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AIRWAYS NEUROGENIC INFLAMMATION*

NEUROGENA INFLAMACIJA DISAJNIH PUTEVA

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Key words

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

Ključne reči

Neurogena inflamacija, Axon reflex, Bolesti pluća, Tahikinini, Supstanca P, Neurokinin A, Neutralna endopeptidaza

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, mucus 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 enkephalina-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 mecha-nism(s) involoving 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.

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 be¬en 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) ín 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 tachykinins are a group of neuropeptides that share the C-terminal sequence Phe-X-Gly-Leu-Met-NH2 (T*able 1*).

Table 1. *Chemical structure of tachykinins*

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

Present evidence suggests that tachykinins, substance P in particular, are involved in sensory neurotransmission. In sensory neurons, substance P is usually accompanied by a second tachykinin, neurokinin 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 gama-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-tachykinins present in the airways, the best studied is substance P. Since its discovery (von Euler and Gaddum, 1931), many studies have demonstrated

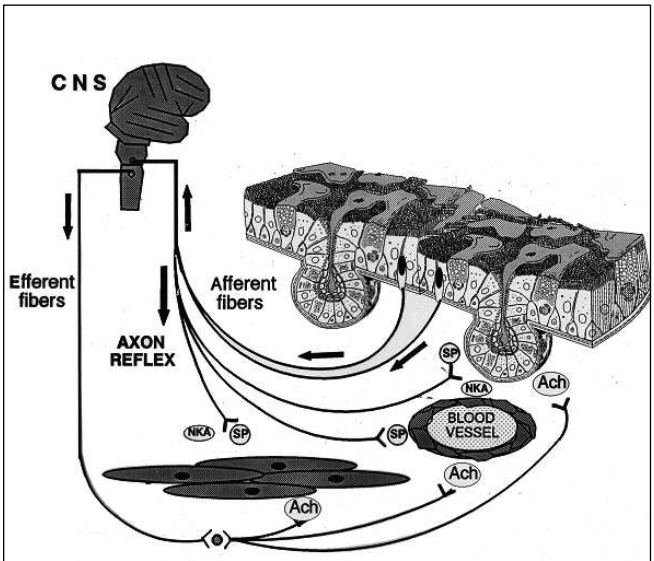

Fig. 1. *Diagrammatic model hypothesized for mechanisms of neutral endopeptidase modulation of airway smooth muscle tonus, gland secretion and blood vessels permeability to substance P (SP) and other tachykinins released from sensory nerves by axon reflex. In airways, many of the release sites of neuropeptides from the sensory nerves in the epithelium in close contact with basal cells which contain the enzyme neutral endopeptidase on their surfaces. When the nerves release substance P, cleavage and inactivation of the SP reduced the concentration of the peptides near sites of release. The remaining SP diffuses toward target cells (in the example, smooth muscles, glands and blood vessels). Neutral endopeptidase on the surface of the airway further reduces the concentration of SP and other neuropeptides in close contact with the receptors of the surface of the effector cell.*

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 po¬tentiating effects on the vagai 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 stimulati¬on 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

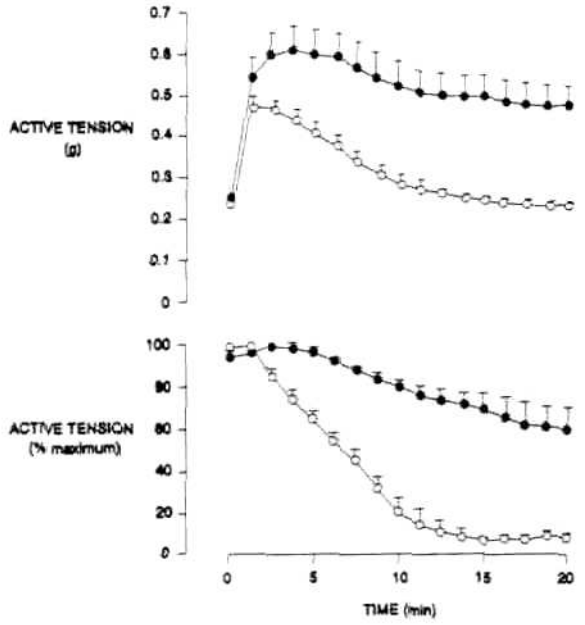

Fig. 2. *Effects of phosphoramidon on the time course and magnitude of bronchial muscle contraction and relaxation after electrical fieid stimulation (EFS, 2 Hz.) in five guinea pigs. The tension (mean ± 1SE) is expressed in g (upper trace) and as the percentage of the maximal tension in each tissue (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 contracti¬ons in control tissues (open circles) (Reproduced from reference 21.)*

