

Allergens, IgE, mediators, inflammatory mechanisms

Evidence that enhanced nasal reactivity to bradykinin in patients with symptomatic allergy is mediated by neural reflexes

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Objective: *The aim of this study was to determine whether allergic inflammation induces nasal hyperreactivity to bradykinin by enhancing neuronal responsiveness.*

Methods: *We compared the response to localized, unilateral nasal challenge with bradykinin in patients with perennial allergic rhinitis and nonallergic subjects, and in patients with seasonal allergic rhinitis challenged in and out of season. Weights of secretions from each nostril were recorded, and levels of albumin and lactoferrin in secretions recovered from each nostril were assayed. Contralateral administration of atropine (0.32 mg) was used to evaluate the role of cholinergic reflexes in nasal hyperresponsiveness to bradykinin.*

Results: *In patients with symptomatic allergy, bradykinin induced greater symptom scores than in asymptomatic atopic or nonallergic control subjects. Moreover, bradykinin caused sneezing in a majority of patients with symptomatic allergy but in none of the asymptomatic atopic or nonallergic control subjects. Only patients with symptomatic allergy showed dose-dependent bilateral increases in secretion weights and levels of the serous glandular marker, lactoferrin. In contrast, bradykinin induced similar increases in ipsilateral, but not contralateral, levels of albumin in all patient populations. Atropine inhibited contralateral secretion and lactoferrin production ($p < 0.05$) in patients with symptomatic allergy.*

Conclusion: *The induction of sneezing and of atropine-inhibitable contralateral glandular secretion demonstrates that allergic inflammation causes nasal hyperreactivity to bradykinin, at least in part, by enhancing neuronal responsiveness. (J Allergy Clin Immunol 1996;97:1252-63.)*

Key words: *Bradykinin, allergic rhinitis, atropine, neural reflexes, cholinergic, glandular secretion, lactoferrin, albumin, vascular permeability, sneezing*

Bradykinin and lysylbradykinin are potent vasoactive peptides that have been implicated as potential mediators of allergic airway diseases. It has been demonstrated that increased levels of kinins are generated in human airway secretions during the inflammatory response to allergen challenge in both the upper^{1,2} and lower^{3,4} airways. Moreover,

Abbreviations used

AUC: Area under the curve
PAR: Perennial allergic rhinitis
SAR: Seasonal allergic rhinitis

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increased levels of kinins are also detected during seasonal allergic rhinitis (SAR)⁵ and during naturally occurring exacerbations of asthma.³

Additional support for a role of kinins in allergic diseases of the airways is provided by provocation studies. In the lower airways bradykinin inhalation causes bronchoconstriction, retrosternal discomfort, and cough in asthmatic subjects,⁶⁻⁸ whereas application of a spray of bradykinin to the nasal mucosa of normal subjects or patients with asymp-

tomatic allergy induces symptoms of nasal irritation, nasal congestion, rhinorrhea, and sore throat.⁹⁻¹²

The mechanisms by which bradykinin induces airway symptoms are not fully understood, but it has been suggested that some actions of bradykinin in the airways may be mediated through stimulation of sensory nerves and induction of neural reflexes.¹³ The capacity of bradykinin to induce nasal irritation, sore throat, retrosternal discomfort, and cough is consistent with the stimulation of sensory nerves. Moreover, the ability of bradykinin to stimulate neural reflexes may be dependent on the presence of airway inflammation, because bradykinin-induced bronchoconstriction is evident only in patients with asthma.⁶⁻⁸

In this study we have used a model of localized challenge of the human nasal mucosa to directly examine the hypotheses that allergic inflammation induces nasal hyperreactivity to bradykinin and that enhanced neural responsiveness contributes to this allergen-induced hyperreactivity. To test these hypotheses, we performed unilateral bradykinin challenge in patients with perennial allergic rhinitis (PAR) and in nonallergic subjects but collected secretions separately from both sides of the nasal cavity. Responses of the two populations were compared with regard to symptoms, secretion weights, and sneezing; and the levels of markers for vascular permeability (albumin) and glandular secretion (lactoferrin) were assessed in recovered secretions. To confirm that differences in bradykinin responsiveness between these populations were due to the effects of allergen exposure and could not be attributed to atopic status per se, we also compared the response of patients with SAR to bradykinin challenges performed both in and out of their allergy season. Finally, to investigate the involvement of neural reflex pathways in responses to bradykinin, we performed a double-blind, crossover study in which atropine was applied to the contralateral nostril to block efferent cholinergic reflexes before bradykinin challenge was performed.

METHODS

Materials

The following materials were purchased: bradykinin (Peninsula Laboratories, Belmont, Calif.); physiologic saline and lactated Ringer's solutions (Kendall McGaw Laboratories, Irvine, Calif.); atropine sulfate, 1 mg/ml (American Regent Laboratories, Shirley, N.Y.); allergen extracts for skin testing (Greer Laboratories, Lenoir, N.C.); white filter cards (no. 190005; Shandon, Pitts-

burgh, Pa.); sheep antibody to human albumin and horseradish peroxidase-conjugated sheep antibody to human lactoferrin (The Binding Site, Inc., San Diego, Calif.); rabbit antibody to human lactoferrin and horseradish peroxidase-conjugated sheep antibody to human albumin (Dako Corp., Carpinteria, Calif.); 2, 2'-azino-bis (3 ethylbenzthiazoline-6-sulfonic acid) and human serum albumin (Sigma Chemical Co., St. Louis, Mo.); and lactoferrin (Calbiochem, San Diego, Calif.).

Subjects

For the first study, we used 19 healthy subjects, seven of whom were male. Ages ranged from 19 to 41 years. Of these subjects, nine (four male) were nonallergic on the basis of a lack of history of allergic symptoms and negative skin test responses to a battery of common allergens (see below). The remaining 10 subjects had PAR, defined as the presence of symptoms for at least 10 months of the year, and positive epicutaneous skin test responses to perennial allergens.

