ORIGINAL CONTRIBUTION

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Mediator release of neuropeptides after nasal provocation in perennial allergic rhinitis patients*

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SUMMARY

Background: *Neuropeptides may play a role in allergic rhinitis including development of vasodilation and vascular leakage, which may result in rhinorrhea and congestion. While neuropeptide release during the immediate allergic response is well known, the role of neuropeptides in the late phase of allergic responses is less well defined.*

Methods: *Eleven subjects with dust mite allergy induced allergic rhinitis were compared to 5 healthy control subjects using nasal allergen provocation. Nasal lavage fluid was analyzed for Substance P, bradykinin, and total protein.*

Results: *Both bradykinin and substance P levels increased in nasal lavage fluid immediately after dust mite allergen challenge of dust mite allergic subjects, the magnitude of increase of both neuropeptides being significantly correlated. There was a greater increase in substance P versus bradykinin 4 to 6 hours after allergen challenge, with a lack of correlation between the late phase increases of these two neuropeptides. The bradykinin increases correlated with the increase in total protein in the nasal lavages of the allergic subjects, whereas the increases in substance P did not correlate with the total protein in the nasal lavages. An increase in nasal eosinophils was only seen in the allergic subjects after allergen provocation.*

Conclusion: *Both bradykinin and substance P appear in nasal lavage fluid 4 to 6 hours after allergen challenge of dust mite allergic subjects, suggesting a role for the neuropeptides in late phase allergic events.*

Key words: bradykinin, nasal lavage fluid, substance P

BACKGROUND

In genetically susceptible individuals, exposure to airborne allergens results in sensitization with the production of specific IgE, migration of inflammatory cells and release of inflammatory mediators. The involvement of inflammatory cells including mast cells, eosinophils, neutrophils, basophils, dendritic cells, and lymphocytes in this process is associated with development of allergic inflammation. Although it is clear that histamine is a major mediator of allergic rhinitis, other mediators such as leukotrienes (LTB4, LTC4), prostaglandins (PGD2, PGF2), neuropeptides (substance P, neurokinin A, calcitonin gene-related peptide), kinins (bradykinin), cytokines (IL-4, IL-5, IL-6, IL-8, IL-10, IL-13) and chemokines play a role.

The neuropeptides and kinins can cause vasodilatation, increased vascular permeability, bronchoconstriction and inflammatory cell migration. Animal studies have demonstrated that neuropeptides have a great potential biologic role in inflammatory mechanisms in the nasal mucosa. There is evidence of release of neuropeptides after allergen provocation during the early phase of allergic responses. Recently, it has been possible to observe mediator release in nasal secretions. Nasal lavage of allergic rhinitis patients performed before and after allergen provocation can detect mediators such as histamine, kinins, leukotrienes LTC4 and LTB4, and TAME-esterase activity, allowing investigation of the pathophysiology of allergic rhinitis [1,2].

These mediators applied alone may provoke clinical symptoms connected with the immediate allergic response including sneezing, rhinorrhea, and pruritis [3,4]. Recent studies [5] using an animal model have demonstrated that substance P released from the nasal mucosa consequent to allergen challenge activated the kinin receptor NK1 and induced allergic nasal symptoms. Histopathology studies have confirmed the presence of regulatory peptide-associated immuno-reactivity in the human nasal mucosa. Peptide immuno-reactive nerve fibers and vasoactive intestinal peptide (VIP), calcitonin gene related peptide (CGRP), substance P, and neurokinin A have been detected in human nasal mucosa [6]. Depolarization of immuno-peptide reactive nerves leads to release of neuropep-

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tides such as substance P and calcitonin gene related peptide [7]. Substance P is a vasodilator, causes vascular leakage, and stimulates mucus secretion from submucosal glands [8-10]. Substance P can induce release of histamine from mast cells [11,12]. Release of histamine and increased vascular permeability are linked to depolarization of sensory nerves [8,13].

Allergic inflammation is thought to stimulate non-adrenergic non-cholinergic nerves. Released neuropeptides may be involved in symptoms of allergic rhinitis. Following allergen exposure, the early allergic response subsides within minutes, but can often be followed hours later by a second response, the late allergic response. Local tissue accumulation of eosinophils, basophils, neutrophils and increased concentration of mediators (IL-4, IL-5, IL-13, VCAM-1, and eotaxins) can be observed during the allergic response. There is no direct evidence that substance P and bradykinin are released into nasal secretions in the human nose several hours after allergen provocation. The aim of this study was to examine the concentration of substance P and bradykinin in nasal lavage fluid 6 hours after allergen provocation.

