

Neuroeffector mechanisms: The interface between inflammation and neuronal responses

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There is a complex relation between inflammation and neural control of the airways. Cholinergic neurotransmission may be enhanced by inflammatory mediators; cholinergic nerves are the dominant neural pathway for bronchoconstriction in humans. Anticholinergic drugs are more effective in acute severe asthma than in chronic asthma, suggesting that cholinergic mechanisms may be important in exacerbations. Several possible abnormalities in adrenergic control in asthma have been proposed and may be caused by the inflammatory process. Adrenergic nerves do not have direct control of airway smooth muscle but may influence bronchomotor tone in several ways, such as adrenergic neural control of the bronchial vasculature or a secondary effect on cholinergic neurotransmission. Nonadrenergic noncholinergic (NANC) mechanisms mediate both bronchoconstriction and bronchodilation, and a defect in NANC bronchodilation has been suggested to operate in severe asthma. Relatively little is known about the properties of airway sensory (afferent) nerves in human beings. They are thought to be involved in symptoms of cough and chest tightness, and the threshold for their activation is lowered in conditions of chronic inflammation. In addition, retrograde activation of sensory nerves by a local axon reflex, resulting in the release of peptides, may contribute to inflammation of the airways. Neurogenic inflammation is probably not relevant to mild asthma, however, but it may be more important in severe disease such as brittle asthma. (J Allergy Clin Immunol 1996;98:S73-83.)

Key words: Adrenergic nervous system, asthma, cholinergic pathways, nonadrenergic noncholinergic pathways, neurogenic inflammation, sensory nerves, symptoms

There is considerable evidence that neural mechanisms contribute to the pathophysiologic basis and symptoms of asthma. There is complex interaction between inflammation and neural control of airways, with effects of inflammatory mediators on neurotransmission and neurotransmitters, which in turn modulate the inflammatory response in the airways.

Autonomic nerves regulate many aspects of airway function, including airway smooth-muscle tone, secretions, blood flow, microvascular permeability, and the migration and release of inflammatory cells.^{1,2} Neural control of human airways is complex, and the contribution of neurogenic mechanisms to airway disease is still debated. Because changes in bronchomotor tone in asthma occur rapidly, it was suggested many years ago that

Abbreviations used

CGRP:	Calcitonin gene-related peptide
L-NAME:	L-N ^G -nitroarginine methyl ester
NANC:	Nonadrenergic noncholinergic
NEP:	Neutral endopeptidase
NK:	Neurokinin
NO:	Nitric oxide
SP:	Substance P
VIP:	Vasoactive intestinal polypeptide

there may be an abnormality in autonomic neural control of the airways, with an imbalance between excitatory and inhibitory pathways, resulting in excessively reactive airways. Several types of autonomic defect have been proposed in asthma, including enhanced cholinergic, α -adrenergic, and nonadrenergic noncholinergic (NANC) excitatory mechanisms, or reduced β -adrenergic and NANC bronchodilator mechanisms. Various abnormalities in airway control have been documented in asthma, and it now seems likely that they are

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effects of the disease or its treatment rather than primary abnormalities.

In addition to classic cholinergic and adrenergic neural pathways, NANC neural mechanisms are present in the airways. Neuropeptides may mediate many of the NANC effects described in airways,³ but it has been recognized that nitric oxide (NO) may also function as a NANC transmitter. Although it was once believed that there were discrete NANC nerves, it is now apparent that NANC effects are mediated by the release of cotransmitters from classic autonomic nerves.

AFFERENT NERVES

At least three types of afferent nerve are recognized in the lower respiratory tract.⁴ Slowly adapting receptors act as stretch receptors in airway smooth muscle, whereas myelinated rapidly adapting (irritant) receptors and unmyelinated C fibers are superficially localized and may be activated by inflammatory and irritant stimuli in the airway lumen. Dolor (pain) is one of the classic signs of inflammation, and sensitization and activation of sensory nerves is a common feature of acute and chronic inflammation. It is therefore likely that sensory nerves are involved in asthmatic inflammation, but in the lower respiratory tract sensory nerve activation may be reflected by symptoms of cough and chest tightness rather than by pain.

