

Attenuation of allergen-evoked nasal responses by local pretreatment with exogenous neuropeptide Y in atopic patients

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Background: Nasal obstruction and rhinorrhea present in allergic rhinitis are at least partly influenced by neuropeptides released from sensory, parasympathetic, and sympathetic nerves. Neuropeptide Y (NPY) is co-localized with norepinephrine in sympathetic perivascular nerves. NPY is released with norepinephrine on sympathetic nerve stimulation and produces long-lasting vasoconstriction of the nasal vascular bed. In addition to vasoconstriction, there is evidence to suggest that NPY modulates the release of transmitters originating from parasympathetic and sensory nerves by acting on prejunctional receptors. Putative therapeutic application of NPY in rhinitis has been recently suggested because intranasal administration of exogenous NPY in human beings reduces nasal airway resistance and vascular permeability without affecting submucosal gland secretion.

Objective: The aim of this study was to determine whether intranasal pretreatment with exogenous NPY could influence the functional responses to subsequent allergen challenge.

Methods: A randomized double-blind, three-way, crossover, placebo-controlled study was performed in 13 patients with allergic rhinitis. Pretreatments with NPY (20 nmol), oxymetazoline hydrochloride (20 nmol), or saline solution (NaCl 0.9%) were administered in one nostril 5 minutes before nasal challenge with grass pollen allergen (10,000 standardized quality units). The number of sneezes and the subjective evaluation of nasal itching, obstruction, and rhinorrhea were recorded. Nasal airway resistance was measured by anterior rhinomanometry, and nasal secretions were weighed. Mean arterial pressure and heart rate were recorded by noninvasive methods.

Results: The number of sneezes, nasal itching, and subjective rhinorrhea evoked by the allergen were similar after the three pretreatments. Subjective and objective increases in nasal airway resistance and mucus production induced by the allergen were significantly ($p < 0.01$) reduced after NPY pretreatment when compared with saline solution or oxymetazoline pretreatment. Mean arterial pressure and heart rate were not changed.

Conclusions: Local pretreatment with exogenous NPY reduces nasal obstruction and mucus secretion evoked by allergen challenge in allergic patients without modification of local itching and number of sneezes. (*J Allergy Clin Immunol* 1996;98:611-6.)

Key words: Neuropeptides, neuropeptide Y, allergic rhinitis, nasal secretion, nasal obstruction

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Nasal airway obstruction and rhinorrhea present during rhinitis are at least partly influenced by biologically active agents released from sensory, sympathetic, and parasympathetic autonomic nerves.^{1,2}

Nasal sensory nerves are involved in protective reflexes such as sneezing, nasal secretion, and congestion.^{3,4} Several biologically active polypeptides (i.e., substance P [SP], neurokinin A, and calcitonin gene-related peptide [CGRP]) have been demonstrated by immunohistochemical techniques in a subgroup of unmyelinated sensory C-fibers.⁵ SP has a secretagogue effect on submucosal glands⁶ and increases microvascular leakage in postcapillary venules.^{7,8} Exogenous SP and

Abbreviations used

ANOVA:	Analysis of variance
CGRP:	Calcitonin gene-related peptide
NAR:	Nasal airway resistance
NPY:	Neuropeptide Y
SP:	Substance P
SQ:	Standardized quality units
VIP:	Vasoactive polypeptide

CGRP induce nasal vasodilatation in human beings.⁹

Vasoactive intestinal polypeptide (VIP) is co-localized with acetylcholine in postganglionic parasympathetic neurons supplying exocrine glands and blood vessels in the nasal mucosa.¹⁰ VIP is released during parasympathetic stimulation or nasal allergen challenge¹¹ and stimulates serous cell secretion. This neuropeptide could be the mediator of noncholinergic nasal vasodilatation.^{12, 13}

