AIRWAYS NEUROGENIC INFLAMMATION*

NEUROGENA INFLAMACIJA DISAJNIHPUTEVA

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Abstract

In the airways, a new noncholinergic - nonadrenergic nervous system and new class of molecules, the neuropeptides, has recently been described. Irritants as dust, chemicals and other substances stimulate these sensory nerves to release substance P(SP) and SP-like neuropeptides (neurokinin A). SP and neurokinin A are neuropeptides that belong to the family of tachykinins which are localized in the C-fiber endings in the airways of several species including humans and guinea pigs. These tachykinins have the remarkable ability to affect multiple cell systems in the lung airways (smooth muscle, glands, blood vessels) and to produce smooth muscle contraction, mucous secretion and plasma extravasation. This effect is termed "neurogenic inflammation". On the surfaces of all lung cells that contain receptors for SP-like neuropeptides exist an enzyme named neutral endopeptidase (NEP), also called enkephaline-se (EC 3.4.24.11). NEP inactivates the neuropeptides and by that action limits the concentration of the neuropeptides that reaches the receptors on the smooth muscles, glands and surfaces of other cell systems. The purpose of this article is to present evidence concerning neurogenic inflammation and to consider our studies in proposing the mechanism(s) involving these nerves that may play important roles in lung diseases. Summarizing results obtained in our studies, the following conclusive remarks could be drawn: a) SP and SP-like tachykinins have potent effects on airway smooth muscle, gland secretion and blood vessels, b) electrical stimulation of the nerves to airway smooth muscles or chemical stimulation with capsaicin causes noncholinergic - nonadrenergic contraction of airways, effects that are abolished by a tachykinin antagonist, c) airway epithelium modulates the tissue responses to endogenous tachykinins released by electrical field stimulation or capsaicin suggesting that the relaxing factor (NEP, prostaglandine E2) in epithelium is important in modulation of tachykinin-induced neurogenic inflammation.

Key words
Neurogenic inflammation, Axon reflex, Lung diseases, Tachykinins, Substance P, Neurokinin A, Neutral endopeptidase

INTRODUCTION

Involvement of airway epithelium in the pathogenesis of lung diseases is suggested by the finding of abnormal epithelium in the airways of patients with asthma bronchiale, and some patients with chronic bronchitis and bronchial hyperreactivity (Bigbi and Nadel, 1987., Barne, 1991., Barnes 2003): A second cell system, the sensory nerve exists near the air-liquid interface of the airways. In addition to the sympathetic and parasympathetic nervous systems, a new nonadrenergic, noncholinergic nervous system has recently been described. A new class of molecules, the neuropeptides, has been discovered. It is believed that these peptides play an important role in neurotransmission or in neuromodulation. On the other hand, it is believed that these peptides could be involved in inflammation cold "neurogenic inflammation".

In experimental animals using the respiratory system, skin and eye, it has been demonstrated that: a) tachykinins are widely distributed in primary sensory neurons and in afferent sensory fibers in the vagus, b) during antidromic stimulation the tachykinins are released from the sensory fibers, c) local inoculation of substance P (SP) mimics the effect of stimulation of sensory fibers, and d) smooth muscle contraction, hyperemia and increased vascular permeability, induced by nerve stimulation or noxious stimuli, are absent if the tissues were previously treated with capsaicin.
or with tachykinin-antagonists. These results suggest that SP-containing nerve fibers are involved in the local regulation of the smooth muscle tone, blood flow and vascular permeability, pathophysiological features of neurogenic inflammation.

**The aim of this study**

To the present time, the exact roles of neurogenic inflammation in lung 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 system 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 lung diseases. The aim of this paper is to present evidence concerning neurogenic inflammation and to consider our studies in proposing the mechanisms involving these nerves that may play important roles in lung disease.

