Plasma extravasation through neuronal stimulation in human nasal mucosa in the setting of allergic rhinitis

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Sanico, Alvin M., Satsuki Atsuta, David Proud, and Alkis Togias. Plasma extravasation through neuronal stimulation in human nasal mucosa in the setting of allergic rhinitis. J. Appl. Physiol. 84(2): 537–543, 1998.—We have previously shown that capsaicin nasal challenge in subjects with allergic rhinitis produces a dose-dependent increase in the albumin content of nasal lavage fluids. In the present set of studies, we determined whether this observation represents plasma extravasation that is neurally mediated. To evaluate whether glandular secretions contribute to the albumin increase in nasal lavage fluids, volunteers with allergic rhinitis were pretreated with atropine or placebo before capsaicin challenge. Atropine significantly reduced the volume of returned lavage fluids and their lysozyme content but increased their albumin and fibrinogen content. To assess the contribution of sensory nerve stimulation, subjects with allergic rhinitis were pretreated in a second study with lidocaine or placebo before capsaicin challenge. Lidocaine significantly attenuated the capsaicin-induced increases in the volume of nasal lavage fluids, as well as their lysozyme and albumin content. To rule out the possibility of a direct effect of lidocaine on blood vessels rather than on nerves, healthy subjects were pretreated in a third study with lidocaine or placebo before bradykinin nasal challenge. Lidocaine did not affect the bradykinin-induced increase in the albumin content of nasal fluids. We conclude that, in allergic rhinitis, high-dose capsaicin induces plasma extravasation in the human nose and that this effect is neurally mediated. This provides more definitive evidence that neurogenic inflammation can occur in vivo in the human upper airway.

capsaicin; neurogenic inflammation

there is abundant evidence in animal models that capsaicin, the pungent principle of hot peppers, can induce plasma extravasation in the airways. In the rat trachea (7, 17, 18, 29, 39) and nasal mucosa (19), for example, extravasation of injected Evans blue dye occurs after capsaicin administration. Similarly, capsaicin challenge induces leakage of intravenously administered blue dye in the guinea pig nasal mucosa (1) and tracheobronchial airways (10, 11). Capsaicin stimulates a subpopulation of nerves consisting mostly of unmyelinated C fibers and some myelinated Aδ fibers (15). Capsaicin-induced plasma extravasation, therefore, has been viewed as evidence for neurogenic inflammation in these animal species (16). This phenomenon has been ascribed to neuropeptides, such as tachykinins, released antidromically from the sensory nerve endings on stimulation (7, 26).

In humans, increased vascular permeability can be manifested by the leakage of serum proteins, such as albumin. This has not been observed by previous investigators who used low doses of capsaicin (5, 13), raising doubts about the occurrence of neurogenic inflammation in human airways (4). In the case of other stimuli, such as allergen and histamine, much higher doses than what would be anticipated on the basis of biochemical calculations are required to induce a measurable in vivo response in humans, when delivered to the nasal mucosa. By using a higher dose of capsaicin, we have found a modest but significant increase in the albumin levels of nasal lavage fluids in subjects with active allergic rhinitis, compared with those with nonallergic rhinitis and with healthy controls (38). Furthermore, we have recently demonstrated that this phenomenon is dose dependent (37).

We propose that the albumin increase in nasal secretions after capsaicin administration represents plasma extravasation. However, some investigators have suggested in the past that albumin in nasal secretions may be partly derived from submucosal glands and, therefore, may not be truly representative of plasma leakage (23, 33). Furthermore, our use of relatively high doses of capsaicin may raise the possibility of nonspecific, nonneuronally mediated, effects. In this report, we present the results of a set of studies that addresses these issues.

In the first study, we applied atropine before capsaicin nasal challenge in allergic subjects. We hypothesized that, if the capsaicin-induced increase in albumin levels in nasal secretions is not of glandular origin, anticholinergic pretreatment will decrease glandular activity but will not decrease the albumin content of nasal lavage fluids. To further evaluate the occurrence of plasma extravasation, we also measured the levels of a larger serum protein, fibrinogen, before and after capsaicin challenge. In the second study, we applied lidocaine before capsaicin nasal challenge. We hypothesized that, if capsaicin induces plasma leakage through neuronal stimulation, nerve blockade will attenuate this effect. Some studies have suggested that lidocaine may attenuate plasma extravasation through vascular endothelial membrane stabilization (40). To rule out this possibility, we performed a third study in which we applied lidocaine before bradykinin nasal challenge of nonatopic subjects. Assuming that plasma leakage induced by bradykinin in these individuals represents a direct effect on vascular receptors (2), we hypothesized that, if lidocaine attenuates capsaicin-induced vascular permeability by neuronal blockade, it should have no effect on bradykinin-induced plasma leakage.
METHODS

