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REVIEW

Neutral endopeptidase modulates neurogenic inflammation

J.A. Nadel

Neutral endopeptidase modulates neurogenic inflammation. J.A. Nadel.
ABSTRACT: A noncholinergic, nonadrenergic nervous system has been described, involving the sensory nerves in the airways. Chemicals, dusts and other irritants stimulate these sensory nerves to release substance P and related neuropeptides. These neuropeptides have the remarkable ability to affect multiple cells in the airways and to provoke many responses including cough, mucus secretion, smooth muscle contraction, plasma extravasation and neutrophil adhesion. This series of effects is termed "neurogenic inflammation."

An enzyme exists on the surfaces of all lung cells that contain receptors for these neuropeptides. This enzyme, neutral endopeptidase (NEP), by cleaving and thus inactivating the neuropeptides, limits the concentration of the neuropeptide that reaches the receptor on the cell surface. Thus, neurogenic inflammatory responses are normally mild and presumably protective in nature. However, when NEP is inhibited pharmacologically (with NEP inhibitors) or by cigarette smoke, respiratory viral infection, or by inhalation of the industrial pollutant toluene diisocyanate, neurogenic inflammatory responses are exaggerated. Delivery of exogenous human recombinant NEP inhibits neurogenic inflammation. Finally, evidence is provided that corticosteroids suppress neurogenic plasma extravasation and that this drug can upregulate NEP in human airway tissue.

Neutral endopeptidase cleaves multiple peptides. Thus, its selectivity resides, at least in part, on its fixed location on the surfaces of specific cells where it can modulate effects of peptides exposed to the cells' surfaces.
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A nonadrenergic, noncholinergic nervous pathway has recently been discovered, and these nerves contain a novel class of molecules, the tachykinins. Among these, the best known is substance P. Because substance P is the best characterized tachykinin, the discussion will focus on this neuropeptide. However, other tachykinins are also released from sensory nerves [1]. In addition, peptides other than tachykinins are also released upon sensory nerve stimulation (e.g. calcitonin gene-related peptide (CGRP)) [2–4]. Sensory nerves in the lower respiratory tract contain substance P immunoreactivity, including nerves in the airway epithelium, smooth muscle, and blood vessels [5, 6]. Substance P can also be detected in the airways by radioimmunoassay in various species [7], including humans [8]. Release of substance P in the airways can be induced by antidromic electrical stimulation of the vagus nerves and by chemical stimulation of the nerves by capsaicin [9]. Cigarette smoke and other chemical irritants are also reported to release substance P [10].

Substance P has potent inflammatory effects, including increased vascular permeability [11–14], neutrophil adhesion [14], vasodilation [15] and exocytosis in some mast cells [16]. In addition, substance P has potent effects on submucosal gland secretion [7], ion transport [17,18], smooth muscle contraction [19–21], cholinergic neurotransmission [22], and cough [23, 24] (fig. 1). These findings provide convincing evidence that tachykinins contained in sensory nerves are powerful mediators of multiple tissue responses in airways. This sequence of events, known as "neurogenic inflammation", mimics various manifestations of asthma and other inflammatory diseases of airways.

In nerves throughout the body, responses to released neuromediators (*e.g.* acetylcholine) are limited by degradative cleavage of the mediators (*e.g.* acetylcholinesterase). Substance P and other tachykinins can be degraded by a variety of enzymes, including neutral endopeptidase [25, 26], kininase II [25, 27], serine proteases [28, 29], mast cell chymase [30], and possibly by acetylcholinesterase ([31]; refuted by [32]). Evidence will be presented that all neurogenic inflammatory responses are modulated by an enzyme, neutral endopeptidase (NEP), also called enkephalinase (EC

