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Eosinophil and airway nerve interactions in asthma

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Abstract

Airway eosinophils are increased in asthma and are especially abundant around airway nerves. Nerves control bronchoconstiction and in asthma, airway hyperreactivity (where airways contract excessively to inhaled stimuli) develops when eosinophils alter both parasympathetic and sensory nerve function. Eosinophils release major basic protein, which is an antagonist of inhibitory M_2 muscarinic receptors on parasympathetic nerves. Loss of M_2 receptor inhibition potentiates parasympathetic nerve-mediated bronchoconstriction. Eosinophils also increase sensory nerve responsiveness by lowering neurons' activation threshold, stimulating nerve growth, and altering neuropeptide expression. Since sensory nerves activate parasympathetic nerves via a central neuronal reflex, eosinophils' effects on both sensory and parasympathetic nerves potentiate bronchoconstriction. This review explores recent insights into mechanisms and effects of eosinophil and airway nerve interactions in asthma.

Keywords

asthma; eosinophil; major basic protein; parasympathetic nerve; sensory nerve

1 | INTRODUCTION

While the association of eosinophils and asthma is well established, the importance of interactions between eosinophils and airway nerves has only recently been appreciated. Airways are densely innervated by sensory and parasympathetic nerves that regulate airway tone and provoke bronchoconstriction (Fig. 1).[1–7] In humans with asthma and in animal models, eosinophils localize to nerves and alter nerve function.[8–11] As a result, airway hyperreactivity develops, where airways contract excessively in response to inhaled stimuli.[12] This review explores key mediators and mechanisms that govern interactions between eosinophils and airway nerves in asthma and in models of airway hyperreactivity induced by antigen exposure, respiratory virus infection, and ozone inhalation. The implications of these interactions for clinical asthma are also discussed.

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DISCLOSURES

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1.1 | Sensory and parasympathetic nerves control airway reflexes

Airway sensory nerves detect stretch (mechanoreceptors) and chemical stimuli (chemoreceptors) and relay information along axons contained within the vagus nerves to the central nervous system via their cell bodies in the nodose and jugular ganglia at the base of the skull.[13,14] The vagus nerves also contain parasympathetic nerve fibers. Parasympathetic nerves travel from the brain to the airways and provide the dominant autonomic control of bronchoconstriction. Parasympathetic nerves induce bronchoconstriction by releasing acetylcholine (ACh), which activates M_3 muscarinic receptors on airway smooth muscle to stimulate contraction.[5] ACh simultaneously activates prejunctional inhibitory M_2 muscarinic receptors that reduce ACh release and limit bronchoconstriction, thus serving as an auto-inhibitory feedback mechanism (Fig. 2).[3,4,15]

Sensory and parasympathetic nerves communicate via a central pathway known as a neuronal reflex. Sensory nerves initiate “reflex bronchoconstriction” through this pathway by stimulating parasympathetic nerve ACh release (Fig. 2). Reflex bronchoconstriction occurs in response to a variety of stimuli, including methacholine,[6] histamine,[16] cold air,[17] exercise,[18] and allergens,[19] and is increased in patients with asthma.[20–22] Changes in either the sensory afferent limb or parasympathetic efferent limb of the reflex pathway can inhibit or exacerbate bronchoconstriction induced by triggering this reflex.

1.2 | Eosinophils increase bronchoconstriction by potentiating ACh release from parasympathetic nerves

Eosinophil granules are filled with highly charged, cationic proteins including major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin.[23] Major basic protein is of particular relevance since it is an allosteric antagonist of parasympathetic nerve M_2 receptors.[24] In the presence of major basic protein, M_2 receptors no longer limit ACh release from parasympathetic nerves. Consequently, loss of M_2 function potentiates parasympathetic nerve-mediated bronchoconstriction. The importance of this mechanism in clinical asthma is underscored by the observation that M_2 receptors are dysfunctional in asthmatics[25,26] and that muscarinic antagonists such as tiotropium, which block parasympathetic nerve signaling, reverse bronchoconstriction.[27,28] M_2 receptor dysfunction also occurs in all animal models of airway hyperreactivity, including in the setting of respiratory virus infections,[29–36] antigen sensitization and challenge,[4,8–11,37–43] ozone inhalation,[44–47] pesticide exposure,[48,49] and obesity.[50] Treatment with the polyanionic substances heparin or poly-L-glutamic acid that neutralize major basic protein,[37] or an antibody against major basic protein,[9,46] preserve neuronal M_2 receptor function and prevent airway hyperreactivity. Furthermore, M_2 receptor and airway function are protected in experimental models by reducing the number of airway eosinophils using an anti-IL5 antibody,[8] or by blocking eosinophil migration into airways with anti-CCR3[41] or anti-very late antigen-4 (VLA-4) antibodies.[38]