Subjects

A total of 23 volunteers (7 men and 16 women) between the ages of 23 and 68 yr participated in the studies. Seventeen subjects were diagnosed as having active allergic rhinitis, with sensitivity to at least two aeroallergens confirmed by skin testing. They gave a history of experiencing chronic symptoms of rhinitis, including rhinorrhea, nasal congestion, and sneezing, during the months when the studies were conducted. The remaining six volunteers had negative skin tests and no history of nasal symptoms. All subjects avoided the use of antihistamines or decongestants for at least 1 wk before the study. All participants gave informed consent, and the study was approved by the Institutional Review Board of the Johns Hopkins Bayview Medical Center.

Nasal Lavage

Nasal secretions were collected by using previously described methods (24). Lavages were performed by using lactated Ringer solution (Kendall McGaw Laboratories, Irvine, CA) prewarmed to 37°C, instilled into both nostrils with a pipette while the head was extended, and then expelled into a plastic collecting basin after ~10 s. The nasal lavage fluids were then transferred into conical tubes and, after their volume was recorded, stored on ice before further processing. These samples were then centrifuged at 2,500 g at 4°C for 15 min, and aliquots of the supernatant were stored at −20°C for later assays.

Albumin Assay

Human serum albumin (HSA) levels in lavage samples were determined by using an enzyme-linked immunosorbent assay (ELISA) as previously described (35). A 96-well ELISA plate was coated with a 1:900 dilution of sheep anti-HSA antibody (The Binding Site, Birmingham, UK) and incubated overnight at 4°C. After the wells were washed with buffer, nonspecific binding sites were blocked with 1% sheep serum and incubated for 30 min at 37°C. The wells were then washed and received appropriate dilutions of the samples and of HSA standard (Sigma Chemical, St. Louis, MO). They were then incubated for 90 min at 37°C. After the wells were washed, a 1:1,200 dilution of horseradish peroxidase-conjugated sheep anti-HSA (Dako, Carpinteria, CA) was added and incubated for another 90 min at 37°C. The wells were then washed, and the reaction was developed by using o-phenylenediamine dihydrochloride (Sigma Chemical); absorbance was read at 490 nm. The assay standard curve for HSA ranged from 1 to 256 ng/ml.

Fibrinogen Assay

Fibrinogen levels in lavage samples were determined with ELISA as above, by using a 1:5,000 dilution of sheep anti-human fibrinogen antibody (The Binding Site) as the primary antibody and a 1:3,000 dilution of horseradish peroxidase-conjugated sheep anti-human fibrinogen (Dako) as the secondary antibody. The assay curve for the fibrinogen standard (Calbiochem, La Jolla, CA) ranged from 2 to 512 ng/ml.

Lysozyme Assay

Lysozyme levels in lavage samples were determined with ELISA as described in Albumin Assay, by using a 1:400 dilution of sheep anti-human lysozyme antibody (Dako) as the primary antibody and a 1:1,500 dilution of horseradish peroxidase-conjugated sheep anti-human lysozyme (The Binding Site) as the secondary antibody. The assay curve for the lysozyme standard (Calbiochem) ranged from 0.5 to 64 ng/ml (36).

Capsaicin Nasal Challenge After Pretreatment With Atropine

Design. Eight subjects with allergic rhinitis received atropine or placebo, 1 wk apart, in a randomized, double-blind, crossover manner, before the administration of capsaicin. To wash out biological components in the nasal secretions before nasal challenge, a total of eight nasal lavages, each by using 10 ml lactated Ringer solution, were performed at the beginning of each study period, and the returned fluids were discarded. Nasal lavages by using 5 ml lactated Ringer solution were thereafter performed at prechallenge and then at 10 min postchallenge with capsaicin. The lysozyme, albumin, and fibrinogen levels in these samples were measured. The volume of returned lavage fluids was also considered an outcome of this study.

