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Regulation of airway neurogenic inflammation by neutral endopeptidase

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ABSTRACT: Airway neurogenic inflammation is caused by tachykinins released from peripheral nerve endings of sensory neurons within the airways, and is characterized by plasma protein extravasation, airway smooth muscle contraction and increased secretion of mucus.

Tachykinins are degraded and inactivated by neutral endopeptidase (NEP), a membrane-bound metallopeptidase, which is located mainly at the surface of airway epithelial cells, but is also present in airway smooth muscle cells, submucosal gland cells and fibroblasts. The key role of NEP in limiting and regulating the neurogenic inflammation provoked by different stimuli has been demonstrated in a large series of studies published in recent years. It has also been shown that a variety of factors, which are relevant for airway diseases, including viral infections, allergen exposure, inhalation of cigarette smoke and other respiratory irritants, is able to reduce NEP activity, thus enhancing the effects of tachykinins within the airways.

On the basis of these observations, the reduction of neutral endopeptidase activity may be regarded as a factor that switches neurogenic airway responses from their physiological and protective functions to a detrimental role that increases and perpetuates airway inflammation. However, further studies are needed to assess the role of neutral endopeptidase down regulation in the pathogenesis of asthma and other inflammatory airway diseases.

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The pathogenesis of chronic inflammatory disorders, such as asthma and chronic bronchitis, is obscure. There is evidence, however, that neural pathways in the airways may be involved in some of the inflammatory responses characterizing these disorders [1]. It has long been recognized that cholinergic parasympathetic nerves play a role in the regulation of airway calibre and secretion of mucus in health and disease [2]. More recently, peptide transmitters released from the peripheral endings of a subset of slowly conducting primary sensory neurons were found to cause many of the inflammatory responses that characterize asthma [3]. These primary sensory neurons, mainly C-fibre and Aδ-fibre polymodal nociceptors, the cell bodies of which are present in the vagal nodose ganglia, supply the conducting airways. The cell bodies of these neurons produce neuropeptides that are transported along the axons and are released from both central and peripheral nerve endings. These neuropeptides are the tachykinins, substance P (SP) and neurokinin (NK)A, and the calcitonin gene-related peptide (CGRP). Although CGRP is colocalized with SP in some sensory nerves and exerts various effects in different organs and tissues, its role in the airways appears to be restricted to vasodilation of large bronchial arteries.

In recent years, attention has been focused on the possible pathophysiological role of tachykinins in asthma, because SP and NKA cause many of the airway responses observed in this disease as a manifestation of neurogenic inflammation [1]. These responses include bronchoconstriction, submucosal gland secretion, vasodilation, plasma protein extravasation, adhesion of leukocytes to the vascular endothelium, potentiation of cholinergic neurotransmission and cough.

Neurogenic inflammation

Neurogenic inflammation is regarded as a protective mechanism called into operation whenever noxious conditions or agents threaten homeostasis of the tissue. The activation by sensory nerve fibres of a local inflammatory protective response was proposed a century ago, on the basis of the observation that in the skin of humans and other mammals neurogenic vasodilation in response to noxious stimuli is independent of central connections of sensory nerve fibres [4, 5]. Subsequently, evidence has accumulated showing that neurogenic inflammation is not restricted to the skin but also occurs in visceral organs,

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including the respiratory tract, eyes, joints and dura mater [6]. The release of sensory neuropeptides from peripheral endings of primary sensory neurons has been referred to as the "efferent" or "local effector" function, as opposed to the afferent (sensory) function of these nerve fibres [7, 8].

The inflammatory response complex, including local vasodilation, plasma protein extravasation, leukocyte and platelet adhesion, and mast cell degranulation, is brought about by neuropeptides released from peripheral endings of sensory neurons upon stimulation of their primary sensory terminals [9]. Sensory neuropeptides, by virtue of their vasodilator action and stimulation of proliferative activity of fibroblasts and endothelial cells [10, 11], are considered to play major roles in the maintenance of tissue trophism, as indicated by the occurrence of skin and corneal lesions [12] and the increased incidence of gastric ulceration [13] in animals in which primary sensory neurons were permanently destroyed in the postnatal period. In contrast to these beneficial and protective actions, activation of primary sensory neurons may cause an exaggerated inflammatory response, leading to neurogenic inflammation. This neurogenic inflammation has been proposed to play a role in various chronic diseases, including arthritis, migraine and asthma [14–16].