Afferent nerves are likely to be activated in asthma by several inflammatory mediators. In addition, mediators of inflammation may sensitize airway afferent nerves, so that they are more readily triggered by endogenous or exogenous irritants. Relatively little is known about the properties of airway afferent nerves. Studies in animals *in vivo* are confounded by the effect of reflexes and anesthetics, and studies in human beings are difficult to conduct for ethical reasons. We have developed an *in vitro* single-fiber model in guinea pig proximal airways to characterize the properties of airway afferent fibers.⁵ We have demonstrated the presence of myelinated A fibers that respond to mechanical stimulation and to low-pH solutions (protons), and unmyelinated C fibers that respond to capsaicin, bradykinin, and hypertonic saline solution but not to histamine, serotonin, or prostaglandins.⁵⁻⁷ Both prostacyclin and platelet-activating factor appear to sensitize afferent nerves to the activating effects of bradykinin.⁷ The capsaicin antagonist capsazepine inhibits capsaicin-induced activation of C fibers, as expected, but also inhibits activation by protons, suggesting that there is

release of an endogenous activator of capsaicin-binding sites.⁸

Chronic inflammation leads to hyperesthesia and to a lowering of the threshold of activation of sensory nerves. The molecular basis of hyperalgesia is an area of active research, although there is little information about it in airway sensory nerves.

Afferent nerves may play an important role in the symptoms of asthma (cough or chest tightness) but may also be involved in the activation of cholinergic reflexes and in the release of proinflammatory peptide neurotransmitters, as discussed later in this article.

CHOLINERGIC MECHANISMS

Because cholinergic nerves are the dominant neural bronchoconstrictor pathway in animal and human airways,¹ there has been considerable interest in determining whether cholinergic mechanisms are exaggerated in asthma or chronic obstructive pulmonary disease. Many triggers that induce bronchospasm (such as sulfur dioxide, prostaglandins, histamine, and cold air) also stimulate afferent receptors, leading to reflex cholinergic bronchoconstriction. Anticholinergic drugs are the most effective bronchodilators in chronic obstructive pulmonary disease. These drugs are effective in the treatment of acute severe asthma but are less useful in chronic asthma, suggesting that cholinergic mechanisms may play a role in exacerbations.

Several mechanisms may contribute to cholinergic bronchoconstriction in asthma.⁹ They include an increase in central vagal cholinergic tone and activation of cholinergic reflexes by way of afferent pathways inside or outside the airways (e.g., esophagus, larynx, or nose). There is evidence, through use of an acoustic reflectance technique to estimate airway caliber, for increased cholinergic tone in patients with asthma.¹⁰ Inflammatory mediators may enhance cholinergic neurotransmission in the airways by facilitating acetylcholine release in parasympathetic ganglia within the airways or from postganglionic nerve terminals.¹¹ These parasympathetic ganglia are surrounded by inflammatory cells, including mast cells, and have an afferent nerve input. There is evidence for facilitated transmission in sensitized animals exposed to allergen.¹² Inflammatory mediators may also facilitate acetylcholine release from postganglionic nerve terminals through an effect on prejunctional receptors.¹¹ For example, thromboxane, prostaglandin D₂, and tachykinins facilitate acetylcholine release from

postganglionic nerves in the airways and thereby exaggerate cholinergic reflex effects.

Patients with asthma show an exaggerated bronchoconstrictor response to cholinergic agonists, but this response is not specific: a similar increased responsiveness is observed with other spasmogens, such as histamine, leukotrienes, or prostaglandins, suggesting that an isolated enhancement of muscarinic receptor function is unlikely.

Muscarinic receptor subtypes

At least three subtypes of muscarinic receptor may be differentiated pharmacologically, and five distant receptor subtypes have now been cloned. Muscarinic receptor subtypes have also been recognized in airways.¹³ Autoradiographic mapping shows that in human airways they are differentially distributed,¹⁴ and *in situ* hybridization shows a corresponding differential gene expression.¹⁵ Inhibitory muscarinic receptors (autoreceptors) have been demonstrated on cholinergic nerves of airways in animals *in vivo* and in human bronchi *in vivo*. These prejunctional receptors inhibit acetylcholine release and may serve to limit vagal bronchoconstriction. Autoreceptors in human airways belong to the M₂-receptor subtype,¹⁶ whereas those on airway smooth muscle and glands belong to the M₃-receptor subtype. Drugs such as atropine and ipratropium bromide, which block prejunctional M₂-receptors and postjunctional M₃-receptors on smooth muscle with equal efficacy, therefore increase acetylcholine release, which may then overcome the postjunctional blockade. As a result, drugs of this type are not as effective against vagal bronchoconstriction as they are against bronchoconstriction mediated by cholinergic agonists, and it may be necessary to reevaluate the contribution of cholinergic nerves when drugs that are selective for the M₃-receptors are developed. The paradoxical bronchoconstriction that is occasionally seen with inhaled anticholinergic agents may result from this effect.