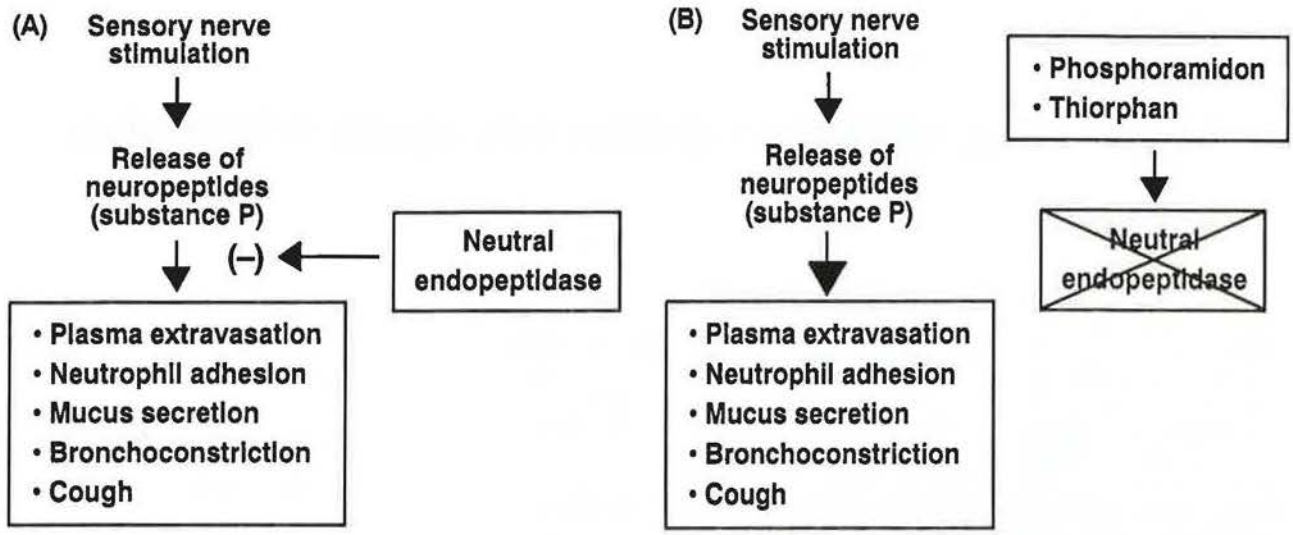

Fig. 1. – (A) Diagram of neurogenic inflammation and modulation by neutral endopeptidase. Sensory nerve stimulation causes the release of neuropeptides which diffuse to target cells and cause tissue responses termed "neurogenic inflammation". Neutral endopeptidase (NEP), an enzyme located on the surfaces of cells containing receptors for these neuropeptides, cleaves and thus inactivates the neuropeptides, thus limiting the tissue responses. The minus sign indicates the inhibitory effect of NEP on tissue responses. (B) Inhibition of NEP (NEP crossed out) pharmacologically (by thiorphan or phosphoramidon) removes the inhibitory effect and thus exaggerates neurogenic inflammatory responses (indicated by the enlarged arrow). Other stimuli such as respiratory viral infection, cigarette smoke, or toluene diisocyanate (TDI) have similar effects.

3.4.24.11). Upon stimulation, neuropeptides are released from the sensory nerves and diffuse to the target cells. Neutral endopeptidase located on the surfaces of these target cells cleaves and inactivates the neuropeptides and thereby limits their effects on cell receptors (fig. 1A). NEP is a novel enzyme in that it controls the accessibility of neuropeptides such as substance P to the receptor of the cell upon which it resides, and thus acts as an autocrine enzyme. Evidence will also be provided that the actions of neuropeptides can be modulated, at least in part, by cleavage near sites of release by basal cells in the epithelium.

Neutral endopeptidase was first discovered in renal tissue [33]. The enzyme was later described in the central nervous system (where it was called "enkephalinase"), and it has been suggested that it modulates opioid actions [34]. Since then, the enzymes from the two sources have been shown to be identical [26, 35, 36]. In a series of studies initiated in 1987 [7], evidence has accumulated that NEP modulates neurogenic inflammation. Evidence will be reviewed that: a) NEP exists in specific airway cells with substance P receptors; b) NEP modulates responses of these cells; c) stimuli that decrease NEP activity cause exaggerated neurogenic inflammatory responses; d) up-regulation of NEP suppresses neurogenic inflammation; and e) exogenously delivered human recombinant NEP also suppresses neurogenic inflammation. This brief review is not intended to cover the entire literature on the subject of neurogenic inflammation. Neutral endopeptidase also cleaves and modulates effects of other peptides; some of these effects in lungs are discussed briefly.

Evidence for presence and location of neutral endopeptidase in airways

Biochemical studies have demonstrated NEP activity in various organs including lungs [37–39]. Neutral endopeptidase activity was found in epithelium, submucosal glands, nerves, and airway smooth muscle [21, 38, 40, 41]. Neutral endopeptidase immunoreactivity has been demonstrated in airway epithelium [37] where it is concentrated in the basal cells [21], in lung tissue [38] where it is located in type II cells (Nadel and Ueki, personal communication), in submucosal glands and ducts, and in postcapillary venules (Nadel, Ueki and Piedimonte, personal communication). Enzyme immunoreactivity was found in every species studied including human, ferret, rat, guinea-pig and dog.

Potentiation of effects of tachykinins and of neurogenic inflammation by pharmacological inhibitors of neutral endopeptidase

It was reasoned that if NEP normally exists on the membranes of cells that contain substance P receptors, and if NEP normally cleaves, and thus inhibits, a significant proportion of the substance P as it diffuses through the tissue to the receptor, then inhibition of NEP by pharmacological inhibitors should potentiate responses to substance P (fig. 1B). Two relatively selective drugs can be used for this purpose, phosphoramidon [42] and thiorphan [43]. Thiorphan is less specific, because it also inhibits kininase II [44].

More recently, a third selective NEP inhibitor (SCH 34826) has been reported [45]. The first tissue studied was ferret trachea. Inhibitors of NEP potentiated the secretagogue effect of substance P [7]. Although other enzymes can cleave substance P, inhibitors of kininase II and serine proteases had no effect on substance P-induced secretion, indicating that the latter enzymes did not play a significant role in degradation of substance P under these conditions. In addition to substance P, NEP inhibitors potentiated the secretagogue effects of a series of related tachykinins (Borson and Nadel, personal communication).