The mechanisms by which neurogenic inflammatory responses may be exaggerated are multiple. Increased synthesis, release and transport of peptide transmitters have been observed in models of arthritis [17]. An increased number of receptors for tachykinins has been detected in bowel tissue affected by chronic inflammatory disease [18]. In addition to these mechanisms, it is possible that normal levels of released peptides acting on normally functioning receptors cannot be removed efficiently from the milieu, leading to exaggerated responses. Termination of the action of neuropeptides is usually accomplished by enzymatic degradation, whereas dilution and reuptake provide negligible contributions.

The activity of peptides in biological systems can be dramatically changed by increasing or decreasing the rate of hydrolysis of their amino acid bonds by peptidases. The great importance of modulating the action of peptide neurotransmitters and hormones by inhibition of their enzymatic degradation is emphasized by the clinical and therapeutic impact of the discovery of inhibitors of angiotensin-converting enzyme (ACE). During the last two decades, a variety of peptides has been recognized to play pivotal roles in a number of physiological and pathophysiological processes. The identification of enzymes involved in peptide degradation and the discovery of selective inhibitors for these enzymes are of primary importance for disclosing mechanisms of disease, and eventually for the design of new therapeutic modalities. In this perspective, the role of enzymes that are able to cleave and inactivate tachykinins may be of primary importance in shifting neurogenic inflammatory responses from their physiological protective functions to negative and detrimental pathophysiological roles.

Neutral endopeptidase

Tachykinins may be degraded *in vitro* by a variety of enzymes, including serine proteases [19], mast cell chymase [20], calpains [21], neutral endopeptidase [22] and ACE [23]. However, *in vivo*, responses to tachykinins appear to be modulated mainly by neutral endopeptidase (NEP) and in a few instances by ACE (which is able to cleave SP, but not NKA [23]). Neutral endopeptidase (also known as metalloendopeptidase, E.C.3.4.24.11), first discovered in the brush border epithelium of the kidney [24], is identical to a peptidase isolated from the rat brain and called enkephalinase for its ability to cleave the small opioid peptides Leu- and Met-enkephalin [25]. NEP is also identical to the common acute lymphoblastic leukaemia antigen (CALLA), also called antigen CD10 or gp100 [26].

NEP is a glycosylated zinc metallopeptidase protein, consisting of 749 amino acid residues [27], a short cytoplasmic domain, a hydrophobic domain that anchors the enzymatic molecule to the plasma membrane, and a large extracellular domain containing the active site [28]. Neutral endopeptidase preferentially cleaves peptides on the amino acid site of hydrophobic residues (phenylalanine, leucine, methionine), and hydrolyses the peptide bonds Gln-Phe, Phe-Phe and Gly-Leu of SP, and Ser-Phe and Gly-Leu of NKA [22]; thus, yielding inactive fragments lacking the carboxyl terminal region, which is necessary for the binding to the tachykinin receptor.

Like other peptidases, NEP is not selective for a given peptide, but it cleaves a variety of substrates of different lengths, including kinins, gastrin-releasing peptide, atrial natriuretic peptide (ANP), enkephalins, endothelins, insulin-B chain, interleukin-1α, and the chemotactic peptide N-formyl-Met-Leu-Phe (fMLP). However, the selectivity of NEP for its substrates is variable. For instance, it has been shown that NEP is very active in cleaving internal peptide bonds of SP, whereas despite its ability to cleave CGRP at several locations, the enzyme to substrate interaction is weak [29]. In addition, despite the ability of NEP to cleave and inactivate a variety of peptides *in vitro*, a certain specificity is observed *in vivo*, probably determined by the expression and tissue distribution of NEP and by the distribution of its potential substrates.

