

Allergens, IgE, mediators, inflammatory mechanisms

Substance P, calcitonin gene-related peptide, and vasoactive intestinal peptide increase in nasal secretions after allergen challenge in atopic patients

Bernard L. Mosimann, MD, Martha V. White, MD, Robert J. Hohman, PhD, Michael S. Goldrich, MD, Helen C. Kaulbach, MD, and Michael A. Kaliner, MD Bethesda, Md.

Background: There is suggestive evidence that neuropeptides participate in allergic reactions. Substance P (SP) and calcitonin gene-related peptide (CGRP) are released by sensory nerves, whereas vasoactive intestinal peptide (VIP) is released mainly by parasympathetic nerves. Both sets of nerves are thought to be stimulated by allergic inflammation. The aim of this study was to assess nasal secretions to determine whether SP, CGRP, and VIP were increased after allergen challenge.

Methods: Eight patients with allergic rhinitis were challenged nasally with 1 mg histamine or increasing doses of allergen. Nasal lavages were collected into a cocktail of protease inhibitors in order to restrict neuropeptide degradation. Radioimmunoassay for SP, CGRP, and VIP were performed on each sample.

Results: All patients had immediate clinical reactions to both histamine and allergen challenges, and seven patients experienced a later allergic reaction. After histamine challenge, SP and CGRP did not increase significantly above baseline in the nasal lavages, whereas VIP did ($p < 0.02$). In contrast, SP, CGRP, and VIP all significantly increased immediately after allergen challenge and returned to baseline within 2 hours. At the clinical peak of the late allergic reaction, SP, but not CGRP or VIP, was increased slightly but significantly ($p < 0.01$).

Conclusions: Thus SP, CGRP, and VIP are found in nasal secretions after allergen challenge, which confirms that neuropeptides are released in human beings during allergic reactions. The selective stimulation of VIP secretion by histamine challenge suggests that histamine-induced cholinergic reflexes induce the release of VIP. These data support the suggestion that neuropeptides may be partly responsible for some of the nasal symptoms of allergy. (*J ALLERGY CLIN IMMUNOL* 1993;92:95-104.)

Key words: Neuropeptides, substance P, calcitonin gene related peptide, vasoactive intestinal peptide, allergic rhinitis, nasal secretion

From the Allergic Diseases Section, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda.

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Reprint requests: Michael A. Kaliner, MD, Allergic Diseases Section, Laboratory of Clinical Investigation, Building 10,

Abbreviations used

SP:	Substance P
CGRP:	Calcitonin gene-related peptide
VIP:	Vasoactive intestinal peptide
RIA:	Radioimmunoassay
LAR:	Late allergic reaction
AU:	Allergy unit

Room 11-C-205, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892.

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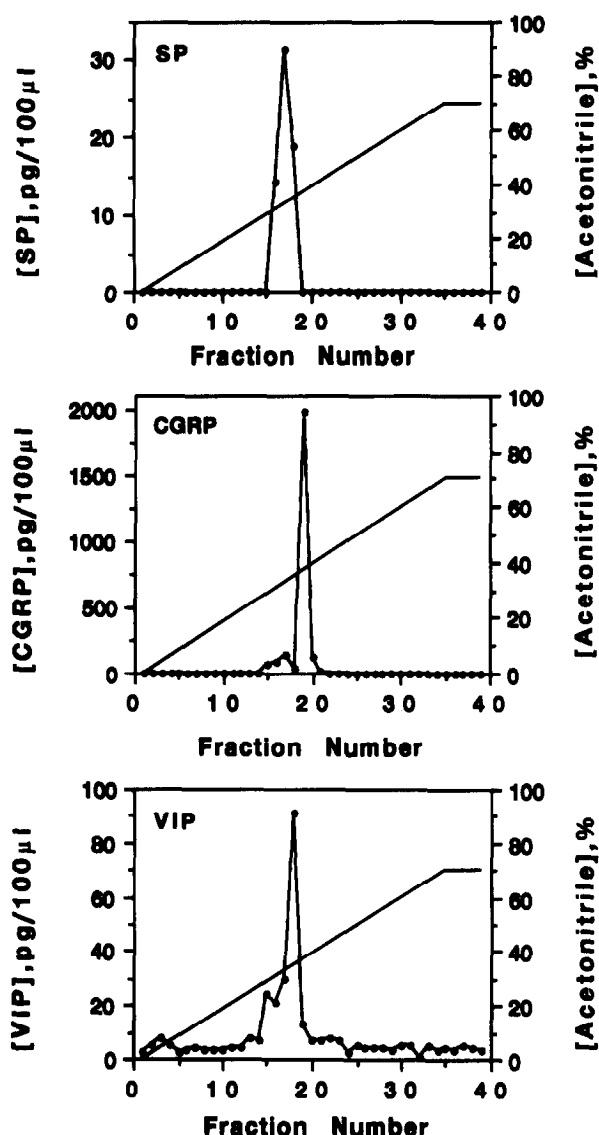


FIG. 1. High-pressure liquid chromatography elution pattern for neuropeptides added to nasal secretions and measured by individual RIA. Immunoreactivity for each neuropeptide was recovered from a narrow range of successive fractions from reverse-phase chromatography. ●—●, Neuropeptide concentration (scale on left of figure); —, acetonitrile gradient (scale on right of figure).