METHODS

Subjects

Eleven subjects (6 female and 5 male) having a mean age of 23.6 ± 3.5 years were investigated. Six patients (3 female and 3 male) age 24.8 ± 3.6 years had perennial allergic rhinitis confirmed by allergen specific IgE to *Dermatophagus pteronyssinus* (> class 2, UniCAP, Phadia, Uppsala, Sweden) and positive skin prick test with a biologically standardized extract of *D. pteronyssinus* (50000 SBU/ml) and negative skin prick tests with other inhalant allergens (Allergopharma, Rheinbek, Germany). Positive skin prick area of the wheal response was at least as large as that of the positive control, histamine 1 mg/ml. All 6 investigated patients experienced rhinitis symptoms immediately and 3 to 10 hours after exposure to house dust mites. All allergy medications were withheld for 2 weeks before the study.

The control group consisted of 5 healthy volunteers (3 female and 2 male) mean age 22.4 ± 2.1 years with negative skin prick tests to inhalant allergens and undetectable or low concentrations (class < 2, UniCAP) of allergen-specific IgE directed to *D. pteronyssinus*.

Informed consent was obtained from all subjects. The study was approved by the local institutional review board of the medical university.

Provocation test

Nasal allergen provocation using an allergen extract of *D. pteronyssinus* (5000 BU/m, Allergopharma) was performed according to the method of Naclerio et al. [14]. Four lavages of each nostril using 5 ml of 0.9% NaCl at room temperature were performed to reduce baseline mediator levels to a stable level. A diluent provocation was then performed. Subsequently 5000 BU of allergen extract was administered into the same nostril by a hand-held nebulizer bottle, which provided 0.1ml per puff. Each challenge was followed by lavage with 0.9% NaCl at room temperature performed at 5, 15, and 30 minutes, and at 1, 2, 4, and 6 hours after the nasal challenge. Nasal lavage fluid was collected into tubes positioned on the same side of the anterior nasal septum as the challenge was performed.

The nasal lavage fluid was immediately put on ice, phosphoramidon added, and centrifuged at 3600g for 10 minutes at 4°C to remove cellular fragments and mucus and then stored at –70°C until analysis. The cell pellet was stained with May-Grünwald-Giemsa and inspected by light microscopy.

Substance P and protein measurement

The concentration of substance P in nasal lavage fluid was determined by RIA (Incstar, Stillwater, USA). The concentration of bradykinin was determined by the method of Proud et al. [15]. Total protein was assayed in each nasal lavage sample by the Lowry method and the neuropeptide to total protein ratio was calculated.

Statistics

Statistical analysis was performed using Statistica for Windows. Wilcoxon's signed rank test was used to compare the allergen-induced mediator release at each time point. Correlations were performed using the Spearman rank method.

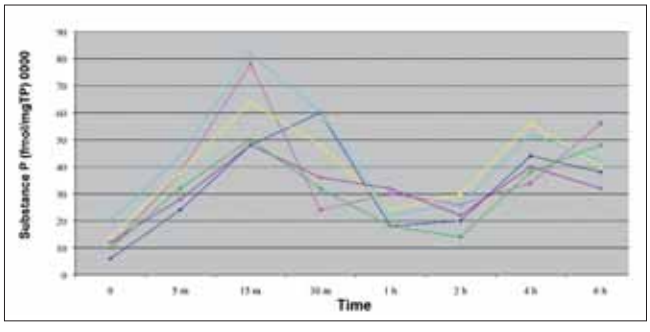

Figure 1. Concentration of substance P in nasal lavage fluid after *D. pteronyssinus* provocation in allergic rhinitis patients.

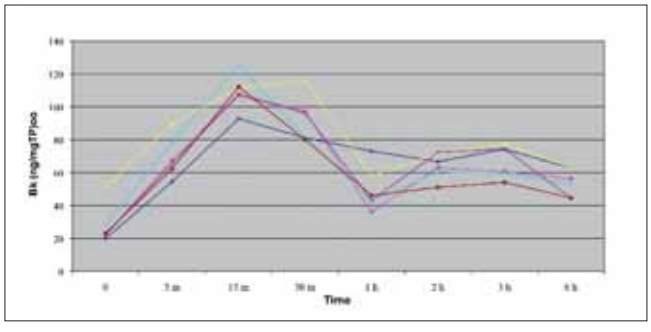

Figure 2. Concentration of bradykinin in nasal lavage fluid after *D. pteronyssinus* challenge in allergic rhinitis patients.

RESULTS

The allergen challenge induced an increase in nasal bradykinin and substance P concentrations. The volume of recovered nasal lavage fluid from allergic rhinitis patients (64.8 ± 8.2%) were comparable to controls (69.3 ± 7.5%). Compared to the levels found after diluent challenge, there was a statistically significant increase in bradykinin and substance P in the first 30 minutes after allergen provocation and a further increase after 4 hours.