The presence of muscarinic autoreceptors has been demonstrated in human subjects *in vivo*. A cholinergic agonist, pilocarpine, which selectively activates M₂-receptors, inhibits cholinergic reflex bronchoconstriction induced by sulfur dioxide in normal subjects, but this type of inhibitory mechanism does not appear to operate in subjects with asthma, suggesting that there may be dysfunction of these autoreceptors.¹⁷ Such a defect in muscarinic autoreceptors may then result in exaggerated cholinergic reflexes in asthma because the normal feedback inhibition of acetylcholine release may be

lost. This mechanism may also explain the sometimes catastrophic bronchoconstriction that occurs with β -blockers in persons with asthma and that, at least in persons with mild asthma, appears to be mediated by cholinergic pathways. Antagonism of inhibitory β -receptors on cholinergic nerves would result in increased release of acetylcholine, which may be switched off in patients with asthma.^{18, 19}

The mechanisms that lead to dysfunction of prejunctional M₂-receptors in asthmatic airways are not certain, but it is possible that M₂-receptors may be more susceptible to damage by oxidants or other products of the inflammatory response in the airways. Recent experimental studies have demonstrated that influenza virus infection in guinea pigs may result in a selective loss of M₂-receptors compared with M₃-receptors as a result of the action of viral neuraminidase on the sialic acid residues on M₂-receptors, which are necessary for high-affinity acetylcholine binding. This loss of M₂-receptors results in a loss of autoreceptor function and enhancement of cholinergic bronchoconstriction.²⁰ This mechanism may contribute to airway hyperresponsiveness after upper respiratory tract virus infections. M₂-receptors are also inhibited by eosinophil major basic protein, indicating that eosinophilic inflammation may result in autoreceptor dysfunction.²⁰

M₁-receptors, which are excitatory, are present in the airway parasympathetic ganglia of animals and may be inhibited by the M₁-selective blocker pirenzepine. The function of these M₁-receptors in regulation of airway tone is not yet certain, but they may play a significant role in controlling the amount of filtering that occurs in parasympathetic ganglia, because these receptors appear to be important in the chronic regulation of ganglionic transmission, whereas the classic nicotinic receptors may be more important in rapid neurotransmission, as in reflexes. Similar receptors are also probably present in human parasympathetic ganglia, because pirenzepine inhibits reflex bronchoconstriction at doses that do not inhibit the direct effect of cholinergic agonists on airway tone.²¹ M₁-receptors may be important in asthma if they facilitate cholinergic reflexes, and they may play a role in nocturnal asthma, in which increased vagal tone at night is important.

ADRENERGIC MECHANISMS

Because β -agonists have a dramatic effect in relieving asthmatic bronchoconstriction, it was logical to suggest that there may be a defect in adrenoceptor function in asthma. Adrenergic

mechanisms involve sympathetic nerves, circulating catecholamines, and α - and β -adrenoceptors. Several possible abnormalities in adrenergic control have been proposed in asthma and may be caused by the inflammatory process.

Adrenergic nerves do not control smooth muscle in human airways directly but may influence cholinergic neurotransmission by means of prejunctional α - or β -receptors.¹ Adrenergic neural control of the bronchial vasculature may be important in asthma, particularly during exercise and cold-air challenge, when the conditioning of inspired air may be important.

Because adrenergic nerves do not directly control airway smooth muscle, it seems probable that circulating catecholamines play a more important role in regulation of bronchomotor tone.²² Although the catecholamines—norepinephrine, epinephrine, and dopamine—are present in the circulation, only epinephrine has physiologic effects, and is secreted by the adrenal medulla. That β -blockers cause bronchoconstriction in patients with asthma but not in normal subjects suggests that adrenergic drive to the airways is important as a defense against bronchoconstriction. In the absence of direct adrenergic innervation, this drive must be provided by circulating epinephrine. However, plasma epinephrine concentrations are not increased in patients with asthma, even during acute exacerbations, suggesting that severe bronchoconstriction is not a stimulus to epinephrine release. Furthermore, plasma epinephrine concentrations are not increased during provoked bronchoconstriction induced by a variety of challenges.