Nine healthy subjects with SAR (5 male), aged 29 to 40 years, were enrolled in the second study. In addition to positive skin test responses to either grass or ragweed, the subjects also had strictly seasonal symptoms. All subjects with seasonal allergy were studied both out of season and during the height of the ragweed or grass pollen season. To ensure that the need for long-term medication would not be a problem during the allergy season, we selected subjects who reported relatively mild seasonal symptoms that were usually controlled with antihistamines on an as-needed basis.

The final arm of the study was designed to assess the contribution of neural reflexes in the responses to bradykinin. In order to do this, five patients with symptomatic allergy who had demonstrated a clear contralateral response to bradykinin challenge in the first two studies were selected. Of these subjects, three (two male) had PAR and two (one male) had SAR and were challenged during the grass pollen season while they had symptoms.

Subjects refrained from taking all medications during the course of these studies. No subject had taken astemizole in the previous 3 months, and glucocorticosteroids were stopped 1 month before challenge. Decongestants were discontinued 24 hours before challenge, whereas antihistamines were withheld for 2 to 5 days before challenge, depending on the characteristics of the antihistamine. All subjects were nonsmokers or had not smoked for at least 1 year. The Institutional Review Board of The Johns Hopkins Bayview Medical Center gave approval for each study, and each subject gave informed consent before participation.

Skin testing

Epicutaneous skin prick tests were performed with the following common allergens: Ragweed, mixed grasses (timothy, orchard, Bermuda), mixed trees (oak, beech, maple, elm), *Dermatophagoides pteronyssinus*, *D. farinae*, cat, dog, mold 1 (*Aspergillus* spp., *Penicillin* spp., *Mucor*

fusarium) and mold 2 (*Alternaria*, *Helminthosporium*, *Cladosporium*, *Botrytis* spp.). In addition, a negative control with the extract vehicle and a positive control with 0.01% histamine were obtained. If epicutaneous skin puncture yielded negative results in the nonallergic subjects, intradermal skin testing with a 1:100 dilution of original extract was performed by injecting 0.05 ml of the diluted extract into the dermis to confirm nonatopic status. A positive reaction was defined as a wheal that was more than 5 mm larger than the wheal induced by the vehicle and more than 10 mm of erythema 15 minutes after application.

Nasal bradykinin challenge protocol

Nasal challenges were performed, and nasal secretions were collected by using white filter paper disks, which were punched from filter cards with an 8 mm hole punch.¹⁴ These disks are each capable of absorbing approximately 50 μ l of fluid. Each disk to be used for collection of secretions was sealed in a 1.5 ml microtube (Sarstedt, Newton, N.C.) and weighed on an analytical balance (Mettler Instruments, Hightstown, N.J.). Bradykinin for nasal provocation was prepared at a concentration of 10 mg/ml in lactated Ringer's solution containing an additional 1.5 gm/L sodium bicarbonate. This solution, which had a pH of 6.5, was sterile-filtered and stored in aliquots at -80° C. Further dilutions were made in sterile isotonic saline solution. All challenge solutions were isosmolar. The protocol used was based on results of pilot studies, indicating that responses to bradykinin challenge were maximal within 2 to 5 minutes after challenge and usually resolved within 10 minutes.

To create a uniform baseline level of secretion and to clear the nose of any residual mucus, each subject, on entering the laboratory, underwent three 10 ml nasal lavages (5 ml per nostril) with 0.9% physiologic saline solution maintained at 37° C. Each lavage was expelled into a collecting basin and discarded. The subjects were then allowed to rest for 5 minutes before a disk containing 50 μ l of saline solution was applied, under direct vision, to the anterior portion of the left nasal septal mucosa by using a surgical headlight, a speculum, and duckbill forceps. The disk was left in place for 1 minute. After removing the challenge disk and waiting 30 seconds, a preweighed collection disk was placed at the same spot on the challenged (ipsilateral) side, and an additional disk was placed at the corresponding location on the opposite (contralateral) side of the septal mucosa for 30 seconds. Disks were then removed, re-sealed in their original microtube, and re-weighed. These disks therefore represented secretions recovered 2 minutes after application of the challenge stimulus. Four minutes and 30 seconds later, a second set of collection disks was applied bilaterally for 30 seconds, removed, re-sealed, and re-weighed to represent secretions recovered 7 minutes after application of the challenge. Beginning 3

minutes later, disks containing increasing doses of bradykinin (1, 3, 10, 30 μ g) were applied at 10-minute intervals, with secretions collected at 2 minutes and 7 minutes after each challenge dose, in a manner identical to that described for the saline challenge. For each challenge, ipsilateral and contralateral secretion weights were calculated from differences between pre- and postchallenge weights of tubes containing disks. For each side of the nose, data are presented as the sum of secretions collected at 2 minutes and 7 minutes after challenge.

Throughout the course of the challenge experiments, subjects maintained a symptom score record for symptoms of runny nose and stuffy nose. Each symptom was graded on a 4-point scale from 0 to 3 (0 = no symptoms, 3 = severe symptoms). Symptom scores recorded at 2 minutes and 7 minutes after each challenge were summed. In addition, sneezes were counted.

Atropine protocol

Beginning 5 minutes after the baseline lavages had been performed, each subject received either atropine (0.32 mg) or saline solution (placebo) administered in 4 puffs over a 20-minute period (1 puff, wait 5 minutes; 1 puff, wait 5 minutes; 2 puffs, wait 10 minutes) from a hand-held spray bottle into the right (contralateral) nostril. This protocol has been shown to effectively inhibit reflex cholinergic secretion induced by histamine,¹⁴ and similar protocols have been used by others to examine cholinergically mediated effects of various stimuli.^{15,16} A disk containing saline solution was then applied to the left nostril according to the protocol outlined previously, and a single dose of 30 μ g of bradykinin was applied 10 minutes later. Collection points were again at 2 and 7 minutes after challenge for both saline solution and bradykinin, and secretion weights and symptom scores were again summed for the two time points for each challenge.