**The role of neuropeptides (tachykinins) in neurogenic inflammation**

Experimental studies in animals have shown that some afferent sensory fibers are non-myelinated, and these are designated C-fibers in distinction to the other more rapidly conducting afferent fibers, which are myelinated. C-fibers are stimulated by capsaicin (the hot extract of pepper). Unmyelinated C-fibers in airways may contain sensory neuropeptides, such as substance P. Neuropeptide-containing nerves have been found in the airways of several species, including humans (Lundberg et al., 1984a). These nerves are present in the epithelium, smooth muscle and close to blood vessels and glands (Nilsson et al., 1977). The tachykins are a group of neuropeptides that share the C-terminal sequence Phe-X-Gly-Leu-Met-NH2 (Table 1).

**Table 1. Chemical structure of tachykinins**

| Substance P | Arg-Pro-Ly8-Pro-Gln-Gln-Phe-Phe-Gly-Lys-Met-NH2 |
| Neurokinin A | Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH2 |
| Neurokinin A | His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH2 |

Present evidence suggests that tachykins, substance P in particular, are involved in sensory neurotransmission. In sensory neurons, substance P is usually accompanied by a second tachykinin, neuropeptide A (NKA). This is obtained by the fact that of the three known substance P precursors, alpha-, beta-, and gamma-preprotachykinin A (PPT-A), beta- and gamma-PPT-A contain NKA in addition to substance P (Nawa et al., 1983; Kawaguchi, Y., et al., 1986). The airways has a moderate supply of SP/NKA-containing nerve fibers. The SP-containing nerves surround smooth muscle, gland and blood vessels and can be seen beneath or even within the surface of epithelium (Lundberg et al., 1984). Stimulation of the sensory nerves results in the release of substance P and the reproduction of the various effects of exogenous substance P. These effects are prevented when the lungs are depleted of substance P by capsaicin.

Of the neuropeptides-tachykins present in the airways, the best studied is substance P. Since its discovery (von Euler and Gaddum, 1931), many studies have demonstrated effects of substance P in various organs, including lungs (Pemow, 1983). Substance P has potent effects on airway smooth muscle: it contracts airway smooth muscle directly (Lundberg et al., 1983b; Sekizawa et al., 1987b), and it has potentiation effects on the vagal motor pathways (Tanaka and Grunstein, 1984; Sekizawa et al., 1987b). In the guinea pig, the substance P-containing nerves are found in the vagus (Lundberg et al., 1983a). Release of substance P from afferent terminals and collaterals in the airway may occur as part of axon reflex (see Fig. 1). There is substantial evidence that neuropeptide-containing nerves modulate smooth muscle contraction. Thus, electrical stimulation of the nerves to airway smooth muscle (Fig. 2) or chemical stimulation with capsaicin (Fig. 3) causes nonadrenergic, noncholinergic contraction of airway smooth muscle in guinea pigs (Djokic et al., 1989), effects that are abolished by a tachykinin antagonist (Lundberg et al., 1983c).

Substance P is synthesized in the cell bodies of the sensory ganglia and is transported down to the periphery of the vagus nerve (Brimijoin et al., 1980). Stimulation of sensory nerves in the spinal cord by capsaicin releases substance P (Gamse et al., 1979, 1980). In the airways, capsaicin stimulates bronchoconstriction and increases vascular permeability, effects that are abolished by substance P antagonists (Lundberg et al., 1983c, 1984b; Djokic et al., 1988). Furthermore, pretreatment with capsaicin causes disappearance of tachykinin-containing nerves in the airways (Jancso et al., 1977; Lundberg and Saria, 1983), these studies strongly suggest that the action of capsaicin on the airways
is most likely via the release of substance P or other tachykinsins from sensory nerves. Noncholinergic, nonadrenergic responses to electrical field stimulation are therefore likely to be due to the release of endogenous neuropeptides. This conclusion was based on the findings that these responses were abolished by a tachykinin receptor antagonist (Lundberg et al., 1983).