α -Receptors, which mediate bronchoconstriction, have been demonstrated in the airways of several species and may be seen only in specific experimental conditions, such as exposure to inflammatory mediators. There is now considerable doubt about the role of α -receptors in the regulation of tone in human airways, however, because it has been difficult to demonstrate their presence functionally or autoradiographically,²³ and α -blocking drugs do not appear to be effective as bronchodilators. It is possible that α -receptors may play an important role in regulating airway blood flow, which may indirectly influence airway responsiveness, and there is some evidence that α -agonists may reduce airway narrowing in exercise-induced asthma.

β -Adrenoceptor function

The possibility that β -receptors are abnormal in asthma has been extensively investigated. The sug-

gestion that there is a primary defect in β -receptor function in asthma has not been substantiated, and any defect in β -receptors is likely to be caused by the disease, perhaps as a result of inflammation or as a consequence of adrenergic therapy. β -Adrenoceptors are localized to many different cell types in the lung and have several functional effects. By means of *in situ* hybridization it is possible to map the expression of the β -receptor gene in human lungs.²⁴ A high level of expression in airway smooth muscle compared with the low density of β -receptors may indicate a rapid turnover of receptors, which may explain why desensitization is difficult to demonstrate in airway smooth muscle.

Some studies have demonstrated that airways from patients who have died of asthma do not relax normally to isoproterenol, suggesting a defect in β -receptor function in airway smooth muscle.^{25, 26} Autoradiographic studies have demonstrated a surprising increase, rather than the expected decrease, in airway smooth muscle β -receptors, however.²⁷ Indeed, there appears to be an inverse correlation between reduced response to isoproterenol and the density and affinity of β -receptors.²⁷ This inverse correlation may indicate uncoupling of airway smooth-muscle β -receptors in fatal asthma, possibly because of phosphorylation of stimulatory G protein as a result of protein kinase C activation in response to inflammatory mediator receptor activation.²⁸ It is unlikely that this mechanism is relevant in less severe asthma because patients with asthma are very sensitive to the bronchodilator action of β -agonists.

NANC BRONCHODILATOR NERVES

In human airways, bronchodilator nerves that are nonadrenergic have been demonstrated *in vitro* by electrical field stimulation and *in vivo* by reflex activation with chemical or mechanical irritation.²⁹ A defect in NANC bronchodilator nerves has been proposed in asthma because this is the only neural bronchodilator pathway in human airways.¹ This defect could arise from an intrinsic defect in these nerves (which seems unlikely) or from a secondary abnormality such as increased breakdown of the neurotransmitter or a defect in the NANC inhibitory receptors. In cats a defect in NANC bronchodilatation has been demonstrated after allergen exposure.³⁰ However, in patients with mild asthma no reduction in the NANC bronchodilator reflex is apparent,³¹ and *in vitro* studies have demonstrated a normal NANC bronchodilator response in airways obtained from lung-transplant donors with mild asthma.³² However,

those findings do not preclude a defect in more severe asthma, when inflammation is more pronounced.

The neurotransmitters of bronchodilator NANC nerves in airways have recently been elucidated. In guinea pig trachea there is evidence that vasoactive intestinal polypeptide (VIP) is at least contributory, because degradation with α -chymotrypsin results in a 50% reduction in the bronchodilator NANC response, and a similar effect is obtained with VIP antiserum.³³ VIP is colocalized with acetylcholine in parasympathetic nerves in airways, and degradation of VIP with α -chymotrypsin results in enhanced cholinergic neural constriction,³⁴ suggesting that VIP acts as a "braking" mechanism for cholinergic nerves. VIP not only acts as a functional antagonist to acetylcholine contraction of airway smooth muscle; it also acts as a "double-braking" system, because in addition it inhibits the release of acetylcholine from cholinergic nerves.¹¹

VIP appears to be localized to cholinergic nerves in human airways and is a potent bronchodilator in human airways *in vitro*.³ A complete absence of VIP-immunoreactive nerves has been described in asthmatic lungs,³⁵ but this absence might be explained by enzymatic degradation of VIP by enzymes released from inflammatory cells. VIP is susceptible to degradation by a variety of enzymes; tryptase derived from mast cells appears to be the most active of these enzymes and may lead to more rapid breakdown of VIP in asthmatic airways. There are doubts as to whether VIP acts as the bronchodilator NANC transmitter in human airways, however, because the neural bronchodilator response is not reduced by α -chymotrypsin as it is in guinea pigs³⁶ and because there is no facilitation of the cholinergic response.³⁷