Disk elution and measurement of mediators

At the end of each experiment, 250 μ l of phosphate-buffered saline containing 40 mmol/L ethylenediaminetetraacetic acid was added to each tube containing collection disks, and tubes were incubated at 4° C for 30 minutes to elute proteins from the disks. For any given challenge stimulus, the eluates from disks used to collect secretions from a given nostril at 2 minutes and 7 minutes after challenge were pooled and then divided into aliquots. The aliquots were stored at -80° C for subsequent determination of lactoferrin, an index of glandular secretion, and albumin, an index of vascular permeability.

Human serum albumin was measured by using an ELISA method. In brief, a 1:900 dilution of a sheep antibody to human albumin was immobilized on 96-well microtiter plates overnight. Plates were then washed,

and nonspecific binding sites were blocked by incubation with 1% sheep serum for 30 minutes at room temperature. After washing the plate, standard albumin or samples at varying dilutions were added to the plate and incubated at 37° C for 90 minutes. After washing, plates were incubated for an additional 90 minutes with a 1:1000 dilution of a second sheep anti-human albumin antibody conjugated to horseradish peroxidase. Plates were then washed, and the reaction was developed with 1 mmol/L 2, 2'-azino-bis-(3 ethylbenzthiazoline-6-sulfonic acid) as a substrate, and the absorbance was read at 410 nm. The assay standard curve ranged from 1 to 256 ng/ml. The ELISA method yielded results in excellent agreement with those obtained by using a previously published radioimmunoassay.¹⁷

Lactoferrin, a marker of serous glandular secretion, was also measured by using a similar ELISA method, as described in detail elsewhere.¹⁸

Statistics

Although data are expressed as means $\pm$ SEM to permit illustration of the range of responses, nonparametric methods were used for statistical analysis because the data obtained were not normally distributed. Within each subject group, analysis of the ability of increasing doses of bradykinin to induce significant effects compared with saline control was performed by using Friedman analysis of variance for repeated measures, and subsequent post hoc analysis was done with the Wilcoxon matched-pairs, signed-rank test between each bradykinin dose and the saline response. In order to compare bradykinin-induced responses between groups, the responses to saline challenge were subtracted from responses to each dose of bradykinin, and the resulting values were summed to provide an overall area under the curve (AUC) value, which is stated in the Results section. To determine significant differences in responses to bradykinin between the perennial and nonallergic populations, the Mann-Whitney U test was used to compare the AUC values across the two groups. Responses to the control saline challenge between nonallergic and perennial groups were also compared by using the Mann-Whitney U test. Comparisons between responses of the patients with SAR challenged in and out of season were performed by using the Wilcoxon matched-pairs, signed-rank test, as were comparisons of the effects of atropine and placebo on the responses of subjects to bradykinin.

RESULTS

Study 1: Responsiveness to bradykinin in nonallergic subjects and patients with PAR

Secretion weight. Although there was a trend for increased bilateral responses to saline challenge in the group with PAR, responses to saline challenge were not significantly different between groups

either ipsilaterally or contralaterally. The effect of bradykinin administration on secretion weights in nonallergic subjects, compared with patients with perennial allergy is shown in Fig. 1, A. Bradykinin induced a significant increase in secretion weights ipsilaterally (challenged side) in both nonallergic subjects ($p < 0.05$) and patients with perennial allergy ($p < 0.01$). There was, however, a marked enhancement of the ipsilateral response to bradykinin in the patients with PAR compared with the nonallergic subjects (AUC = 60.0 ± 17.9 mg vs 16.6 ± 4.2 mg, respectively; $p < 0.01$). Moreover, although a pronounced, dose-dependent increase in secretion weights was seen in the patients with PAR, secretion weights in the normal population showed a modest increase after the first dose of bradykinin and then remained essentially flat. Bradykinin also induced a significant contralateral increase in secretion weights in the population with perennial allergy (AUC = 46.2 ± 13.9 mg; $p < 0.01$) but not in the nonallergic group (AUC = 8.0 ± 4.0 mg; $p = 0.52$). The magnitudes of the ipsilateral and contralateral responses in patients with perennial allergy were not different.

Lactoferrin levels. The effects of bradykinin challenge on the levels of the serous glandular marker, lactoferrin, in secretions recovered from nonallergic subjects and patients with PAR are shown in Fig. 1, B. There were no differences between groups in either ipsilateral or contralateral lactoferrin levels after saline challenge. Bradykinin induced significant ipsilateral (AUC = 3.56 ± 1.83 μ g/ml; $p < 0.05$) and contralateral (AUC = 3.26 ± 0.97 μ g/ml; $p < 0.05$) increases in lactoferrin in the patients with PAR but did not stimulate significant lactoferrin production in nonallergic subjects (ipsilateral AUC = -0.87 ± 1.42 μ g/ml; contralateral AUC = 0.68 ± 0.63 μ g/ml). The magnitudes of the ipsilateral and contralateral responses in patients with perennial allergy were not different.

Albumin levels. Albumin secretion in response to bradykinin challenge is shown in Fig. 1, C. Control responses to saline challenge were not different between patients with perennial allergy and nonallergic subjects. Bradykinin application caused striking and significant increases in albumin levels ipsilaterally in both the nonallergic group (AUC = 535 ± 85.9 μ g/ml; $p < 0.001$) and the perennial allergic group (AUC = 508 ± 206 μ g/ml; $p < 0.001$), consistent with the ability of bradykinin to increase vascular permeability. Ipsilateral increases in albumin levels were not significantly different between the two populations ($p = 0.2$).