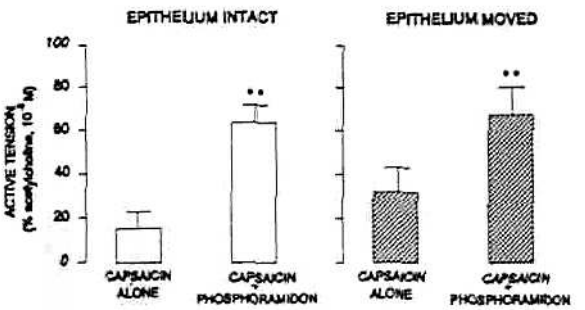

Fig.3*. Noncholinergic. nonadrenergic contractile responses to capsaicin (1.5 x 10^{-8}M) in guinea pig bronchi with and without the epithelium, in the absence and presence of neutral endopeptidase inhibitor phosphoramidon. Data are expressed as percentage of muscle contraction (mean ± 1SE) induced by 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.)*

is most likely via the release of substance P or other tachykinins from sensory nerves. Noncholinergic, nonadrenergic responses to electrical field stimulation are therefore likely to be doe 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., efferent 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 hydrolyses many different peptides, including enkephalins (*Malfroy et al.*, 1978), tachykinins (*Almenoff and Orlowski*, 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 et al.*, 1985). Thus, much of the specificity and degree of action is determined by the selectivity of the peptide released and by the location of the peptidases. Airway smooth muscle responses to substance P, neurokinin A and neurokinin Â (*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 tachykinins in airways. A recent study demonstrated that i.v. phosphoramidon potentiated capsaicin-induced bronchoconstriction (*Thompson and Sheppard*, 1988, *Dusser et al.*, 1989). Finally, because substance P-induced secretion is potentiated by NEP inhibitors, glandular NEP plays a significant role in modulating airway secretion (*Borson et al.*,1987).

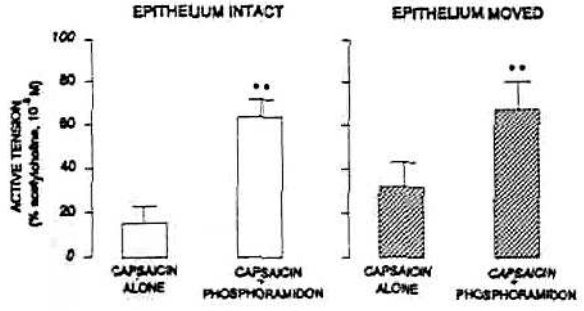

Fig. 4 *Effects of various enzyme inhibitors on the noncholinergic, nonadrenergic contractile response to EFS at 2 Hz in bronchi from five guinea pigs. Data are expressed as the percentage of the control contraction (mean ± 1SE) induced by EFS in each tissue. Captopril, bestatin, leupeptin and physostigmine (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.)*

Neutral endopeptidase is a membrane-bound peptidase that was initially identified in the kidney (*Kerr and Kenny*, 1974) and later in the brain (*Malfroy et al.*, 1978). Subsequently, enzymes from the two sources were shown to be identical (*Malfroy and Schwartz*, 1982). NEP-like activity is present in the lungs and airways of guinea pigs (*Sheppard, et. al.*, 1988) and other species including humans

