MINIREVIEW

Peptidase Modulation of Airway Effects of Neuropeptides (43616)

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Neuropeptides are small peptides synthesized in nerve cell bodies, transported by axoplasmic flow, and stored in the terminal ramifications of axon dendrites. Stimulation of peptidergic nerves, either sensory or motor, can lead to the release of these peptides into the microenvironment. The neuropeptides so released have the capacity to mediate a number of distinct physiological effects and therefore fit into the broad category of neurotransmitters. Immunohistochemical analyses of tissues from both the central and peripheral nervous systems have demonstrated the presence of numerous structurally distinct peptides with potential neurotransmitter function.

Although much is known about the biology of neuropeptides in general (1–5), our understanding of the role of these molecules in lung tissues is limited (6, 7). However, our appreciation of the role of neuropeptides as modulators of lung function has grown substantially over the past 5 years. Neuropeptides have been implicated as factors governing the growth and differentiation of the lung during embryogenesis (8, 9), regulating the tone of the pulmonary vasculature (10, 11), and modulating both the growth of certain forms of lung cancer (12, 13) and airway secretions (14). Substantial progress has been made in understanding the role of neuropeptides in modulating airway patency and therefore potentially contributing to the pathogenesis of asthma and related disorders. This Minireview will focus on the latter aspect of neuropeptide function in the lung. Furthermore, although numerous neuropeptides have been identified throughout the body, the bulk of the information currently available about neuropeptides in the lung concerns the contractile peptides substance P (SP) and neurokinin A (NKA) and the inhibitory peptide vasoactive intestinal peptide (VIP). There is reason to believe that the excitatory physiological effects of SP and NKA are counterbalanced by the inhibitory effects of VIP. This Minireview will deal predominantly, but not exclusively, with these three peptides.

Studies of the modulation of airway obstruction by neuropeptides have established that the physiological effects of SP, NKA, and VIP are limited by their degradation at or near the site of their release. These peptides are all subject to cleavage by neutral endopeptidase (NEP; E.C. 3.4.24.11); SP (but not NKA or VIP) is also subject to cleavage and inactivation by angiotensin-converting enzyme (ACE; E.C.3.14.5.1). In isolated systems, VIP can be cleaved and inactivated by mast cell-derived chymase and tryptase. Not only are these neuropeptides subject to enzymatic degradation, good evidence suggests that the expression or activity of the enzymes that cleave them is also modulated. For example, in an inflammatory microenvironment, such as the asthmatic lung, the loss of NEP activity could enhance the phlogistic responses elicited by SP and NKA. Ordinarily, the inhibitory actions of VIP counteract the effects of SP and NKA. However, when the inactivation of SP and NKA is inhibited because NEP is inactive, the contractile and microvascular effects of SP and NKA may be so great as to prevent a counterbalancing action of VIP. This loss of the potential homeostatic effects of VIP may be especially devastat-

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ing since loss of NEP activity does not prevent degradation of VIP by other enzyme systems, such as mast cell chymase and tryptase. As a result, airway inflammation has the potential to differentially enhance the prophlogistic effects of SP/NKA and to diminish the homeostatic effects of VIP.

In this Minireview we will consider (i) the identity of the neuropeptides present in the airways and their specific locus of expression; (ii) the enzymatic inactivation of SP, NKA, and VIP (i.e., which enzymes are known to inactivate these peptides and how the peptides are cleaved); (iii) the stimuli responsible for the release of the neuropeptides; and (iv) the inflammatory modulation of neuropeptide cleavage in the lung. The relationships among these various aspects of neuropeptide physiology in the lung are shown in Figure 1.

Location of Neuropeptides in the Airways

Multiple neuropeptides have now been identified in the lungs and airways. These include—but are not limited to—SP, NKA, calcitonin gene-related peptide, VIP, gastrin-releasing peptide, enkephalin, neuropeptide Y, somatostatin, neuropeptide K (NPK), and peptide histidine isoleucine/methionine. Neuropeptides are present (i) co-localized with acetylcholine and norepinephrine in parasympathetic and sympathetic nerve varicosities; (ii) in and around airway ganglia; (iii) in C fiber afferent nerves; and (iv) in neuroendocrine cells. Distinct patterns exist for each peptide. The distribution of each peptide also varies with the species studied and with the location in the tracheobronchial tree. This discussion will be limited to the tachykinins and VIP. The reader is referred to a recent review article (15) for information on the localization of other neuropeptides.

Tachykinins. The tachykinins are a family of small neuropeptides that share a common amidated carboxyl-terminal amino acid sequence: Phe-X-Gly-Leu-Met-NH_2. At least three mammalian tachykinins have been identified: SP, NKA, and neurokinin B. However, only SP and NKA have been localized to the lungs and airways. Both SP and NKA are derived from the *PPT-I* gene (16, 17), which can be alternatively spliced to yield at least three distinct forms of *PPT-I* mRNA: α, β, and γ. SP is produced by translation of all three forms, whereas NKA is produced only from the β- and γ-forms. In addition, recent reports indicate that three other NKA-related peptides—$NKA_{3\text{-}10}$, NPK, and neuropeptide-γ—can be produced by differential posttranslational processing of the β- and γ-forms (18). NPK and neuropeptide-γ are N-terminally extended forms of NKA.

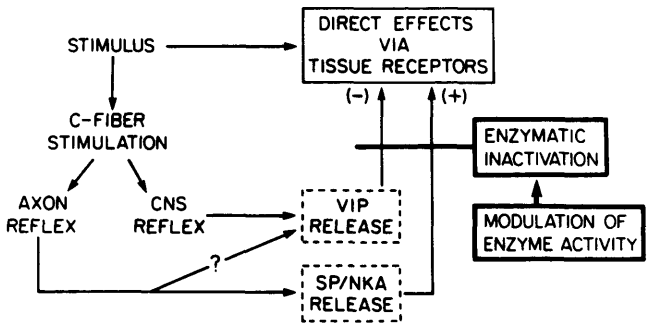

Figure 1. Potential interactions between evocative stimuli and secondarily released SP, NKA, and VIP.

SP has been localized to C fiber afferent nerves in the airways of several species, including humans. This neuropeptide is synthesized in the cell bodies of these neurons, which lie in the nodose and thoracic dorsal root ganglia, and is transported by axoplasmic flow down these nerves to the lungs. In rats and guinea pigs, SP-immunoreactive nerves are found in the airways from the pharynx down to the peripheral bronchioli, although their concentration is greatest in the larger bronchi (19, 20). SP-immunoreactive nerves have even been demonstrated at the alveolar level in some species (21). Measurements of SP content from extracts of lungs and airways confirm this distribution; SP content is greatest in the main stem bronchi, though measurable amounts are also obtained from peripheral lung samples (21, 22). In the airways, SP-immunoreactive nerves are seen close to and within the respiratory epithelium, near blood vessels, within the bronchial smooth muscle layer, and around but not in tracheobronchial ganglion cells (19, 20). SP-containing nerves are also present in the pulmonary vasculature (23).