1.3 | Nerve-associated eosinophils cause M_2 receptor dysfunction

Eosinophil migration to airway nerves is critical for the development of M_2 receptor dysfunction and airway hyperreactivity. In histologic airway sections from patients who died of acute asthma[11] and in experimental models of antigen- and ozone-induced airway

inflammation,[9,39,42,43,45,51] eosinophils are found clustered around nerve axons and parasympathetic ganglia. This association is not simply due to more eosinophils in airways overall. Eosinophils are dis-proportionally associated with nerves (~2-fold greater number), compared to blood vessels and airway parenchyma, and the number of eosinophils along nerve axons corresponds with the degree of M_2 receptor dysfunction.[11]

Airway nerves actively recruit eosinophils by releasing chemokines such as eotaxin-1. Eotaxin-1 binds eosinophil CCR3 receptors and is a potent mediator of eosinophil migration.[52] Parasympathetic and sensory nerves constitutively express eotaxin-1 at baseline and increase expression after antigen challenge.[41,53] These changes may be mediated by IL4, which increases eotaxin-1 expression in a cholinergic neuroblastoma model of parasympathetic nerves. Furthermore, in antigen-sensitized and challenged guinea pigs, CCR3 antagonists reduce eosinophils around nerves without affecting the number of eosinophils in lung tissue overall, indicating that CCR3 specifically regulates eosinophil migration to nerves in vivo. Importantly, CCR3 antagonists also block airway hyperactivity.[41] Thus, the proximity of eosinophils to airway nerves is critical for mediating their effects.

TNF-α is another cytokine that regulates eosinophil recruitment to nerves, albeit through multiple mechanisms. TNF-α promotes eosinophil chemotaxis and survival by stimulating eotaxin and IL5 production, respectively.[54,55] Accordingly, eosinophil migration to nerves and subsequent development of airway hyperreactivity after antigen challenge and after ozone exposure are blocked by the TNF-α receptor antagonist etanercept.[42,56] TNF-α also directly decreases M_2 receptor expression in airway parasympathetic nerves independent of eosinophils. Therefore, TNF-α induces airway hyperreactivity through direct and indirect effects on airway nerves.

After eosinophils are recruited to airway nerves, they bind to neuronally expressed adhesion proteins VCAM-1 and ICAM-1 via complementary receptors VLA-4 and CD11b, respectively.[57] VCAM-1 is constitutively expressed by parasympathetic nerves, whereas ICAM-1 is upregulated in response to TNF-α or IFN-γ.[58] Similar de novo expression of ICAM-1 or VCAM-1 occurs on IMR-32 cholinergic neuroblastoma cells in response to TNF-α or IFN-γ,[58] and on sensory nerves in response to nerve growth factor.[53] The physiologic importance of these adhesion proteins is supported by studies showing that adhesion proteins are upregulated in the lungs after antigen sensitization,[59] and that blocking eosinophil binding to VCAM-1 or ICAM-1 prevents airway hyperreactivity in antigen challenged guinea pigs[38] and monkeys in vivo.[60]

The corticosteroid dexamethasone also reduces airway hyperreactivity after antigen sensitization in guinea pigs by down-regulating parasympathetic nerve ICAM-1 expression, which reduces eosinophil-nerve adhesion.[58] This model provides additional evidence for the importance of physical interactions between eosinophils and airway nerves since the dose of dexamethasone that prevents airway hyperreactivity decreases the number of eosinophils associated with nerves, but not eosinophils in broncho-alveolar lavage or in airway tissue.