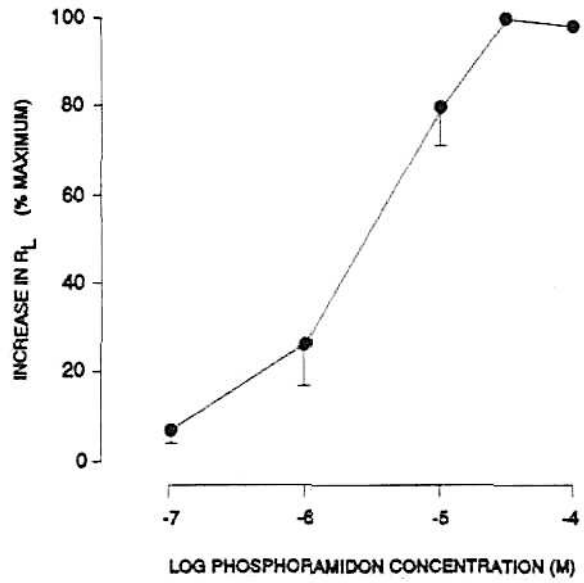

***Fig. 5**. Effects of administration (90 breaths) of increasing concentrations of aerosolized phosphoramidon on aerosolized substance P-induced bronchoconstrictor responses (10^{-4}M , 7 breaths) in 5 anesthetized guinea pigs. Total pulmonary resistance (RL, means ± 1SE) is expressed as percent of maximum increase in RL above response observed without phosphoramidon. Maximum effect of phosphoramidon was observed at 3x 10^{-5}M in every animal (Reproduced from reference 25.).*

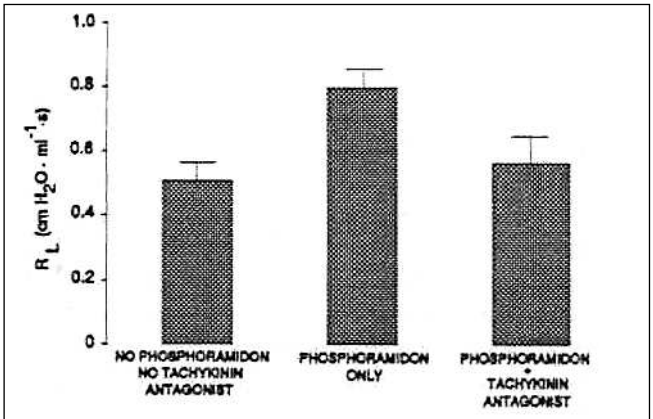

***Fig. 6.** Effects of tachykinin antagonist [D-Arg1, D-Pro2, D-Trp7,9, Leu11]-substance P on potentiation by phosphoramidon of atropine-résistant response to vagus nerve stimulation m anesthetized guinea pigs. Response to vagus nerve stimulation (10 V, 2 Hz, 5 ms for 20 s) was measured either 15 minutes after administration of aerosolized 0.9% NaCl solution (90 breaths) alone or 15 minutes after administration of aerosolized phosphoramidon (10^{-4}M, 90 breaths) in absence and in presence of pretreatment by [D-Arg1, D-Pro2, D-Trp7,9, Leu11]-substance P. Total pulmonary resistance (RL) is mean ± 1SEM of data collected from 5 guinea pigs in each condition. * Significant differences ($p<.01$) between animals treated with phosphoramidon alone and the 2 other groups. No difference existed between responses observed in animals treated with phosphoramidon in presence of tachykinin antagonist and control animals ($p>.5$) (Reproduced from reference 25.)*

(*Borson et. al*., 1986, *Johnson et. al*., 1985, *Kenny et. al*., 1985, *Llorens and Schwartz*, 1981, *Sekizawa et. al.,* 1987). 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. a1*., *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, the aerosolized substances that reached the lungs deposited mainly in the airways (*Bates et. al.,* 1966). Therefore, by using 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 administered 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 endopeptidaselike 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-résistant 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 peptidase inhibitor. Finally, the potentiating effect of phosphoramidon on the atropine-résistant 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 a1*., 1989).

As opposed to intravenous injection of substance P, which may induce bronchoconstriction at very low concentrations (*Andersson and Persson*, 197.), *Dusser,*1989) shows

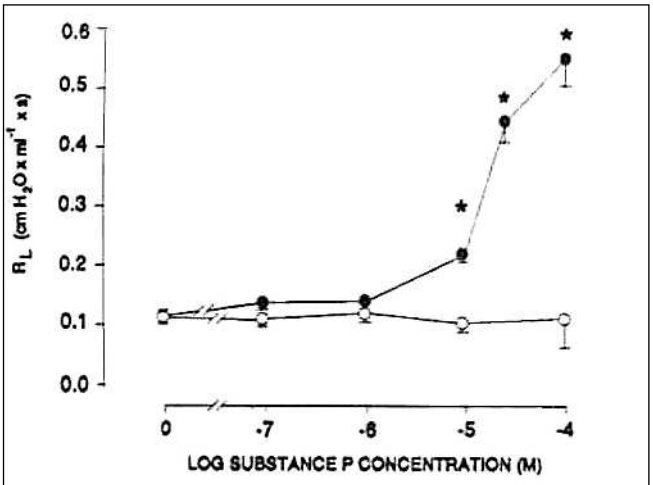

Fig. 7. *Dose-response curves to aerosolized substance P (7 breaths) in the absence (open circles) or 15 minutes after the administration of aerosolized phosphoramidon (10^{-4}M, 90 breaths, closed circles) in anesthetized guinea pigs (n=5 for each condition). Total pulmonary resistance (RL) is expressed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

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 in¬ability 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-Argl,D-Pro2,D-Trp7,9,Leul 1)-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 vagai nerve stimulation. (D-Argl,D-Pro2,D-Trp7,9, Leull)-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.