There are some species-related differences in the amount and distribution of SP in the airways. SP content is greatest in airways from rats and guinea pigs (21, 22). In addition, it appears that in larger mammals, such as cats and humans, fewer SP-immunoreactive fibers are present in the airway epithelium (22). Age-related changes in the distribution of SP-immunoreactive nerves have been documented. Examination of autopsy specimens from children 3 years of age or less has revealed SP-containing nerves in airways from bronchi all the way to the alveolar ducts (24). In contrast, in airways from persons aged 10 years and older, SP-containing nerves are present in bronchi but are only rarely observed in smaller airways (although general neuronal markers do confirm their presence at this site).

NKA is co-localized to the same nerve terminals as SP and has a similar distribution (25, 26). When tachykinins are extracted from the lungs and airways of rodents, about two thirds as much NKA-like immunoreactivity (NKA-LI) as SP-like immunoreactivity (SP-LI) is recovered (27). While this difference may reflect the actual content of each peptide, it is also possible that the extraction procedure results in differential loss of NKA and SP.

High-performance liquid chromatography of ex-

tracts of airway tissues from guinea pig trachea show three peaks of NKA-LI (28, 29). One peak corresponds to the retention time of authentic NKA and another to the retention time of NPK. The third peak of activity, termed eledoisin-like, has a retention time corresponding to that of NKA_{3-10}. Neuropeptide-γ has not yet been detected in lung extracts, although it is present in extracts of other organs (30). Thus both NPK and NKA_{3-10} appear to be present in the airways.

VIP. VIP is a 28-amino acid peptide that was originally described in extracts of pig duodenum. Nerves with VIP immunoreactivity have been found in the airways of most mammalian species studied (15, 22). VIP immunoreactivity is localized both to nerves and to ganglia in the airway wall, is often, though not exclusively, co-localized with acetylcholine (15), and has been observed in ganglion cells from the trachea to the intrapulmonary bronchi. In adult human lungs, VIP-like immunoreactivity is found as branching networks of fibers from major bronchi down to bronchioli as small as 200 μm; however, the number of VIP-immunoreactive nerves decreases with distance into the lung (24). VIP-immunoreactive fibers are found within the smooth muscle and in association with vessels and mucous glands (21, 22, 31, 32). In some species, VIP has been localized to nerves running beneath the epithelium, though these subepithelial fibers appear to be of sensory origin (15). The presence of VIP in the airways has been confirmed by measurements of the VIP content of extracts of airway tissue (21, 33).

Like those of SP, the content and distribution of VIP in the airways differ significantly according to the species studied. VIP-containing nerve fibers are most abundant in cat airways (22), whose VIP content is about 10 times that found in rat or guinea pig airways (21). In smaller mammalian species, VIP-containing nerves are found mostly in association with mucous glands and are less abundant in airway blood vessels and airway smooth muscle. In larger species, including humans, the degree of VIP innervation in smooth muscle and in blood vessels is greater (22). There are also age-related changes in the location of VIP-containing nerves in the airways. In airways from children less than 3 years of age, VIP is localized to ganglia (prominent in young children) and to nerves from the bronchi, bronchioli, respiratory bronchioli, and alveolar ducts (24). In humans more than 10 years of age, VIP-containing nerves do not extend beyond bronchioli. Gepetti *et al.* (34) reported a decrease in VIP content (as measured both by radioimmunoassay and by immunofluorescence) in rat airways with increasing age.

Changes in the distribution of VIP-immunoreactive nerves have also been reported in airways (or airway biopsies) from patients with airway disease. Ollerenshaw *et al.* (35) reported few, if any, VIP-containing nerves in the airways of asthmatic patients; in contrast, such nerves were found in other subjects. The VIP content of airways of children who died of acute respiratory distress syndrome did not differ from that of airways in age-matched control children who died of nonrespiratory causes (33).

Peptidase Cleavage of Neuropeptides in the Airways

Peptidase Cleavage of NKA. The airway effects of SP, NKA, and VIP are limited in part by enzymatic degradation by NEP. NEP is a membrane-bound ectoenzyme found on a variety of cells constitutive to the airway wall. This location is ideal for the exertion of a regulatory effect on peptide mediators released into the extracellular microenvironment. NEP is anchored to the membranes of cells through a small hydrophobic domain near the amino terminus; the glycosylated hydrophilic extracellular domain contains the zinc-requiring active site (36). NEP cleaves peptides preferentially at the amino-terminal side of the hydrophobic amino acid residues Phe, Leu, Ile, Val, Tyr, and Trp. NEP, purified from the brush border of the kidney, hydrolyses NKA primarily at Gly^8-Leu^9 and Ser^5-Phe^6 (Fig. 2). High-performance liquid chromatography analysis of peptides recovered after the introduction of radiolabeled NKA into the perfusate of tracheally perfused guinea pig lungs suggests that lung NEP cleavage of NKA occurs at the same sites (37). A cleavage site with less favorable kinetics has also been described at Phe^6-Val^7 (38). The K_m for NKA obtained from renal brush border is 113 μM and the k_{cat}/K_m for NEP with NKA as substrate exceeds that for SP. Among 22 putative substrates for NEP, NKA and SP were cleaved with the greatest efficiency (38, 39). An important observation is that NEP cleavage of NKA results in biologic inactivation of the peptide; the products of NKA cleavage by NEP are markedly less potent bronchoconstrictors than intact NKA (40). NKA is not efficiently cleaved by ACE prepared from kidney (36) or lung (41), but is a substrate for the corpus striatum isozyme of ACE.

Importance of NKA Cleavage in Its Physiologic Actions. The physiologic relevance of NKA inactivation by enzymatic hydrolysis has been deduced from studies of the effects of enzyme inhibitors on the phys-

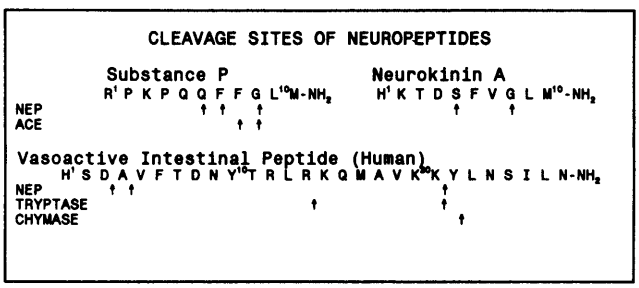

Figure 2. Established sites for proteolytic cleavage of SP, NKA, and VIP. Only sites whose kinetics are consistent with physiologic activity are shown.

iologic action of exogenously administered peptides. When given intravenously to anesthetized, mechanically ventilated guinea pigs, NKA is a more potent bronchoconstrictor than SP (36, 42, 43). NKA-induced bronchoconstriction is significantly enhanced when the NEP inhibitors thiorphan and SCH32615 are administered (40, 44, 45). NEP inhibition with phosphoramidon has been shown to enhance NKA-induced bronchoconstriction throughout the tracheobronchial tree and to enhance microvascular leakage in proximal intrapulmonary airways (46). These same inhibitors enhance NKA-induced pulmonary constriction in tracheal superperfused lungs (37). In this model, the addition of a combination of other inhibitors—including inhibitors of aminopeptidases, serine proteases, and ACE—along with NEP inhibitors failed to enhance NKA-induced bronchoconstriction beyond the level provided by NEP inhibitors alone. These results suggest that these other enzyme systems are not relevant for NKA-induced bronchoconstriction in this system (37). Likewise, ACE inhibitors have no effect on NKA-induced bronchoconstriction in the intact guinea pig (45). Further evidence against a regulatory role for aminopeptidases is provided by the observation that the C-terminal fragments of NKA, which would be the products of aminopeptidase cleavage of NKA, are bronchoconstrictors as potent as NKA itself (40).