Thus, because NEP, tachykinins and tachykinin receptors are widely present in airway tissues of different animal species, many researchers have paid attention to the importance of NEP in modulating airway responses to tachykinins. In this article, we will review the evidence for the role of NEP in regulating airway responses to tachykinins, and we will discuss the putative role of airway sensory nerves and neurogenic inflammation in the pathogenesis of asthma.

Effect of NEP inhibition on airway responses to tachykinins and neurogenic inflammation

The initial observation that NEP inhibitors potentiate the macromolecule secretion evoked by SP from submucosal glands of the ferret trachea [30, 31] provided the hypothesis that NEP may be important in the regulation of airway responses to exogenously administered tachykinins. This observation also raised the possibility that NEP metabolism is of critical importance in modulating the activity of endogenously released tachykinins, and hence of neurogenic inflammation [1]. These ideas were proven to be true in a large series of *in vitro* and *in vivo* studies based on the use of NEP inhibitors, thiorphan and phosphoramidon, and of recombinant NEP (rNEP) in a series of experimental models. The secretion of mucus from submucosal

glands, evoked by exogenous tachykinins, is mediated by NK_1 receptors and is markedly potentiated by NEP inhibitors. This effect has been observed in several mammalian species, including human [32, 33].

Tachykinins potently contract airway smooth muscle. This contractile response is mediated mainly by NK_2 receptors and is regulated by NEP activity. Evidence for this regulation is provided by the observation that NEP inhibition by phosphoramidon shifts the concentration-response curve to NKA and SP to the left in guinea-pig [31], ferret [34] and human airway smooth muscle [35]. Similarly, the suppression of NEP activity potentiates the increase in total pulmonary resistance caused by tachykinins in guinea-pigs [36] and humans [37, 38]. In guinea-pigs, bronchoconstriction in response to inhaled SP is accompanied by cough, an effect which is also increased by NEP inhibition and decreased by pretreatment with aerosolized rNEP [39].

Exudation of a protein-rich plasma into the affected tissues is a key feature of airway neurogenic inflammation [40] and SP is exceedingly potent in causing increased vascular permeability in rodent airways [41]. All of these effects of SP are exaggerated by the suppression of NEP activity [42], and are reduced or abolished by SP antagonists [43, 44]. Hence, it is quite reasonable to postulate that NEP modulates the activity of SP on airway vascular permeability and plasma exudation that occur in rodents in response to airway irritants. Finally, it has been demonstrated that NEP inhibitors potentiate other important aspects of airway neurogenic inflammation, including SP-induced increase in cholinergic neurotransmission [34], and vasodilation evoked by tachykinins in the rat airway microcirculation [45].

Altogether, these findings provide powerful evidence that tachykinins released from sensory nerves mediate multiple effects in the airways, and that NEP activity may modulate effects of tachykinins in a variety of target cells. It has also been reported that the inhibition of NEP caused by aerosolized phosphoramidon is accompanied by a significant increase in airway smooth muscle tone in asthmatic subjects; thus, suggesting that tachykinins and NEP may participate in the regulation of resting airway calibre [46]. However, these observations conflict with previous results obtained using a similar experimental approach [38]. A more recent study demonstrates that NEP inhibition by inhaled thiorphan does not affect resting airway calibre in asthma [47].

There is a large body of evidence that NEP also modulates the airway response to endogenously released tachykinins. Electrical stimulation of the vagus nerves or administration of capsaicin, the pungent agent contained in the plants of the genus *Capsicum*, release tachykinins from sensory nerve endings and evoke a complex response in the airways, that includes vasodilation, plasma protein extravasation, leukocyte adhesion, cough, noncholinergic bronchoconstriction, and secretion of mucus [48–51]. The noncholinergic contraction of guinea-pig bronchi evoked by electrical field stimulation [52] and other airway responses mediated by endogenously released tachykinins [53, 54] are potentiated by the NEP inhibitors, phosphoramidon and leucine-thiorphan. Interestingly, in human isolated small bronchi, capsaicin causes a mild contractile effect that is evident after NEP inhibition [55]. The observation that the NEP inhibitor, thiorphan, potentiates bronchoconstriction in response to inhaled sodium metabisulphite in normal subjects [56] provides circumstantial evidence that bronchoactive NEP substrates are released in human airways in response to sodium metabisulphite, and that NEP modulates the airway response to this indirect bronchoconstrictor agent.