Eosinophil degranulation is triggered by adhesion to either neuronal ICAM-1 or VCAM-1, followed by an additional stimulus from neurons such as the release of reactive oxygen

species.[61,62] This secondary neuronal signal is essential since formaldehyde-fixed neurons bind eosinophils, but do not induce degranulation. However, binding ICAM-1 or VCAM-1 does augment other eosinophil functions such as leukotriene release, even in the absence of neuronal mediators.[62] Thus, adhesion alone activates eosinophils while degranulation requires an additional neuronally released stimulus.

1.4 | Eosinophils increase sensory innervation, neuropeptide expression, and reflex bronchoconstriction

The effects of eosinophils are not limited to the autonomic parasympathetic pathway. Eosinophils alter sensory nerve structure and regulation of reflex bronchoconstriction as well. Recently, we found that transgenic mice with airway eosinophilia driven by over-expression of IL5 from airway epithelium have markedly increased airway sensory innervation compared to wild-type mice. Innervation in eosinophil-deficient mice and eosinophil-deficient mice with elevated airway IL5 was similar to that in wild-type mice, indicating that eosinophils, not IL5, mediate changes in nerve structure (unpublished observation). Mice with more airway sensory nerves had increased reflex bronchoconstriction. Thus, increased innervation is linked to airway hyperreactivity. Airway biopsies from humans with eosinophilic asthma also had more sensory innervation compared to non-asthmatics, suggesting that eosinophils have similar effects on nerve structure in humans (unpublished observation).

Eosinophil-mediated changes in sensory nerve structure are accompanied by alterations in neuropeptide expression. Neuropeptides, such as substance P, are small signaling molecules that are synthesized and secreted by sensory nerves. Nerves increase substance P expression in response to allergens,[63] ozone,[64] and respiratory viruses,[65] and blocking the substance P receptor neurokinin-1 (NK1) suppresses airway hyperreactivity.[35,39,64,66] Eosinophils mediate an increase in neuronal substance P after allergens and consequently, changes in neuronal substance P after allergens are absent in eosinophil-deficient mice (unpublished observation). Substance P causes airway hyperreactivity in several ways. Substance P activates NK1 receptors on parasympathetic nerves, which potentiates neuronal ACh release,[67] and NK1 receptors on eosinophils, which triggers the release of major basic protein.[68] Substance P also stimulates bronchoconstriction directly by activating NK1 receptors on airway smooth muscle.[69,70]

Neuropeptides also promote eosinophil recruitment. For example, eosinophil chemotaxis occurs along neuropeptide concentration gradients[71] and neuropeptides enhance eosinophil responsiveness to other chemotactic factors such as platelet activating factor and leukotriene B4.[72] In turn, neuropeptides stimulate eosinophils' entry into tissues by upregulating ICAM-1,[73] VCAM-1,[74] and E-selectin[75] binding molecules on vascular endothelium. This effect can be modeled in vivo by stimulating nerves in rat airways. Nerve stimulation increases eosinophil adherence to endothelium and is blocked by an antagonist of NK1.[76] Thus, neuropeptides promote eosinophil recruitment by increasing eosinophil responsiveness and by modulating local tissue environments.

Eosinophils influence sensory nerve function by altering the threshold for nerve activation. Specifically, sensory nerves are more sensitive to depolarization by capsaicin, ATP, and

electrical stimulation after exposure to major basic protein and eosinophil peroxidase.[77,78] The effects of granule proteins are completely eliminated by the neutralizing polyanions heparin or poly-L-glutamic acid, further establishing that sensory nerves and eosinophils exert bidirectional effects on each other's function.

1.5 | Distinct eosinophil populations exert different effects in airways

Eosinophils perform a broad range of functions in the airways that have reshaped perceptions that they solely have cytotoxic effects in asthma.[36,79–84] Their interactions with airway nerves and their role in mediating airway hyperreactivity are no exception. For example, Wicher et al. recently demonstrated that eosinophils paradoxically reduce airway hyperreactivity 3 days after ozone exposure in guinea pigs despite initially causing airway hyperreactivity after 1 day.[56] Distinct eosinophil populations mediate these divergent effects. Initially, ozone triggers the release of eosinophil major basic protein from airway resident eosinophils, which results in M_2 dysfunction and airway hyperresponsiveness. In contrast, an influx of newly divided bone marrow-derived eosinophils arrives 3 days later and attenuates airway hyperreactivity at this time point. TNF-α and IL5 mediate eosinophilopoiesis and the recruitment of protective eosinophils in nonsensitized hosts after ozone. However, this mechanism is fundamentally altered by antigen sensitization. Eosinophils fail to expand in bone marrow after ozone in antigen-sensitized animals. Consequently, newly divided bone marrow-derived eosinophils do not arrive in the airways and ozone-induced airway hyperreactivity persists in sensitized animals 3 days after exposure.[85] These findings have important implications for asthmatics exposed to environmental ozone. Ozone is a well-established precipitant of asthma exacerbations and approximately half of all asthmatics are sensitized to aero-allergens.[86] Atopic status may be an important determinant of treatment response in these patients.