Following these observations, a series of studies reported that NEP inhibitors potentiate the effects of substance P on smooth muscle contraction *in vitro* [20, 46–49] and *in vivo* [50–53] (fig. 2). Like the secretory responses, smooth muscle responses to all tachykinins are potentiated by NEP inhibitors [21, 48]. Inhibitors of kininase II, aminopeptidases, and serine and thiol proteinase did not potentiate substance P-induced smooth muscle contraction [20]. In one study, potentiation of smooth muscle contraction by neurokinin A was greater than by substance P in ferret trachea [21], while in another study, substance P-induced smooth muscle contraction was potentiated more by NEP inhibition than neurokinin A [54]. These differences are unexplained, but they may be due to differences in accessibility of the NEP to receptors in different tissues or species. Neutral endopeptidase inhibitors also potentiated the effects of tachykinins on cholinergic neurotransmission [20].

Substance P also promotes plasma extravasation in airways [11, 55], and NEP inhibitors potentiate substance P-induced plasma extravasation [55]. Substance P has recently been shown to cause cough in very low concentrations in awake guinea-pigs, an effect that is potentiated by aerosols of NEP inhibitors [23]. In airway epithelium, substance P increases short-circuit current [17] and stimulates chloride secretion [18], effects that are potentiated by NEP inhibitors [18]. All of these studies show that NEP inhibitors potentiate substance P actions and suggest that NEP localized to the surfaces of cells that are targets for substance P actions limits the responses to substance P in each of the airway tissues studied (submucosal glands, smooth muscle, postcapillary venules, sensory and motor nerves).

Neutral endopeptidase inhibitors also potentiate the effects of neuropeptides released from sensory nerves in airways by retrograde electrical nerve stimulation or by capsaicin. Thus, capsaicin-induced cough is exaggerated by aerosolized leucine-thiorphan or phosphoramidon in guinea-pigs [23]. Similarly, phosphoramidon potentiated smooth muscle responses to vagus nerve stimulation [20, 51]. Neurogenic plasma extravasation in response to electrical nerve stimulation or to capsaicin is potentiated by NEP inhibitors [14, 56–58] (fig. 3). Neutrophil adhesion to postcapillary venular endothelium induced by electrical nerve stimulation or by capsaicin is also potentiated by phosphoramidon [14]. Curiously, the rapid adherence of neutrophils to the vascular endothelium is followed by a gradual re-entry of the neutrophils into the circulation (half-life of adherence, approximately 4 h) [56].

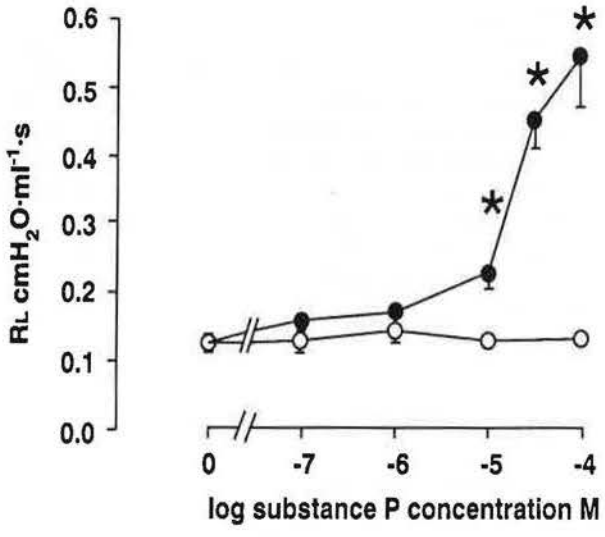

Fig. 2. – Concentration-response curves to aerosolized substance P (7 breaths) in the absence (open circles) or 15 min after the administration of aerosolized phosphoramidon (10^{-4} M, 90 breaths; closed circles) in anaesthetized guinea-pigs (n=5 for each condition). Total pulmonary resistance (R_L) is expressed as means±SEM. * :significant differences between control animals and animals treated with aerosolized phosphoramidon (P<0.005). (Reproduced with permission from Dusser *et al.* [51]).