Peptidase Cleavage of SP. *NEP.* In the lung, SP is inactivated by enzymatic cleavage by two enzymes, NEP and ACE. NEP, prepared from the brush border of the pig kidney, hydrolyzes SP at three sites: Gln^6-Phe^7, Phe^7-Phe^8, and Gly^9-Leu^{10} (Fig. 2) (47). NEP efficiently cleaves and inactivates SP with a K_m of 31.9 μM. It hydrolyzes 1 mM SP at a rate of 7.7 μmol/min/mg. This cleavage is completely inhibited in the presence of 1 μM phosphoramidon.

ACE. ACE prepared from rat lung hydrolyzes SP at the Phe^8-Gly^9 bond with a k_{cat}/K_m 60-fold lower than that reported for ACE hydrolysis of angiotensin I (48). A second cleavage site with less favorable kinetics has been reported at Gly^9-Leu^{10} (49). In human lung preparations, Phe^8-Gly^9 cleavage occurs three times more frequently than Gly^9-Leu^{10} cleavage (41). Similar SP cleavage sites have been documented with use of ACE prepared from human kidney (50, 51), although when enzymes from this renal source are used, cleavage at Phe^8-Gly^9 is preferred by a ratio of 4:1 to cleavage at Gly^9-Leu^{10}. ACE cleaves 0.1 mM SP at a rate of 1.2 μmol/min/mg, and this action is completely inhibited by 1 μM captopril. The products of NEP or ACE hydrolysis of SP at Gly^9-Leu^{10} are inactive in anesthetized, mechanically ventilated guinea pigs (52).

Dipeptidyl(amino)peptidase IV. In human and rat plasma, SP is cleaved sequentially at Pro^2-Lys^3 and Pro^4-Gln^5 by dipeptidyl(amino)peptidase IV (DAP-IV; E.C.3.4.14.5), which is specifically inhibited by diprotin (53). The resulting products $SP_{3\text{-}11}$ and $SP_{5\text{-}11}$ are more potent bronchoconstrictors (52) than intact $SP_{1\text{-}11}$, but each can be rapidly inactivated in plasma by aminopeptidase M (54). Rats genetically deficient in DAP IV are more sensitive to the salivary effects of SP than are normal rats; this difference indicates that DAP IV can be physiologically significant for SP metabolism. The significance of DAP IV in limiting the pulmonary action of SP is unclear.

Chymase. SP is cleaved at Phe^7-Phe^8 by chymase derived from canine mastocytoma cells (55), but not by tryptase derived from the same cells. The k_{cat}/K_m for the action of chymase on SP is 3.9×10^4—a value significantly lower than the corresponding value for NEP or ACE. Thus it appears that SP is not a favored substrate for chymase.

Importance of SP Cleavage in Limiting Its Physiologic Action. The physiologic importance of NEP and ACE as regulators of the pulmonary action of SP appears to depend on the route of administration of the peptide to the lung. When SP is presented to the lung via the vasculature, its airway effects are enhanced (56, 57) by the inhibition of ACE. The further enhancement of SP-induced pulmonary constriction when a NEP inhibitor is added to the ACE inhibitor indicates modulation of this process by NEP. NEP inhibition with phosphoramidon or thiorphan, but without captopril, augments SP-induced increases in lung resistance in anesthetized, mechanically ventilated guinea pigs (56, 58). However, when SCH32615 is used to inhibit NEP, the SP response is not augmented unless ACE is also inhibited (59). Since SCH32615 is a very specific NEP inhibitor (60) (i.e., has no significant effect on ACE) while thiorphan has some anti-ACE activity, it appears that NEP and ACE are physiologically competitive in their capacity to degrade SP.[2] In the presence of thiorphan and captopril (given to inhibit NEP and ACE), the detection of SP-LI in arterial blood after intravenous administration of SP is enhanced (56). Recovery is not enhanced by a specific NEP inhibitor alone (59), but is enhanced by captopril alone (56). Thus, when administered by the vascular route, ACE appears to control the quantity of SP in the circulation, while NEP has an effect on activity independent of circulatory level.

In contrast, when SP is presented via the airway lumen, NEP alone appears to be important in modulating its bronchoconstrictor activity. Addition of the NEP inhibitor thiorphan or SCH32615 alone to the

[2] In this discussion, except as noted, we have assumed that enhancement of neuropeptide action by phosphoramidon, thiorphan, or SCH32615 is evidence for cleavage by NEP (EC 3.4.24.11). However, there is increasing evidence for a distinct phosphoramidon, and to some extent, a thiorphan, sensitive activity known as endothelin-converting enzyme (ECE; 61–63). It is not known at this time if ECE can cleave SP, NKA, or VIP. If it is discovered that ECE can cleave SP, NKA or VIP, the surety of the conclusions drawn about NEP from experiments using these two inhibitors is somewhat tempered.

perfusate of isolated tracheally perfused lungs increased both the bronchoconstriction induced by a bolus of SP added to perfusate as well as recovery of the intact peptide from the lung effluent. In contrast, captopril had no effect (37). NEP inhibitors also increase the bronchoconstriction induced by inhaled aerosolized SP (64). In contrast, ACE inhibitors do not alter these responses (43). In isolated guinea pig, ferret, and human airways, inhibition of NEP but not ACE activity augments SP-induced contractions (65–67).

Inhibitor studies have indicated that NEP also modulates nonbronchoconstrictor actions of SP that occur at or near the airway epithelium. In addition to their effects on SP-induced airway obstruction, NEP inhibitors have been shown to augment SP-induced tracheal secretion of macromolecules (68) and NEP inhibitors nebulized to the airways augment SP-induced cough (69). Further evidence that airway NEP modulates cough (induced by nebulization of SP to the airway epithelial surface) is the decrease in SP-induced cough in guinea pigs pretreated with nebulized human recombinant NEP (70). SP (71) and capsaicin (72) increase the ciliary beat frequency of tracheal epithelial cells, and inhibitors of NEP (but not those of serine proteases, ACE, or aminopeptidases) increase the ciliary beat frequency of cultured rabbit epithelium—an effect suggesting endogenous SP release (73). SP induces chloride secretion in cultured canine tracheal epithelial cells (74); NEP inhibition increases the capacity of SP to elicit this response, while agents capable of inhibiting aminopeptidases or serine proteases do not. These observations suggest that in cultured epithelial cells only NEP is present in a locus that is physiologically competitive for SP receptors.

Cleavage of VIP. VIP is known to be cleaved by NEP, mast cell tryptase, and mast cell chymase, which are active at neutral pH in the extracellular microenvironment. NEP hydrolyzes VIP at several sites, with the kinetics most favorable for human recombinant NEP cleavage of VIP at the Asp^3-Ala^4, Ala^4-Val^5, Lys^{21}-Tyr^{22}, and Ser^{25}-Ile^{26} bonds. Cleavage sites with less favorable kinetics are present at the Arg^{12}-Lys^{13} and the Gln^{16}-Met^{17} bond (75). Studies in isolated tracheal perfused lungs have demonstrated that NEP hydrolysis fragments of VIP are not physiologically active (76).