In the sympathetic nerves, norepinephrine is considered to be the classical mediator of nasal vasoconstrictor effects in perivascular fibers.¹⁴ Recent immunohistochemical studies and functional observations have suggested that both adrenergic and nonadrenergic mechanisms may occur in the peripheral sympathetic control of several vascular beds, including the nasal mucosa.¹⁵ One candidate for nonadrenergic effects is neuropeptide Y (NPY), a 36-amino acid peptide that is co-localized with norepinephrine in sympathetic postganglionic perivascular nerves in several organs, including the nasal mucosa in various species.¹⁶ NPY is released with norepinephrine from nasal sympathetic nerves and produces long-lasting vasoconstriction of the nasal vascular bed.¹⁵ In addition to vasoconstriction, both NPY and α_2 -adrenoceptor agonist modulate the functional effects of transmitters released from parasympathetic and sensory pathways.^{17, 18} NPY also inhibits its own release, as well as release of other transmitters, such as norepinephrine¹⁹ and acetylcholine²⁰ by acting on prejunctional receptors.²¹

Because neuropeptides from both sensory and parasympathetic nerves most likely participate in allergic reactions,¹¹ the effect of NPY and α_2 -adrenoceptor agonist on the functional responses to allergen challenge remains to be studied in patients with allergy. The aim of this study was to determine whether intranasal pretreatment with exogenous NPY or the α_2 -adrenoceptor agonist oxymetazoline could influence the functional responses to subsequent allergen challenge.

METHODS

Subjects

Thirteen subjects (8 men and 5 women) between the ages of 27 and 45, who had nasal allergy to grass pollen, were studied outside of their allergy season. The experimental protocol was approved by the ethical committee of our institution, and informed consent was obtained from all subjects. Allergic rhinitis was confirmed by history and positive skin prick test results. None of the patients had asthma or any symptoms of rhinitis at the time of the study. All medications were stopped 3 weeks before the study.

Nasal pretreatment and challenge

NPY, oxymetazoline, and saline solution pretreatments were prepared simultaneously by a laboratory assistant who was not involved in the study. Pretreatment solutions were kept in small polyethylene vials at -80°C until the experiment. Each vial had a code number corresponding to one patient and a type of pretreatment. The secret code was given to the investigators when all experiments were completed. Thus pretreatments were delivered to one nostril in a double-blind, randomized, controlled manner. NPY, (20 nmol; Novabiochem, L  ufelfingen, Switzerland) with 0.8% Tween-20 (Pierce Chemical Co., Rockford, Ill.), oxymetazoline hydrochloride (20 nmol; Merck & Co., Inc., Whitehouse Station, N.J.), or saline solution (NaCl 0.9%) was administered in a total volume of 200 ml of saline solution with a hand-held nebulizer. Five minutes after the administration of the pretreatment, nasal challenge was performed with grass pollen allergen (10,000 standardized quality units [SQ] in a volume of 100 ml of saline solution; A.L.K., H  rsholm, Denmark) in the homolateral nostril. Blood pressure and heart rate were recorded by noninvasive methods.

Symptom recordings

Subjective evaluation of the intensity of local itching, nasal obstruction, and rhinorrhea was obtained by means of a visual analog scale (graded from 0 to 5, where 0 represents absence of symptoms, and 5, severe intensity of symptoms); and the number of sneezes was recorded. Nasal symptoms were recorded 5 minutes before and 5 minutes after the pretreatment administration. Then, symptom scores were obtained 5, 10, 20, 30, and 60 minutes after the nasal challenge.

Nasal secretions

Nasal secretions were collected after each symptom recording from each nostril separately by nose blowing in preweighed tissues.

Rhinomanometry

Nasal airway resistance (NAR) was measured by anterior rhinomanometry (Rhinotest MP 441; EVG Electronic, Vertriebs, Germany) after each nose blowing. Data were obtained after 10 breathings, and the