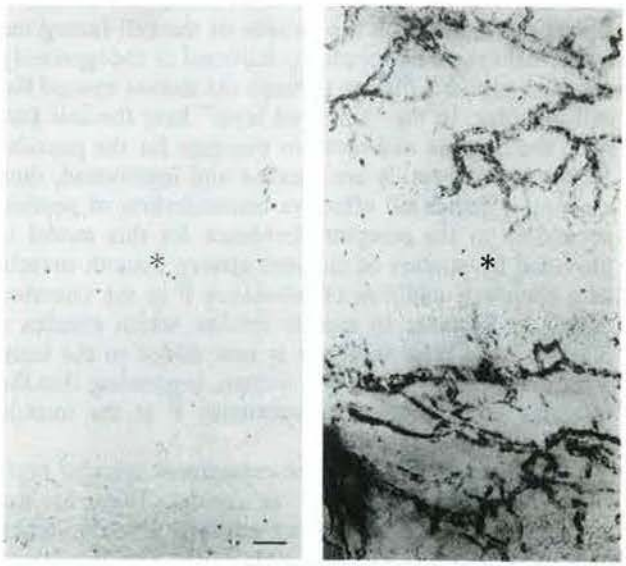

Fig. 3. – Light micrographs of whole mounts of tracheas from a pathogen-free rat (left) and from an infected rat (right) that received an injection of capsaicin (75 $\mu g \cdot kg^{-1}$, *i.v.*) 5 min before fixation. Few monastral blue-labelled blood vessels are present in the trachea of the pathogen-free rat, but they are abundant in the trachea of the infected rat. Asterisks mark cartilaginous rings. Bar = 100 µm. (Reproduced with permission from Piedimonte *et al.* [57]).

In spite of potent and prolonged neutrophil adhesion (half-life of adhesion approximately 4 h), no significant chemotaxis occurred. Only after treatment with phosphoramidon did significant chemotaxis occur, and even under these conditions, chemotaxis was only mild [56]. These results are compatible with the reports that substance P is only very weakly chemotactic for neutrophils *in vitro* [59–61]. Both neutrophils [62] and tracheal postcapillary venules (Nadel, Ueki and Piedimonte, personal observation) contain NEP; their individual contribution to neutrophil adhesion is unknown. One study showed that phosphoramidon inhibition of NEP abolished the ability of neutrophils to migrate toward f-L-methionyl-L-leucyl-L-phenylalanine (fMLP) *in vitro* in Boyden chambers [63]. Neutrophils, postcapillary venular endothelium and epithelial cells contain NEP and could modulate the effects of neutrophil adhesion and/or movement. Dissecting the roles of these different cells could provide interesting new insights into the mechanisms of neutrophil movement into tissues. From all of the studies of capsaicin and electrical nerve stimulation in various tissues, we conclude that the effects of endogenously released sensory neuropeptides, like the effects of exogenously delivered substance P, are potentiated by NEP inhibitors.

Because NEP is located on the surfaces of cells that contain substance P receptors, Iwamoto *et al.* [64] studied the effect of NEP inhibitors on the binding of substance P to receptors in homogenates of rat ileum. The results suggested that NEP regulates the binding of substance P to its receptor by decreasing the amount of substance P available to the receptor, without significantly changing the affinity or the number of receptors.

The following sequence of events is proposed to explain the findings with NEP inhibitors on target cells: NEP exists on the surfaces of target cells, with the active site of the enzyme on the outside of the cell facing the interstitial space. Exogenously delivered or endogenously released peptides diffuse through the tissues toward the cell receptor. In the "unstirred layer" near the cell surface, the enzyme and receptor compete for the peptide. Molecules of peptide are cleaved and inactivated, thus creating a decreased effective concentration of peptide accessible to the receptor. Evidence for this model is provided by studies of isolated airway smooth muscle in a chamber: addition of substance P to the chamber causes an increase in muscle tension which reaches a plateau. If an NEP inhibitor is now added to the bath, a further increase in tension occurs, suggesting that the effective concentration of substance P at the muscle receptor has increased.

The exact conditions of the experiment are also critical to the interpretation of the results. Take, for example, the effect on airway smooth muscle: when capsaicin or electrical stimulation of the nerves releases neuropeptides, the smooth muscle contraction is reduced by a pharmacological antagonist to substance P [21], indicating that substance P or a related tachykinin was responsible for the contraction. This neurogenic inflammatory response is markedly potentiated by inhibitors of NEP (phosphoramidon, thiorphan) but is unaffected by an inhibitor of kininase II (captopril) [20, 46]. One explanation for this is that NEP exists in high concentrations in tissues of the airways such as epithelium and smooth muscle, but only little kininase II exists in these tissues. On the other hand, when substance P is injected *i.v.*, both captopril and phosphoramidon potentiated the bronchomotor responses [65]. The effect of captopril can be explained by the fact that kininase II exists in high concentrations on pulmonary endothelial cells [38]. The data presented by Shore *et al.* [65] suggest that substance P is cleaved and inactivated by kininase II mainly in the pulmonary endothelium and by NEP mainly in the airway tissue itself (*e.g.* epithelium, smooth muscle), and this is compatible with the major sites of localization of the two enzymes.

When aerosolized substance P is inhaled or when the sensory (vagus) nerves in the airways are stimulated to release neuropeptides, the responses of submucosal glands [7] and smooth muscle [20, 21, 49, 51] are modulated by NEP but not by kininase II. However, during neurogenic inflammation, responses of the vascular bed itself (such as vascular permeability and neutrophil adhesion) may very well be modulated by both NEP and by kininase II, because both enzymes reside in the vascular endothelium.

Although the primary effects of neurogenic inflammation are likely to derive from locally released neuropeptides in the airways, other peptides may arrive from the circulation and secondarily affect airway tissues. For example, when the vascular permeability in airways increases, kininogen from blood may arrive in the lungs and become activated to form kinins. Kininase II in the vascular endothelium (along with NEP in the vascular endothelium and in the airway tissues) may play an important role in kinin modulation.