Neuropeptides are neurotransmitters secreted by unmyelinated nociceptive C fibers (tachykinins, calcitonin gene-related peptide [CGRP], neurokinin A, gastrin-releasing peptide), parasympathetic nerve endings (vasoactive intestinal peptide [VIP], peptide histidine methionine), and sympathetic nerve endings (neuropeptide Y). The potential roles of these mediators in airway homeostasis and disease have been reviewed recently.¹⁻⁴

Substance P (SP) is an undecapeptide of

the tachykinin family (peptides with the common C-terminal sequence Phe-X-Gly-Leu Met-NH₂). SP is often found as a cotransmitter with neurokinin A (NKA) and CGRP. In human nasal mucosa, SP-containing nerve fibers are found in high density in arterial vessels and to some extent in venous vessels, gland acini, and epithelium.⁵ SP receptors (NK₁ receptors) are located in epithelium, glands, and vessels.⁵ SP stimulates secretion from submucosal glands,⁵ increases nasal blood flow,^{6, 7} and induces microvascular leakage in postcapillary venules.^{8, 9} Whether SP and other tachykinins may induce degranulation of nasal mast cells in human beings is still uncertain.¹⁰⁻¹³

CGRP, a 37-amino-acid peptide, is a cotransmitter with SP.¹⁴ In the nose CGRP receptors are found in high concentration on small muscular arteries and arterioles.¹⁵ CGRP is probably involved in vasomotor (vasodilation) responses but has no recognized role in glandular secretion.

VIP, a 28-amino-acid peptide, is abundant in the nasal mucosa.¹⁶ The distribution of VIP-ergic fibers in human airways corresponds to the distribution of cholinergic nerves.¹⁷ In the human nose VIP receptors are located on arterial vessels, submucosal glands, and epithelial cells.¹⁶ VIP stimulates serous cell secretion,¹⁶ dilates nasal vessels,¹⁸ and may regulate mucociliary clearance.¹⁹ Antiinflammatory actions have also been attributed to VIP.²⁰

These various effects of SP, CGRP, and VIP on the nasal mucosa suggest that these mediators could participate in allergic (or nonallergic) rhinitis. The aim of this study was to determine whether increased amounts of SP, CGRP, and VIP could be recovered from nasal lavages after histamine and allergen nasal challenge in atopic patients.

METHODS

Subjects

Eight adult subjects with allergic rhinitis (four men and four women) between the ages of 29 and 41 were studied outside of their allergy season, after informed consent was obtained. No subject had symptoms of rhinitis at the time of the study, and all allergy medications had been withheld for 3 weeks before the study. Allergic rhinitis was defined clinically as symptoms of rhinitis after exposure to relevant allergens and was confirmed by positive results of skin prick tests with appropriate aeroallergens.

Nasal challenge

To preliminarily verify that neuropeptides could be recovered from nasal lavages after allergen challenge,

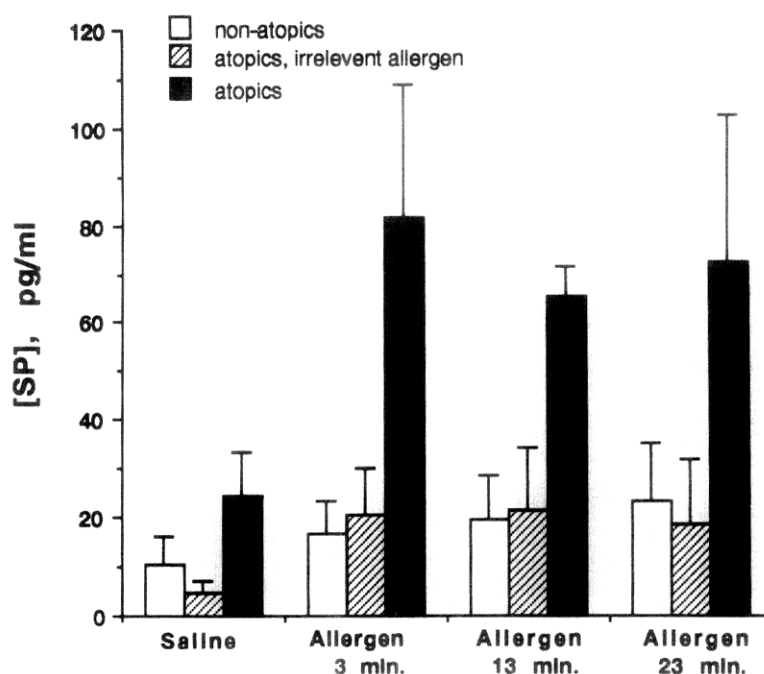


FIG. 2. SP levels in nasal lavages from four atopic patients challenged with relevant allergen and four nonatopic patients and three atopic patients challenged with irrelevant allergen. The results (mean $\pm$ SEM) are shown 3, 13, and 23 minutes after challenge with allergen (10,000 AU/ml) and 10 minutes after saline challenge.