There is compelling evidence that NO may function as a NANC transmitter. NO is synthesized from L-arginine by way of an NO synthase isoenzyme that is localized to peripheral nerves. An inhibitor of NO synthase, L-N^G-nitroarginine methyl ester (L-NAME), reduces the bronchodilator response to electrical field stimulation in guinea pigs by 50%, suggesting that both NO and VIP contribute to the neural bronchodilator response in this species.³³ L-NAME also enhances the cholinergic neural response in this species.³⁸ In human airways L-NAME almost abolishes the neural bronchodilator response *in vitro*; this effect of L-NAME is reversed by L-arginine but not by D-arginine, adding further support to the view that NO is the transmitter.³⁹ L-NAME also increases the cholinergic neural response in human airways,

confirming that NO may modulate cholinergic contraction in human airways.³⁷ NO may be degraded more rapidly by superoxide anions released from inflammatory cells, and this factor may contribute to increased cholinergic reflex bronchoconstriction in asthma.

SENSORY NEUROPEPTIDES

Airway C fibers contain several neuropeptides, including substance P (SP), neurokinin (NK) A, and calcitonin gene-related peptide (CGRP), which may be released from sensory nerves on retrograde activation of the nerve through a local (axon) reflex mechanism. Release of neuropeptides from airway sensory nerves is involved in various models of airway hyperresponsiveness in rodents and mediates bronchoconstrictor NANC neural responses. Sensory neuropeptides may also play an important role in mucosal immunity, and evidence is now emerging for complex interactions between neuropeptides and the immune system.⁴⁰

Tachykinins

SP and NK_A belong to the tachykinin family and activate different subtypes of tachykinin receptor. NK_A is a potent constrictor of human bronchi *in vitro* and is considerably more potent than SP, indicating that an NK₂-receptor is present on airway smooth muscle; its presence has been confirmed by the use of selective agonists and antagonists. NK_A is particularly potent in peripheral human airways.

SP is a potent inducer of microvascular leakage and mucus secretion from submucosal glands and goblet cells in airways and is potent than NK_A in this respect, suggesting that NK₂-receptors are involved. SP is potent in stimulating goblet-cell secretion,⁴¹ and this characteristic may be important in asthma, because goblet cells are the only source of mucus glycoproteins in peripheral airways.

Tachykinins are degraded predominantly by neutral endopeptidase (NEP) (EC 3.4.24.11, enkephalinase), which is localized to airway epithelium. Epithelium removal greatly exaggerates the constrictor effect of tachykinins *in vitro*.⁴² Specific inhibitors of NEP, such as phosphoramidon and thiorphan, enhance the contractile response to tachykinins to the same extent as epithelial removal, and no further effect of epithelium removal can be demonstrated, suggesting that metabolism of tachykinins by epithelium enzymes accounts for this effect of epithelium.⁴² These observations may be of relevance to asthma, because if epithelium is

shed or if NEP activity is down-regulated, as has been observed in some viral infections and after oxidant exposure or with some cytokines, such as interleukin-1 β ,⁴³⁻⁴⁵ then tachykinins released from sensory nerves would not be degraded as effectively. As a result, their effects—such as bronchoconstriction, microvascular leakage, and mucus secretion—would be exaggerated.

CGRP

CGRP is colocalized with tachykinins in airway C fibers. CGRP is a potent dilator of bronchial vessels *in vitro* and causes a long-lasting increase in blood flow in the canine trachea *in vivo*.⁴⁶ Receptors for CGRP are localized predominantly to airway blood vessels in animal and human airways,⁴⁷ suggesting that CGRP may contribute to the hyperemia that is characteristic of asthmatic airways.

Neurogenic inflammation

Release of peptides from sensory nerves in the airways may result in inflammation of the airways. This effect is readily demonstrated in rodents and some other species, but whether it is relevant in human airways is not yet certain. In a chronic animal model of allergen-induced airway hyperresponsiveness, depletion of sensory neuropeptides, by previous treatment with capsaicin, results in abrogation of the hyperresponsiveness,⁴⁸ whereas in an acute model of allergen exposure capsaicin pretreatment has no effect.⁴⁹ This difference suggests that sensory neuropeptides may become more important in chronic inflammation.