The selectivity of effects of NEP on various peptide substrates is determined in part by its selective ability to cleave at certain amino acid sequences. The C-terminal six amino acids and the amide moiety of tachykinins are responsible for receptor recognition and activation of tachykinins [66]. The enzyme cleaves peptides on the amino side of hydrophobic residues. A major site of cleavage of substance P by NEP is between positions 9 and 10 [25], producing the N-terminal fragment SP_{1-9}, which does not stimulate smooth muscle contraction [20, 49] or gland secretion [7]. Furthermore, the C-terminal fragment of substance P was shown to be a chemoattractant for neutrophils, but the N-terminal fragment was not [61].

Confirmation that NEP inhibitors potentiate substance P-induced airway smooth muscle contraction by inhibiting the breakdown of the active peptide is provided by studies that showed that substance P infused into guinea-pig lung is rapidly cleaved into inactive peptide chains that would be predicted by the known actions of NEP, and this breakdown is prevented by inhibitors of NEP [49]. As mentioned previously, other enzymes are capable of cleaving tachykinins; inhibitors of these enzymes did not affect smooth muscle contraction by neurogenic inflammation in healthy animals. However, under pathological conditions, such enzymes may become

available to cleave the peptides. For example, mast cell enzymes only have access to the neuropeptides when they are released. However, when mast cell granules are released, these enzymes could play important roles in modulating neurogenic inflammation. In the case of other enzymes that do not cleave the C-terminal end of the peptide, the cleavage event may potentiate rather than inhibit the responses. For example, SP_{4-11} is reported to be a more potent chemoattractant than substance P itself [61].

In addition to substance P, NEP also cleaves and, therefore, modulates effects of other inflammatory mediators such as kinins [67, 68], neurotensin [25, 69], enkephalins [70], the synthetic chemotactic peptide fMLP [46, 62], and endothelin [71–73]. Thus, the specificity of NEP resides in part in the selectivity of cleavage sites in some peptides that act as favourable substrates for the enzyme. However, the selectivity of NEP also resides in the specific locations (*i.e.* surface of specific cells) where NEP is expressed, where the enzyme can cleave only peptides presented to the surface of the cell.

Potentiation of neurogenic inflammation by stimuli that decrease neutral endopeptidase

Because chemical inhibition of NEP exaggerates neurogenic inflammatory responses, we tested the hypothesis that various damaging inhaled materials act, at least in part, by inhibiting NEP activity. We found that respiratory viral infections [47, 52, 55, 57], cigarette smoke [53], and the industrial pollutant toluene diisocyanate [50] all exaggerate neurogenic inflammatory responses, and they decrease NEP activity. In each of these conditions, the fact that pharmacological inhibition of NEP causes little or no further exaggeration of neurogenic inflammatory responses provides further evidence that decreased NEP activity is responsible for the exaggerated responses. We have studied viral infection [47, 52], toluene diisocyanate inhalation [50], and cigarette smoke inhalation [53] on smooth muscle responses. In the case of respiratory infections, we have also shown potentiation of neurogenic extravasation [55, 57].

Role of the airway epithelium in neutral endopeptidase modulation

Removal of the airway epithelium has been shown to potentiate the smooth muscle contractile responses to substance P, effects which the authors attributed to the elimination of an epithelial relaxant factor [74, 75]. However, there is biochemical [40] and immunocytochemical [21] evidence that the airway epithelium contains substantial NEP, and SEKIZAWA and co-workers [21] have provided evidence that the potentiated substance P response in ferret airway smooth muscle that occurs when the epithelium is removed is due to the removal of NEP that is present in the epithelium. In this sense, NEP can be considered to be an epithelial "relaxant factor", in that it cleaves and thus inactivates exogenously delivered peptides (*e.g.* in a muscle chamber or when delivered by aerosol). These results, incriminating the loss of NEP in the enhancement by epithelial removal of substance P-induced airway smooth muscle contraction, have been confirmed [76]. Furthermore, removal of the airway epithelium also enhances airway smooth muscle contraction due to neurotensin [69], and the authors provided evidence that the enhanced responses were due to removal of epithelial NEP. It is not surprising that NEP could limit substance P-induced responses to exogenous neuropeptides, because the epithelium has access to the peptides as they diffuse through the epithelium on their way to the smooth muscle. It is more surprising that smooth muscle contractile responses to neuropeptides released from the sensory nerves by capsaicin or by electrical field stimulation are also potentiated when the airway epithelium is removed, but these results can be explained by the anatomical locations of NEP and of the sensory nerves containing the neuropeptides. In the epithelium, immunofluorescent studies show that NEP is concentrated in the basal cells in all species studied, with very little NEP in ciliated cells (fig. 4) [21] (Nadel and Ueki, personal communication). The localization of substance P-containing sensory nerves has been studied in detail in the rat trachea [6]. Substance P immunoreactivity was localized to a plane at the base of the epithelium. Morphometric analysis of the trachea visualized by electron microscopy showed that the majority of the