Apstrakt

U disajnim putevima je, ne tako davno, otkrivena treća komponenta autonomnog nervnog sistema neholinergični-neadrenergični nervni sistem, kao i nova klasa supstanci neuropeptidi. Iritansi kao što je prašina i brojne hemijske supstance stimulišu senzorna nervna vlakna na oslobađanje supstance P (SP) i SP-sličnih neuropeptida (neurokinin A). SP i neurokinin A pripadaju subklasi neuropeptida nazvanih tahikinini koji su lokalizovani u C-vlaknima disajnih puteva čoveka i više životinjskih vrsta. Tahikinini poseduju sposobnost da deluju na mnoge tkivne sisteme u disajnim putevima (glatki mišić, žlezde, krvni sudovi) i uzrokuju bronhokonstrikciju, sekreciju mukusa i ekstravazaciju plazme. Ovi efekti su nazvani "neurogena inflamacija". Na površini ćelija koje sadrže receptore za SP i njoj slične neuropeptide, postoji enzim pod imenom neutralna endopeptidaza (NEP, Enkefalinaza, EC 3.4.24.11). NEP biološki inaktivira neuropeptide i time reguliše koncentraciju neuropeptida i njihov efekat na glatki mišič, žlezde i druge ćelijske sisteme. Cilj ovog rada je da prezentira ulogu neurogene inflamacije u svetlu najnovih svetskih saznanja uključujuči i autorove radove, i predloži mehanizme u kojima SP-senzorni nervi mogu imati značajnu ulogu u patogenezi bolesti pluća. Sumirajući rezultate dobijene u našim brojnim istraživanjima, utvrđeno je, da: a) SP i SP-slični tahikinini poseduju snažan efekat na glatki mišić, sekreciju žlezda i krvne sudove, b) električna stimulacija senzornih nerava ili hemijska stimulacija kapsicinom uzrokuje neholinergičnu – neadrenergičnu kontrakciju disajnih puteva, efekat koji je moguće blokirati tahikinin-antagonistima, c) intaktni epitel disajnih puteva modulira tkivne odgovore na endogene tahikinine što ukazuje da relaksantni faktori (NEP, prostaglandin E2) u epitelu poseduju značajnu ulogu u modulaciji tahikinin-indukovane neurogene inflamacije.

REFERENCES

1. *Almenoff', Y., Orlowki. M.* : Membrane-bound kidney neutral metalloendopeptidase: Interaction with synthetic substances, natural peptides, and inhibitors. Biochemistry, 1983; 22, 590-599.

2. *Barnes PJ..*: Neurogenic inflammation in airways. Int Arch Allergy Appl Immunol. 1991;, 94: 303-309

3. *Barnes P.J.*: Neurogenic inflammation in the airways, NeuroImmune Biology. 2003; Volume 3, 437-446

4. *Butler, C.A., Liam G. Heaney, L,.G.*: Neurogenic Inflammation and Asthma ,Inflammation & Allergy - Drug Targets .2007; Volume 6, Number 2, , pp. 127-132(6)

5. *Babior. B.M.)*: Oxygen-dependent microbial killing by phagocytes, N. Engl. J. Med., 1978; 298, 659-668.

6. *Bigbi. T.D.. Nadel, J.A.*: In: Gallin, J. I., Goldstein, I.M., Snyderman, R., eds. Inflammation:basic principles and clinical correlates. New York: Raven Press, 1987; 679-694.

7. *Borson, D.B., B.Malfroy, M. Gold, J. Ramachandran, and Nadel,J.A.*: Tachykinins inhibit enkephalinase activity from tracheas and lungs of ferrets (Abstract). Physiologist, 1986; 29, 174.

8. *Borson, D.B., J.J. Brokaw, K. Sekizawa, D. M. McDonald, and J.A. Nadel* : Neutral endopeptidase and neurogenic inflammation in rats with respiratory infections, J. Appi. Physio!, 1989; 66, 2653-2658.