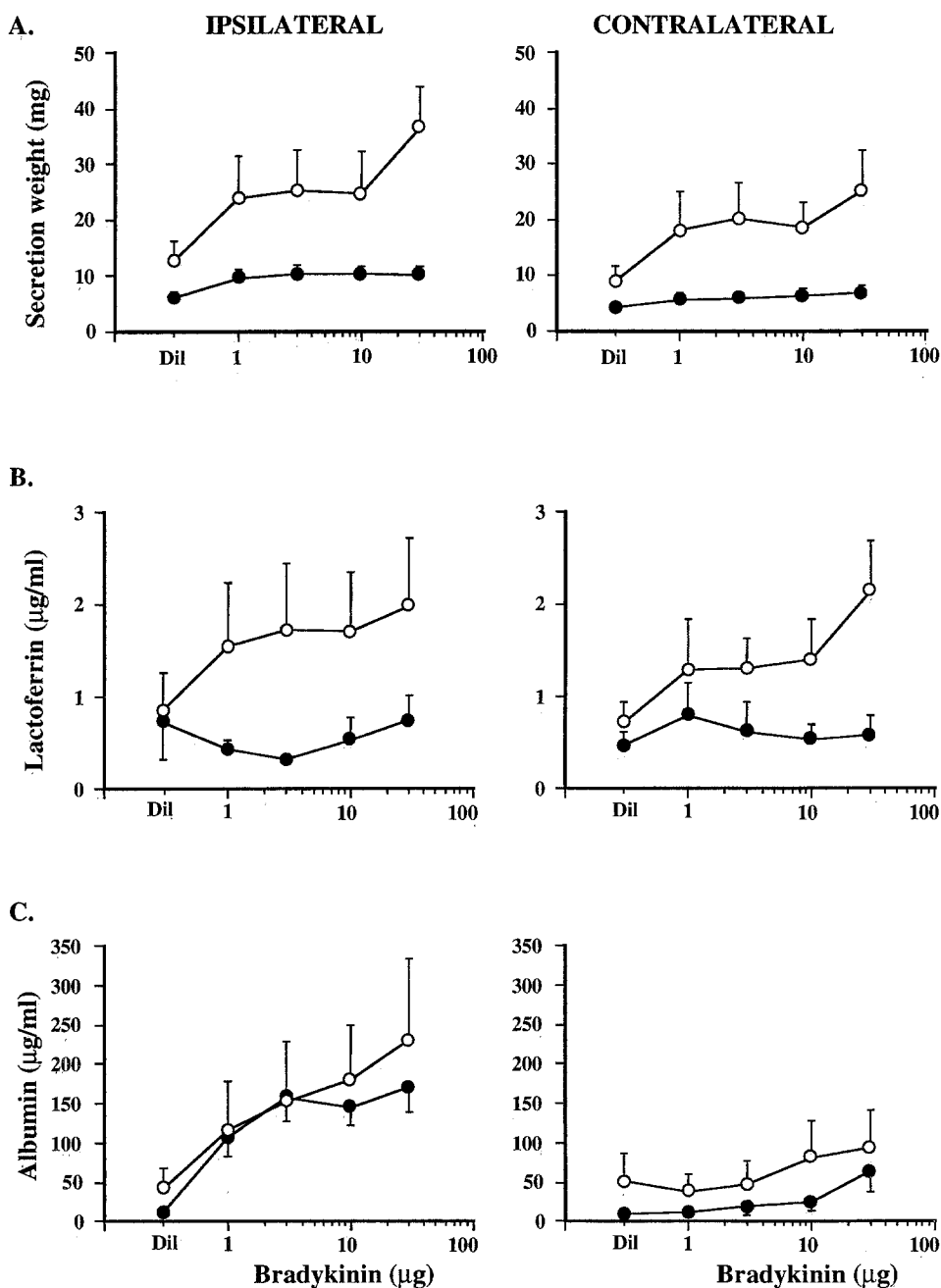


FIG. 1. Effect of nasal provocation with bradykinin in nonallergic subjects (filled circles; $n = 9$) and patients with perennial allergy (open circles; $n = 10$). Panels at left represent ipsilateral responses, and panels at right show contralateral responses. **A**, Secretion weights. Bradykinin induced a significant increase in ipsilateral secretions in both patients with perennial allergy ($p < 0.01$) and nonallergic subjects ($p < 0.05$), although the response was significantly greater in the group with perennial allergy ($p < 0.01$). Bradykinin induced significant contralateral responses in patients with perennial allergy ($p < 0.01$) but not in the nonallergic subjects. **B**, Lactoferrin levels. Bradykinin induced significant ($p < 0.05$ in each case) increases in ipsilateral and contralateral lactoferrin secretions in the patients with perennial allergy but not in the nonallergic subjects. **C**, Albumin levels. Bradykinin induced significant ($p < 0.001$ in each case) increases in ipsilateral albumin secretions in the patients with perennial allergy and nonallergic subjects, which were not significantly different between the two groups. Modest increases in contralateral secretion of albumin achieved significance for nonallergic subjects ($p < 0.001$) but not for patients with perennial allergy ($p = 0.06$).

TABLE I. Effect of unilateral bradykinin provocation on symptom scores in patients with PAR and nonallergic subjects

	Runny nose		Stuffy nose	
	Saline	AUC	Saline	AUC
Ipsilateral				
Nonallergic subjects	0	1.00 ± 0.53	0.22 ± 0.22	0.78 ± 0.36
Patients with PAR	1.40 ± 0.45*	6.30 ± 1.25*	1.20 ± 0.39*	5.90 ± 1.51*
Contralateral				
Nonallergic subjects	0	0.11 ± 0.11	0.22 ± 0.15	0.78 ± 0.57
Patients with PAR	1.10 ± 0.48*	4.00 ± 1.01*	1.20 ± 0.47	3.70 ± 1.10

Results are expressed as means ± SEM. AUC values are derived by subtracting the responses to saline solution from the responses to each dose of bradykinin and summing the values.

* $p < 0.05$ for patients with perennial allergy versus nonallergic subjects by Mann-Whitney U test.

Increases were seen in contralateral albumin levels in both populations but were very modest in comparison with the ipsilateral increases. Contralateral increases achieved statistical significance ($p < 0.001$) for nonallergic subjects ($AUC = 56.4 \pm 43.4 \mu\text{g/ml}$) but not for patients with perennial allergy ($AUC = 81.1 \pm 33.8 \mu\text{g/ml}$; $p = 0.06$). The magnitudes of the increases were not different between the two groups.