SP was measured in nasal lavages from four atopic patients and four nonatopic patients at 3, 13, and 23 minutes after challenge with 10,000 AU/ml of allergen. SP was also measured after challenge of three atopic patients with irrelevant allergens (atopic subjects challenged with a pollen to which they were not sensitized; no positive history, negative skin tests). CGRP was measured in nasal lavages from the same four atopic patients and three of the nonatopic patients at 3, 13, and 23 minutes after challenge with 10,000 AU of allergen.

Once optimal conditions for the collection of nasal secretions had been established, nasal challenge for assessment of neuropeptide levels was performed as described previously.²¹ Briefly, nasal lavage fluid was collected through 8 F rubber catheters inserted 3 to 4 cm along the floor of the nasal cavity and attached to a suction trap. After the nasal cavity was washed with four 4 ml saline prewashes, the challenge protocol formally began. On the first day of the study the patients were challenged with saline solution and then 1 mg of histamine. On the second day, the subjects were challenged sequentially with saline and increasing concentrations of allergen (1,000, 10,000, and 100,000 AU/ml).

All nasal challenge solutions were delivered to one side of the nasal cavity from a hand-held nebulizer bottle. The volumes of the challenge solutions were 0.4 ml for histamine and 0.13 ml for allergen. Histamine challenge was followed 10 minutes later by a 4 ml saline

lavage. Saline lavages were obtained 3 and 13 minutes after allergen challenges, at 1000 AU/ml and 10,000 AU/ml. Saline lavages were obtained 3, 13, and 23 minutes and 2, 4, 6, 8, and 14 hours after the 100,000 AU/ml allergen challenge. Neuropeptides were assayed only in samples collected through 2 hours after antigen challenge unless a late allergic reaction (LAR) occurred (defined as a later recurrence of symptoms after complete resolution of the symptoms associated with the immediate reaction). The lavage corresponding to the peak of the symptomatic late reaction was then assayed for neuropeptides. Three subjects were challenged with rye grass, three with ragweed, one with oak, and one with cat.

Nasal lavage fluids were collected on ice, and a cocktail of protease inhibitors was immediately added (final concentration): captopril (Bristol-Myers-Squibb Co., Princeton, N.J.) 10 μ mol/L; phosphoramidon (Peninsula Laboratories, Inc., Belmont, Calif.) 10 μ mol/L; leupeptin (Boehringer Mannheim Corp., Indianapolis, Ind.) 10 μ mol/L; puromycin (Calbiochem Corp., San Diego, Calif.) 100 μ mol/L; and aprotinin (Calbiochem) 400 KU/ml. The nasal lavages were frozen at -70° C until they were assayed.

Clinical score

A clinical score that ranged from 0 to 12 was obtained from patients before each nasal lavage (itching 0 to 3, rhinorrhea 0 to 3, sneezing 0 to 3, and congestion 0 to 3).

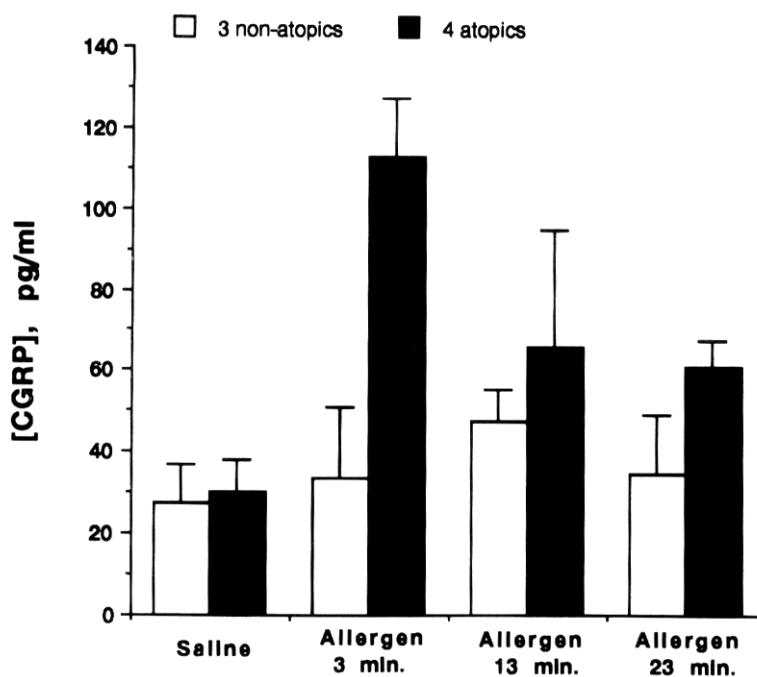


FIG. 3. CGRP levels in nasal lavages from four atopic patients challenged with relevant allergen and three nonatopic patients challenged with the same allergen. The results (mean $\pm$ SEM) are shown 3, 13, and 23 minutes after challenge with allergen (10,000 AU/ml) and 10 minutes after saline challenge.