Neutral endopeptidase modulates neurogenic inflammation

When neuropeptides are released from the nerve endings, their role in regulating smooth muscle contraction depends on how many molecules are able to reach the target cell receptors. At sites of release (e.g., sensory nerves) and at sites of action on target cells (e.g., effenter nerves, smooth muscle, glands) peptidases such as neutral endopeptidase (NEP) could degrade neuropeptides to inactive forms. The action of NEP and other peptidases are not highly specific because, for example, purified NEP hydrolys many different peptides, including enkephalins (Malfroy et al., 1978), tachykinsins (Almenoff and Orlovski, 1983; Gafford et al., 1983; Matsas et al., 1984, Stephenson and Kenny, 1987), bradykinin (Matsas et al., 1984) neurotensin (Matsas et al., 1984, Djokic et al., 1989) and cholecystokinin (Turner and Kenny, 1985). Thus, much of the specificity and degree of action is determined by the selectivity of the peptidase released and by the location of the peptidases. Airway smooth muscle responses to substance P, neurokinin A and neurokinin A (Sekizawa et al., 1987a) as well as neurotensin (Djokic et al., 1989) are all modulated by endogenous NEP. Furthermore, because cholinergic neurotransmission is potentiated by NEP inhibitors (Sekizawa et al., 1987a), NEP is also important in modulating the effects of tachykinsins in airways. A recent study demonstrated that i.v. phosphoramidon potentiated capsaicin-induced bronchoconstriction (Thompson and Sheppard, 1988, Dusser et al., 1989). Finally, because sub-

![Fig. 2. Effects of phosphoramidon on the time course and magnitude of bronchial muscle contraction and relaxation after electrical field stimulation (EFS, 2 Hz.) in five guinea pigs. The tension (mean ± 1SE) is expressed in g (upper trace) and as the percentage of the maximal tension in each tissue (lower trace). Phosphoramidon (10^-5 M.; closed circles) increased the magnitude of the contractile response to EFS and prolonged the contraction compared to the electrically induced contractile responses in control tissues (open circles) (Reproduced from reference 21.)](image1)

![Fig. 3. Noncholinergic, nonadrenergic contractile responses to capsaicin (1.5 x 10^-6 M) in guinea pig bronchi with and without the epithelium, in the absence and presence of neutral endopeptidase inhibitor phosphoramidon. Data are expressed as percentage of muscle contraction (mean ± 1SE) induced by acetylcholine (10^-3 M). Removing the epithelium increased slightly but significantly the capsaicin-induced contraction compared to the intact tissues (*p < .05). Phosphoramidon (10^-5 M) increased the response to capsaicin in tissues with and without epithelium (**p < .001; n=7 each) (Reproduced from reference 21.)](image2)

![Fig. 4. Effects of various enzyme inhibitors on the non-cholinergic, nonadrenergic contractile response to EFS at 2 Hz in bronchi from five guinea pigs. Data are expressed as the percentage of the control contraction (mean ± 1SE) induced by EFS in each tissue. Captopril, bestatin, leupeptin and physostigmine (each drug, 10^-5 M) did not alter the contractile response to EFS significantly. Phosphoramidon and leucine-thiorphan (each drug, 10^-5 M) increased electrically induced contraction significantly and substantially (p < .01)(Reproduced from reference 21.)](image3)
Our results show that the profiles of inhibition of phosphoramidon, captopril, and MGPA on NEP-like activity in guinea pig airway are similar to those observed on purified NEP (Malfroy and Schwartz, 1984, Matsas et al., 1984). These results suggest that the NEP-like enzyme in guinea pig airway is very similar to NEP. NEP hydrolyses substance P and other tachykinins in vitro (Kerr and Kenny, 1974). This peptidase cleaves substance P at different sites but mainly between the 9 and the 10 positions, generating NH2-terminal fragments that are inactive or much less potent than substance P on airway smooth muscle in guinea pigs and other species (Mizrahi et al., 1985, Sekizawa et al., 1987) as well as on the majority of systems (Mizrahi et al., Watson, 1984). Phosphoramidon is a potent and selective inhibitor of NEP with a nanomolar affinity constant (Hudgin et al., 1981) (Fig. 5).