Symptom scores and sneezing. Subjective symptom scores for rhinorrhea and nasal congestion in response to bradykinin challenge are summarized as AUC values in Table I. Consistent with their history of chronic allergic symptoms, patients with PAR reported significantly increased ipsilateral ($p < 0.01$) and contralateral ($p < 0.05$) rhinorrhea after saline challenge compared with the nonallergic subjects, as well as increased ipsilateral ($p < 0.05$), but not contralateral, nasal congestion. In the group of patients with perennial allergy, bradykinin challenge resulted in striking further increases in symptom scores for rhinorrhea and congestion both ipsilaterally ($p < 0.001$ in each case) and contralaterally ($p < 0.05$ in each case). By contrast, bradykinin challenge did not significantly increase nasal congestion or rhinorrhea on either side of the nasal mucosa in the nonallergic subjects.

Bradykinin challenge did not cause sneezing in any of the nine nonallergic subjects but had significant effects on the patients with perennial allergy. Although one patient with perennial allergy sneezed in response to saline challenge, bradykinin induced sneezing in seven of the 10 patients with perennial allergy ($AUC = 2.30 \pm 1.32$; $p < 0.05$ compared with nonallergic subjects). The effects of bradykinin on sneezing in the group with perennial allergy were not dose-related.

Study 2: Responsiveness to bradykinin in patients with seasonal allergy challenged both in and out of season

Secretion weight. The effects of bradykinin provocation on secretion weights in patients with seasonal allergy challenged in and out of season are shown in Fig. 2, A. Ipsilateral and contralateral secretion weights in response to saline challenge were not different in subjects challenged in season compared with those challenged out of season. Bradykinin induced a significant, dose-dependent increase in secretion weights ipsilaterally (challenged side), both out of season and in season ($p < 0.001$ in each case). There was, however, a marked enhancement of the ipsilateral response to bradykinin when the subjects were challenged in season compared with out of season (AUC in season = $53.9 \pm 18.0 \text{ mg}$ vs out of season = $16.6 \pm 3.2 \text{ mg}$; $p < 0.01$). Bradykinin also induced a significant contralateral increase in secretion weights in subjects challenged in season ($AUC = 33.2 \pm 10.4 \text{ mg}$; $p < 0.01$) but not out of season ($AUC = -0.9 \pm 3.6 \text{ mg}$).

Lactoferrin. There were no differences in ipsilateral or contralateral lactoferrin levels between groups after saline challenge (Fig. 2, B). In subjects challenged during their allergy season, bradykinin induced significant increases in ipsilateral ($AUC = 2.42 \pm 0.21 \mu\text{g/ml}$; $p < 0.01$) and contralateral levels of lactoferrin ($AUC = 2.89 \pm 0.66 \mu\text{g/ml}$; $p = 0.05$). The same subjects challenged out of season showed no significant secretion of lactoferrin on either side of the nasal mucosa (ipsilateral $AUC = -0.11 \pm 0.16 \mu\text{g/ml}$; contralateral $AUC = 0.99 \pm 0.36 \mu\text{g/ml}$).

Albumin levels. There were no differences in ipsilateral or contralateral albumin levels after