The number of SP-immunoreactive nerves may be increased in the airways of patients who die of asthma,⁵⁰ although assays of SP have not confirmed this observation.⁵¹ This increase could be the result of chronic inflammation, and trophic factors (such as nerve growth factor) may be released by inflammatory cells, resulting in proliferation of sensory nerves in the airways. Inflammatory signals, such as cytokines, may stimulate increased synthesis of tachykinins in sensory neurons. There is evidence for an increase in SP in the bronchoalveolar lavage fluid and sputum of patients with asthma.^{52, 53}

Bradykinin may be particularly important in activating sensitized sensory nerves, and, when inhaled by patients with asthma, it induces marked dyspnea and coughing like those of an asthma attack.⁵⁴ Bradykinin is formed from plasma, which exudes into the asthmatic airway lumen as a result of microvascular leakage. Activation of these sensory nerves may then cause antidromic stimulation

of branches of the nerves, with release of their neuropeptides by way of an axon reflex.⁵⁴ Some evidence in support of this proposed mechanism is the protective effect of a tachykinin antagonist on the bronchoconstrictor response to inhaled bradykinin in asthma.⁵⁵

There may be an increased responsiveness to sensory neuropeptides in asthmatic airways. Northern analysis of asthmatic lungs indicates an increased amount of NK₁-receptor messenger RNA, which may result from the effects of various cytokines on NK₁-receptor gene transcription, whereas NK₂-receptor expression is normal.⁵⁶

It is possible that NEP activity is reduced in asthmatic airways, either as a result of epithelial shedding or after exposure to virus infections, oxidants, or cytokines. An inhaled NEP inhibitor, thiorphan, potentiates the bronchoconstrictor effect of inhaled NK_A in human beings, indicating the presence of NEP on the surface of human airways,⁵⁷ and NEP has been localized immunocytochemically and its gene expression determined by *in situ* hybridization in human airways.⁵⁸ An NEP inhibitor, acetorphan, does not cause bronchoconstriction in patients with asthma, however, indicating that there may not be increased basal release of tachykinins in asthmatic airways.⁵⁹

The role of neurogenic inflammation in asthma may only become evident when specific blockers, such as tachykinin antagonists, are used in patients with differing severities of asthma. Currently there is no direct evidence that neurogenic inflammation is important, at least in mild asthma. However, it is possible that it plays a role in some patients with more severe chronic forms of asthma, such as brittle asthma, which is difficult to control with antiinflammatory therapy.⁶⁰

THERAPEUTIC IMPLICATIONS

Cholinergic mechanisms appear to be very important in acute exacerbations of asthma because anticholinergic agents are effective bronchodilators in these situations. In contrast, anticholinergic agents are less effective in the treatment of chronic asthma, with the implication that cholinergic neural mechanisms play a less important role in that condition.

If neurogenic inflammation is important in asthma, then drugs that modulate inflammation may have potential benefit in asthma therapy. There are several strategies to inhibit neurogenic inflammation.⁶¹

Tachykinin receptor antagonists may be of benefit. Because the inflammatory effects of

tachykinins are mediated by NK₁-receptors, NK₁-selective antagonists would be of particular benefit. Recently, nonpeptide and selective NK₁-receptor antagonists have been discovered. One such antagonist, CP-96,345, is very effective in blocking the airway microvascular leakage due to SP infusion in guinea pigs and also blocks vagal nerve-mediated, cigarette smoke- and bradykinin-induced leakage, although allergen-induced plasma extravasation is not inhibited.^{62, 63} Tachykinin antagonists for asthma therapy are currently being studied in clinical trials.