A number of different stimuli has been shown to release sensory neuropeptides from peripheral endings of sensory neurons. These include alterations of the internal milieu, such as changes in hydrogen ion concentration or osmolarity, recreational or industrial pollutants, such as cigarette smoke or toluene diisocyanate (TDI) irritants such as mustard oil, and pro-inflammatory mediators, including bradykinin, histamine, peptidoleukotrienes, prostanoids, platelet-activating factor (PAF), eosinophil cationic protein (ECP), and lipoxins [7, 32, 57]. NEP inhibitors have been repeatedly shown to increase the release of tachykinins and to exaggerate the neurogenic inflammatory responses evoked by these mediators.

Distribution of sensory nerves, tachykinin receptors and NEP in the airways

Critical factors that govern specificity in the enzymatic degradation of endogenously released peptides are the tissue distribution of the enzyme peptidase and that of its substrate. The skeletal neuromuscular junction forms a specialized unit, in which acetylcholinesterase clusters with nicotinic receptors are in close proximity to the nerve terminal. Sensory nerve endings and effector cells expressing tachykinin receptors do not appear to be arranged in a similar manner. There is no evidence of a specialized unit between the sensory nerve terminal and effector cells, and the sensory peptide transmitter is presumed to diffuse from the site of release to the site of action. Peptidases specifically involved in the cleavage of sensory neuropeptides may be located at any site between these two points. It is also possible that the peptidase is colocalized in the same cells as the tachykinin receptors, and that both of these entities compete for agonist-binding. This latter hypothesis is supported by the observation that the response to SP is smaller in cells expressing both NK_1 receptors and NEP than in cells expressing only the NK_1 receptor or NEP [58].

Sensory nerves containing tachykinins make a dense network of fibres beneath the epithelium of rodent airways [59]. Sensory nerves fibres are also present in the vicinity of smooth muscle cells and submucosal gland cells and around arterial vessels, whereas they are apparently absent around venules [59]. In human tissues, SP-containing sensory nerves are less abundant than in rodents, but they exhibit a similar pattern of distribution [60, 61]. Morphological [62] and functional evidence [32] indicates the presence of NK_1 or NK_2 receptors for tachykinins in submucosal glands, in endothelial and smooth muscle cells.

The distribution of NEP has been examined by means of specific antibodies, by measuring its activity, or by its labelling with selective inhibitors. NEP is conspicuously present in the kidney, lungs, discrete brain regions, reproductive organs, bone marrow, intestine and lymph nodes [28]. The broad substrate specificity and the almost ubiquitous distribution of NEP suggest roles in the cleavage of

different peptides involved in several functions. In the lungs, NEP is abundantly expressed in epithelial cells [63]. Less, although functionally relevant, NEP has been found in fibroblasts, smooth muscle cells and submucosal gland cells [63]. The presence of NEP in the target cells for tachykinin action, such as fibroblasts and smooth muscle cell explains why even after the removal of the major source of NEP (*e.g*. the epithelium) NEP inhibitors still potentiate the nonadrenergic noncholinergic contraction evoked by electrical stimulation or by capsaicin [52].

It is possible that tachykinins released from sensory nerve terminals are cleaved by NEP, either during their diffusion through the tissue or at effector cell sites. Because of the high concentration of NEP in the epithelium, it is not surprising that removal of epithelium leads to potentation of the bronchoconstrictor responses evoked by exogenous tachykinins [52]. Shedding of epithelial cells is commonly found in bronchial biopsies of patients with severe asthma [64]. Reduction of peptidase activity in epithelial cells by various agents known to aggravate asthma has also been reported [65–67]. These observations are of particular interest, because they depict a possible scenario for the involvement of exaggerated neurogenic inflammatory responses as a consequence of decreasing NEP activity in asthma [68]. Moreover it has recently been shown that NEP expression in the airway epithelium of bronchial biopsies from atopic asthmatics is inversely related both to asthma symptoms and to bronchial hyperresponsiveness [69]. This observation provides further circumstantial evidence that decreased NEP activity may be involved in the occurrence of asthma symptoms and airway hyperresponsiveness.