Mast cell proteases have also been shown to hydrolyze VIP. Canine mastocytoma tryptase cleaves human VIP with favorable kinetics at the Arg^{14}-Lys^{15} and Lys^{20}-Lys^{21} bonds (55); the k_{cat}/K_m for this hydrolysis is 2.2×10^5 sec^{-1} M^{-1}, and the K_m is 3.3 mM. These kinetic parameters compare favorably to those of other substrates screened for susceptibility to human skin and lung mast cell proteases (77). Chymase, obtained by purified carbon from canine mastocytoma cells, has favorable kinetics for cleaving VIP at Tyr^{22}-Leu^{23} (55). The K_m of 3.3 mM and the k_{cat} of 179 sec^{-1} for this cleavage give a k_{cat}/K_m of 5.4×10^4 sec^{-1} M^{-1}. While this k_{cat}/K_m is not as high as that obtained for tryptase, it is consistent with physiologically relevant inactivation of VIP by chymase. In this regard, it is important to note that the products of hydrolysis of VIP by tryptase or chymase fail to relax vascular, gastrointestinal, or tracheal smooth muscle (55) or tracheal perfused lungs (76).

Modulation of VIP Responses by Enzymatic Cleavage. VIP-induced pulmonary relaxation can be attenuated by the addition of exogenous mast cell tryptase or chymase in the ferret (78) and by the addition of exogenous chymotrypsin or papain in the guinea pig (79). VIP-induced relaxation was not augmented in cat airways by a combination of inhibitors devoid of a NEP inhibitor (80), but was augmented in guinea pig bronchial tissues by soybean trypsin inhibitor (79) and in guinea pig tracheal rings by inhibitors of NEP (81); in the latter tissue, inhibitors of ACE and aminopeptidases were ineffective. Other studies in guinea pig tracheal rings demonstrate the importance of NEP in modulating the effects of VIP; when the NEP level is decreased by removal of the airway epithelium, the enhancement of VIP-induced relaxation is equivalent to that observed upon treatment of the epithelial intact airways with NEP but not ACE inhibitors (82). In human bronchial rings, VIP-induced relaxation is potentiated by a combination of serine protease and NEP inhibitors (83). In isolated tracheal perfused guinea pig lungs, combinations of enzyme inhibitors augment VIP-induced pulmonary relaxation, while single-agent inhibitors are ineffective (76). Effective combinations of inhibitors also increase the amount of intact VIP recovered from lung effluent. Analysis of hydrolysis fragments and inhibitor profiles allows the identification of NEP and a tryptic enzyme with a cleavage profile identical to that of mast cell tryptase. These enzymes are similar to those responsible for limiting VIP-induced pulmonary relaxation.

Other Neuropeptides. NEP also appears to be important in modulating the bronchoconstrictor activity of other neuropeptides. Changes in pulmonary resistance induced by $NKA_{3\text{-}10}$ (40) and by NPγ (84) are increased when guinea pigs are pretreated with the NEP inhibitor SCH32615. In contrast, the bronchoconstriction induced by NPK is not altered by NEP inhibitors (84), even though this peptide has the same carboxyl terminal sequence as NKA, $NKA_{3\text{-}10}$, and NPγ.

Role of Neuropeptides in Modulating Airway Responses

C fibers are stimulated both by exogenous substances, such as inhaled irritants, and by endogenous substances, such as inflammatory mediators. A list of such agents is given in Table I. Once stimulated, C fibers transmit information to the central nervous sys-

Table I. C Fiber Stimulants

Exogenous	Endogenous
Cigarette smoke	Histamine
Capsaicin	Cholinergic agonists
Formaldehyde	Bradykinin
TDI	Leukotrienes
Acrolein	Prostaglandins
SO_2	Platelet-activating factor
Ether	Arachidonic acid

tem, where reflex responses may be evoked. In addition, under many if not all circumstances, tachykinins are released from the peripheral ends of these afferents into the airway microenvironment, where they can alter airway tone. A single bronchoconstrictor stimulus can, therefore, have two effector arms: a direct effect, in which the stimulus acts directly on the target tissue, and a secondary effect, in which the stimulus prompts the release of neuropeptides, which then elicit a response in the target organ (Fig. 1). In the airways, bronchoconstriction and microvascular leak are the most extensively studied responses resulting from the release of neuropeptides. Because a given stimulus may simultaneously have both a direct and a neuropeptide-mediated effect, a number of approaches have been developed to unmask the role of neuropeptides in modulating airway responses. It is important to review these general strategies before considering the various physiologic roles attributed to the neuropeptides (Fig. 3).

Strategies to Detect Neuropeptide Release. Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) has the capacity to release neuropeptides into the microenvironment. When capsaicin is given as an acute experimental reagent (acute capsaicin administration), neuropeptides are released and their physiological effects examined. In contrast, when capsaicin is given repeatedly in large doses (85) (chronic capsaicin depletion), neuropeptides are depleted from sensory nerves. Investigators have examined the role of neuropeptides by comparing the response to a stimulus in normal control animals with that in animals depleted of neuropeptides by chronic capsaicin treatment. An altered response is attributed to the effects of depleted neuropeptides. It is important in evaluating experimental regimens that use this protocol to consider that all neuropeptides may not be depleted by capsaicin treatment and that capsaicin treatment itself may induce inflammation that could alter airway responses. Because of this latter problem, most investigators allow at least 1 week to elapse after administering the last dose in a series required for chronic capsaicin depletion to use animals in an experiment.

Because neuropeptides are inactivated in the microenvironment by peptidases, another strategy used to examine their role has been the evaluation of the effects of peptidase inhibitors in a given physiologic setting. For example, if a given stimulus exerts its physiologic effects by directly constricting airway smooth muscle and at the same time releasing neuropeptides that modulate this direct effect, then peptidase inhibitors, which prevent the inactivation of the neuropeptides of note, should amplify the effects of the secondarily released neuropeptides. When this strategy is used, it is important to remember that many neuropeptides may be subject to cleavage by multiple peptidases and that all potentially important peptidases must be inhibited in order to achieve a physiologic effect.

The recent discovery of a number of specific and selective neuropeptide antagonists at various neuropeptide receptors (86–96) has allowed another approach to the assessment of the role of various neuropeptides in

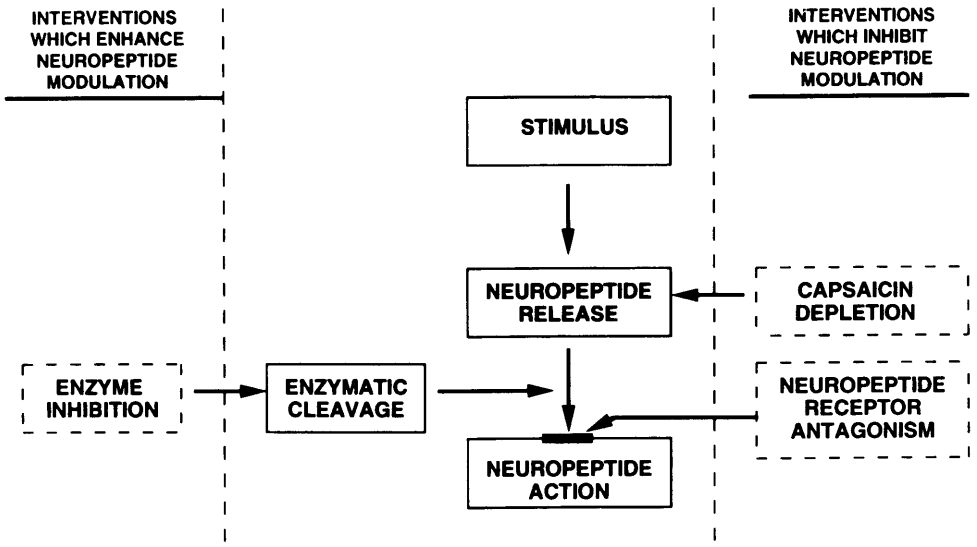

Figure 3. Schematic diagram of approaches used to implicate neuropeptides as modulators of airway responses.