In the central nervous system, opiates inhibit the release of SP, and enkephalins act as the endogenous modulators of SP-ergic neurones. Opiates are effective in inhibiting NANC constriction of guinea pig airways in vitro and in vivo, and this effect is mediated by μ -opioid receptors on sensory nerve endings that inhibit the release of tachykinins.⁶¹ Similarly, opioids inhibit neurogenic microvascular leakage in guinea pig airways and mucus secretion induced by capsaicin in human bronchi. The central inhibitory neurotransmitter, γ -aminobutyric acid, is similarly effective on NANC bronchoconstriction. Several other prejunctional receptors, including neuropeptide Y, α_2 -adrenergic, VIP, galanin, and adenosine A₂ receptors, also inhibit sensory neuropeptide release in airways. These prejunctional modulator effects appear to be attributable to the opening of a common potassium channel.⁶⁴ Nedocromil sodium also modulates excitatory NANC responses in guinea pig bronchi.⁶⁵

Another approach is to prevent activation of airway sensory nerves. Cromolyn sodium and nedocromil sodium may have antiasthma effects by preventing activation of sensory nerves, and both of these agents are extremely effective in inhibiting bradykinin- and metabisulfite-induced bronchoconstriction and dyspnea in patients with asthma.⁶⁶ The loop diuretic furosemide given by inhalation is also effective in the same challenges as cromolyn sodium and may have a similar mechanism of action on sensory nerves.⁶⁷

CONCLUSIONS

It is now apparent that neural networks in the airways are complex and play an important, integrative role in normal airways. It is likely that in the acute and chronic inflammatory processes that characterize asthma, neural function is disrupted, and there is increasing evidence that neural mechanisms contribute to the pathophysiologic basis of asthma. Sensory nerve activation may be important in causing the common symp-

toms of cough and chest tightness, and studies on the mechanisms of activation, sensitization, and growth of sensory nerves are now needed. Although cholinergic mechanisms appear to be little involved in the control of airways in chronic asthma, but they become important in exacerbations possibly because of dysfunction of muscarinic autoreceptors, reduction in the braking affect of NO, or reflex activation. There is still uncertainty about the role of adrenergic mechanisms in asthma, but the potent bronchoconstrictor effect of β -blockers provides evidence that adrenergic mechanisms must have a critical protective function. Whereas there is little evidence for dysfunction of β -receptors in mild or moderate asthma, in vitro studies suggest that β_2 -receptors in airway smooth muscle may become uncoupled in fatal asthma.

Many different neuropeptides have now been identified in human airways, but the physiologic role of these peptides is obscure. It is possible that these peptides are less important in acute functional changes but may be involved in trophic control of the airways. Although these peptides may play only a small role under normal conditions, they may assume a much more important role in disease. The role of neurogenic inflammation, due to the release of peptides such as tachykinins from sensory nerves, is still uncertain in asthma. It is not likely that blocking of neurogenic inflammation will have a major effect on asthma control, because many other inflammatory mechanisms are also operative. However, it is possible that neurogenic inflammation may be important in some patients, such as those with brittle or unstable asthma; this possibility will require careful assessment.

Future studies of asthma will probably increasingly emphasize the chronic changes that occur in the airways. This research may focus on structural changes in nerves (proliferation in response to nerve growth factors), altered ion channel function, altered synthesis of neurotransmitters, and altered expression of autonomic receptors (e.g., NK₁-receptors). With molecular tools it will be possible to examine the mechanisms of these changes and thus to new therapeutic approaches.

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DISCUSSION

J. G. Widdicombe. Peter Barnes said that neurogenic inflammation in humans might be important in some forms of asthma, but surely the first place to look is in the airways of healthy humans, for example the nose. After all, the rodents in which it has been studied are more or less healthy, and the triple response in the skin is in healthy skin.

P. J. Barnes. Neurogenic inflammation has not been clearly demonstrated in the human nose. Capsaicin applied to the nose induces sneezing but not plasma exudation, whereas in guinea pigs plasma exudation is readily demonstrated after nasal application of capsaicin.

J. G. Widdicombe. What about patients with rhinitis?

P. J. Barnes. Even in patients with rhinitis there is no evidence that neurogenic inflammation is important.

R. Pauwels. There are actually no data for the lower airways.

P. J. Barnes. It may be that in severe asthma these neurogenic inflammatory mechanisms might become important, because there may be reduced expression of NEP, more NK₁-receptors, increased synthesis of tachykinins, and proliferation of tachykinin-containing nerves. These changes may become apparent only in very severe disease, but in those patients investigations are difficult to conduct with appropriate techniques.

S. T. Holgate. Can you talk about the drug studies that have focused on antagonists? How confident you are about those studies, in terms of their conclusion to reject the antagonists?

P. J. Barnes. Studies with the nonsedative tachykinin antagonist FK224 did not show any effect in asthma, but in patients with chronic bronchitis there was a reduction in coughing and sputum production. Other studies with more potent NK₁-receptor antagonists have not shown inhibition of induced bronchoconstriction, but there is some reduction in exercise-induced asthma.