Radioimmunoassay

Radioimmunoassay (RIA) reagents were purchased from Peninsula Laboratories and included human SP; iodine 125 [Tyr⁸]-SP, 10 μ Ci; polyclonal rabbit antiserum to SP; human CGRP; ¹²⁵I-[Tyr⁶]-CGRP, 10 μ Ci; polyclonal rabbit antiserum to CGRP; human VIP; ¹²⁵I-VIP, 10 μ Ci; polyclonal rabbit antiserum to VIP; goat anti-rabbit γ -globulin serum; and normal rabbit serum. According to the manufacturer's specifications, the SP antiserum had 74.5% cross-reactivity with SP [3-11], 12.4% with SP [4-11] and SP [5-11], and 4.8% with SP [6-11] but had very limited cross-reactivity (<0.01%) with SP [7-11], SP [8-11], SP [9-11], neurokinin A, and neurokinin B. The CGRP antiserum had a cross-reactivity of 100% with rat CGRP. The VIP antiserum had a cross-reactivity of less than 0.001% with VIP [10-28] and peptide histidine methionine.

All the reagents were diluted in RIA buffer (19 mmol/L monobasic sodium phosphate, 81 mmol/L dibasic sodium phosphate, 0.05 mol/L NaCl, 0.01% NaN₃, 0.1% bovine serum albumin, 0.1% Triton X-100, pH 7.4). Standard SP and CGRP solutions that ranged from 1 to 12 pg per tube and VIP standard solutions that ranged from 1 to 100 pg per tube were prepared. Samples and standards 100 μ l were pipetted into individual tubes to which was added 100 μ l of rabbit anti-SP, anti-CGRP, or anti-VIP (dilution 1:3). For CGRP and VIP determinations, 0.5% Triton X-100 was added to each sample. After an overnight incubation at 4° C, 0.01 μ Ci ¹²⁵I-SP, ¹²⁵I-CGRP, or ¹²⁵I-VIP was added (15,000 to 20,000 cpm) to each tube. After a second overnight incubation at 4° C, 100 μ l of polyclonal goat

anti-rabbit γ -globulin and 100 μ l of nonimmune rabbit serum were added for 2 hours at room temperature. RIA buffer (500 μ l) was added, the tubes were centrifuged at 1700 g for 40 minutes at 4° C, and the supernatant was aspirated. The pellet was counted in a gamma scintillation counter (Auto Gamma 5550, Packard, Meriden, Conn.). The linear portion of the standard curve was analyzed by linear regression, and the amount of peptide per tube was interpolated. To determine whether nasal secretions affected the RIA, standard curves for each peptide were also prepared in pooled nasal lavage fluids and compared with standards run in buffer. Standard curves for SP were not affected by nasal lavage fluid, whereas standard curves for CGRP and VIP were slightly shifted to the left. The VIP curve was shifted the most impressively, but always in parallel to the curve in RIA buffer.

Total protein

Total protein was assayed in each nasal lavage sample (BCA Protein Assay, Pierce, Rockford, Ill.), and a neuropeptide to total protein ratio was calculated.

Thin-layer chromatography

To assess the efficacy of the protease inhibitors, 50 μ l of pooled nasal lavages from atopic patients, which were obtained after allergen challenge, were incubated at 37° C for 15 minutes with 6 nCi ¹²⁵I-SP (887 cpm), 2.8 nCi ¹²⁵I-CGRP (1817 cpm), or 6.25 nCi ¹²⁵I-VIP (2121 cpm) in the presence or absence of protease inhibitors. The nasal lavages were developed on a silica gel plate

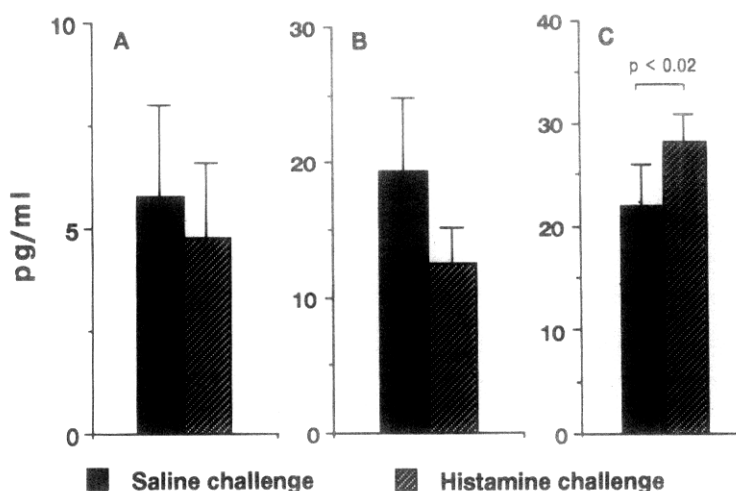


FIG. 4. Neuropeptide levels in nasal lavages collected 10 minutes after challenge with saline or 1 mg histamine. A, SP; B, CGRP; C, VIP ($n = 8$).

(Whatman, Clifton, N.J.) with 33% N-butanol, 33% pyridine, 25% water, and 9% glacial acetic acid. The plates were dried, and each column was divided into 12 fractions. The radioactivity of each fraction was measured in a gamma counter. Thin-layer chromatography of the radiolabeled neuropeptides showed that intact SP migrated with an R_f of 0.58, and CGRP and VIP each had an R_f of 0.7. Migration of neuropeptides in the presence and absence of inhibitors was compared. In the absence of inhibitors, counts per minute decreased by 54% for SP, 20% for CGRP, and 75% for VIP within 15 minutes. In the presence of inhibitors, counts per minute decreased by 12% for SP, 0% for CGRP, and 12% for VIP.