a physiologic setting. If neuropeptides are of physiologic importance in modulating a response, then the difference of experimental results with and without a specific neuropeptide antagonist can be attributed to action at this receptor. With this approach it is important to remember that many neuropeptide receptors are promiscuous; that is, they can transduce signals, albeit with varying efficiency, from a number of neuropeptide agonists.

A final approach to determining the role of neuropeptides in a given response is the measurement of their release into the microenvironment by means of specific immunoassays. Failure to detect a peptide in a given setting does not mean that it is not of physiological significance. For example, the local concentrations at the site of release may be high enough to effect a response but not high enough to be detectable in biologic fluids. Alternatively, a peptide may be cleaved after it acts; in this case, it could have an effect but not be detectable. Finally, a peptide may be detected in biologic fluids, but its site of release and action may be distinct; in this case, its detection may not indicate a physiological role.

Because of the limitations of the various methods used to discern the effects of neuropeptides, it is common to use more than one of these approaches at a given time.

Evidence that Various Stimuli Release Neuropeptides. *Capsaicin.* In isolated guinea pig bronchi, capsaicin induces airway constriction (97–99), and there is strong evidence that tachykinins mediate this event. Pretreatment with phosphoramidon (97) or thiorphan (99) has been shown to enhance the contractile response elicited by capsaicin. Furthermore, both SP-LI and NKA-LI can be detected in organ baths in which tracheas are incubated with capsaicin (99). In the guinea pig trachea, there is a close correlation between the contractile response to capsaicin and the content of SP-LI (98). In isolated vascularly perfused guinea pig lungs, pulmonary arterial injection of capsaicin results in a concentration-dependent increase in the pressure required to ventilate the lungs; this effect is neural in origin, as it is blocked by tetrodotoxin (100). In the presence of phosphoramidon, capsaicin infusion is associated with the appearance of NKA-LI. In the tracheally perfused guinea pig lung, capsaicin treatment results in an increase in tracheal perfusion pressure, and both NKA-LI and SP-LI can be detected in lung perfusate (27). Both the physiological effects of capsaicin and the amount of SP-LI and NKA-LI detected are enhanced by treatment with a NEP inhibitor but not by treatment with captopril. Chronic depletion with capsaicin ablates the acute airway response to capsaicin (27). When NEP inhibitors were not present, capsaicin-induced contraction was apparent in four of nine samples of isolated human bronchi (66); when phosphoramidon, but not captopril, was included in the organ bath, all of five samples tested responded to capsaicin treatment.

In intact anesthetized rats given Evans blue dye into the circulation, capsaicin treatment is associated with increased recovery of the dye from the trachea, which suggests an increase in airway vascular permeability. This effect is blocked by the specific NK1 receptor antagonist CP-96,345 (101). In awake guinea pigs, capsaicin-induced cough is enhanced in the presence of leucine-thiorphan and phosphoramidon (69), a result suggesting that this response is also mediated by tachykinins.

Taken together, these data indicate that SP and/or NKA may serve as the physiological effector molecule when capsaicin is administered to the airways. The relative importance of these two tachykinins is not known at this time. Studies with specific and selective antagonists of the NK1 and NK2 receptors will be required for determination of the relative importance of each of these peptides.

Vagal nerve stimulation. Vagal nerve stimulation has the capacity to release both contractile and bronchodilator neuropeptides. Retrograde stimulation of C fibers carried in the vagus may result in the release of SP and NKA, while VIP may be released from cholinergic nerves. Similarly, electrical-field stimulation (EFS) of isolated airways stimulates all nerves in the preparation, both afferent and efferent.

The contractile response resulting from EFS in isolated guinea pig bronchi appears to be partly mediated by tachykinins. Both thiorphan (99) and phosphoramidon (97) enhance the response, while captopril, leupeptin, and bestatin have no effect. NEP inhibitors have similar effects in the ferret trachea (67). In addition, in the latter tissues, the EFS-induced contractile response in the presence of leucine-thiorphan is inhibited by [D-Pro_2, D-$Trp_{7,9}$]SP, a tachykinin receptor antagonist. When vagal stimulation rather than EFS is examined in guinea pig trachea, a biphasic response is evident (102, 103). The first, rapid phase is atropine sensitive. The second phase is slow in onset, can be prevented by chronic tachykinin depletion with capsaicin, and can be inhibited by pretreatment with SKF 104353, WY 48252, or ICI 198615, all inhibitors of the action of leukotriene D_4 (102). This inhibition suggests that interactions between tachykinins and leukotrienes may be important in the mediation of bronchoconstriction.

In the guinea pig, graded vagal nerve stimulation in the presence of atropine induces a graded bronchoconstrictor response. This response is enhanced in the presence of phosphoramidon and is inhibited by [D-Arg_1, D-Pro_2, D-$Trp_{7,9}$, Leu_{11}]SP, a tachykinin receptor antagonist (64). Inhibitors of ACE do not influence this response (104). Taken together, these data suggest that

the atropine-resistant component of the vagal stimulation response is mediated by SP release and that the release occurs endobronchially where ACE concentrations are low. However, each of two relatively selective NK2 receptor antagonists, MEN10207 and MEN10376, inhibits this response by about 50% (88). Since SP and NKA can each stimulate NK1 and NK2 receptors, it will be necessary to determine the effects of more selective antagonists, individually and together, on atropine-resistant vagal nerve responses.

There is much less information about neural responses that result in smooth-muscle relaxation. In the guinea pig trachea, relaxation responses induced by EFS are unaffected by the tryptic inhibitors aprotinin and soybean trypsin inhibitor. However, the relaxant responses are depressed by thiorphan. This effect can be prevented by chronic capsaicin depletion. The authors of the study yielding this information (79) interpreted it to mean that the inhibitory neurotransmitter was not VIP. However, if VIP is cleaved by more than one distinct enzyme system, the data are compatible with the hypothesis that VIP is released by EFS. The actions of VIP may not be "direct"; rather, VIP may stimulate the synthesis of nitric oxide, which itself induces airway relaxation (105, 106).