J. P. Seale. I was interested to see on your slides that glutamate is a major transmitter, and I wondered what is known about the particular subtype of receptor that is involved in the effector mechanism for glutamate. This seems to be a relatively new area of research.

P. J. Barnes. We have not been able to find glutamate receptors in the airway. Glutamate is the central transmitter of C-fibers, and it can work on different types of glutamate receptor, such as N-methyl-D-aspartate receptors. The area has not been thoroughly explored in airway nerves, but it may be relevant in chronic asthma and cough.

K. F. Chung. With regard to the tachykinin antagonists in asthma, it is important that the right studies are done because if tachykinins are important in terms of neurogenic inflammation and resulting symptoms, the agents need to be tested in patients who have the symptoms.

P. J. Barnes. Asthma symptoms due to sensory nerve activation may be divorced from neurogenic inflammation. Sensory nerves may be activated to cause coughing, chest tightness, and cholinergic reflex effects, without the necessity for neurogenic inflammation. Thus tachykinin antagonists may have effects on sensory symptoms.

K. F. Chung. But if tachykinin receptors were to be up-regulated in asthma and NEP activity reduced?

P. J. Barnes. The available evidence suggests that tachykinin antagonists do not affect symptoms in asthma, but they may be involved without neurogenic inflammation.

R. Pauwels. With regard to the effects of inflammation on the neuronal control of the airways, another possibility would be that inflammatory cells are coming into the airways and actually taking part in the neuronal responses. We found that one of the reasons why there is a big difference between rat strains with regard to the response to NK_A or SP is that one has mast cells that are responsive and the other one does not. The distinct possibility, then, would be that, for example in people with asthma, mast cells are actually involved in the reaction to tachykinins. That could explain the difference between healthy people and people with asthma in their sensitivity to neuropeptides.

P. J. Barnes. I agree that an interaction between nerves and inflammatory cells is a possibility. In addition, inflammatory cells themselves can make neuropeptides; for example lymphocytes can synthesize enkephalins, which can down-regulate inflammation in some systems, and eosinophils can express SP.

A. Laitinen. What effect do you think do glucocorticosteroids have on NEP? There are contradictory results in cell cultures with regard to this effect.

P. J. Barnes. The effect of glucocorticoids on NEP in airways is controversial. Borson DB et al. (Am J Physiol 1991;260:L83-9) showed that steroids increase NEP expression in epithelial cells, but this could not be confirmed by Proud D et al. (Am J Respir Cell Mol Biol 1994;11:57-65.) Sterk looked at NEP expression in asthmatic airways (in biopsy specimens) and found increased levels in patients with asthma who were taking steroids compared with those not taking steroids. Your question is very important, because steroids may down-regulate neurogenic inflammation through several mechanisms. For example, steroids inhibit NK₁-receptor expression and so could return the increased expression of NK₁-receptors to normal. Steroids can also inhibit the synthesis of inflammatory mediators, such as bradykinin, prostaglandin I₂ and cytokines, which could drive the hyperexcitability in asthma. Although steroids may not have direct effects on nerves, they may down-regulate neurogenic inflammation in the same way that they down-regulate other types of inflammatory process.

A. A. Norris. Is there any evidence that different types of inflammation can up-regulate or down-regulate neuronal mechanisms?

P. J. Barnes. Neurogenic mechanisms may be important in chronic bronchitis, which is charac-

terized by coughing and mucus hypersecretion. Kuo H-P et al. (*Am J Physiol* 1992;7:L161-7) has shown that tachykinins are potent in causing goblet cell discharge and submucosal gland secretion and that cigarette smoke can activate neurogenic inflammation in the airways. A study in Japan with the tachykinin antagonist FK224 showed some clinical benefit in patients with chronic bronchitis (Ichinose M et al. *Lancet* 1992;340:1248-51). It is important to stress that

neurogenic inflammation is a fairly nonspecific type of process and could occur in any type of inflammatory disease. With regard to down-regulation of inflammation, there is some evidence that CGRP may be antiinflammatory in arthritis models. It seems to have a protective effect, and the inflammation may increase with CGRP antagonists. It is also likely that other neurotransmitters, such as VIP and related peptides, may be antiinflammatory.