High-pressure liquid chromatography

To assess the specificity of the anti-SP, anti-CGRP, and anti-VIP antibodies, 1 ml of nasal lavage that contained 13 ng of SP, 13 ng of CGRP, or 10 ng of VIP was chromatographed on a POROS reverse phase column (PerSeptive Biosystems, Cambridge, Mass.). The column was washed for 1 minute (flow rate = 5 ml/min.) with solvent A (water + 0.05% trifluoroacetic acid), and proteins were eluted with a linear gradient of 0% to 70% solvent B (acetonitrile + 0.05% trifluoroacetic acid) over 5 minutes. Fractions (0.2 min/fraction) were collected and lyophilized. The fractions were resuspended in buffer, and SP, CGRP, and VIP were measured by RIA. One hundred percent of SP immunoreactivity was recovered from three successive high-pressure liquid chromatography fractions (Fig. 1, A), 80% of CGRP immunoreactivity was recovered from one fraction (Fig. 1, B), and 100% of VIP immunoreactivity was recovered from four fractions (Fig. 1, C). Thus the RIA recognized only the neuropeptides that were eluted in a very narrow range.

Statistical analysis

Data were expressed as means $\pm$ SEM. Clinical scores and neuropeptide levels in nasal lavage fluids

(expressed in picograms per milliliter) were compared by Wilcoxon's signed-rank test.

RESULTS

Clinical response to nasal challenge

The total clinical score after saline challenge was 0.88 ± 0.4 . All eight patients responded clinically to histamine challenge with a clinical score of 5.25 ± 1 ($p < 0.01$). The total clinical score after allergen challenge with 1000 AU/ml was 6.1 ± 1 ($p < 0.01$ compared with saline challenge) and 5.2 ± 1 ($p < 0.01$ after 10,000 AU/ml). Three and 13 minutes after challenge with 100,000 AU/ml, the scores were 7.5 ± 1 ($p < 0.006$) and 6.5 ± 0.9 ($p < 0.006$), respectively. Seven patients experienced a symptomatic LAR 2 to 14 hours after the last dose of allergen, which was characterized by a recurrence of congestion (plus other symptoms) experienced during the immediate allergic reaction. The highest total score during the LAR was 3.1 ± 0.6 ($p < 0.02$).

Neuropeptides in nasal lavages after histamine and allergen challenges

In the preliminary experiments, all four atopic patients challenged with 10,000 AU/ml of relevant allergen had immediate allergic symptoms with a score of 8 ± 0.6 and an increase in SP in their nasal secretions (81.8 ± 27.3 pg/ml compared with a baseline value of 24.4 ± 9.3 pg/ml), whereas the four nonatopic subjects and three atopic patients who were challenged with irrelevant allergens did not experience allergic symptoms and had no increase in SP in their nasal secretions (Fig. 2). The four atopic patients challenged with allergen also had increased CGRP levels, whereas the three nonatopic control pa-

