bronchoconstriction may ensue.

of cloned muscarinic receptor subtypes, and the application of in situ hybridisation techniques, should also point the way to studying differential regulation of muscarinic receptor expression in disease.

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P J BARNES
 Department of Thoracic Medicine
 National Heart and Lung Institute
 London SW3 6LY

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