Significantly greater concentrations of both substance P and bradykinin were seen in nasal lavage fluid immediately (15 minutes) after allergen provocation, with substance P levels of 61.67 ± 15.5 fmol/ml and bradykinin levels of 126.34 ± 18.67 nmol/l noted (Figures 1 and 2).

A positive correlation was seen between substance P and bradykinin levels during the immediate allergic response ($r = 0.62$, $p < 0.005$). This observation was not seen during the late phase reaction. After 4 hours, a second increase occurred in levels of bradykinin and substance P in nasal lavages, but the bradykinin increased only moderately as the level at 4 hours of substance P was 44.0 ± 8.5 fmol/ml and the level of bradykinin was 64.59 ± 11.65 nmol/l, whereas at 6 hours the level of substance P was 38.0 ± 8.3 fmol/ml and the level of bradykinin was 61.85 ± 12.54 nmol/l. The control subjects had only a slight elevation of substance P and bradykinin at 5 minutes and 15 minutes after nasal provocation (Figures 3 and 4).

A significant correlation between bradykinin and total protein was observed at 15 minutes ($r = 0.66$); 30 minutes ($r = 0.62$); 4 hours ($r = 0.72$); and 6 hours ($r = 0.68$) after nasal allergen challenge. There was no correlation between substance P and total protein at the following times: 15 minutes ($r = 0.15$); 30 minutes ($r = 0.26$); 4 hours ($r = 0.31$); and 6 hours ($r = 0.19$) after allergen challenge.

An influx of inflammatory cells accompanied the late allergic reaction. Eosinophils were present in all nasal washes from patients with perennial allergic rhinitis. The total number of eosinophils was significantly greater in perennial allergic rhinitis patients than in the control subjects (Table 1).

Table 1. Eosinophils in nasal lavage fluid after allergen challenge.

	Eosinophils (% eos/total cell count)					
Time	0	15 min	1 hour	2 hours	4 hours	6 hours
Perennial allergic rhinitis patients	24.4 ± 16.6	35.1 ± 14.8	38.6 ± 21.5	35.8 ± 24.4	44.6 ± 24.7	42.6 ± 26.5
Control group	0.1	0.2	0.1	0.1	0.1	0.2

DISCUSSION

Bradykinin is a vasoactive neuropeptide formed as a cleavage product from low and high molecular-weight kininogens by kallikreins. Because mast cell tryptase possesses kallikrein-like activity, the local generation of kinins within the nose after nasal allergen challenge may be related to mast cell degranulation [15]. Presence of bradykinin has a positive correlation with vascular permeability. Bradykinin also has a role in regulating chemokine receptors in nasal epithelial cells from patients with allergic rhinitis.

Interestingly, bradykinin stimulates production of IL-8 by cultured human airway muscle cells [16] and airway epithelial cells [17]. IL-8 is a potent stimulus for eosinophil recruitment in vitro in the human nasal mucosa [18]. Significant correlation between the levels of kinins and IL-8 in the lavage samples was observed after grass pollen challenge in one study [19].

Few studies have been performed to evaluate the possible involvement of neurogenic mechanisms in human nasal allergic inflammation.

This study was performed during a period when patients were having relatively few symptoms. The presence of eosinophils in all nasal lavages from perennial allergic rhinitis patients confirmed the presence of ongoing allergic inflammation. There were almost no eosinophils in the recovered nasal lavages from subjects in the control group.

The present study describes the nasal lavage substance P release in allergic rhinitis patients 4 and 6 hours after an aller-

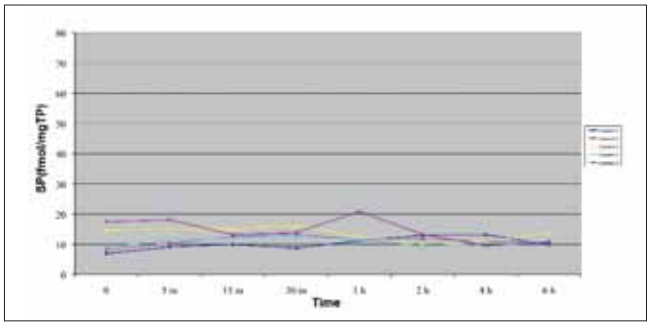

Figure 3. Concentration of substance P in nasal lavage fluid after *D. pteronyssinus* allergen challenge in controls.