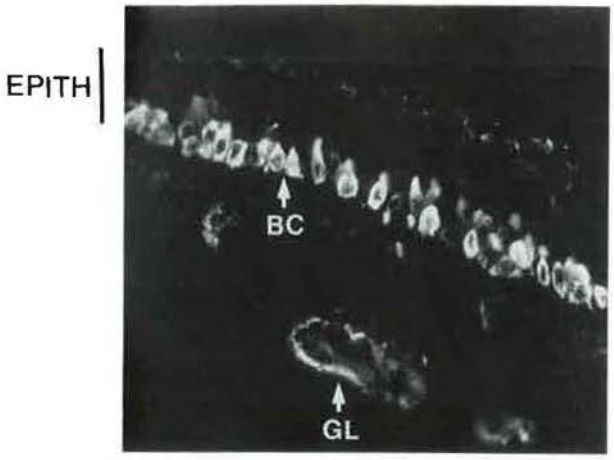

Fig. 4. – Immunocytochemical localization of neutral endopeptidase in rat trachea, showing staining with fluorescein-labelled neutral endopeptidase antibody in basal cells (BC) of the epithelium (EPITH) and glands (GL).

sensory nerve profiles were in the epithelium, and these were almost all in close association with the basal cells (figs 5 & 6). Thus, the basal cells appear to play an important role in modulating neurogenic inflammatory responses by cleaving neuropeptides near their sites of release. Conceivably, NEP located in the perineurium of the vagus nerves (Nadel and Ueki, personal observation) may play a similar role. In other species, sensory nerves are also present near airway structures other than basal cells. Differences among species in loci of

neuropeptide release will affect the relative importance of epithelial NEP in modulating neurogenic inflammatory responses. In a species, the distribution of sensory nerve endings and NEP may also vary depending on the size of the airway.

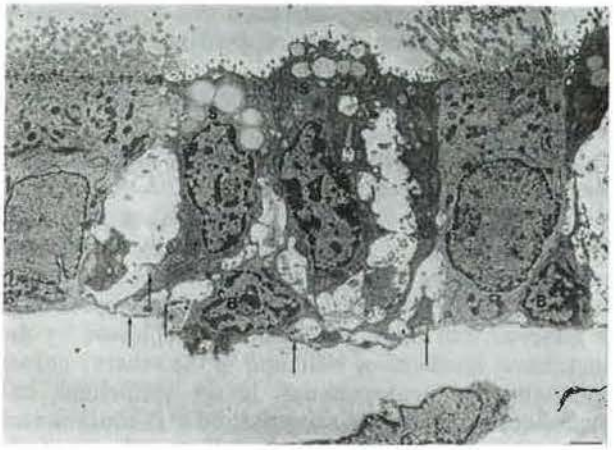

Fig. 5. – Electron micrograph of tracheal epithelium showing at least five intra-epithelial neurites (arrows), all of which are located at the base of the epithelium. Also visible are several prominent changes resulting from 2 min of vagal stimulation: the shrunken appearance of the secretory cells (S), the clustering of secretory granules at the apex of secretory cells, and the conspicuous widening of the spaces next to secretory cells and basal cells (B) and beneath the epithelium. The ciliated cells appear to be unaffected by the vagal stimulation. Bar = 2 μm. (Reproduced with permission from McDonald *et al.* [6]).

The preferential localization of the varicose sensory nerve endings next to the basal cells provides a logical explanation for the effect of epithelial removal on neurogenic inflammatory responses in airway smooth muscle (*i.e.* the procedure removes NEP that normally modulates substance P near its site of release). The increased neurogenic inflammatory responses of airway smooth muscle [47, 52] and of vascular permeability [55, 57] during viral respiratory infections may also be due to direct viral effects on the epithelium where the virus replicates. Similarly, toluene diisocyanate is a very reactive compound, so its inhalation is likely to produce profound effects on epithelial cells [50]. The effect of cigarette smoke on neurogenic responses is believed to be due to free radicals generated by the smoke [53]. Upon inhalation, the free radicals effects first encounter the epithelium, where they could inactivate NEP.

If the epithelium were the only airway tissue that contained NEP, it would be anticipated that NEP inhibitors would not increase bronchomotor responses to substance P after removal of the epithelium, and this was true in one study [76]. However, because airway smooth muscle contains substantial NEP in ferrets [21] and in other species including humans, rats, and guinea-pigs (Nadel and Ueki, personal communication), it is not surprising that various studies showed that after removal of the airway epithelium, NEP inhibitors still potentiated the bronchomotor effects of tachykinins [21], neurotensin [69], and capsaicin [77], albeit less.