NEP modulates neurogenic inflammation evoked by different stimuli

A number of stimuli causes neurogenic inflammation in the airways. Some of them bear a particular relevance for human diseases. The industrial pollutant, TDI, is the causative agent of a form of occupational asthma. The mechanisms by which TDI causes asthma are not well understood, but there is evidence that TDI contracts guinea-pig bronchial smooth muscle by releasing tachykinins from sensory nerve endings [70]. Similarly, cigarette smoke inhalation produces a remarkable plasma protein extravasation in the airway mucosa of rodents, which is completely abolished by chemical denervation of sensory neurons with capsaicin or by blockade of NK_1 tachykinin receptors [49]. Increased tonicity of the fluid covering the epithelium is considered to be the stimulus that triggers exercise-induced asthma [71]. Hypertonic media release sensory neuropeptides, and thus evoke inflammatory responses in the airways [72]. The observation that NEP inhibitors potentiate these effects indicates the ability of NEP to limit inflammation caused by these agents.

Inhalation of allergens is a common cause of airway narrowing and inflammation in asthmatic patients. The involvement of neurogenic inflammation in the bronchomotor and inflammatory responses to allergen has been extensively evaluated in experimental animals, using different experimental approaches based on sensory denervation with high doses of capsaicin, NEP inhibition, or highly selective nonpeptide antagonists for NK_1 [73] and NK_2 [74] tachykinin receptors. Allergen inhalation causes protein plasma extravasation and increases total pulmonary resistance in sensitized guinea-pigs. Both the vascular and the bronchomotor responses to allergen are potentiated by NEP inhibition and are reduced by tachykinin receptor antagonists [75–78]. These observations suggest that mediators released from airway inflammatory cells in response to antigen activate sensory nerves to release neuropeptides that contribute to airway narrowing and plasma extravasation. When NEP activity is inhibited, tachykinins are less rapidly inactivated and accumulate in the tissue, thus contributing to the exaggerated responses. This interpretation is further supported by the observation that ovalbumin-induced plasma extravasation in the nasal mucosa of sensitized guinea-pigs is decreased by the NK_1 receptor antagonist and is increased by the NEP inhibitor phosphoramidon [79].

In contrast to these data in rodents, recent data have shown that inhalation of the NEP inhibitor, thiorphan, does not affect either the early or the late asthmatic bronchoconstriction to allergen inhalation in asthmatic subjects, suggesting that tachykinins may not be involved in these allergic asthmatic responses in humans [47].

A key role for NEP in the control of inflammation in different organs has recently been demonstrated [80]. In mice in which the gene coding NEP has been disrupted by homologous recombination and gene-targeting (NEP knockout mice), baseline increased levels of plasma extravasation were observed in skin, airway, gastrointestinal and urinary tract tissue [80]. The increase in plasma extravasation was completely abolished by a tachykinin NK_1 receptor antagonist and by a bradykinin B_2 receptor antagonist. These observations support the hypothesis that NEP downregulation results in increased kinin levels, leading to inflammation in several tissues, including airways.

Another mechanism by which NEP could achieve bronchoprotection in asthma is suggested by the study of Harrison *et al*. [81]. These authors demonstrated that the NEP inhibitor, phosphoramidon, enhanced human fibroblast proliferation in a dose-dependent manner, thus suggesting that the structural abnormalities enhanced by neuropeptides are modulated by NEP.