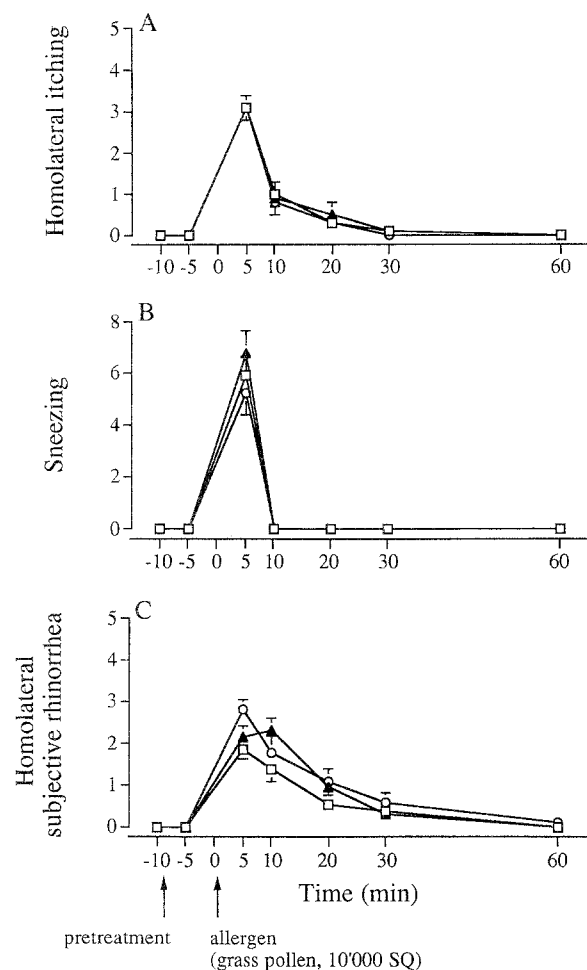


FIG. 1. Time-course variations of symptom score measured by a visual analog scale (graded from 0 to 5, where 0 represents absence of symptom, and 5, severe intensity of symptoms) for homolateral itching (**A**), number of sneezes (**B**), and subjective homolateral rhinorrhea (**C**) induced by intranasal allergen challenge after pretreatment with saline solution (open circles), oxymetazoline (filled triangles), and NPY (open squares) ($n = 13$).

mean resistance value (expressed in $\text{Pa} \cdot \text{sec} \cdot \text{cm}^{-3}$) for each nostril was calculated at a pressure of 150 Pa.

Statistical analysis

Data are given as means $\pm$ SEM. Statistical differences in symptom scores, NAR, and nasal secretions were estimated by using one-way analysis of variance (ANOVA).

RESULTS

Effects of NPY, oxymetazoline, and saline solution before nasal challenge

The intranasal spray of NPY, oxymetazoline, or saline solution did not induce local irritation, and no sneezing was observed. Heart rate and blood pressure remained unchanged (not shown). None

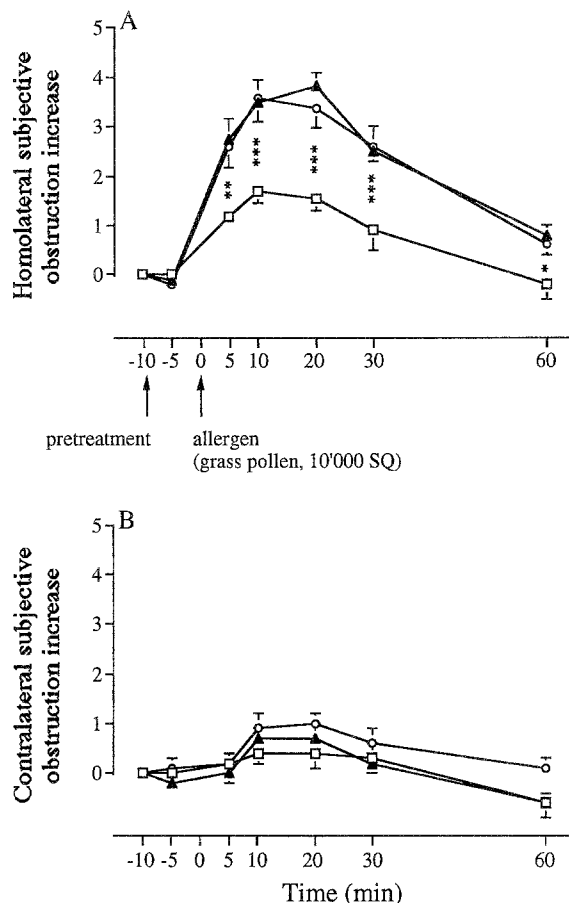


FIG. 2. Time-course variations of symptom score measured by a visual analog scale (graded from 0 to 5, where 0 represents absence of symptom, and 5, severe intensity of symptoms) for homolateral nasal obstruction (**A**) and contralateral nasal obstruction (**B**) induced by intranasal allergen challenge performed after pretreatment with saline solution (open circles), oxymetazoline (filled triangles), and NPY (open squares) ($n = 13$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (one-way ANOVA).

of the subjects studied reported any rhinorrhea or nasal obstruction (Figs. 1 and 2). Rhinomanometry recordings did not show any modification of NAR after the administration of NPY, oxymetazoline, or saline solution (Fig. 3), and no mucus was collected (Fig. 4).

