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Muscarinic receptors in human airways

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Muscarinic receptors play a double role in airway disorders, mediating an increase in mucus secretion, as well as constriction of smooth muscle. Cholinergic activity of the lung is more pronounced in large than in peripheral airways; in the nose parasympathetic stimulation leads to hypersecretion and vasodilation. This article reviews the differences in muscarinic subreceptors in the upper and lower airways and discusses the effectiveness of anticholinergic agents in blocking parasympathetic stimulation at these sites. (J ALLERGY CLIN IMMUNOL 1995;95:1065-8.)

Key words: Muscarinic, anticholinergic, upper airways, lower airways, pharmacology, asthma, rhinitis

Muscarinic antagonists have proved useful in the treatment of obstructive respiratory disorders and various forms of rhinitis. In this review the role of muscarinic receptors and their antagonists in human airway disease will be discussed.

To date five subtypes (M1 to M5) of muscarinic receptor have been cloned. All are typical of G proteins, with an extracellular N terminal, three extracellular loops, seven transmembrane loops, three intracellular loops, and an intracellular carboxyl terminal.^{1,2} Receptors encoded by the M1, M3, and M5 genes are positively coupled to phospholipase C, whereas receptors encoded by the M2 and M4 genes are negatively coupled to adenylyl phosphate.² Three muscarinic receptor subtypes (M1 to M3) have been identified pharmacologically (Table I). All three can be inhibited nonspecifically by atropine. The M1 receptor, which is located primarily in neuronal tissues such as the

cerebral cortex and autonomic ganglia, is specifically stimulated by McN-A-343 and inhibited by pirenzepine and telenzepine. The M2 receptor, which is located primarily in the heart where it mediates cholinergically induced bradycardia, is an autoreceptor stimulated by pilocarpine and antagonized by gallamine, AF-DX 116, and methoctroline. The M3 receptor, which is located primarily in the glands and smooth muscle, is antagonized by 4-diaphenyl acetoxymethylpiperidine methiodide (4-DAMP) and hexahydrostiladifenidol.³⁻⁵

MUSCARINIC RECEPTORS IN THE LUNG

Cholinergic fibers travel down the vagus nerve into the lower airways and synapse in parasympathetic ganglia located in airway walls. Short postsynaptic fibers travel from these ganglia to airway smooth muscle and glands. Stimulation of the vagus nerve in animals leads to release of acetylcholine from nerve terminals, with activation of muscarinic receptors on smooth muscle and glands with resultant bronchoconstriction and mucus secretion. These effects are inhibited by muscarinic receptor antagonists such as atropine or ipratropium bromide.⁶⁻⁹ Cholinergic innervation is greater in large than in peripheral airways,¹⁰ al-

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TABLE I. Pharmacologic properties of muscarinic receptors

Receptor subtype	Agonist	Antagonist
M1	McN-A-343	Pirenzepine Telenzepine
M2	Pilocarpine	Gallamine AF-DX 116 Methoctromine
M3	—	4-DAMP Hexahydrosiladifenidol

though cholinergic receptors are found in both large and small human airways.¹¹ In humans cholinergically induced bronchoconstriction seems to involve primarily large airways, whereas β -agonists equally inhibit large and small airway constriction.¹² Thus one would expect that small airway obstruction would be more responsive to β -agonists than anti-cholinergic compounds. Alternatively, in chronic obstructive pulmonary disease, which is characterized by airway damage resulting in irreversible airway obstruction, relaxation of vagal tone may still result in improvement in airway caliber.

In situ hybridization studies have revealed the presence of messenger RNA for M2 and M3 receptors but not M1, M4, or M5 receptors in human airway smooth muscle.¹³ Pharmacologic studies with cultured airway smooth muscle cells confirm the presence of functional M2 and M3 receptors.¹⁴ In contrast, autoradiographic studies have shown that airway glands contain both M1 and M3 receptors at a ratio of 1:2, whereas alveolar walls contain M1 receptors (Table II).¹¹