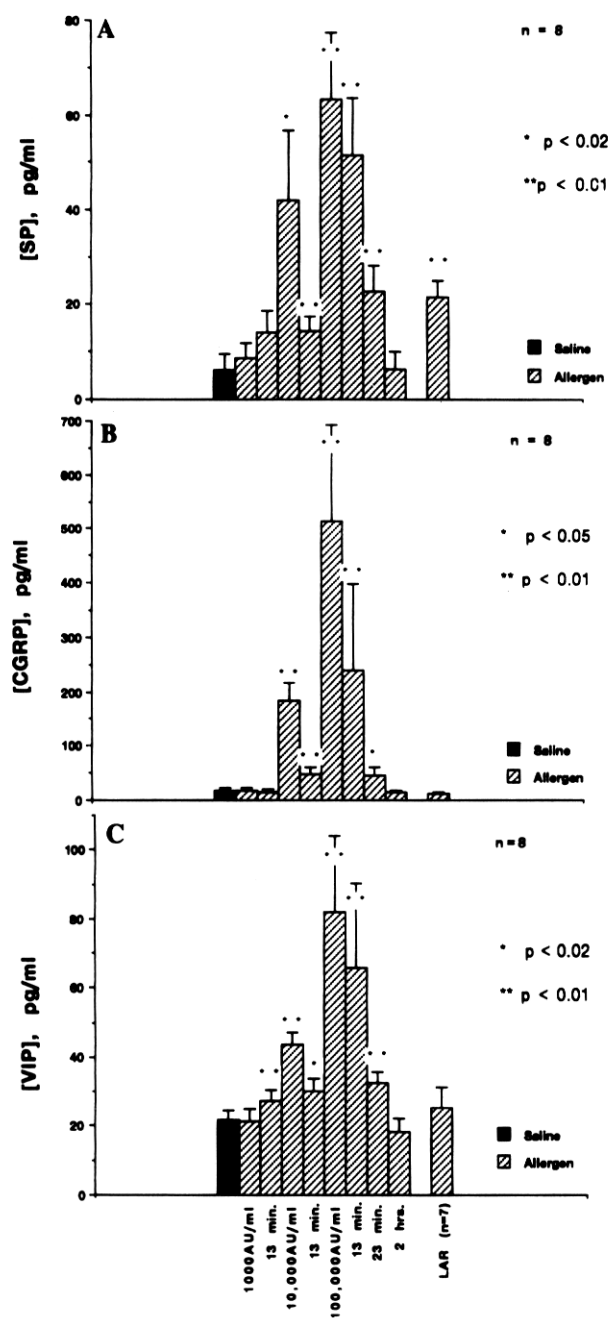


FIG. 5. Neuropeptide levels in nasal lavages after allergen challenge. Eight subjects were challenged with saline followed by antigen (1000, 10,000, and 100,000 AU/ml). Neuropeptides were assayed in lavages taken 10 minutes after saline challenge and at 3 and 13 minutes after antigen challenge. In addition, each neuropeptide was also measured in lavages performed at 23 minutes and 2 hours after the 100,000 AU/ml challenge and at the symptomatic peak of the LAR (experienced by seven of the eight subjects). A, SP; B, CGRP; C, VIP.

tients did not (Fig. 3). None of these changes were statistically significant, probably because of the restricted number of subjects analyzed.

Basal SP levels (after saline challenge) from the

eight atopic patients studied next were at the limit of detection of the RIA: 0 to 13 pg/ml (mean 5.4 ± 2.5). After histamine challenge (Fig. 4, A) or administration of the lowest dose of allergen (1000 AU/ml [Fig. 5, A]), SP did not increase significantly: 4.7 ± 1.8 pg/ml and 14.0 ± 4.5 pg/ml, respectively. After allergen exposure at 10,000 AU/ml, a significant increase of SP ($p < 0.02$) was detected: 42.0 ± 14.7 pg/ml. Three minutes after 100,000 AU/ml, the increase was more pronounced: 63.3 ± 14.2 pg/ml ($p < 0.01$). This increase was still significant 10 and 20 minutes later: 51 ± 12.3 pg/ml ($p < 0.01$) and 22.6 ± 5.66 pg/ml ($p < 0.01$), respectively, but not 120 minutes later (Fig. 5, A). The levels of SP in the lavages obtained during LAR in seven patients were slightly but significantly increased: 20.6 ± 3.3 pg/ml ($p < 0.01$). To determine whether the previous histamine challenge affected the allergen challenge, three additional atopic patients who had not received histamine were challenged with 100,000 AU of relevant allergen. SP levels after saline challenge, immediately after allergen challenge, and 2 hours later were 8.5 ± 0.8 pg/ml, 69.1 ± 10.6 pg/ml, and 9.7 ± 4.8 pg/ml, respectively. These results are not significantly different from those obtained in the patients studied 1 day after histamine challenge. Thus it seems unlikely that the response to antigen was affected by the preceding challenge with histamine.

The level of CGRP after saline challenge was 17.0 ± 4.3 pg/ml. After histamine challenge (Fig. 4, B) and 3 minutes after allergen challenge with 1000 AU/ml (Fig. 5, B), CGRP did not increase significantly. Three minutes after challenge with 10,000 AU/ml, a significant increase of CGRP was detected: 184.9 ± 31.9 pg/ml ($p < 0.01$). Three minutes after challenge with 100,000 AU/ml, the level of CGRP was 512 ± 180.3 pg/ml ($p < 0.01$). Twenty minutes later, CGRP was still significantly increased at 44.1 ± 7.5 pg/ml ($p < 0.05$). Two hours after the last dose of allergen was given, the level of CGRP was at baseline. No increase of CGRP could be detected in the lavages obtained during the LAR (Fig. 5, B).

The level of VIP after saline challenge was 21.9 ± 2.6 pg/ml. After histamine challenge, a slight but significant increase of VIP (Fig. 4, C) was demonstrated ($p < 0.02$). Three minutes after allergen challenge with 1000 AU/ml, no increase of VIP could be detected. Thirteen minutes after challenge with 1000 AU/ml, VIP levels increased to 27.1 ± 3.3 pg/ml ($p < 0.01$). Three minutes after challenge with 10,000 AU/ml, VIP

was at 43.4 ± 3.8 pg/ml ($p < 0.01$). Three minutes after allergen challenge with 100,000 AU/ml, the level of VIP was 81.9 pg/ml ± 22.1 ($p < 0.01$), and 10 and 20 minutes later the increase of VIP was still significant: 65.7 ± 24.7 pg/ml ($p < 0.01$) and 32.4 ± 3.1 pg/ml ($p < 0.01$). Two hours after the last dose of allergen was given and during the LAR, the levels of VIP were at baseline (Fig. 5, C).

In order to determine whether the increased neuropeptide levels were only a reflection of increased secretion of total protein, a neuropeptide-total protein ratio was calculated for each nasal lavage. The peak increase of SP and CGRP levels in lavage fluids after allergen challenge occurred concomitantly with the highest neuropeptide-total protein ratios (Fig. 6, A and B). Thus the SP and CGRP increases represented an increased secretion of these peptides in excess of the increased protein secretion. The VIP-total protein basal ratio (after the first saline lavage) on the other hand, was already high and did not increase further after allergen challenge (Fig. 6, C).

Individual increases of the three neuropeptides in nasal lavages obtained after the 100,000 AU/ml allergen challenge are presented in Fig. 7. Seven of the eight patients showed an impressive increase in SP and CGRP levels after allergen challenge. Five also had a dramatic increase in VIP. Although there was a trend for the highest levels of neuropeptide to correlate with the highest clinical scores, the correlation failed to achieve statistical significance.