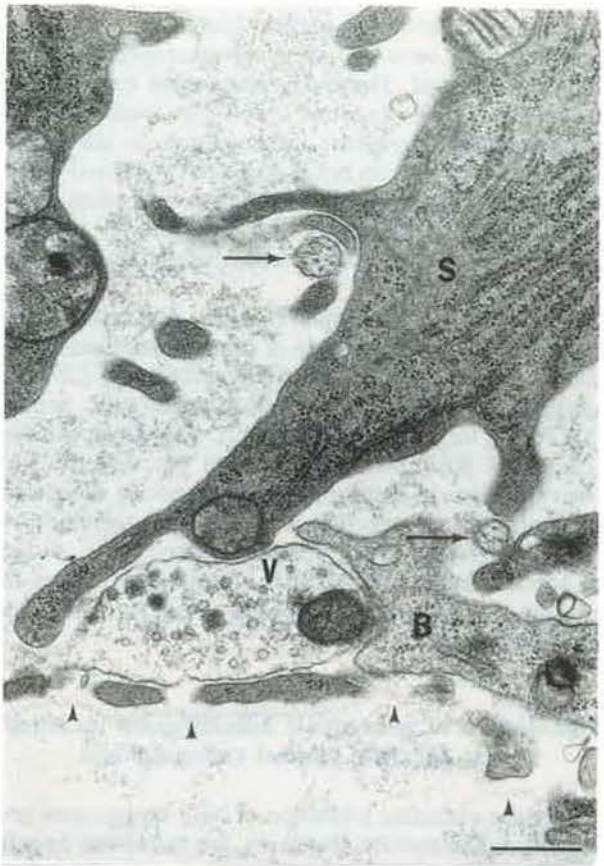

Fig. 6. – A region of figure 5 enlarged here to show a neuronal varicosity (V), which contains both small clear vesicles and large dense-cored vesicles, and two tiny neurites without vesicles (arrows) at the base of the tracheal epithelium. Arrowheads mark the basal lamina of the epithelium. The extracellular space between epithelial cells and the space beneath the epithelial basal lamina are conspicuously enlarged as a result of vagal stimulation for 2 min. B: epithelial basal cell; S: epithelial secretory cell. Bar = 0.5 μm. (Reproduced with permission from McDonald *et al.* [6]).

Based on present information, we suggest that NEP modulates neurogenic inflammatory responses both at sites of neuropeptide release and at sites of neuropeptide action. The data for this "model" are compelling but indirect. Future studies should be directed to the establishment of the mechanisms of this modulation. Utilization of *in situ* hybridization and immunogold localization may provide further information on the effects of various interventions and diseases on NEP at specific sites. In addition to modulation of airway smooth muscle contraction, airway epithelial NEP may modulate the effect of peptides that relax airway smooth muscle, and it may modulate peptide effects on other airway tissues (*e.g.* glands, blood vessels).

Effects of increased neutral endopeptidase on neurogenic inflammation

If decreased NEP causes exaggerated responses, we reasoned that increased NEP should suppress neurogenic inflammatory responses. Because human NEP has

recently been cloned [78], we were able to study the potential therapeutic uses of this enzyme. We found that aerosolized recombinant human NEP inhibited cough produced by both endogenous and exogenous tachykinins [24] (fig. 7). Recombinant human NEP has also been used successfully to prevent neuropeptide responses in other organs. IWAMOTO *et al.* [79], showed that various tachykinins cause increased plasma extravasation in guinea-pig skin, effects that were potentiated by NEP inhibitors and they showed that significant NEP activity existed in the skin. Recombinant human NEP inhibited substance P-induced plasma extravasation in a dose-dependent fashion [80].

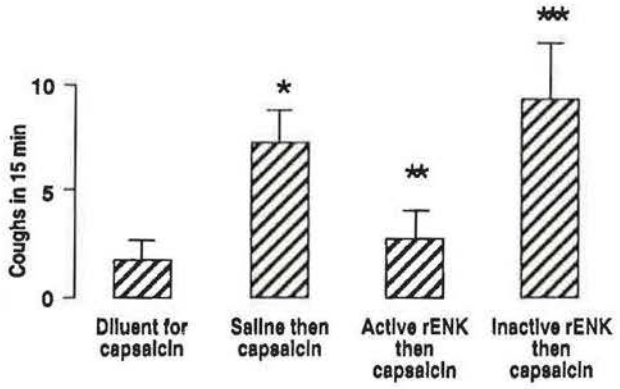

Fig. 7. – Effects of capsaicin, recombinant enkephalinase dialysed in the absence of liposomes (rENK), and inactivated enkephalinase in pathogen-free guinea-pigs. One group of five animals was exposed to Hepes-buffered saline followed by the diluent for capsaicin, which did not cause significant cough. Another group of animals was exposed to hydroethylpiperazine ethanesulphonic acid (Hepes)-buffered saline followed by capsaicin (10^{-13} M), which caused significant coughing (two left columns). In a blind study in which the observer did not know whether the animal had received active or inactive enkephalinase, six other animals in each group received aerosols of either active or inactive enkephalinase (33 μg, 5 min) followed by capsaicin (10^{-13} M). Active enkephalinase prevented the response to capsaicin, but inactive enkephalinase did not significantly decrease the response to capsaicin (two right columns). *:$p<0.05$ compared with the responses to the diluent for capsaicin alone; **:$p<0.05$ compared with the response to capsaicin alone; ***:$p<0.05$ compared with the response to capsaicin after active enkephalinase. (Reproduced with permission from KOHROGI *et al.* [24]). In this figure, "enkephalinase", an analogous term to "neutral endopeptidase", is used.