The role of eosinophils during a respiratory virus infection is similarly dependent on the sensitization status of the host. While respiratory viruses cause airway hyperreactivity in both nonsensitized and antigen-sensitized guinea pigs, virus-induced airway hyperreactivity is only mediated by eosinophils in antigen-sensitized animals.[36] Eosinophils' effects in sensitized animals require the presence of CD8 T cells.[34] CD8 T cells bind to eosinophils directly to induce degranulation[87] and secrete cytokines that modulate eosinophils' responses.[88,89] Other noncanonical eosinophil functions are observed during viral infections as well, including antigen presentation to CD4 T cells[90,91] and nitric oxide generation that contributes to antiviral immune defense.[79]

These studies highlight the evolving understanding that eosinophils have diverse phenotypes and functions. This concept builds in large part on the foundational work of Leeet al.,[92] and is particularly evident in the lung. As Mesnil et al. elegantly illustrated, the lungs of mice at steady state contain a population of resident eosinophils that are IL5-independent and express Siglec-F^{int} $CD62L^{+}CD101^{low}$.[93] In contrast, a second population of IL5-dependent, Siglec-F^{hi} $CD62L^{-}CD101^{hi}$ eosinophils is recruited to lungs after house dust mite exposure. It is likely that these unique populations affect nerve function in different ways, particularly given that nerve function is preserved by blocking IL5-dependent eosinophils in some models (e.g. antigen-sensitized guinea pigs),[8] but not in others (e.g. nonsensitized guinea

pigs exposed to ozone).[56] The relationships between eosinophil phenotypes and airway nerve function in asthma are an area of active, ongoing investigation.

1.6 | Implications of eosinophil-nerve interactions for asthma phenotypes

Asthma is categorized into subgroups, or phenotypes, based on clinical features and biomarkers.[94] Phenotypes identify groups with common immunologic mechanisms that drive disease and are used clinically to identify patients who may benefit from advanced therapies. Examples of such therapies include antibodies against IL5 (e.g. mepolizumab and reslizumab) and the IL5 receptor (e.g. benralizumab), which have been found to reduce the number of asthma exacerbations and systemic corticosteroid use in eosinophilic asthmatics.[95–98] Several other treatments may enter clinical use soon, including antibodies directed against IL13, IL4 receptor-alpha, and thymic stromal lymphopoietin (TSLP).[99–101] However, none of these agents were developed with eosinophil-nerve interactions specifically in mind. Indeed, targeted therapies that prevent eosinophil migration and binding to nerves have the potential to reduce airway hyperreactivity and improve airway function. These outcomes are distinct from exacerbations, contribute to patients' daily symptom burden, and are insufficiently treated by current anti-IL5 therapies. Prime candidates include antagonists against CCR3 and ICAM-1 that have shown promise in preclinical animal models.[41,60] Furthermore, existing therapies that block parasympathetic nerve-mediated bronchoconstriction, such as tiotropium, may have even greater efficacy in eosinophilic asthmatics with increased airway innervation. Therefore, it may be valuable to consider increased innervation as a distinct asthma phenotype in future clinical trials.

2 | SUMMARY

Interactions between eosinophils and airway nerves lead to the development of airway hyperreactivity and excessive bronchoconstriction that are characteristic of asthma. Nerves release chemokines that actively recruit eosinophils, and stimulate eosinophil degranulation and release of major basic protein, which potentiates parasympathetic nerve-mediated bronchoconstriction. Eosinophil mediators also increase sensory nerve-induced reflex bronchoconstriction by stimulating nerve growth and neuropeptide expression. Therefore, eosinophils worsen bronchoconstriction through effects on both sensory afferent and parasympathetic efferent pathways. Interactions between airway nerves and eosinophils provide a rich opportunity for development of therapies that treat excessive bronchoconstriction in asthma.