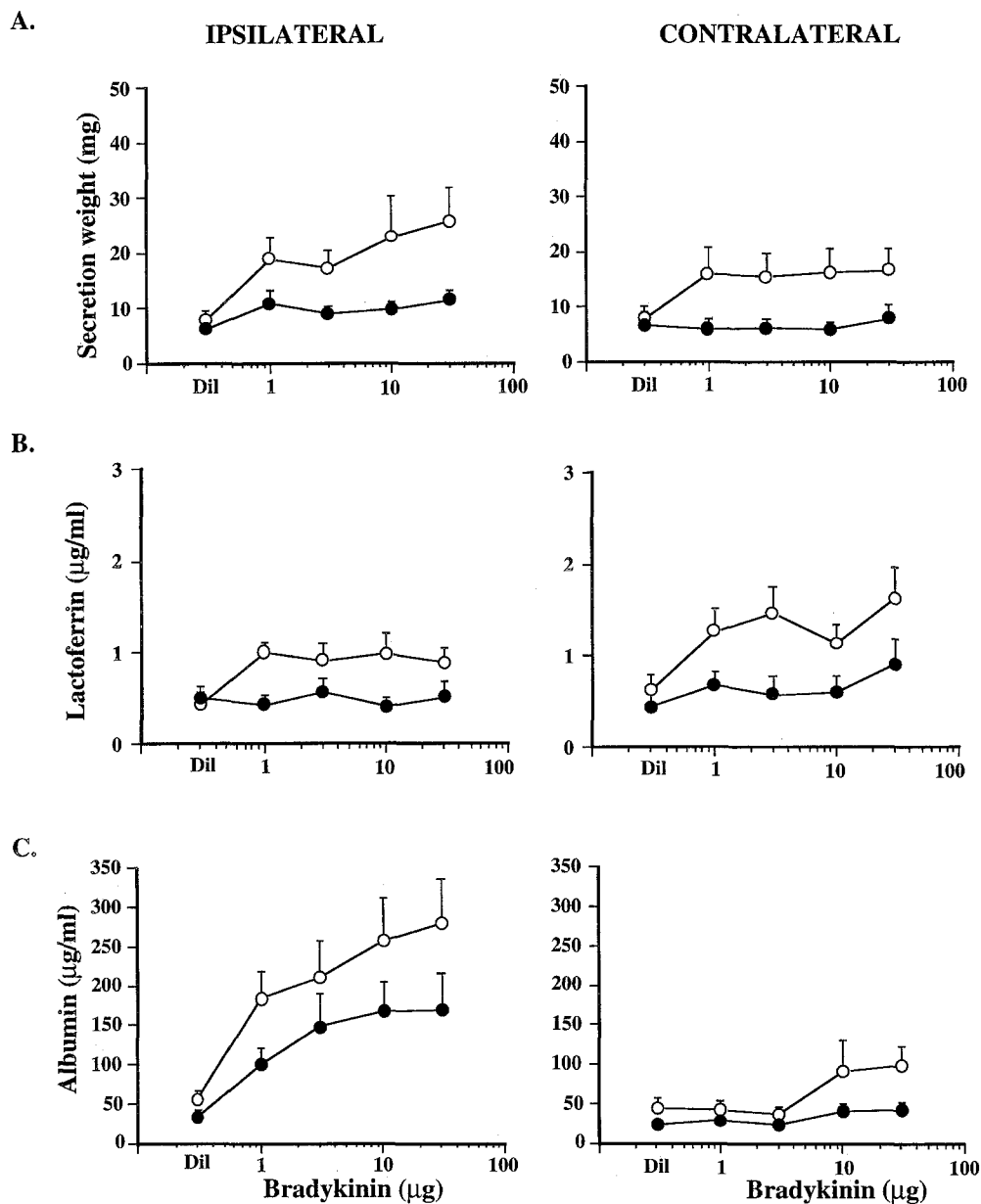


FIG. 2. Effect of nasal provocation with bradykinin in patients with seasonal allergy ($n = 9$) challenged in season (open circles) and out of season (filled circles). Panels at left represent ipsilateral responses, and panels at right show contralateral responses. **A**, Secretion weights. Bradykinin induced significant increases ($p < 0.001$ in each case) in ipsilateral secretions in subjects challenged both in season and out of season, but the increase was significantly greater during the season ($p < 0.01$). A significant increase in contralateral secretion weights was seen in the subjects challenged in season ($p < 0.01$) but not out of season. **B**, Lactoferrin levels. Bradykinin did not stimulate either ipsilateral or contralateral production of lactoferrin when subjects were challenged out of season. When subjects were challenged in season, bradykinin induced significant increases in ipsilateral ($p < 0.01$) and contralateral ($p < 0.05$) lactoferrin levels. **C**, Albumin levels. Bradykinin induced significant ($p < 0.001$ in each case) increases in ipsilateral albumin secretions in the subjects challenged both in and out of season, which were not significantly different between the two groups. Bradykinin challenge did not cause a significant contralateral secretion of albumin in subjects challenged in season or out of season.

TABLE II. Effect of unilateral bradykinin provocation on symptom scores in patients with seasonal allergy challenged in and out of season

	Runny nose		Stuffy nose	
	Saline	AUC	Saline	AUC
Ipsilateral				
Out of season	0.38 ± 0.35	1.13 ± 0.52	0.78 ± 0.32	1.11 ± 1.26
In season	0.44 ± 0.29	5.56 ± 1.60*	1.56 ± 0.56	2.67 ± 0.90
Contralateral				
Out of season	0.38 ± 0.35	0.50 ± 0.47	0.33 ± 0.24	3.11 ± 1.50
In season	0.67 ± 0.33	3.67 ± 1.12*	2.00 ± 0.44*	4.22 ± 0.98

Results are expressed as means ± SEM. AUC values are derived by subtracting the responses to saline solution from the responses to each dose of bradykinin and summing the values.

* $p < 0.05$ for in season versus out of season as determined by Wilcoxon matched-pairs, signed-rank test.

saline challenge between subjects challenged in season or out of season (Fig. 2, C). Bradykinin challenge induced a dose-dependent ipsilateral increase in albumin levels in subjects challenged both in season (AUC = $707 \pm 158 \mu\text{g/ml}$; $p < 0.001$) and out of season (AUC = $454 \pm 104 \mu\text{g/ml}$; $p < 0.001$), and the magnitudes of these changes were not significantly different. There was no significant increase in contralateral albumin levels after bradykinin provocation in subjects challenged either in season (AUC = $88.1 \pm 61.9 \mu\text{g/ml}$) or out of season (AUC = $42 \pm 22.3 \mu\text{g/ml}$).

Symptom scores and sneezing. Symptom scores for rhinorrhea and nasal congestion are summarized as AUC values in Table II. Consistent with the subjects' relatively mild symptoms during the season, relatively few differences were seen in responses to saline solution in and out of the season. Although nasal congestion scores in response to saline provocation were not different on the ipsilateral side between subjects challenged in season or out of season, contralateral scores were significantly greater ($p < 0.05$) in subjects challenged in season. Symptom scores for rhinorrhea in response to saline provocation were not different between subjects challenged in season or out of season either ipsilaterally or contralaterally. Bradykinin induced significant increases in both ipsilateral ($p < 0.01$) and contralateral ($p < 0.05$) symptom scores for rhinorrhea in subjects challenged in season but not out of season. In addition, there were significant increases in nasal congestion reported by the patients with seasonal allergy challenged in season on the ipsilateral ($p < 0.001$) and contralateral ($p < 0.001$) sides, whereas the same subjects challenged out of season had no such responses.

The numbers of sneezes after bradykinin chal-

lenge tended to be higher in subjects challenged in season compared with the same subjects challenged out of season, but this did not attain significance ($p = 0.07$). Bradykinin challenge induced sneezing in four of nine subjects challenged in season (AUC = 1.44 ± 0.8) but in no subjects challenged out of season.

Comparison of normal subjects and patients with allergy out of season. The nasal responses of normal subjects and of patients with seasonal allergy challenged out of season were essentially the same. Neither normal subjects nor patients with seasonal allergy challenged out of season had any significant increases in lactoferrin levels or in contralateral secretion weights. Ipsilateral increases in secretion weights were not different between the two groups ($p = 0.96$), nor were ipsilateral ($p = 0.45$) or contralateral ($p = 0.35$) albumin responses. Symptomatic responses between the groups also showed no differences.

Study 3: Effect of contralateral atropine treatment on responses to bradykinin

Application of atropine (0.32 mg) to the contralateral nostril before provocation with $30 \mu\text{g}$ of bradykinin virtually abolished bradykinin-induced increases in contralateral secretion weights ($p < 0.05$) and lactoferrin secretion ($p < 0.05$) when compared with vehicle pretreatment (Fig. 3). Bradykinin induced significant increases in ipsilateral secretion weights ($p < 0.05$) and lactoferrin levels ($p < 0.05$), which were not significantly different between vehicle and atropine treatments.