Antigen challenge. Repeated challenge with specific antigen elicits a state of airway hyperresponsiveness. The experimental strategies described earlier in this section have been used to examine the role of neuropeptides in modulating airway hyperresponsiveness. The results have been mixed. Repeated antigen challenge can be shown to induce elevated airway resistance and hyperresponsiveness in the guinea pig. Lai (107) and Ingenito and co-workers (108) were unable to demonstrate an effect of chronic capsaicin depletion on airway responses or responsiveness to antigen challenge in the guinea pig. Ingenito and associates documented the adequacy of chronic capsaicin depletion by showing decreased tissue levels of SP-LI and NKA-LI. In contrast, Matsuse and colleagues (109) demonstrated that chronic capsaicin depletion blunted the hyperresponsiveness induced by repeated antigen exposure in guinea pigs. Similarly, Manzini *et al.* (110) documented a protective effect of chronic capsaicin depletion on antigen-induced bronchospasm in the guinea pig. Lotvall and co-workers (111) detected no effect of chronic capsaicin depletion on the microvascular response to chronic antigen challenge, while Lundberg *et al.* (112) found such an effect. Although the data gathered by these various groups are discordant, there is probably a simple explanation for the discordance. Antigen exposure induces inflammation and also may initiate the local release of tachykinins. The inflammatory microenvironment may vary substantially with the precise details of the immunization procedure and the genetic background of the animals studied. As outlined below in the section on inflammatory modulation of neuropeptide processing, differences in the capacity of the cells resident in the inflammatory lesion to cleave neuropeptides may substantively alter the modulatory role of neuropeptides. It is likely that such differences are reflected in the results of the various studies cited above.

Dry gas hyperpnea. Dry gas hyperpnea is a somewhat unusual stimulus in that almost all of its effects appear to be due to neuropeptide release. Hyperpnea-induced bronchospasm is diminished in guinea pigs receiving chronic capsaicin treatment. In contrast, phosphoramidon enhances bronchoconstrictor responses (113). However, the potential role of sensory neuropeptide release during hyperpnea-induced bronchoconstriction in human subjects with exercise-induced asthma remains uncertain.

Inflammatory mediators. ***Histamine.*** Addition of histamine to the perfusate of isolated tracheally perfused lungs causes an increase in the levels of SP-LI and NKA-LI detected in the effluent (114), and addition of the NEP inhibitor thiorphan augments these levels. Thiorphan also causes an increase in the change in airway opening pressure induced by histamine, particularly at low doses of histamine. Taken together, these results suggest that tachykinins released by histamine contribute to the bronchoconstrictor response to histamine, at least under circumstances in which NEP degradation of SP and NKA is inhibited. Experiments with tachykinin receptor antagonists will be required for confirmation of the contribution of tachykinins to the response in normal airways. However, such a contribution is suggested by data from experiments in guinea pigs subjected to chronic capsaicin pretreatment. Two groups of investigators have demonstrated a decrease in the bronchoconstriction induced by histamine in anesthetized guinea pigs after chronic capsaicin pretreatment (115, 116).

Cholinergic agonists. Experiments performed in isolated perfused lungs with methacholine yield results similar to those obtained with histamine. Methacholine increases levels of SP-LI and NKA-LI, and thiorphan enhances bronchoconstrictor responses to methacholine. Saria *et al.* (28) have demonstrated that addition of a ganglionic stimulant to the perfusate of vascularly perfused guinea pig lungs also results in the release of materials with SP-LI and NKA-LI. Finally, chronic capsaicin pretreatment attentuates bronchoconstrictor responses to methacholine in guinea pigs *in vivo* (116).

Leukotrienes. Leukotriene D_4 causes the release of materials containing SP-LI and NKA-LI from isolated tracheally perfused guinea pig lungs (114) and in isolated guinea pig trachea (117). We were not able to augment leukotriene D_4-induced changes in airway opening pressure in the isolated tracheally perfused lung with thiorphan, but responses to leukotriene D_4 in this preparation may reflect events occurring in the lung

periphery. In contrast, in the guinea pig trachea, contraction induced by leukotriene D_4 is partially inhibited by a tachykinin receptor antagonist (117) and is augmented by the NEP inhibitor thiorphan (118).

Other mediators. Prostaglandin I_2 *contracts* the guinea pig bronchus. This effect can be inhibited by chronic capsaicin depletion and by treatment with an NK2 receptor antagonist (119). It has been suggested that this prostaglandin I_2-induced contraction is in part mediated by endogenous release of tachykinins. Arachidonic acid also induces contractions of isolated guinea pig bronchial rings, and these contractions are augmented by thiorphan (120) and inhibited by chronic capsaicin depletion (121). Although platelet-activating factor (114, 122) can induce the release of SP-LI and NKA-LI, inhibition of NEP does not enhance the physiologic effects of platelet-activating factor administration in isolated perfused guinea pig lungs. This observation suggests that the tachykinins released do not contribute to the contraction induced.

Inflammatory Modulation of Neuropeptide Degradation

There is reason to believe that the inflammatory microenvironment differs from the normal microenvironment in its capacity for enzymatic degradation of neuropeptides and thus influences the capacity of neuropeptides to elicit biologic responses in the airways. In this section we consider (i) the evidence for changes induced by different forms of inflammation in the activity of the enzymes (particularly NEP) that normally degrade neuropeptides; (ii) the mechanisms that account for these changes in NEP activity; and (iii) the importance of other constitutive peptidases (which may be unmasked when NEP action is altered) in modulation of neuropeptide responses.

Evidence for Alterations in NEP Activity Under Conditions Associated with Airway Inflammation. Exposure to a variety of agents known to induce inflammation in the airways is associated with decreased NEP activity. In the airways of rodents, NEP activity decreases with respiratory viral infections; after exposure to toluene 2,4-diisocyanate (TDI), a plasticizer used extensively in industry; after inhalation of cigarette smoke; and after exposure to ozone. In contrast, anti-inflammatory agents, such as corticosteroids, increase NEP activity. In most studies, physiologically relevant changes in NEP activity have been measured as alterations in the capacity of thiorphan or phosphoramidon to augment airway responses to endogenous or exogenous neuropeptides in intact animals. However, NEP activity, as measured by specific substrate cleavage, has also been evaluated in homogenates of airway tissue derived from these animals.

Respiratory infections. Ferret tracheal rings cultured *in vitro* with human influenza virus type A (123) or obtained from animals inoculated *in vivo* with this virus (124) showed a stronger contractile response to SP than did tracheal rings from uninfected controls. The ability of phosphoramidon and thiorphan to augment contractile responses to SP was more pronounced in control rings than in rings infected with virus (123, 124). Indeed, after treatment with phosphoramidon, contractile responses to SP did not differ between groups. Likewise, an increased responsiveness to SP and a decreased ability of phosphoramidon or thiorphan to enhance the pulmonary resistance response to SP in guinea pigs infected with Sendai virus (125) were consistent with diminished endogenous NEP activity. Similar results were obtained when tracheal vascular permeability was used as an index of response in rats exposed naturally to *Mycoplasma pneumoniae*, coronavirus, or Sendai virus (126). These data provide indirect evidence for decreased NEP activity in the airways and are consistent with the hypothesis that diminished degradation of SP is responsible for increased functional responses to this peptide during viral infections. NEP-like activity was decreased in tracheal homogenates from animals with experimental viral infection (123, 125, 126). When the trachea was further dissected into epithelium, submucosa, and smooth muscle, the decrease in NEP activity was found to be restricted to the epithelial layer (125). This locus of alteration in NEP activity may help explain the observation that, whereas contractile responses to SP are augmented in virus-infected ferret trachea, mucous secretory responses to the tachykinins are not altered (124).