Abbreviations:

ACh	acetylcholine
NK1	neurokinin-1 receptor
VLA-4	very late antigen-4

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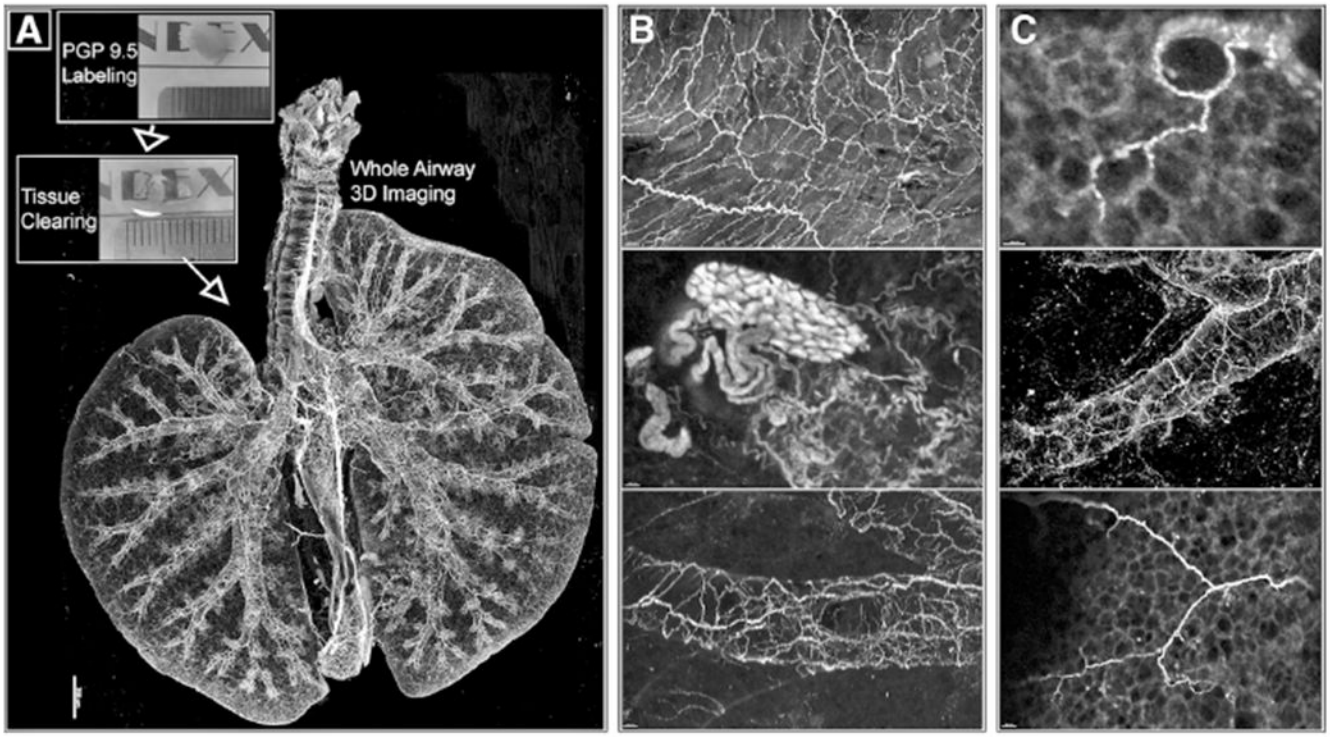

FIGURE 1. Sensory and parasympathetic nerves form a complex network in the airways.
(A) Optically cleared mouse lungs labeled with neuronal marker PGP9.5. Images obtained by confocal microscopy. (B and C) Magnified images of nerves along airways and a cluster of nerve cell bodies (ganglia; B middle image). Reprinted with permission of the American Thoracic Society. Copyright © 2017 American Thoracic Society, from Scott et al.[7]