DISCUSSION

In this study we have used a technique in which relatively low doses of bradykinin are applied to a localized area of the nasal mucosa with filter paper

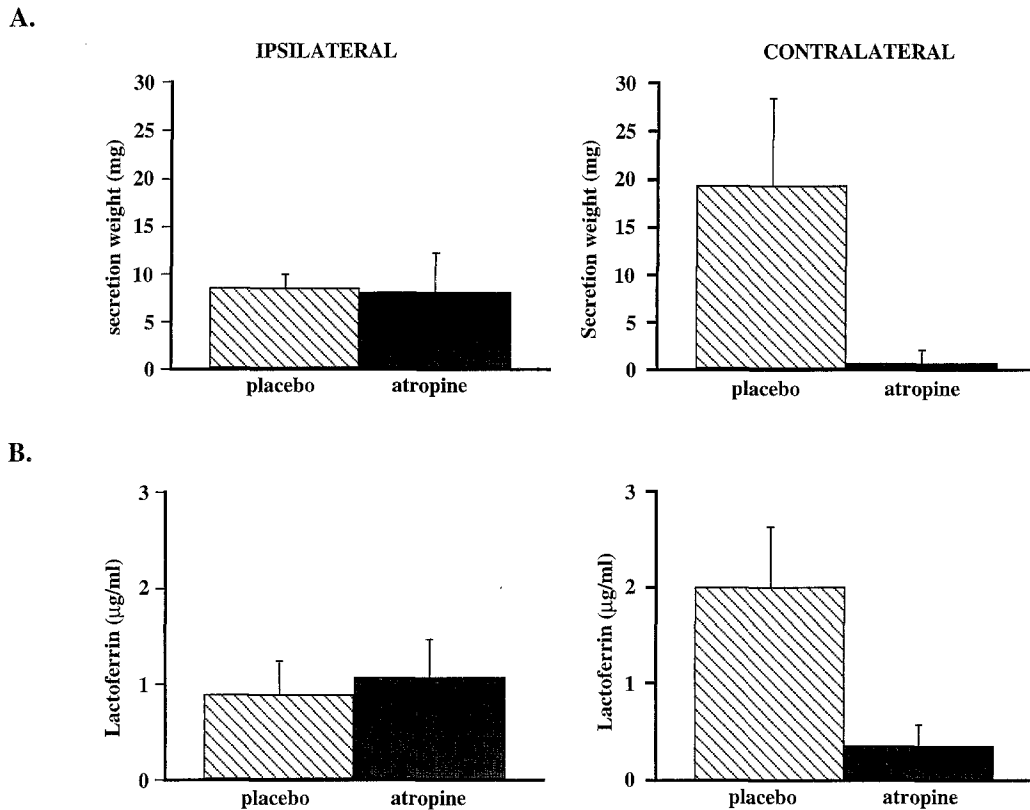


FIG. 3. Effects of pretreatment of the contralateral nostril with atropine (0.32 mg) or placebo on the ipsilateral and contralateral glandular responses to unilateral challenge with a single dose (30 µg) of bradykinin. Panels at left represent ipsilateral responses, and panels at right show contralateral responses. **A.** Secretion weights. **B.** Lactoferrin levels. Atropine significantly ($*p < 0.05$ in each case) attenuated the contralateral increases in secretion weights and lactoferrin levels induced by bradykinin provocation without altering ipsilateral responses.

disks. This technique, which has also been used to study the effects of provocation with other stimuli, such as histamine, methacholine, and allergen,^{14, 19} permits the objective measurement of the weight of resultant nasal secretions not only at the site of stimulation but also at other sites, including a corresponding location in the contralateral nostril. Moreover, elution of proteins from collection disks permits the measurement of proteins that serve as indices of different components of nasal lining fluid, such as glandular secretion and vascular leakage.

We compared the response to bradykinin provocation in normal, nonallergic subjects to that in patients with PAR, a condition associated with chronic allergic inflammation of the nasal mucosa. Patients with PAR displayed a striking hyperresponsiveness to bradykinin compared with normal subjects. This hyperresponsiveness was reflected not only by increased subjective symptom scores but also by the induction of responses not observed in the normal subjects. Thus although bradykinin

did not cause sneezing in any of the normal subjects, an observation consistent with previous reports,^{9, 12} a pronounced sneezing response was seen in the patients with perennial allergy. In addition, bradykinin not only caused a striking increase in secretion weights from the challenged (ipsilateral) nostril in patients with perennial allergy, compared with normal subjects, but also caused hypersecretion from the nonchallenged (contralateral) nostril of patients with perennial allergy, a response that was absent in the normal population. The increased secretion weights on both the ipsilateral and contralateral sides reflected primarily enhanced glandular secretion, because there were matching increases in the serous glandular marker, lactoferrin,¹⁸ whereas there were no differences between the two patient populations in ipsilateral or contralateral levels of the vascular marker, albumin. The increases seen in both populations in contralateral levels of albumin require some comment. These levels were very modest compared with ipsilateral increases and are

probably due to modest vascular leakage caused by the irritant effect of repeated placement of dry disks in the contralateral nostril. We have recently shown that repeated application of disks in this manner can induce similar modest increases in albumin levels.²⁰

These data differ somewhat from those of Baraniuk et al.²¹ who compared the reactivity of small groups of patients with perennial allergy and normal subjects to spray challenges with bradykinin and collected secretions by lavage and suction by using indwelling catheters. These authors found no difference in bradykinin-induced sneezing between the two groups, nor did they report any difference in the symptoms noted by the two populations. Their challenge method did not permit the analysis of secretion weights, but they demonstrated increased ipsilateral and contralateral levels of total protein and mucus glycoproteins in response to bradykinin challenge in the group with perennial allergy. Interestingly, in contrast to the data in this study, they saw no differences in contralateral levels of lysozyme, an alternative index of serous glandular secretion, and found lower levels of albumin in ipsilateral secretions of the group with perennial allergy. The reasons for these discrepancies are not clear but may reflect differences in the model used, the size of subject groups, or the criteria used for subject selection. Of the six patients with PAR in the study by Baraniuk et al.,²¹ three were treated with topical corticosteroids, which were withdrawn for only 48 hours before challenge. It is possible that residual effects of the corticosteroids may have modified serous glandular responses in these subjects and could also explain the lower levels of albumin seen in the population with perennial allergy.^{22, 23}