Role of NEP in the metabolism of kinins

Because kininogens and kallikrein are present in the airways and because these molecules [82], as well as kinins, are increased in the bronchoalveolar lavage fluid from asthmatics challenged with allergen [83, 84], kinins have been suspected to play important roles in asthma [85]. Bradykinin is an algesic agent that causes inflammatory responses by acting directly on specific receptors in effector cells and indirectly by releasing a variety of agents [86], including sensory neuropeptides [87]. When applied locally to the airways, bradykinin causes plasma extravasation and bronchoconstriction, mainly by activating cholinergic reflexes and releasing tachykinins from sensory nerves endings [88]. Although bradykinin is cleaved by ACE and NEP [22, 23], increased total pulmonary resistance and airway wall oedema caused by local application of bradykinin are exaggerated following NEP inhibition [89, 90]. The observation that bradykinin B_2 receptor an-tagonists and tachykinin NK_1 receptor antagonists are equally potent in reducing antigen-evoked

plasma extravasation indicates that kinins that are released by antigen activate sensory nerves [75].

Bradykinin also increases vascular permeability in human upper airways [91], and causes bronchoconstriction in asthmatics, in part by activating cholinergic reflexes [92]. A mixed antagonist for NK_1 and NK_2 receptors has been shown to reduce bronchoconstriction in response to inhaled bradykinin in asthmatics, thus suggesting that part of the bradykinin-evoked increase in total pulmonary resistance is due to local release of tachykinins [46–93]. These observations indicate that kinins activate inflammatory and bronchomotor responses in human airways, involving the release of tachykinins and similar to those observed in experimental animals. However, further studies are needed to establish whether this pharmacological information has pathophysiological relevance in the mechanism of human allergic diseases.

Conditions that affect airway NEP activity

There is a growing evidence that NEP activity can be affected by a variety of factors. Reduction in NEP activity leads to exaggeration of the inflammatory response evoked by peptides released from peripheral endings of sensory nerves. Therefore, NEP downregulation may be regarded as a factor that switches neurogenic inflammation from its physiological trophic and protective function to a detrimental role that increases or perpetuates the severity of different disease states.

Several studies have suggested that a number of pathogens, including Sendai virus and influenza virus, and respiratory irritants, including cigarette smoke, TDI, hypochlorous acid and acrolein, can decrease airway NEP activity. In rats with respiratory infections, it has been shown that plasma extravasation induced by SP is markedly increased and that the NEP inhibitor, thiorphan, significantly potentiates SP-induced plasma extravasation in pathogen-free rats but not in infected rats, suggesting a reduction of NEP activity in the airways of the infected animals [42, 66]. This evidence is further supported by the observation of a significantly lower content of NEP in airways obtained from infected rats [42].

Cigarette smoke causes airway inflammation and hyperresponsiveness in humans. It has been shown that cigarette smoke causes neurogenic inflammation through the release of endogenous tachykinins [49]. By showing that cigarette smoke solution inhibits NEP activity from guinea-pig tracheal homogenates in a concentration-dependent manner, Dusser *et al.* [67] further extended the knowledge of the mechanism by which cigarette smoke causes neurogenic inflammation. These findings have been confirmed by the recent observation showing that airway plasma extravasation induced by smoke inhalation in guinea-pigs is blocked by an NK_1 receptor antagonist, and it is potentiated by the NEP inhibitor, phosphoramidon [94].

TDI and hypochlorous acid decrease NEP activity and selectively potentiate tachykinin-evoked responses in guinea-pig airways [65]. Recently, it has been shown that sulphur mustard, an alkylating agent causing airway irritation and asthma-like symptoms, decreases NEP activity in the tracheal epithelium of the guinea-pig [95]. A similar inhibitory effect on NEP activity is exerted by hydrogen peroxide [96]. Because it is widely recognized that viral infections, cigarette smoke and industrial or air pollutants cause asthma exacerbations, these observations further support the hypothesis that NEP and neurogenic inflammation are involved in the pathogenesis of asthma.

It has been hypothesized that corticosteroids are able to increase the activity and the expression of NEP. This hypothesis is supported by the observations that glucocorticoids increase the expression of NEP in transformed epithelial cells from human bronchi [97], and markedly reduce neurogenic plasma extravasation in rat airways [98]. However, a recent study provides evidence against the upregulating effect of glucocorticoids on NEP activity, because dexamethasone decreased plasma extravasation induced by capsaicin without affecting NEP activity in the rat trachea [99]. Consistent with the hypothesis that glucocorticosteroids upregulate NEP in the airways is the finding that NEP expression is enhanced in airway epithelium from atopic asthmatic patients who regularly use inhaled glucocorticosteroids [69].