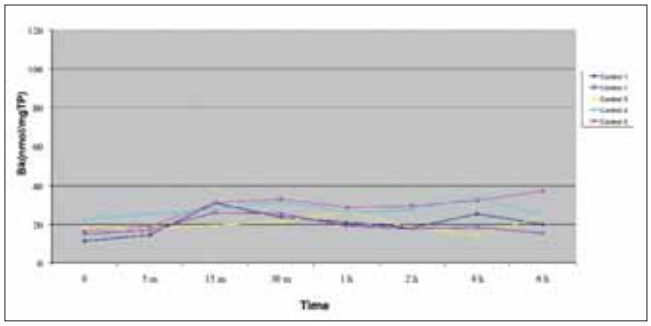

Figure 4. Concentration of bradykinin in nasal lavage fluid after *D. pteronyssinus* allergen challenge in controls.

gen challenge in perennial allergic rhinitis patients sensitized to house dust mites. However, bradykinin release during late phase allergic responses has been reported in seasonal allergic rhinitis patients [3,14]. The authors observed an increase in levels of bradykinin, although these were less than during immediate allergic responses [1]. An increase in Substance P in nasal secretions immediately after allergen challenge has also been shown in another study [2].

This study demonstrates that substance P and bradykinin can be recovered in significantly increased concentrations from nasal lavage fluid after *D. pteronyssinus* allergen challenge. This increase in levels of neuropeptides was associated with elicitation of symptoms induced by the allergic reaction. Controls challenged in the same manner did not have significantly increased substance P and bradykinin in their nasal lavage fluid or clinical symptoms. Minor changes of bradykinin concentration in nasal lavage fluid can also potentially arise from the ability of house dust mite proteases to activate the kinin-kallikrein system and generate kinins [20].

A second increase of substance P in nasal lavage fluid was observed 4 to 6 hours after allergen challenge, the substance P increase being more pronounced than the bradykinin increase. The substance P increase was not associated with changes in vascular permeability and plasma extravasation, which was associated perhaps with bradykinin formation. Total protein concentration was correlated with bradykinin levels but not with substance P levels.

Explanations of this phenomenon include the possibility of an increased number of substance P containing unmediated C-fibres in the nasal mucosa of allergic rhinitis patients who were assessed with allergen challenge [21]. Allergen inhalation induced synthesis of substance P in C nerve fibers in one study [22]. There is a study suggesting that any release of substance P by allergen or even capsaicin is too small to elicit a significant release of histamine but is great enough to release chemo attractant cytokines [23]. Allergic reactions could also lead to release of nerve growth factor (NGF), which can activate nerve terminals [24].

The intensity of the allergic inflammation caused by allergen inhalation leads to increased release of neuropeptides and an increase in plasma leakage and glandular secretion. The prominent role of a cellular component within the late phase allergic response may result in reduced changes in bradykinin concentrations from nasal lavages. For example, allergic inflammation in guinea pig bronchi leads to the induction of substance P production in large-diameter vagal sensory nerves [22]. Peripheral terminals of these nerves are insensitive to stimuli such as bradykinin and capsaicin. Although increased eosinophil numbers were observed in nasal lavage, it is unknown if there is an influence of these eosinophils on release of neuropeptides. Similar results have been reported by others who have concluded that eosinophils do not affect the release of substance P in vivo [25]. The results of this study differ from results of a study, which failed to detect any increase in substance P after allergen challenge. However, other investigations have found an increase in substance P concentration in nasal secretions a few minutes after allergen provocation [3,14,15,26].

The role of neuropeptides including substance P in allergic and non-allergic rhinitis has been further confirmed by a variety of studies [3,7,9,13,27]. Whether substance P is a principle mediator responsible for symptoms observed in patients with allergic rhinitis remains to be determined. This phenomenon has been demonstrated in a study using an animal model [5,28]. There is substantial evidence, which suggests that substance P liberated from sensory nerve endings is involved in chronic allergic and non-allergic rhinitis symptoms. Some vascular changes including vasomotor tone, may partially be due to neurokinin A co-released with substance P.

A role for substance P in inducing release of histamine from human nasal mucosa thereby connecting neurogenic and immunologic mechanisms of pathophysiological changes in nasal mucosa of allergic patients has been reported [23,27]. Substance P can modulate chemotaxis of eosinophils and neutrophils, and also proliferation and activation of B and T lymphocytes, mast cells, and macrophages [28]. Substance P, which may be released by a variety of stimuli including allergens, capsaicin, cold air, and ozone, may also augment the release of cytokines.

Neuropeptides released by intranasal capsaicin administration can provoke greater nasal symptoms during the pollen season than afterward, suggesting a relationship between neuropeptides, symptoms and allergic inflammation. Further studies are required to determine how changes in the inflammatory environment could influence bradykinin and substance P release.

CONCLUSION

This investigation demonstrates that intranasal allergen challenge performed on dust mite allergic patients induces substance P and bradykinin release not only during the early phase of the allergic response but also during the late phase response. These results suggest that both mediators play a role in allergic inflammation in the human nasal mucosa.

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