From the particle sizes that were generated by the ultrasonic nebulizer, we can predict that in our study, aerosolized phosphoramidon as an aerosol, it is likely that we inhibited NEP-like enzyme preferentially in the conducting airways, although we cannot exclude the possibility that phosphoramidon also diffused in the blood circulation. Thus, if exogenous substance P locally administrated in the airway or endogenous tachykinins are inactivated by NEP-like enzymes in the airways, then aerosolized phosphoramidon should potentiate SP-induced effects by preventing the degradation of this peptide locally in the airways. This hypothesis is compatible with the findings that in vitro phosphoramidon decreased the rate of substance P degradation by guinea pig lung membranes and increased the binding of substance P to its receptors on bronchial membranes (Stimler-Gerard, 1987). In our study, aerosolized phosphoramidon increased neither the baseline resistance nor the response to acetylcholine. Therefore the possibility that the effects of phosphoramidon were nonspecific is unlikely.

**The role of neutral endopeptidase in tachykinin-induced bronchoconstriction**

Airway neutral endopeptidase-like enzyme modulates tachykinin-induced bronchoconstriction in vivo. Was found that aerosolized substance P has no effect on airway resistance in control anesthetized guinea pigs (Dusser et al., 1989) (Fig. 6). However, in the presence of the aerosolized NEP inhibitor, phosphoramidon, aerosolized SP produces a concentration-dependent increase in resistance (Fig. 6). Additionally, phosphoramidon potentiates the atropine-resistant responses to vagus nerve stimulation. Furthermore, after phosphoramidon, the responses to vagus nerve stimulation develop slowly and are much more prolonged than in the absence of the peptide inhibitor. Finally, the potentiating effect of phosphoramidon on the atropine-resistant response to vagus nerve stimulation is abolished by a tachykinin receptor antagonist (Dusser et al., 1989) (Fig. 6). NEP-like activity is present in guinea pig airway. We conclude from these results that airway NEP-like enzyme normally prevents the bronchoconstriction from occurring after the administration of aerosolized substance P and that airway NEP-like enzyme modulates the effects of endogenous tachykinins released by noncholinergic vagus nerve stimulation in vivo in guinea pigs (Dusser et al., 1989).

As opposed to intravenous injection of substance P, which may induce bronchoconstriction at very low concentrations (Andersson and Persson, 1977), Dusser, 1989 shows...
that in the absence of phosphoramidon, he was unable to provoke an increase in airway resistance with concentrations of aerosolized substance P as large as 104M (Fig. 7). The inability to provoke bronchoconstriction by substance P, given as an aerosol, was also observed in humans (Fuller et al., 1987). However, our study demonstrates that by inhibiting airway NEP-like enzyme, aerosolized substance P induces a potent and longlasting increase in resistance in a concentration-dependent fashion. From our findings, we conclude that under normal conditions, airway NEP-like enzyme cleaves substance P very efficiently, preventing significant concentrations of the active exogenous peptide from binding to the smooth muscle receptors. Although it is difficult to estimate the concentrations of substance P present at the airway smooth muscle receptors after the administration of seven breaths of aerosolized substance P, it is likely that they were much lower than the concentrations of the aerosolized solution. Thus, from the effect of phosphoramidon on the response to aerosolized substance P, we infer that the NEP-like enzyme might also modulate the effect of low concentrations of endogenously released tachykinins. Therefore, we also studied the effects of aerosolized phosphoramidon on the noncholinergic responses to vagus nerve stimulation (Fig. 7). The increase in airway resistance induced by vagus nerve stimulation in the presence of atropine in the guinea pig has been previously reported to be related to the release of endogenous tachykinins (Dusser et al., 1989). This conclusion was based on the demonstration that these responses were completely abolished when guinea pigs were pretreated with (D-Arg1,D-Pro2,D-Trp7,9,Leu1)-SP, atachykinin antagonist, as well as when the animals were pretreated with capsaicin, which induced a depletion in capsaicin-sensitive SP-containing nerves (Lundberg and Saria, 1983, Lundberg et al., 1983). These suggest that the effect of phosphoramidon was mediated via tachykinins and that airway NEP-like enzyme modulates the effects of endogenous tachykinins released by vagus nerve stimulation. (D-Arg1,D-Pro2,D-Trp7,9, Leu1)-SP is a competitive antagonist that inhibits SP-induced bronchoconstriction (Lundberg and Saria, 1983). This antagonist inhibits both types of tachykinin receptors, the SP-P and SP-E type receptors and, therefore, is not specific for substance P but also inhibits other tachykinins (Watson, 1983). Because nerve endings contain substance P and other tachykinins all of which may be cleaved by NEP (Matsas et al., 1984), it is impossible to determine from these results which endogenous tachykinins are modulated by airway NEP-like enzyme.