9. *Borson, D. B., JJ. Brokaw, K. Sekizawa. D. McDonald, and J.A. Nadel* : Virai infection increases permeability response to substance P (SP) by decreasing tracheal neutral endopeptidase (NEP), FASEB J., 1988; 2, 1382.

10. *Borson. D.B., R. Corrales. S. Varsana, M. Gold, N. Viro. G. Caughey J. Ramachandran and J.A. Nadel*:, Enkephalinaseinhibitors potentiate substanceP-induced secretion of35S04-macromolecules from ferret trachea. Exp. Lung. Res. 1989; 12, 21-36.

11. *Bradford, M.M.* : A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Bio-chem. 1976; 72, 248-254.

12. *Brimijoin. S., Lundberg, J.M..Brodin, E., Hokfelt. T., Nilsson, G.*: Axonal transport of substance P in the vagus and sciatic nerves of the guinea-pig. Brain Res., 1980; 191, 443-457.

13. *Carp, H., and A. Janoff* : Potential mediator of inflammation.Phagocyte-derived oxidants suppress the ela stase inhibitory capacity of alpha 1-proteinase inhibitor in vitro, J.Clin. Invest.,1980; 66, 987-995.

14. *Donnerer,, J., and Holzer-Petsche,U.*: Neurogenic Inflammation in Health and Disease NeuroImmune Biology, 1980; Volume 8, pages 169-189.

15. *Djokić, Lj.T.. Djokić, T.D., Dusser, D.J.. Nadel, J.A.*: Regulation of endogenous tachykinins actions by enkephalinase-like enzyme: role of airway epithelium. Abstracts, Tenth Congress of the Yugoslav Pharmacological Society, Neum, ,1988; pp. 58.

16. *Djokić. T.D.. Borson, D.B.. Graf, P.D., Nadel. J.A.* : Neutral endopeptidase inhibitors potentiate capsaicin and electrically-induced noncholinergic contraction in guinea pig bronchi, American Review of Respiratory Disease. 1988; Vol. 137, 4 p.202.

17. *Djokić. T.D.. Dusser, D.J., Djokić. Lj., Borson. D.B.. Nadel. J.A.*: Virai infection increases airway responsivenessto substance p, capsaicin and electrical stimulation by decreasing enkephalinase. Abstracts, XIV Congress of UYPS with international participation, Beograd,1988; pp. 109, 4, 52.

18. *Djokić. T.D.. Dusser. D.J., Djokić, Lj., Borson, D.B.,Nadel, J.A.* : Decreased enkephalinase-like enzyme in viral infection increases bronchial respon-siveness to endogenous tachykinins. Abstracts. Tenth Congress of the Yugoslav Pharmacological Society, Neum,1988; pp. 59.

19. *Djokič, T.D., Dusser, D.J., Borson, D.B.. Nadel, J.A.*, : Neutral endopeptidase modulates neurotensin-induce airway contraction. Journal of Applied Physiology, 1989; 66(5): 2338-2343.

20. D*jokić, T.D., Sekizawa, K., Borson, D.S., Nadel, J.A.* : Neutral endopeptidase inhibitors potentiate substance p-induced contraction in rat and ferret ileum and duodenum. American Journal of Physiology, Gastrointett. Liver Physiology, 1988; 19: 639-643.

21. *Djokić. T.D., Borson, D.B., Dusser, D.J.. Nadel, J.A.,* : Inhibitors of Neutral Endopeptidase Potentiate Electrically and Capsaicin -Induced Noncholinergic Contraction in Guinea Pig Bronchi. Journal of Pharmacology and Experimental Therapeutics, 1988; Vol. 248, No. 1: 7-11.

22. *Dooley. M.M.. and W.A. Pryor* : Free radical pathology: inactivation of human alpha-1-proteinase inhibitor by products from the reaction of nitrogen dioxide with hydrogen peroxide and the etiology of emphysema, Biochem. Biophys. Res. Commun., 1982 ; 106, 981-987.

23. *Dusser. D.J.. Jacoby. D.B.. Djokić. T.D.. Borson. D.B. Nadel. J. A.*: Virus induces airway hyperres-ponsiveness to tachykinins: role of neutral endopeptidase. J. Appi. Physio!., 1989; 67(4), 1504-1511.