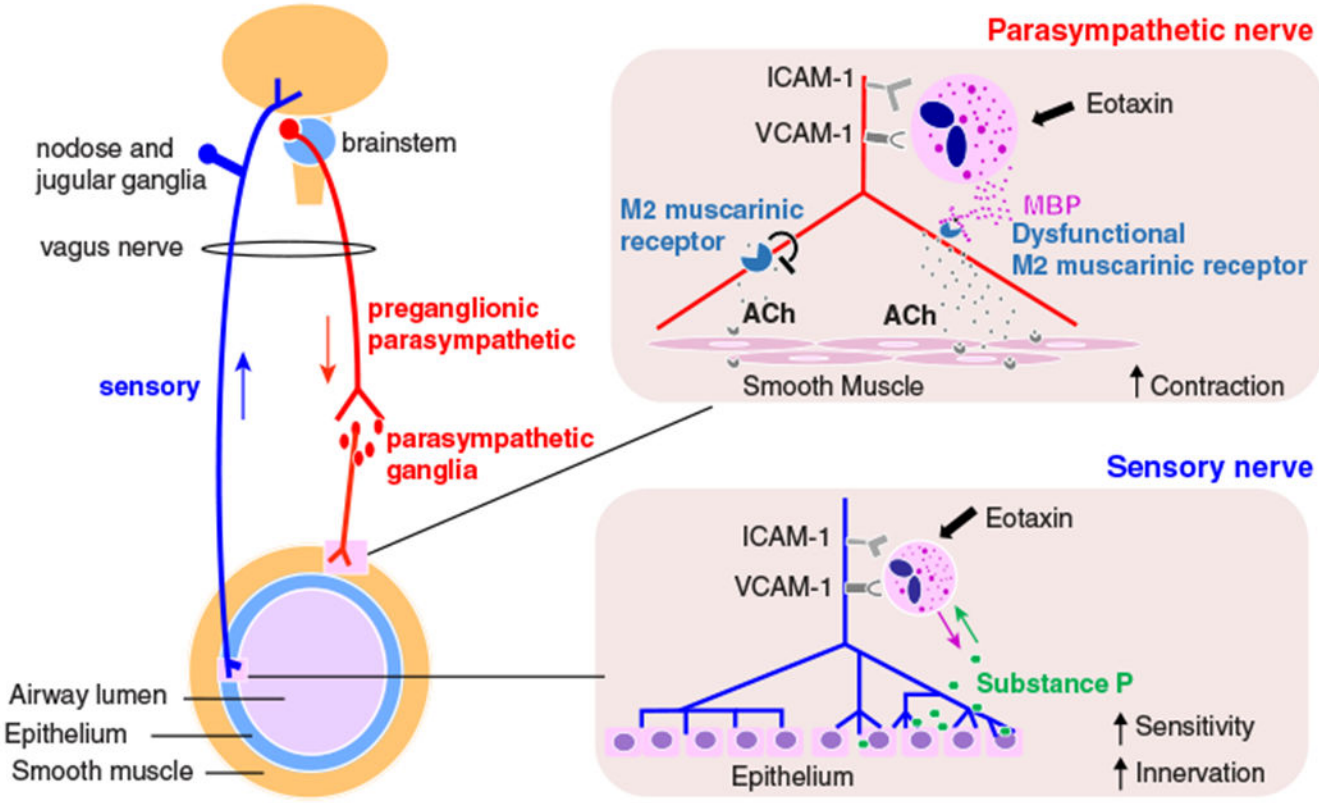

FIGURE 2. Eosinophils cause airway hyperreactivity by altering sensory and parasympathetic nerve function.

Parasympathetic nerves release ACh that activates M_3 muscarinic receptors to induce bronchoconstriction. ACh simultaneously activates presynaptic inhibitory M_2 muscarinic receptors, which reduce further ACh release and serve as an auto-inhibitory feedback mechanism. Sensory nerves can also trigger bronchoconstriction by activating parasympathetic nerves via a central neuronal reflex pathway. In asthma, eosinophils are recruited to nerves by eotaxin-1 and release major basic protein (MBP) that is a parasympathetic nerve M_2 receptor antagonist. Loss of M_2 receptors' inhibitory feedback results in excessive ACh release and increased bronchoconstriction. Eosinophils affect sensory nerve function as well by inducing nerve growth and increasing sensory neuropeptides such as substance P, which results in increased bronchoconstriction