DISCUSSION

This study demonstrates that SP, CGRP, and VIP can be recovered in significantly increased concentrations in nasal secretions after relevant allergen challenge in atopic patients. The increased neuropeptide secretion was specifically associated with elicitation of an allergic reaction because nonatopic subjects and atopic subjects challenged with irrelevant allergens did not have increased neuropeptides in their secretions. Interestingly, the pattern of increased neuropeptide secretion was the same for all three peptides studies. Each showed a rapid, brief, and allergen-dose-dependant elevation. This pattern suggests that mediators liberated during the acute allergic reaction stimulate sensory nerves to liberate SP and CGRP, and reflexively cause parasympathetic nerve endings to liberate VIP. Histamine has been proposed to be capable of stimulating sensory nerve endings.^{22, 23} In the present study, his-

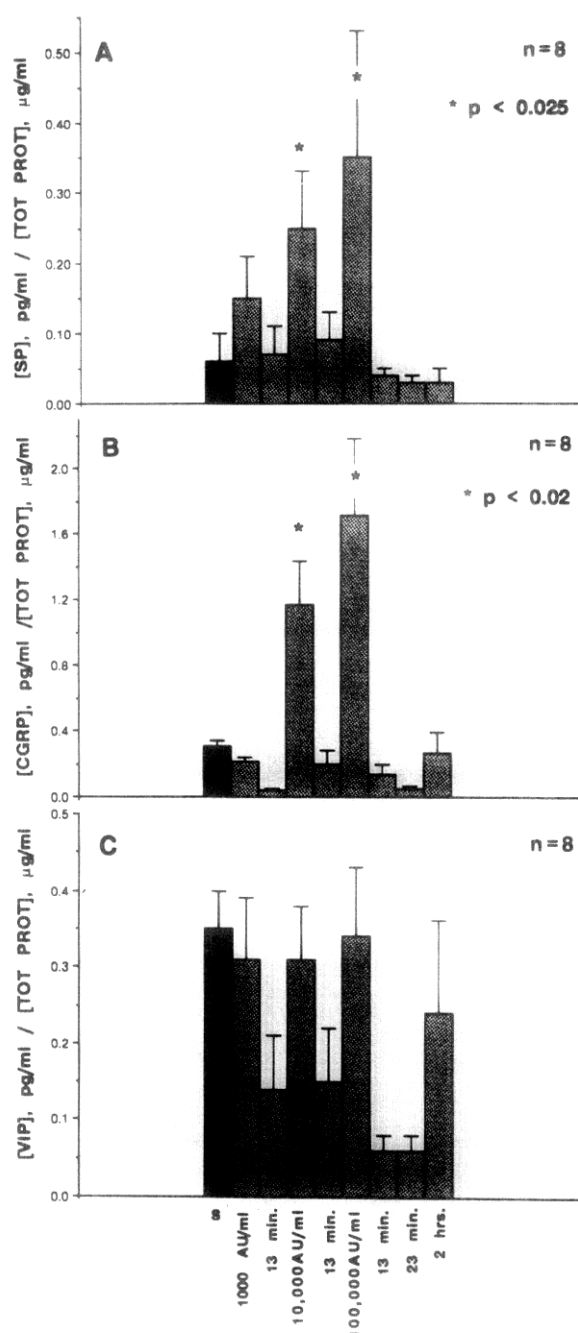


FIG. 6. Neuropeptide-total protein ratios in nasal lavages after allergen challenge. A, SP-total protein; B, CGRP-total protein; C, VIP-total protein.

tamine challenge did not induce either SP or CGRP increases but did cause a significant elevation of VIP. This observation suggests that nasal mucosal sensory nerves (containing SP and CGRP) may not be stimulated by histamine in human beings, whereas parasympathetic nerve endings are. However, exogenous and endogenous histamine may stimulate different nerves, and there is no way of knowing the actual concentra-