24. *Dusser D.J. Djokić T.D. Borson, D.B. and Nadel. J.A.*: Free radicals from cigarette smoke induce bronchoconstrictorhyperresponsivenessto substance p by inactivating airway neutral endopeptidase. Fasseb Journal, 1989; 3: A534.

25. *Dusser. D.J.. Vmeno. E.. Craf. P.D.. Djokić. T.D., Borson. D.B., and Nadel J.A.*: Airway neutral endo-peptidase-like enzyme modulates tachykinin-induced bronchoconstriction in vivo. Journal of Applied of Physiology, 1988; 65(6): 2585-2591.

26. *Dusser, D.J., Djokić, T.D., Borson. D.B., and Nadel, J.A.* : Cigarette smoke induce bronchoconstrictor hyperresponsiveness to substance p and inactivates airway neutral endopeptidase in the guinea pig. Jou¬rnal of Clinical Investigation, Volume, 1989; 84:900-906.

27. *Dusser, D.J., J.A. Nadel.. K. Sekizawa, P.D. Graf. and D.B. Borson* : Neutral endopeptidase and angiotensin converting enzyme inhibitors potentiate kinin-in-duced contraction of ferret trachea. J. Pharmacol. Exp. Ther.1988; 244, 531-536.

28. *Empey. D.W.. L.A.. Laitinen. L. Jacobs, W. M., Gold, and J. A.. Nadel* :, Mechanisms of bronchial hyper-reactivity in normal subjects following upper respiratory tract infection. Am. Rev. Respir. Dis. 1976; 113, 131-139.

29. *Fuller. R.W., Dixon, C.M.S., Barnes, P.J.* ; Bronchoconstrictor response to inhaled capsaicin in humans. J. Appi. Physiol., 1985; 85, 1080-1084.

30. *Gafford, J.T., Skidgel, P.A., Eraos, E.G.. Hersh, L.B.*: Human kidney "enkephalinase", a neutral endopeptidase that cleaves active peptides. Biochemistry, 22, 3265-3271.

31. *Camse. R.,Molnar. A.,Lembeck, F.)*: Substance P release from spinal cord slices by capsaicin. Life Sci., 1979; 25, 629-636

32. *Guy F. Joos, G.F. and Pauwels, R.A.*: Pro-inflammatory effects of substance P: new perspectives for the treatment ofairway-diseases?Trends-in-Pharmacological-Sciences,-2000;131-133.

33. *Camse, R., Holzer, P. Lembeck.F.*: Decrease of substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. Br. J. Pharmacol., 1980; 68, 207-213.

34. *Calvan, L., C.-H.Huang. A.W. Prestayko, J. T. Stout. J.E. Evans, and S.T. Crooke* : Inhibition ofbleo-mycin-induced DNA breakage by Superoxide dismu-tase, Cancer Res., 1981; 41, 5103-5106.

351. *Hudgin, R.L., S.E. Charleson, M. Zimmerman, R. Mumford, and P.L. Wood* : Enkephalinase: selective peptide inhibitors. Life Sci.1981; 29, 2593-2601.

36. *Jancso. G.. Kirali. E.. and Jancso-Cabor.A.*): Phannacologically induced selective degeneration of chemo-sensitive primary sensory neurons. Nature (Lond.),1977; 270, 741-743.

37. *Johnson, A.R.. J. Ashton. W.W. Schulz, and E.G. Eraos* : Neutral metalloendopeptidase in human lung tissue and cultured cells. Am. Rev. Respir. Dis. 1985 ; 132, 564-568.

38. *Kawaguchi, Y., Hoshimaru, M., Nawa, H., Nakanishi, S.* : Sequence analysis of cloned cDNA for rat substance P precursor: existence of a third substance P precursor. Biochem Biophys Res. Commun, 1985; 139, 1040-1046.

39. *enny. A.J., M.A. Bowes, N.S. Gee, and R. Matsas.* : Endopeptidase-24.11 : a cell-surface enzyme for metabolizing regulatory peptides. Biochem. Soc. Trans. 1985; 13:293-295.

40. M.A.. and A.J. Kenny : The purification and specificity of a neutral endopeptidase from rabbit kidney brush border. Biochem. J. 1974; 137, 477-488.

41. *Kohrogi. H.. P.D. Graf, K. Sekizawa. D. B. Borson, and J.A. Nadel* : Neutral endopeptidase inhibitors potentiate substance P. And capsaicin-induced cough in awake guinea pigs. J. Clin. Invest. 1988; 82, 2063-2068.