TDI. Acute exposure to TDI, a widely used plasticizer, augments the changes in pulmonary resistance induced by intravenous administration of SP. Phosphoramidon causes only a slight augmentation of the bronchoconstrictor response to SP in TDI-exposed guinea pigs compared with the augmentation of SP responses observed in control guinea pigs. These data suggest either a lower level of activity or a lesser importance of NEP than of other enzymes in degrading SP under these conditions. In support of lower level activity as the explanation, the authors also noted a decrease of approximately 30% in the phosphoramidon-inhibitable capacity of tracheal homogenates from TDI-exposed animals to degrade a radiolabeled NEP substrate (127).

Inhalation of 1–3 ppm of TDI for 1 hr enhances airway responsiveness to intravenously administered acetylcholine as well as SP in guinea pigs. The fact that this increased responsiveness is abolished after pretreatment with capsaicin or administration of an SP receptor antagonist suggests mediation by tachykinins. We have reported previously that another cholinergic agonist, methacholine, causes release of SP and NKA into the effluent of isolated, tracheally perfused guinea pig lungs and that the NEP inhibitor SCH32615 augments the

contractile response to methacholine in this preparation (114). These findings suggest that most of the bronchoconstriction induced by acetylcholine results from a direct effect of this agonist on airway smooth muscle, but that tachykinins are released by this stimulus and degraded before they can exert a physiological effect. It is possible that a TDI-induced decrease in NEP activity in the airways permits the tachykinins released by acetylcholine to exert a greater physiologic effect (128). In addition, TDI itself is capable of constricting guinea pig bronchial rings *in vitro*—an effect that is also mediated by tachykinins (129). Whether these effects of TDI on the release and degradation of tachykinins are in any way related to the ability of TDI to induce asthma after repeated occupational exposures remains to be established.

Cigarette smoke. Dusser *et al.* (125) have demonstrated that inhalation of cigarette smoke enhances the bronchoconstrictor response to inhaled SP in guinea pigs. In experiments conducted by these investigators, inhaled aerosolized SP at concentrations up to 100 μM had no effect on pulmonary resistance in control animals but resulted in 2- and 3-fold increases in pulmonary resistance in animals that had breathed the smoke from one and two cigarettes, respectively. Notably, phosphoramidon increased the bronchoconstriction induced by SP in control animals, as the authors had reported previously (64), but not in animals that had been exposed to smoke from two cigarettes. The authors also measured the ability of homogenates of guinea pig tracheas to degrade leu-enkephalin. Bubbling of increasing amounts of cigarette smoke through the assay buffer caused a progressive decrease in the phosphoramidon-inhibitable degradation of this peptide by the tracheal homogenates. The authors interpreted these results to indicate that inhalation of cigarette smoke inactivated the airway NEP that normally limits SP-induced bronchoconstriction. However, the NEP activity in tracheas of animals exposed to cigarette smoke *in vivo* was not measured. In a preliminary report, Kwon *et al.* (130) showed that levels of NEP activity and NEP mRNA in trachea and lungs of guinea pigs sacrificed 5 or 30 min after exposure to 50 puffs of cigarette smoke were not significantly different from levels in air-exposed animals. One potential explanation for this apparent discrepancy is that the NEP whose inhibition results in enhanced physiologic responses to inhaled SP represents only a small fraction of the total NEP in the lungs and airways.

Many of the airway responses to cigarette smoke, including increased airway vascular permeability (131) and increased macrophage cytoplasmic motility (132), are not observed in animals receiving chronic capsaicin treatment and hence may be mediated by tachykinins released from C fibers. Prolonged exposure to cigarette smoke causes increases in tussive responses to capsaicin in guinea pigs (133). The role of alterations in NEP activity in these events merits consideration, particularly in light of observations that cough induced by SP is augmented by NEP inhibition (69).

Ozone. In guinea pigs as well as many other species, exposure to ozone results in the recruitment of neutrophils to the airways and increased responsiveness to inhaled aerosolized bronchoconstrictor agonists such as methacholine (134). Murlas *et al.* (135) recently reported that inhaled ozone also increases the responsiveness to intravenously administered SP. Furthermore, whereas phosphoramidon increased the changes in specific airway resistance induced by SP in air-exposed animals (as had been reported by Thompson and Sheppard [58]), this NEP inhibitor had no effect on SP responses after exposure of guinea pigs to 3 ppm of ozone for 2 hr. One explanation for these data is that ozone enhances airway responses to SP by inactivating NEP in the airways. Consistent with this hypothesis, the NEP activity in tracheal tissue from ozone-exposed animals was slightly but significantly lower than that in tracheal tissue from air-exposed control animals (135). Alternatively, ozone exposure may increase the activity of other enzymes (e.g., ACE) that are important in the degradation of SP to such an extent that the presence or absence of NEP makes little difference. However, if the expression of ACE were enhanced, ozone exposure might be expected to result in a decreased rather than an increased SP response (136).

Allergic inflammation. There is increasing evidence for a role of tachykinins in the bronchoconstriction induced by allergen in sensitized animals (109, 118). However, the possibility that sensitization and challenge result in changes in NEP activity in the airways has not been extensively explored. In humans, the administration of thiorphan causes increased airway responses, as indicated by changes in maximal expiratory flow rates after inhalation of aerosolized NKA by healthy subjects (137). In addition, NKA-induced bronchoconstriction is greater in asthmatic than in nonasthmatic subjects (138). However, a recent preliminary report suggests that the augmented NKA responses of asthma are not related to decreased NEP activity; asthmatics responded to thiorphan with an increase in NKA-induced bronchoconstriction similar in magnitude to that in normal subjects (139).

Anti-inflammatory agents (corticosteroids). Whereas in each of the above situations a stimulus evoking inflammation in the airways has been associated with *decreased* NEP activity, anti-inflammatory agents—particularly corticosteroids—have been shown to *increase* the magnitude of NEP activity in airway tissues. Borson and Gruenert (140) reported that transformed human airway epithelial cells grown in culture express NEP activity. Addition of budesonide or dexamethasone to the culture medium resulted in an in-

crease by up to 5-fold in NEP activity and NEP mRNA in these cell cultures. Although the effect of dexamethasone treatment *in vivo* on NEP expression in airway tissues has not yet been reported, the increase in vascular permeability effected by intravenous capsaicin was reduced in rats treated with dexamethasone for 8 to 120 hr (141). Because capsaicin exerts its effects by releasing tachykinins and capsaicin-induced changes in airway vascular permeability are augmented by NEP inhibitors, one explanation for these data is that the decrease in vascular permeability was the result of an increase in NEP. The subsequent demonstration by these authors that treatment of rats with combined NEP and ACE inhibitors prevented the effect of dexamethasone (142) provided evidence in favor of this hypothesis. Adrenalectomy reduces and dexamethasone treatment increases pulmonary ACE activity in rats (143); thus, even endogenous levels of corticosteroids may be important in the regulation of SP responses in the airways by modulating ACE degradation.

Mechanisms for Changes in NEP Activity. The data summarized so far indicate changes in NEP activity without establishing how these changes may occur. At the onset of airway inflammation, a number of changes may alter NEP activity within the airways. These are summarized in Table II and discussed below.