Other pathophysiological roles of NEP in the lung

A variety of peptides are produced in the lungs or delivered to the lungs from the circulation or *via* the airways. NEP contained in airway epithelial cells or in vascular endothelial cells may have an important role in the metabolism of these peptides and may affect their function.

The *N*-formylated oligopeptides, produced by bacteria, are potent chemotactic agents for neutrophils and contribute to the neutrophilic inflammatory response to bacteria. Because fMLP is cleaved by NEP [28], it has been hypothesized that NEP is involved in the regulation of the inflammatory response to bacterial infections in the airways. Evidence for this hypothesis is provided by the observation that inhibition of NEP present in the cell membrane of neutrophils potentiates the chemoattractant activity of fMLP [100].

Bombesin-like peptides, including the mammalian gastrin-releasing peptide (GRP), are potent mitogens for normal bronchial epithelial cells and neuroendocrine cells. Small cell lung cancer, which is considered to be derived from the transformation of neuroendocrine cells, also responds to these peptides. An autocrine loop exists in the small cell carcinomas that secrete bombesin-like peptides, express bombesin receptors and are stimulated to proliferate by these peptides [101]. Neutral endopeptidase efficiently cleaves GRP. The growth of bombesin-like peptide-dependent carcinomatous cells is blocked by NEP and is increased by NEP inhibition [101]. Because cigarette smoke inactivates bronchial epithelial cell surface NEP and small cell carcinomas occur almost exclusively in cigarette smokers, it has been hypothesized that the cigarette smoke-dependent decrease in NEP activity may be causally related to the proliferation of small cell carcinomas of the lung [101, 102].

Endothelins are potent vasoconstrictor peptides produced by endothelial cells. Endothelin-1 may be also produced by bronchial epithelial cells [103], and causes contraction of airway smooth muscle of guinea-pigs [104] and humans [105]. NEP cleaves endothelins [106]. The observations that removal of the endothelium and NEP inhibition increase the bronchomotor response to endothelin-1, whereas recombinant human NEP reduces the response to endo-

thelin, indicate that NEP plays a major role in the metabolism of endothelin-1 [107]. These observations have recently been confirmed and extended [108]. The finding of increased expression of endothelin immunoreactivity in the epithelial cells of asthmatics [109] indicates the possible role of endothelins and their cleavage in diseases that lead to airway narrowing.

Concluding remarks

In the treatment of asthma, there are some limited options. The relief from bronchoconstriction is commonly achieved by β_2-adrenergic agonists, which act directly on airway smooth muscle, whereas airway inflammation, which is a prominent feature of asthma, is controlled by "nonspecific" anti-inflammatory drugs, such as corticosteroids. These considerations emphasize the inadequacy of our current understanding of the pathophysiology of asthma. Accordingly, therapy is not usually targeted selectively to the causal mechanism of the symptoms. It is probable that many factors contribute to the development of disease, either acting independently or arranged in a cascade of events. The release of peptide transmitters from sensory nerve endings by exogenous or endogenous stimuli, by provoking neurogenic inflammation, may be part of this cascade.

NEP may play a central role in the mechanisms governing the integrity of the airways and the modulation of airway neurogenic inflammation in asthma and other inflammatory disorders of the airways. Sensory nerve activation usually exerts a protective action for the integrity of the tissue against injury. Cigarette smoke, hypertonicity of the tracheobronchial fluid, air pollutants, antigen and endogenous mediators released by these stimuli may activate this protective function. However, when NEP is downregulated or when the airway epithelium (which contains measurable amounts of NEP) is damaged, the unopposed action of sensory neuropeptides may lead to exaggerated inflammation.

The discovery of selective and potent antagonists of tachykinin receptors, suitable for use in human studies, will provide the opportunity to test the validity of this hypothesis in a clinical setting. Strategies directed to increase the action of neutral endopeptidase, including the administration of recombinant neutral endopeptidase, may have relevance for the treatment of airway disorders.

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