In the human eye (donors), substance P-induced contraction of the iris was potentiated by NEP inhibitors [81]. In the rabbit eye, recombinant human NEP prevented substance P-induced contraction of the iris [82]. These findings suggest that recombinant NEP might be useful in the treatment of symptoms of diseases involving inflammatory peptides such as substance P and other peptides such as bradykinin [67, 68] that are cleaved by NEP.

Glucocorticoids are known to suppress inflammation, but the mechanisms are generally unknown. Because these substances are known to up-regulate the synthesis of some peptidases (*e.g.* [83]), we examined their effects on neurogenic plasma extravasation. We found that dexamethasone reduced, in a dose-dependent fashion, the magnitude of plasma extravasation produced in rat airways by neurogenic inflammation, and our evidence suggests that this is due, at least in part, to an increase in NEP activity [58]. That corticosteroids are capable of up-regulating the expression of NEP was demonstrated directly in human tracheal epithelial cells [84].

Discussion

From various findings, we hypothesize that the sensory nervous system and its neurogenic inflammatory responses act as follows: a large number of foreign materials in the airways stimulate the airway sensory nerves to release substance P and other related neuropeptides. We suggest that these neurogenic responses are normal and protective. Gland secretion, chloride transport (and associated water movement toward the airway lumen), and cough may assist in the dilution and clearance of the irritant. The short-lived increase in vascular permeability [13] tends to dilute the stimulus. Neutrophils become adherent to the endothelium but do not move into the tissue [56]. Thus, the sequestered neutrophils are readily accessible for movement, but only if a further, chemotactic stimulus occurs. Because of the presence of NEP, the tissue responses are small and, unless the stimulus is great, not clinically manifest. However, when NEP is down-regulated or inactivated (*e.g.* by cigarette smoking), stimuli that activate the sensory nerves now produce exaggerated responses (because the released substance P is not cleaved), and the responses may become clinically evident.

Many studies of sensory nerve release of neuropeptides have focused on substance P. However, neurokinin A [1] and calcitonin gene-related peptide (CGRP) [2–4] are also present in these nerves, and many also play important roles in neurogenic inflammation. Neurokinin A is a favourable substrate for NEP [85], but CGRP appears to be less favourable [86]. Thus, the roles of NEP in modulating neurogenic inflammation are complicated: effects of some neuropeptides (*i.e.* substance P and neurokinin A) would be predicted to be markedly altered by the presence of NEP on target cells, while the effects of CGRP may be less affected.

The exact roles of neurogenic inflammation in disease remain to be elucidated. It is likely that multiple cells and multiple mediators are involved in inflammation and that they interact. Because of the multiple cell systems stimulated by neuropeptides exemplified by substance P and the wide array of stimuli that could activate the sensory nerves to release these mediators, this system may play important roles in the pathogenesis of chronic diseases in airways.

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L' endopeptidase neutre module l'inflammation neurogène. J.A. Nadel.

RÉSUMÉ: L'on a décrit un système nerveux non cholinergique et non adrénergique, qui implique les nerfs sensitifs dans les voies aériennes. Les produits chimiques, les poussières et d'autres irritants, stimulent ces nerfs sensitifs à libérer la substance P et des neuropeptides associés. Ces neuropeptides ont la capacité remarquable d'agir sur de multiples cellules des voies aériennes et de provoquer de nombreuses réponses, notamment la toux, la sécrétion de mucus, la contraction de muscles lisses, l'extravasation plasmatique, et l'adhésion des neutrophiles. Cette série d'effets est appelée "l'inflammation neurogène".

A la surface de toutes les cellules pulmonaires existe un enzyme qui contient des récepteurs pour ces neuropeptides. Cet enzyme, qu'on appelle l'endopeptidase neutre (NEP), entraîne la scission et donc l'inactivation des neuropeptides, et limite la concentration du neuropeptide qui atteint les récepteurs à la surface cellulaire. Dès lors, les réponses inflammatoires neurogènes sont normalement faibles, et probablement de caractère protecteur. Toutefois, lorsque l'on inhibe la NEP par des moyens pharmacologiques, c'est-à-dire par les inhibiteurs de la NEP ou par la fumée de cigarette, l'infection virale respiratoire, ou l'inhalation de polluants industriels ou encore de diisocyanate de toluène, les réponses inflammatoires neurogènes sont accentuées. L'administration de NEP recombinante humaine exogène inhibe l'inflammation neurogène. Finalement, il existe des preuves que les corticoïdes inhibent l'extravasation plasmatique neurogène, et qu'ils peuvent réguler vers le haut la NEP dans les tissus des voies aériennes humaines.

L'endopeptidase neutre clive de multiples peptides. Dès lors, sa sélectivité réside, au moins partiellement, sur sa situation fixe à la surface des cellules spécifiques où elle peut moduler les effets des peptides parvenant au niveau des surfaces cellulaires.

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