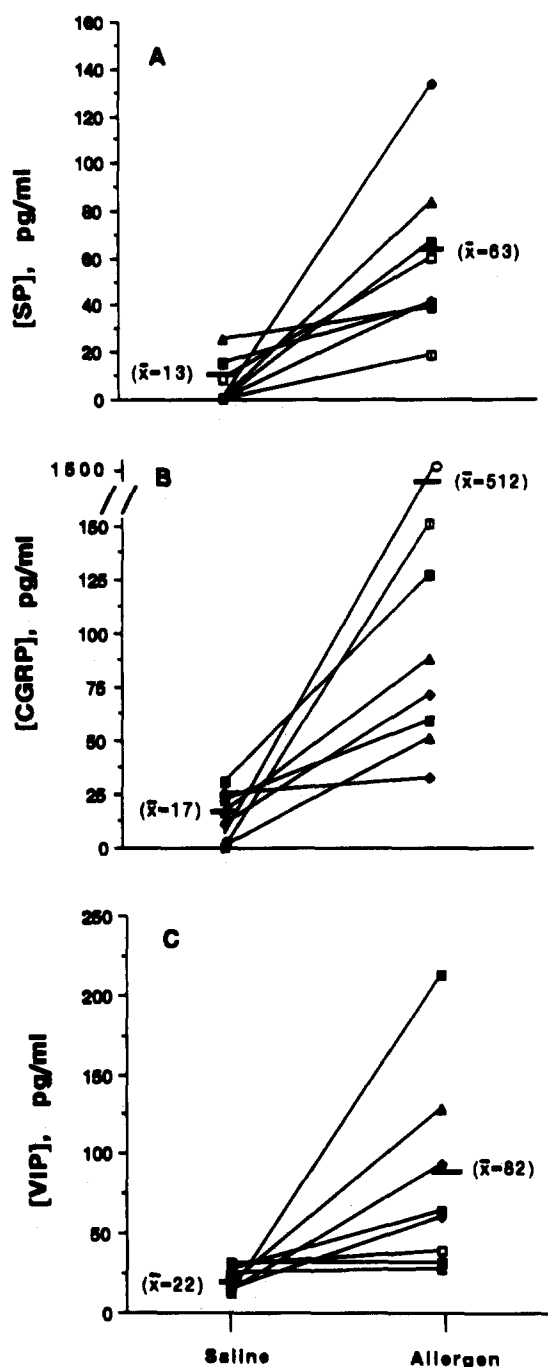


FIG. 7. Neuropeptide levels 10 minutes after saline challenge and 3 minutes after allergen challenge (100,000 AU/ml) for each subject (bars represent mean). A, SP; B, CGRP; C, VIP.

tion of histamine released endogenously. It is possible that higher concentrations of histamine might have induced SP or CGRP release. The exogenous dose of histamine used in this study causes a nasosial cholinergic reflex, which in earlier studies elicited a contralateral, atropine-inhibitable secretory response,²⁴ which could explain the VIP release.

Seven patients experienced an LAR 2 to 14 hours after the last dose of allergen was given, and SP was the only neuropeptide to increase in lavages collected at those times. Although the increase in SP was significant, it was relatively small and only one lavage per patient was assayed. It is possible that more lavages might have revealed increases in the other neuropeptides and perhaps other increases in SP as well.

Our data differ somewhat from results of previous attempts to measure neuropeptides in human nasal secretions. Walker et al.²⁵ found no elevation of SP in nasal secretions from atopic subjects after a single application of 1000 protein nitrogen units of allergen, whereas CGRP was significantly elevated 15 to 60 minutes (but not immediately) after challenge.²⁵ Two other studies failed to detect any increase in SP after allergen challenge.^{26, 27} In these studies the secretions were collected onto filter paper disks or by a nose-blowing method, and no peptidase inhibitors were added. In one of these studies, CGRP was also measured in nasal secretions after nasal allergen challenge, and no increase could be detected.²⁷ More recently, however, Nieber et al.,²⁸ using Bacitracin as protease inhibitor, found increased SP concentrations in nasal secretions after allergen challenge. Interestingly, their data are quite comparable to ours. There are no previous reports of VIP elevations in nasal secretions, but increased plasma levels have been found after nasal challenge with allergen.²⁶ In a related study, an increase in SP in nasal secretions has been reported after nasal serotonin challenge in normal subjects.²⁹

There are several reasons that may explain discrepancies between the reported data and the increased levels noted herein. Our data show that elevation of neuropeptides in secretions is allergen-dose-dependent and that sufficient allergen must be applied to the mucosa to observe the phenomenon. The increase is of brief duration, and frequent lavages must be obtained to define the peak of neuropeptide secretion. Finally, several endogenous peptidases found in secretions can degrade neuropeptides. These include neutral endopeptidase³⁰ and angiotensin-converting enzyme.³¹ Both peptidases have been shown to be present in the nasal mucosa and are also found in nasal secretions.^{32, 33} Basal concentrations of neuropeptides in our study were higher than those in some previous reports, and this could be due to the use of peptidase inhibitors. However, the SP levels were at the limit of detection of the RIA, possibly because of the high concentration of

neural endopeptidase in the nasal epithelium. Although neuropeptides can be hydrolyzed in secretions before nasal collections are recovered, an adequate cocktail of protease inhibitors is essential to prevent further degradation in the test tube. Thus use of protease inhibitors probably accounts for the recovery of neuropeptides in this experiment.

Whether neuropeptides are responsible for some of the symptoms observed in patients with allergic rhinitis remains to be determined. Specific neuropeptide antagonists, some of which may soon be available for clinical trials,³⁴ are needed to address this question. Substantial evidence suggests that neuropeptides liberated by sensory nerve endings are involved in chronic nonallergic rhinitis. Capsaicin, the pungent essence of chili peppers, is known to deplete sensory nerves of their SP and CGRP content.³⁵ Stjärne et al.³⁶ have shown that application of capsaicin to the nasal mucosa of patients who have chronic nonallergic rhinitis produces significant and long-lasting relief of symptoms.³⁶ These results have been confirmed by Lacroix et al.,³⁷ who were able to demonstrate that the decrease in symptoms correlated with a significant decrease of CGRP in the nasal mucosa.³⁷

In conclusion, we have shown that increased SP, CGRP, and VIP are secreted after allergen provocation. Thus the data indicate that neuropeptides may be involved in the events related to allergic rhinitis.

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