Although the exact function of the various muscarinic receptors in the lungs is still under investigation, it is probable that M1 receptors enhance cholinergic transmission because low-dose pirenzepine is able to block bronchoconstriction induced by sulfur dioxide but not by methacholine.¹⁵ These data suggest that pirenzepine does not act on smooth muscle but on some proximal portion of the cholinergic pathway to enhance transmission, presumably M1 receptors on autonomic ganglia. In contrast, M3 receptor stimulation probably induces bronchoconstriction. M2 receptors in the gut act to inhibit the release of acetylcholine from cholinergic neurons.¹⁶ In guinea pig lung, M2 receptors are located prejunctionally on postganglionic parasympathetic nerves and have a powerful inhibitory influence on acetylcholine release.¹⁷ The observation that pilocarpine inhibits SO_2 -

induced bronchoconstriction in normal but not in asthmatic humans¹⁸ also suggests the presence of inhibitory M2 receptors in humans and further suggests that asthmatic patients may have dysfunctional M2 receptors. Thus agents that specifically block the bronchoconstrictive effects of M3 receptor stimulation but not the beneficial effects of M2 receptor stimulation might prove more efficacious than nonspecific, anticholinergic agents in the treatment of asthma and chronic obstructive pulmonary disease.

MUSCARINIC RECEPTORS IN THE NOSE

In the human nasal mucosa cholinergic fibers traverse the vidian nerve, innervating serous and mucous cells of submucosal glands, arteries, veins, and sinusoids.¹⁹⁻²¹ The nasal mucosa is capable of a limited repertoire of responses: congestion (induced by vasodilation, vascular permeability, and sinusoidal pooling), sneezing and itching (induced by sensory nerve stimulation), and secretion of mucus derived both from glandular cells and vascular permeability. Both nasal secretion and vasomotor tone are under parasympathetic regulation. The ability of atropine to block both hypersecretion and vasodilation induced by parasympathetic stimulation suggests that these actions are mediated through cholinergic muscarinic receptors.²²⁻²⁸

M1, M2, and M3 receptors have been pharmacologically characterized in human inferior nasal turbinate mucosa by means of receptor-binding assays. With the use of [³H] (–) QNB, total muscarinic receptor binding is approximately 688.4 ± 49.6 fmol/mg protein (B_{max}) with a K_d of 1.47 ± 0.13 nmol/L.²⁹ [³H]-pirenzapine, which is an M1 receptor antagonist, binds 45% of the total QNB-binding sites. Autoradiographic analysis suggests that M1 and M3 receptors coexist in nasal mucosal glands, with M3 receptors predominating (Table III).²⁹

Nasal challenge with cholinergic agonists such as methacholine produces hypersecretion in normal, perennial allergic and nonallergic rhinitis subjects, which indicates that glandular secretion is mediated by muscarinic receptors.^{24, 30-32} Cholinergic nasal responses are increased in seasonal atopic subjects, even when tested outside of the relevant pollen season, and are augmented further by exposure to allergen.³³ Probably because of this increased cholinergic responsiveness in atopic persons, inhibition of methacholine-induced nasal secretion by (Atrovent) is less pronounced in atopic compared with normal subjects. Functional studies of methacholine-induced glandular secretion from

TABLE II. Location and function of muscarinic receptors in human lung

Receptor subtype	Location	Function
M1	Autonomic ganglia, airway glands, alveolar walls	Enhances transmission
M2	Smooth muscle	Autoreceptor, decreases transmission
M3	Smooth muscle, airway glands	Bronchoconstriction

TABLE III. Location and function of muscarinic receptors in human nose

Receptor subtype	Location	Function
M1	Mucous and serous glands	Secretion
M2	Not present	
M3	Mucous and serous glands	Secretion
	Blood vessels	? Vasodilation

explanted human nasal mucosa confirm that both M1 and M3 but not M2 receptors mediate secretion from both serous and mucous glands of the nose. As expected from the autoradiographic data, M2 receptor antagonists do not affect methacholine-induced serous or mucous cell secretion in explanted nasal mucosal tissues.²⁹