**CONCLUSION**

Summarizing results obtained in our studies investigated the neurogenic inflammation induced by neuropeptides and the role of neutral endopeptidase in a modulation of tachykinins-induced bronchial responses, the following conclusive remarks could be drawn:

- substance P and other tachykinins have potent effects on airway smooth muscle, gland secretion and blood vessels,
- electrical stimulation of the nerves to airway smooth muscle or chemical stimulation with capsaicin causes nonadrenergic, noncholinergic contraction of airway smooth muscle, effects that are abolished by a tachykinin antagonist,
- in airways, neutral endopeptidase is located on the surfaces of multiple cells including epithelium, smooth muscle, glands and nerves, thus providing multiple potential sites for degrading neuropeptides when they are released,
- airway epithelium modulates the tissue responses to endogenous tachykinins released by electrical field stimulation or capsaicin suggesting that the relaxing factor (neutral endopeptidase, prostaglandine E2) in epithelium is important in modulation of tachykinin-induced neurogenic inflammation. On the base of our results, we hypothesize that the sensory nervous system and its neurogenic inflammatory responses of smooth muscle, blood vessels and glands secretion act as follows: a large number of foreign substances and irritants in the airways stimulates the airway afferent sensory nerves involving the axon reflex to release substance P and other related neuropeptides - tachykinins. 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 tends to dilute the stimulus. Because of the presence of neutral endopeptidase, the tissue responses are small and, unless the stimulus is great, not clinically manifest. However, when neutral endopeptidase is down-regulated or inactivated (e.g. by viral infection), stimuli that activate the sensory nerves now produce exaggerated responses because substance P and related tachykinins are not cleaved, and the responses may become clinically evident.

**Fig. 7.** Dose-response curves to aerosolized substance P (7 breaths) in the absence (open circles) or 15 minutes after the administration of aerosolized phosphoramidon (10^4M, 90 breaths, closed circles) in anesthetized guinea pigs (n=5 for each condition). Total pulmonary resistance (RL) is expressed as means ± 1SE. *Significant differences between control animals and animals treated with aerosolized phosphoramidon (p <. 005) (Reproduced from reference 25.)

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**Conclusions**

The study demonstrated that neutral endopeptidase (NEP) plays a crucial role in the modulation of tachykinins, specifically substance P, in the airways. NEP cleaves substance P efficiently, preventing the formation of high concentrations necessary for bronchoconstriction. The absence of NEP, either by pretreatment with capsaicin or by the use of a specific antagonist, abolished the bronchoconstrictive responses to substance P. This suggests that NEP modulates the effects of endogenous tachykinins released by airway stimulation.

- Substance P and other tachykinins are potent bronchoconstrictors.
- Neutral endopeptidase (NEP) is a critical enzyme in the airways, cleaving substance P and other tachykinins.
- The presence of NEP prevents the formation of high concentrations of active substance P, thus protecting against bronchoconstriction.
- Endogenous tachykinins released by electrical field stimulation can induce bronchoconstriction, but neutral endopeptidase modulates these responses.

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**Data Analysis**

The data was analyzed using statistical methods to determine the significance of the differences observed between control and treated groups. The results showed a significant decrease in total pulmonary resistance (RL) in the aerosolized phosphoramidon treatment group compared to the control group, indicating the role of NEP in modulating tachykinin-induced bronchoconstriction.
REFERENCES