42. *ttle, J.W., W.J. Hall, R.G. Douglas. Jr., G. S. Mudholkar. D.M. Speers, and K. Palei* : Airway hyperre-activity and peripheral airway disfunction in influenza A infection. Am. Rev. Respir. Dis1978 ; 118, 295-303.

43. *Lrens, C., and J.-C. Schwartz* : Enkephalinase activity in rat peripheral organs, Eur. J. Pharmacol.,1981; 69, 113-116.

44. *Lorens. C.B., B. Malfray, J.-C. Schwartz, G. Gacel, B. P. Roques, J. Roy, L. Morgat, F. Javoy -Agid, and Y. Agid*: Enkephalin dipeptidyl carboxypeptidase (enkephalinase) activity: selective radioassay properties, and regional distribution in human brain. J.Neurochem.1982; 39, 1081-1089.

45. *Lubereg, J.M., A. Saria, E. Brodin, S. Roseti, and K. Folkers*: A substance P antagonist inhibits vagally induced increase in vascular permeability and bronchial smooth muscle contraction in the guinea pig. Proc. Nati. Acad. Sci., USA, 1983; 80, 1120-1124.

46. *Lundberg, J.M., T. Hokfelt. C.-R. Martling, A. Saria, and C. Cuello* : Substance P-immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. Cell. Tissue Res., 1984; 235, 251-261.

47. *Malfroy, B., and J.-C. Schwartz.*: Properties of "enkephalinase" from rat kidney: comparison of dipeptidyl carboxypeptidase and endopeptidase activity. Biochem. Biophys. Res. Commun.1982 ; 106: 276-285.

48. *Malfroy, B.. P.P. Schoßeid, W.-J. Kuang. P. H. Seeburg. A.J. Mason, and W. Henzel(W¡)*, Molecular cloning and amino acid sequence of rat enkephalinase, Biochem. Biophys. Res. Commun., 1988; 144, 59-66.

49. *Malfroy, B., J.P. Swerls. A. Guyon. B.P. Roques, and J.-C. Schwartz*: High-affinity enkephalin-degrading peptidasein brain is increased after morphine. Nature Lond., 1978; 276, 523-526.

50. *Motsas. R., A. J. Kenny, and A.J. Turner* :The metabolism ofneuropeptides,the hydrolysis ofpeptides, including enkephalins, tachykinins and their analogues, by endopeptidase-24.11. Biochem. J., 1984; 223, 433-440.

51. *McDonald. D.M.*: Respiratory tract infections increase susceptibility to neurogenic inflammation of the rat trachea. Am. Rev, Respir. Dis., 1988; 137, 1432-1440.

52. *Minor. T.E., E.C. Dick. A.N. Demeo. J.J. Ouelette, M. Co hen, and C.E. Reed* : iruses as précipitants of asthmatic attacks in children. J.Am. Med. As-soc.,1974 ; 227,292-298.

53. *Mizrahi, J., S. Dion, P. D'Orléans-Juste, E. Escher. G. Drapeau, and D. Regoli.*: Tachykinin receptors in smooth muscles: a study with agonists (substance P, neurokinin A) and agonists. Eur. J. Pharmacol.1985; 118: 25-36.

54. *Nawa.H., Hirose.T.. Takashima.H., Jnayama.S.. Nakanishi, S.*): Nucleotide sequences of cloned cDNA for two types of bovine brain substance P precursor. Nature, 1983; 306, 32-36.

55. *Nilsson. G.. Dahlberg. K., Brodin. E.. Sundler. F., Strand berg. K.*: Distribution and constrictor effect of substance P in guinea-pig tracheobronchial tissue. In Substance P, ed. by U.S. von Euler and B.Pemow, Raven Press, New York, 1977; 57-61.

56. *Nadel, J.A., Djokić, T.D., Djokič, Lj.T., Börsen, D.B* : Enkephalinase inhibitor potentiates neurotensin-induced contraction in guinea pig bronchi. F ASEB Journal, 1988; Vol. 2, 5, p. 1383.

57. *Nadel JA.*: Neutral endopeptidase modulates neurogenic inflammation. Eur Respir J 1991; 4: 745-754.

58. Nadel : Tachykinins inhibit enkephalinase activity from tracheas and lungs of ferrets (Abs¬tract). Physiologist 1986; 29, 174.

59. *Orlowski, M.. and S. Wilk* :, Purification and specificity of a membrane-bound metalloendopeptidase from bovine pituitaries, Biochemistry,1981; 20, 4942-4950.