Nasal congestion, which is induced by vasodilation of nasal vessels, can also be induced by electrical stimulation of parasympathetic fibers or with methacholine challenge and is partially inhibited by atropine.^{24, 28, 34} It is possible that vasoactive intestinal peptide, which is colocalized with acetylcholine in parasympathetic fibers,²⁹ may be responsible for that portion of parasympathetically induced vasodilation that is atropine resistant.¹⁹ Autoradiographic studies of [³H]-QNB binding to human inferior turbinate mucosa reveals the presence of muscarinic receptors on nasal blood vessels, although they are less numerous than those on glands. Binding is inhibitable by 4-DAMP but not by pirenzepine or AF-DX 116, which indicates that all of these muscarinic receptors are of the M3 subtype.²⁹ In situ hybridization studies demonstrate the presence of M3 receptor messenger RNA in vascular endothelial cells but not in vascular smooth muscle.³⁶

Thus the data indicate that human nasal glandular secretion and vascular tone are regulated by muscarinic receptors. M3 receptors predominate in the nose and are found on blood vessels, as well as in both serous and mucous glands. Serous and mucous glands are also regulated by M1 receptors. M2 receptors, which are known to act as negative feedback autoregulators in other organs, apparently do not exist in the human nasal mucosa. Therefore

selective M3 antagonists, which may prove to be especially useful in asthma, may not offer any additional benefit compared with nonselective, anticholinergic agents for the treatment of rhinitis.

REFERENCES

1. Bonner TI. New subtypes of muscarinic acetylcholine receptors. *Trends Pharmacol Sci* 1989;10(suppl):11-5.
2. Buckley NJ, Bonner TI, Buckley CM, Brann MR. Antagonist binding properties of five cloned muscarinic receptors expressed in CHO-K1 cells. *Mol Pharmacol* 1989;35:469-76.
3. Barnes PJ, Minette PA, MacLagan J. Muscarinic receptor subtypes in lung. *Trends Pharmacol Sci* 1988;9:412-6.
4. MacLagan J, Barnes PJ. Muscarinic pharmacology of the airways. *Trends Pharmacol Sci* 1989;10(suppl):88-9.
5. Minette PA, Barnes PJ. Muscarinic receptor subtypes in airways: function and clinical significance. *Am Rev Respir Dis* 1990;141:S162-5.
6. Barnes PJ. Cholinergic control of airway smooth muscle. *Am Rev Respir Dis* 1987;136:42-5.
7. Barnes PJ. State of art. Neural control of human airways in health and disease. *Am Rev Respir Dis* 1983;134:1289-314.
8. Richardson JB. Nerve supply to the lungs. *Am Rev Respir Dis* 1979;119:785-802.
9. Widdicombe JG, Karlsson JA, Barnes PJ. Cholinergic mechanisms in bronchial hyperresponsiveness and asthma. In: Kaliner MA, Barnes PJ, Persson CGA, eds. *Asthma: Its pathology and treatment*. New York: Marcel Dekker. 1990: 327-56.
10. Barnes PJ, Basbaum CB, Nadel JA. Autoradiographic localization of autonomic receptors in airway smooth muscle: marked differences between large and small airways. *Am Rev Respir Dis* 1983;127:758-62.
11. Mak JCW, Barnes PJ. Autoradiographic visualization of muscarinic receptor subtypes in human and guinea pig lung. *Am Rev Respir Dis* 1990;141:1559-68.
12. Ingram RH, Wellman JJ, McFadden ER, Mead J. Relative contribution of large and small airways to flow limitation in normal subjects before and after atropine and isoproterenol. *J Clin Invest* 1977;59:696-703.
13. Mak JCW, Baraniuk JN, Barnes PJ. Localization of mus-