Decreased NEP. The epithelium is a rich source of NEP within the airways, as has been demonstrated both immunohistochemically (144) and by measurements of NEP activity in homogenates of this tissue (145). Epithelial damage or sloughing, such as that seen in biopsy samples from patients with asthma, could result in loss of one of the primary cell types expressing this enzyme. Indeed, numerous reports indicate that removal of the airway epithelium increases the contractile responses of airways to SP and decreases the augmentation in SP responses normally observed after addition of NEP inhibitors (67, 97, 146, 147). Although the epithelium is a major target of respiratory viruses, it is unlikely that epithelial sloughing is responsible for increases in SP responses in virus-infected animals. Epithelial sloughing, if it occurred, would also cause an increase in acetylcholine responses (148), whereas these responses are unchanged in virus-infected animals (123). Nevertheless, it is possible that viral infection altered NEP at a transcriptional level in these experiments.

Table II. Mechanisms for Alterations in NEP Activity in the Airways During Inflammation

Decreased activity	Increased activity
Loss of cells expressing NEP through •Epithelial sloughing or desquamation	Increase in cells expressing NEP •Neutrophilia •Airway fibrosis •Smooth-muscle hypertrophy
Inactivation of NEP •Oxidants •Endogenous inhibitors?	
Loss of NEP from the cell surface •Endocytosis of cell membrane •Proteolytic clipping	Increased NEP on the cell surface •Translocation from intracellular compartments
Decreased transcription •Protein kinase C activation	Increased transcription •Corticosteroids
Alternative splicing	

Oxidants produced during the inflammatory process can inactivate NEP. When the chemical oxidant *N*-chlorosuccinimide was incubated with guinea pig tracheal homogenates, the capacity of the tissue to degrade a NEP substrate was markedly diminished (125). The ability of cigarette smoke to decrease NEP activity in the airways appears to be related to oxidants in the smoke. When the antioxidant superoxide dismutase was administered to guinea pigs before exposure to cigarette smoke, the smoke exposure-enhanced responsiveness to SP normally induced by this intervention was no longer observed. The investigators did not determine whether or not the ability of phosphoramidon to augment SP-induced bronchoconstriction was also restored.

Changes in the inflammatory microenvironment may also result in loss of NEP from the surface of airway cells, as has been indicated by a variety of studies. Neutrophils incubated with phorbol esters in order to activate protein kinase C show lower NEP activity than neutrophils incubated in the presence of buffer alone (149, 150). Although phorbol esters activate neutrophils and although the oxidative products of activated neutrophils may account for the loss of NEP activity, the authors of one study tried to prevent the decrease by using a panel of enzyme inhibitors, antioxidants, and hydroxyl radical scavengers, all of which were without effect (149). However, if the exposed cells were lysed within 5 min of the addition of phorbol ester, all NEP activity was recovered. These results suggest that neutrophils stimulated with phorbol esters internalize NEP by endocytosis. A similar process appears to take place in human airway epithelial cells exposed to hypochlorous acid (151), which causes a dose-related decrease in NEP activity measured in whole cells but not in cell membranes or in cells after lysis by sonication. Internalization of plasma membrane containing NEP by HOCl could account for these results. HOCl has also been shown to cause decreased NEP activity and increased contractile responses in tracheal rings; thus, this mechanism may also be important to NEP functional responses in whole airways. Recent reports indicate that corticosteroids are capable of restoring the NEP activity of these cells after treatment with HOCl (152).

In addition to internalization of cell membrane, proteolytic cleavage of the enzyme from the cell surface may result in the loss of NEP in the inflammatory setting. Patients with adult respiratory distress syndrome and some other lung diseases have higher levels of NEP activity in serum than do healthy subjects, in whom these levels are very low (153). It was hypothesized that neutrophil elastase might be responsible for cleavage of this membrane-bound enzyme and its subsequent release into the circulation.

Inflammation may also alter NEP at the transcriptional level. For example, the phorbol ester 12-O-tetradecanoylphorbol 13-acetate decreases the expression of NEP mRNA transcripts in rabbit synovial fibroblasts and mammary epithelial cells (154). 12-O-Tetradecanoylphorbol 13-acetate acts mainly by increasing protein kinase C, an effect that is shared by many of the mediators released during inflammation of the airways. Alternative splicing of the NEP gene from human lung has also been demonstrated. NEP activity of COS cells transfected with the alternatively spliced gene exon 16 del was markedly lower than that of cells expressing authentic enzyme (155). The extent to which alternative splicing occurs in normal versus inflamed lungs is not yet known.

Increased NEP. Although there is substantial evidence for decreased NEP activity associated with many acute inflammatory responses in the airways, the possibility that NEP activity can increase in this setting, perhaps as a result of chronic inflammation, should also be explored, especially as an increase in NEP activity is associated with chronic inflammatory conditions in other tissues (156). The neutrophil expresses NEP activity associated with the plasma membrane. While the number of neutrophils in an inflamed airway may be small in comparison with numbers of other constitutive cells that express this enzyme, neutrophils may become an important source of enzyme in the local microenvironment in which epithelial NEP is inactive or missing. The hypertrophy or hyperplasia of fibroblasts and smooth-muscle cells in the airways and air spaces that is observed during some chronic inflammatory states may also result in an overall increase in airway NEP, since both cell types express this enzyme (157). In addition, the NEP activity of neutrophils increases in response to many inflammatory mediators, including C5a and the cytokines tumor necrosis factor and granulocyte-macrophage colony-stimulating factor (150, 158), apparently as the result of translocation of enzyme from intracellular membrane compartments to the cell surface. The ability of inflammatory mediators to similarly alter NEP activity in airway cells remains to be examined.

Induction of New Enzymes. While NEP and ACE appear to be the major enzymes responsible for degradative modulation of tachykinin responses in the airways of healthy animals, these peptides can also be cleaved by a variety of other enzymes. In the absence of NEP activity, degradation of neuropeptides by other enzymes (not yet identified with certainty) may increase. Alternatively, it is possible that inflammation influences the access of other enzymes to peptides released by C fibers in the lung. Increased permeability of the airway vasculature, such as that seen in response to numerous inflammatory mediators, would allow an influx of plasma proteins to the airway interstitium. The plasma is a rich source of both aminopeptidases and DAP-IV, which are capable of degrading SP (36, 54). In fact, the half-life of SP in guinea pig or human plasma is on the order of seconds (39, 159, 160), though NKA is less susceptible to degradation by plasma enzymes (39). Neutrophil influx occurs with many forms of inflammation, and activated neutrophils have an increased capacity to degrade SP through the action of cathepsin G (161) as well as NEP. Though neutrophils express NEP on their surfaces, their ability to degrade SP is related primarily to cathepsin G activity. Mast cell activation or degranulation would result in increased concentrations of chymase, which is also capable of degrading SP (55) in the airway microenvironment.

Summary

SP and NKA are potent endogenous bronchoconstrictors, whereas VIP is a potent endogenous bronchodilator. There is abundant evidence that these neuropeptides are released in the lung in a variety of conditions and that they have the capacity to modulate the bronchoactivity of the same stimuli that release them. On many occasions, their bronchoactive effects are masked by their degradation at or near the site of their release. However, when the microenvironment is modified to decrease their cleavage, they can express enhanced physiologic effects. Although it appears that the human asthmatic lung may be an environment in which the effects of neuropeptides can be amplified, the role of neuropeptides in the pathogenesis of airway obstruction remains speculative.

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