Responses to nasal allergen challenge

Allergen challenge induced marked functional responses in all subjects studied. The first clinical response was homolateral itching, followed by sneezing and then rhinorrhea (Fig. 1). Itching and sneezing lasted for 5 minutes after the nasal challenge, whereas homolateral subjective rhinorrhea lasted for 30 minutes (Fig. 1). Intensity and duration of itching, sneezing, and homolateral subjective

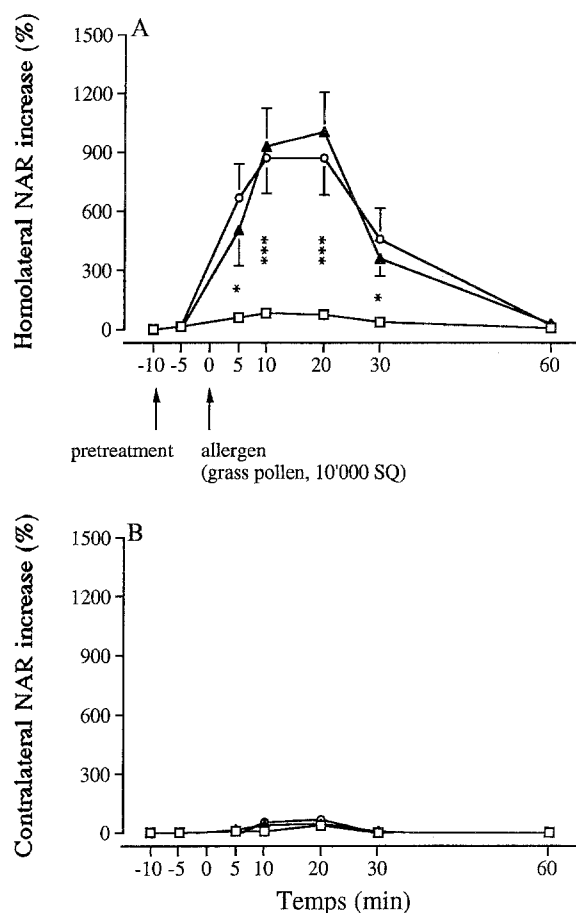


FIG. 3. Time-course variations of NAR, measured by anterior rhinomanometry, (see Methods) in the homolateral nostril (**A**) and the contralateral nostril (**B**) induced by intranasal allergen challenge performed after pretreatment with saline solution (open circles), oxymetazoline (filled triangles), and NPY (open squares) ($n = 13$). * $p < 0.05$, *** $p < 0.001$ (one-way ANOVA).

tive rhinorrhea were not significantly different after the three pretreatments studied (Fig. 1).

Subjective homolateral nasal obstruction induced by the allergen challenge was significantly attenuated ($p < 0.01$) after NPY pretreatment when compared with oxymetazoline or saline solution pretreatment (Fig. 2, *A*). Moderate but similar increases of subjective nasal obstruction were recorded in the contralateral nostril 10 minutes after the allergen challenge (Fig. 2, *B*). Rhinomanometry recordings revealed marked reduction of the NAR augmentation induced by the allergen after pretreatment with exogenous NPY (Fig. 3, *A*). Small NAR increases were recorded in the contralateral nostril within 10 minutes after the allergen challenge. The contralateral NAR increase was similar for the three pretreatments studied (Fig. 3, *B*). None of the