To determine whether the hyperresponsiveness that we observed in patients with PAR was related to the presence of allergic inflammation or was a reflection of atopic status per se, we extended our studies to perform the first comparison of the nasal response to bradykinin in subjects with seasonal allergy while they had symptoms (in season) and while they were free of symptoms (out of season). When the subjects were challenged out of season, their responses were similar to those observed for normal subjects. Indeed, statistical analysis showed no differences between the responses to bradykinin provocation of nonatopic subjects and atopic subjects without symptoms in terms of symptoms, secretion weights, and biochemical markers, a finding consistent with earlier studies in which spray challenge models were used.^{9, 12} By contrast, when

the same atopic subjects were challenged during the allergy season when they had symptoms, they demonstrated hyperreactivity similar to that observed in patients with perennial allergy. Subjective symptom scores were significantly increased compared with those reported when subjects were challenged out of season, and there was an increased incidence of bradykinin-induced sneezing, although this failed to achieve statistical significance. In addition to significantly increased ipsilateral responses in terms of secretion weights and lactoferrin production, but not albumin levels, patients with seasonal allergy challenged in season showed striking contralateral increases in secretion weights and lactoferrin levels. Thus hyperresponsiveness of the nasal mucosa to bradykinin is not an inherent feature of atopic status but requires the presence of active allergic rhinitis. Moreover, bradykinin is, to our knowledge, unique among chemical mediators in its ability to discriminate between patients with active allergic inflammation and non-allergic control subjects in terms of the induction of sneezing and contralateral glandular secretion. By contrast, histamine induces sneezing and contralateral reflexes in both allergic and nonallergic subjects,¹⁴ whereas methacholine is incapable of inducing a contralateral response, regardless of the allergic status of the subject.

Several possibilities exist to explain how allergic inflammation may modify the response of the nasal mucosa to bradykinin. It is possible that inflammation is associated with decreased expression or activity in the nasal mucosa of peptidases that are capable of degrading bradykinin. It seems unlikely, however, that our current data can be explained by loss of peptidases or by a reduction in epithelial barrier function, both of which would allow bradykinin greater access to its sites of action, because albumin levels in secretions are not altered in patients with symptomatic allergy compared with control who are free of symptoms, implying that access of bradykinin to vascular receptors is unaltered in the setting of allergic inflammation.

Another potential explanation is that allergic inflammation may alter responsiveness to bradykinin by increasing kinin receptor expression. It has been demonstrated that IL-1, which is generated at sites of allergic inflammation,²⁴ can increase the number of bradykinin receptors on human synovial cells.²⁵ If the expression of bradykinin receptors on target tissues were induced during allergic inflammation, this could help to explain the hyperresponsiveness to bradykinin seen in patients with allergy.

Further studies are necessary to examine this possibility.

The ability of bradykinin to induce contralateral secretion in patients with allergy is of particular interest. One possible explanation for this enhanced contralateral secretion is that the sensitivity of a neural reflex pathway to bradykinin had been upregulated. Contralateral secretory responses to histamine have been definitively shown to be due to neural reflexes, because unilateral vidian neurectomy on the side contralateral to the challenge site obliterates the secretory response.²⁶

It has been shown that bradykinin can directly activate sensory nerve endings in animal models.^{27,28} Moreover, there is clear precedent from animal models to suggest that inflammation can enhance neural reflex responses. For example, antigen challenge of sensitized guinea pigs has been shown to induce a marked enhancement of neural responses to electrical field stimulation, as monitored by contraction of airway smooth muscle.²⁹

There is also precedent for potential neural actions of bradykinin in the lower airways of human beings. Bradykinin is a potent bronchoconstrictor in patients with asthma but not in normal subjects.⁶⁻⁸ Despite its potent effects *in vivo*, application of bradykinin to airway smooth muscle *in vitro* causes, at best, a modest contraction,^{8,30} implying that responses obtained *in vivo* are mediated through indirect mechanisms. The ability of ipratropium bromide to attenuate the bronchoconstrictive effects of bradykinin⁸ implies a possible neural component of this response to bradykinin.

Our current data clearly confirm that neural responses to bradykinin are increased in the setting of chronic allergic inflammation. The increased capacity of bradykinin to stimulate sneezing in patients with symptomatic allergy points to upregulation of neural responses in these individuals. Moreover, our finding that the muscarinic receptor antagonist, atropine, applied contralaterally, abolishes both the increase in secretion weights and the increase in lactoferrin levels provides clear evidence that the contralateral secretory response is mediated by an efferent cholinergic pathway and is consistent with the finding that contralateral application of ipratropium bromide inhibits bradykinin-induced increases in contralateral levels of total protein and glycoconjugates.¹⁶ Activation of a central reflex by bradykinin could then also explain enhanced ipsilateral secretions in response to bradykinin in these individuals.

In summary, we have demonstrated that patients

with symptomatic allergic rhinitis demonstrate nasal hyperresponsiveness to bradykinin. Taken together, our data show that this hyperresponsiveness is mediated, at least in part, by increased sensory nerve responses to bradykinin, leading to increased sneezing and the initiation of central cholinergic reflexes that are responsible for inducing bilateral glandular secretion. The unique ability of bradykinin to stimulate these responses only in subjects with active inflammation suggests that the inflammatory process modifies neuronal responsiveness to this peptide in a selective manner. This upregulation could, conceivably, involve increased expression of bradykinin receptors on sensory nerves, a selective reduction in the threshold necessary to stimulate an action potential, or an increased firing rate of the nerve in response to bradykinin. Further studies are clearly necessary to delineate which components of allergic inflammation induce the increased neural responses to bradykinin and to define how neural responsiveness is altered.

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