- carinic receptor subtype mRNAs in human lung. *Am J Respir Cell Mol Biol* 1992;7:344-8.
14. Widdop S, Daykin K, Hall IP. Expression of muscarinic M2 receptors in cultured human airway smooth muscle cells. *Am J Respir Cell Mol Biol* 1993;9:541-6.
 15. Lammers JW, Minette P, McCusker M, Barnes PJ. The role of pirenzepine-sensitive (M1) muscarinic receptors in vagally mediated bronchoconstriction in humans. *Am Rev Respir Dis* 1989;139:446-9.
 16. Kilbinger H, Wessler I. Inhibition of acetylcholine of the stimulation-evoked release of [^{3} H] acetylcholine from the guinea-pig myenteric plexus. *Neurosci Lett* 1980;5:1331-40.
 17. Faulkner D, Fryer AD, MacLagan J. Post-ganglionic muscarinic inhibitory receptors in pulmonary parasympathetic nerves in guinea-pig. *Br J Pharmacol* 1986;88:181-7.
 18. Minette PAH, Lammers J, Dixon CMS, McCusker MT, Barnes PJ. A muscarinic agonist inhibits reflex bronchoconstriction in normal but not in asthmatic subjects. *J Appl Physiol* 1989;67:2461-5.
 19. Lundberg JM, Anggard A, Emson PG, Fahrenkrug J, Hokfelt T. Vasoactive intestinal polypeptide and cholinergic mechanisms in cat nasal mucosa: studies on choline acetyltransferase and release of vasoactive intestinal polypeptide. *Proc Natl Acad Sci USA* 1981;78:5255-9.
 20. Cauna N. Blood and nerve supply of the nasal lining. In: Proctor DF, Andersen IB, eds. *The nose: upper airway physiology and the atmospheric environment*. Amsterdam: Elsevier, 1982:45-69.
 21. Klassen ABm, van Megen JB, Kuijpers W, van den Brock P. Autonomic innervation of the nasal mucosa. *Orl J Otorhinolaryngol Relat Spec* 1988;50:32-41.
 22. Borum P. Nasal methacholine challenge: a test for the measurement of nasal reactivity. *J ALLERGY CLIN IMMUNOL* 1979;63:253-7.
 23. Eccles R, Mygind N. Physiology of the upper airways in allergic disease. *Clin Rev Allergy* 1985;3:501-16.
 24. Anggard A. The effects of parasympathetic nerve stimulation on the microcirculation and secretion in the nasal mucosa of the cat. *Acta Otolaryngol (Stockh)* 1974;78:98-105.
 25. Nomura Y, Matsuura T. Distribution and clinical significance of the autonomic nervous system in the human nasal mucosa. *Acta Otolaryngol (Stockh)* 1972;73:493-501.
 26. Eccles R, Wilson H. The parasympathetic secretory nerves of the nose of the cat. *J Physiol* 1973;230:213-23.
 27. Malm L. Vasodilation in the nasal mucosa of the cat and the effects of parasympatholytic and β -adrenergic blocking agents. *Acta Otolaryngol (Stockh)* 1973;76:277-82.
 28. Eccles R, Wilson H. The autonomic innervation of the nasal blood vessels of the cat. *J Physiol (Lond)* 1974;238:549-60.
 29. Okayama M, Mullol J, Baraniuk JN, et al. Muscarinic receptor subtypes in human nasal mucosa: characterization, autoradiographic localization, and function in vitro. *Am J Respir Cell Mol Biol* 1993;8:176-87.
 30. Borum P, Gronberg H, Brofeldt S, Mygind N. Nasal reactivity in rhinitis. *Eur J Respir Dis* 1983;128(suppl):65-71.
 31. Borum P, Larsen F, Mygind N. Nasal methacholine provocation and ipratropium therapy of perennial rhinitis. *Acta Otolaryngol Suppl (Stockh)* 1979;360:35-9.
 32. Brofeldt S, Mygind N, Sorensen CH, Teadman AS, Marriott C. Biochemical analysis of nasal secretions induced by methacholine, histamine, and allergen provocations. *Am Rev Respir Dis* 1986;133:1138-42.
 33. White MV. Nasal cholinergic responsiveness in atopic subjects studied out of season. *J ALLERGY CLIN IMMUNOL* 1993;9:278-87.
 34. Gadlage R, Behnke EE, Jackson RT. Is the vidian nerve cholinergic. *Arch Otolaryngol* 1975;101:422-5.
 35. Baraniuk JN, Okayama M, Lundgren JD, et al. Vasoactive intestinal peptide in human nasal mucosa. *J Clin Invest* 1990;86:825-31.
 36. Baraniuk JN, Kaliner MA, Barnes PJ. Muscarinic M3 receptor mRNA in situ hybridization in human nasal mucosa. *Am J Rhinol* 1992;6:145-8.