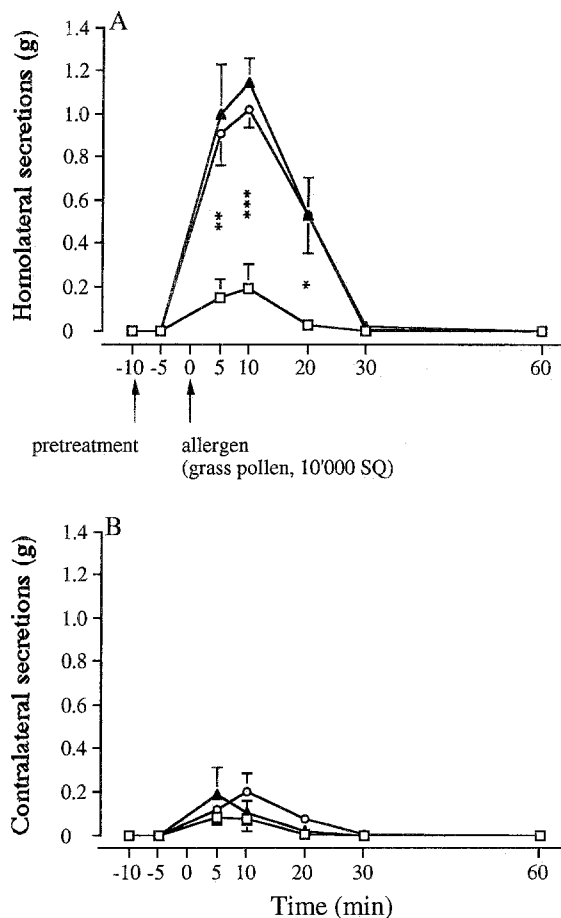


FIG. 4. Time-course variations of nasal secretions (in grams) collected from the homolateral nostril (**A**) and the contralateral nostril (**B**) in response to allergen challenge performed after pretreatment with saline solution (open circles), oxymetazoline (filled triangles), and NPY (open squares) ($n = 13$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (one-way ANOVA).

patients studied reported any late allergic reaction symptoms for 12 hours after the allergen challenge.

The amount of nasal secretions collected from the homolateral nostril after allergen administration was significantly smaller after the NPY pretreatment (Fig. 4, *A*). Some secretions were also collected from the contralateral nostril. The amounts were similar for the three pretreatments studied (Fig. 4, *B*).

DISCUSSION

This study shows that local pretreatment with exogenous NPY, but not oxymetazoline or saline solution, significantly reduces nasal obstruction and mucus secretion evoked by subsequent allergen challenge. In contrast, local itching, sneezing,

and subjective rhinorrhea were similar after NPY, oxymetazoline, and saline solution pretreatment.

The protective effect of NPY on both vasodilatory and secretory responses to the allergen could be due to several mechanisms. Exogenous NPY may have caused local vasoconstriction because intranasal application of this neuropeptide reduces NAR and vascular permeability.²² Bradykinin is a mediator released in allergic rhinitis,²³ which stimulates nociceptive sensory nerves. Because NPY pretreatment significantly reduces the ability of bradykinin to generate nasal secretions,²² similar mechanisms may have occurred in this study with allergen challenge.

Some evidence suggests that histamine released during an allergic reaction could stimulate sensory nerve endings, as well as parasympathetic fibers, through a naso-nasal reflex.^{5, 24, 25} Thus increased concentrations of SP, CGRP, and VIP were found in nasal secretions after allergen challenge.^{11, 26} NPY modulates parasympathetic pathways in lower airways^{17, 18} and sensory neuropeptide release, most likely by acting on prejunctional receptors.²⁷⁻²⁹ Thus pretreatment with NPY could have reduced both vascular and exocrine responses to the allergen challenge through prejunctional inhibitory mechanisms on both sensory and parasympathetic nerves. To confirm this hypothesis, the effect of NPY pretreatment on SP, CGRP, and VIP release during allergic reaction should be studied.

Subjective and objective recordings have revealed a contralateral response to the allergen challenge. The occurrence of a naso-nasal sensory-parasympathetic reflex has been well documented.²⁵ Pretreatment with NPY did not influence the contralateral functional responses to the allergen challenge, suggesting no systemic absorption of exogenous NPY. A recent clinical study has shown very low transnasal systemic absorption of exogenous NPY in human beings.³⁰

In conclusion, local pretreatment with exogenous NPY reduces nasal obstruction and mucus secretion evoked by allergen challenge in allergic patients without modification of local itching and sneezing. The recent development of NPY analogs may have putative therapeutic applications in allergic rhinitis.

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