60. *Pernow. B.*): Role of tachykinins in neurogenic inflammation. J.Immunol., 1985; 135, 2, 812s-815s.

61. *Petersson R.P., Ardill,J.E.S., Johnston, B.T., Heaney,L.G., Lorcan P A McGarvey, L.P.A.*: Increased tachykinin levels in induced sputum from asthmatic and cough patients with acid reflux.Thorax 2007; 62:491-495

62. *Richardson,J.D., Vasko, M.R.*,: Cellular Mechanisms of Neurogenic Inflammation The Journal of Pharmacology and Experimental Therapeutics. 2002; September 1, vol. 302 no. 3 839-8455.

63. *Saban, R.. E.C. Dick. R.I. Fishleder, and C. K. Buckner* : Enhancement by parainfluenza 3 injection of contractile responses to substance P and capsaicin in airway smooth muscle from the guinea pig. A. Rev. Respir. Dis., 1987; 136, 586-591.

64.. *Sekizawa, K., J. Tamaoki, J.A. Nadel, and D. B. Börsen* : Enkephalinaseinhibitor potentiates substance P- and Electrically induced contraction in ferret trachea. J.Appl. Physiol., 1987; 63, 1401-140J.

65. *Sekizawa. K., J. Tamaoki. P.D. Graf. C.B. Basbaum, D.B. Börsen, and J.A.Nadel* : Enkephalinase inhibitcir potentiates mammalian tachykinin-induced contraction in ferret trachea. J. Pharmacol. Exp. Ther.,1987; 243l, 1211-1217.

66. *Shechter. Y., Y. Burstein, and A. Patshornik*:, Selective oxidation of methionine residues in proteins, Bioche-miiitry, 1975; 14, 4499-4503.

67. *Sheppard, D., Thompson. J.E.. Scypinski, L., Dusser, D.J, Nadel., J.A, Börsen, D.B,* : Toluene di-iscicyanate increases airway responsiveness to substance P and decreases airway enkephalinase. J. Clin. Invest.,1988; 81, 1111-1115.

68. *Shore, S.A., N.P. Slimler-Gerard, S.R. Coals, and J.M. Draz.en* : Substance P-induced bronchoconstriction in the guinea pig enhancement by inhibitors of neutra 1 metalloendopeptidaseand angiotensin-converting en:zyme, Am. Rev. Respir. Dis., 1988; 137.,331-336.

69. *Skidgel. R.A., A. Engelbrecht, A.R. Johnson, and E.G. Eraos* : Hydrolysis of substance P and neurotensin by converting enzyme and neutral endoproteinase, Peptides (NY), 1984; 5, 769-776.

70. *Slephenson, S.L., Kenny, A.J.*): Metabolism of neuro peptides. Biochem. J., 1987; 241, 237-247.

71. *Stimler-Gerard, N.P.* : Neutral endopeptidase-like enzyme controls the contractile activity of substance P in guinea pig lung, J. Clin. Invest., 1987; 79, 1819-1825.

73. *Stimler-Gerard. N.P.* : Neutral endopeptidase-like enzyme controls the contractile activity of substance P in guinea pig lung. J. Clin. Invest., 1987; 79, 1819-1825.

74. *Tanaka. D.T., and M.M.* Grunsìein : Mechanisms of substance P-induced contraction of rabbit airway smooth muscle, J. Appi. Physiol., 1984; 57, 1551-1557.

75. *Thompson, J.E. and Sheppard.D.* (I988): Phosphoramidon potentiates the increase in lung resistance mediated by tachykinins in guinea pigs. Am. Rev. Respir. Dis.1988 ; 137:337-340.

76. *Turner, A.J., Matsas, R., Kenny, A.J.* (1985): Are there neuropeptide-specific peptidases? Biochem., Pharmacol.,34, 1347-1356.

77. *Watson,* S.P.):Are the proposed substance P receptor subtypes, substance P receptors? Life Sci., 1984; 25, 797-808.

78. *William J. Meggs*, W.M..: Neurogenic Inflammation and Sensitivity to Environmental ChemicalsEnvironmental Health Perspectives, 1993; Volume 101, Number 3, 243-248.

79. *von Eule*r, U.S., Gaddum. J.H): An unidentified depressor substance in certain tissue extracts, J. Physiol. (Lond.), 1931.;72, 74-87.

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