Atropine pretreatment. A total of 1.2 mg atropine was administered into both nostrils through a metered nasal spray that delivered 75 µl per actuation (coefficient of variation: 10%). Atropine was given as a 1 mg/ml solution (American Regent Laboratories, Shirley, NY) in four divided doses (2 sprays each nostril per dose), 5 min apart. A lower dose of atropine did not effectively attenuate the glandular secretory response to 100 µg capsaicin nasal challenge in a pilot study. Normal saline solution (Abbott Laboratories, Chicago, IL) spray served as placebo.

Capsaicin spray challenge. A total of 100-µg capsaicin (Sigma Chemical) was administered via metered nasal spray 10 min after the last dose of atropine or placebo. Capsaicin was given as a single dose into both nostrils as a 0.67 mg/ml solution (2 mM), dissolved in 5% EtOH and 5% Tween 80 (Sigma Chemical). In our previous dose-response study (37), the same diluent was used in the administration of 1-µg capsaicin nasal challenge, which did not produce any significant albumin increase in nasal lavage fluids. We thus do not expect any significant nonspecific effect of this vehicle in the present study.

Capsaicin Nasal Challenge After Pretreatment With Lidocaine

Design. Ten subjects with allergic rhinitis received lidocaine or placebo, 1 wk apart, in a randomized, double-blind, crossover manner, before the administration of capsaicin. Preliminary lavages were performed at the beginning of each study period, as in the first protocol. Nasal lavages by using 5 ml lactated Ringer solution were thereafter performed at prechallenge and then at 30 min postchallenge with capsaicin. In this protocol, the lysozyme and albumin levels in the pre- and postcapsaicin lavage samples were measured, and the volume of the returned fluids was recorded.

Lidocaine pretreatment. A total of 90 µg lidocaine (Sigma Chemical) was administered into both nostrils via nasal spray as a 10% solution dissolved (wt/vol) in normal saline, given in three divided doses, 2 min apart. To prolong the duration of action of lidocaine, we administered two sprays of the α-adrenergic agonist and topical vasoconstrictor oxymetazoline HCl (0.05%) before the application of the local anesthetic or the normal saline solution, which served as placebo. Oxymetazoline has been previously shown to have no effect on plasma extravasation induced by other stimuli such as bradykinin or histamine (9, 41).

Capsaicin spray challenge. A total of 100 µg capsaicin was administered via nasal spray into both nostrils as described.
in the first protocol, 5 min after the last dose of lidocaine or placebo.

**Bradykinin Nasal Challenge After Pretreatment With Lidocaine**

**Design.** Six healthy nonatopic subjects received lidocaine or placebo, 1 wk apart, in a randomized, double-blind, crossover manner, before the administration of bradykinin. Healthy subjects were used in this protocol to avoid any confounding possibility of neuronal involvement in bradykinin-induced plasma leakage. This may occur in subjects with allergic rhinitis, as suggested by their development of tachyphylaxis after repeated bradykinin nasal challenges (34). On the other hand, healthy subjects do not exhibit this effect. Preliminary lavages were performed at the beginning of each study period, as in the previous protocols. Nasal lavages by using 5 ml lactated Ringer solution were thereafter performed at prechallenge and then at 30 min postchallenge with bradykinin. The albumin levels in these samples were measured, and the volumes of returned lavage fluids were recorded.

Lidocaine pretreatment. A total of 90 µg lidocaine was administered as a 10% solution into both nostrils via nasal spray, as described in the second protocol. Normal saline spray served as placebo.

Bradykinin spray challenge. A total of 20 µg bradykinin (Peninsula Laboratories, Belmont, CA) was administered 5 min after the last dose of lidocaine or placebo, as a 0.13 mg/ml solution (0.1 mM) dissolved in normal saline. This dose of bradykinin was chosen with the expectation, based on previous studies (30), that it would result in an amount of albumin in nasal lavage fluids similar to that observed after capsaicin challenge in our second protocol. Challenges were similarly delivered into both nostrils by using a metered nasal spray.

**Data Analysis**

Nonparametric statistics were applied. Values are presented as medians with 75 and 25th percentiles. Friedman's analysis of variance was first applied to detect significant differences within each protocol. Provided that this analysis yielded significant results, pre- and postchallenge values were compared by using Wilcoxon signed-rank paired test (by using StatView 4.5 software, Abacus Concepts, Berkeley, CA) to evaluate the effects of capsaicin or bradykinin administration. For evaluation of the effects of atropine or lidocaine vs. placebo pretreatment, the postcapsaicin outcomes were compared by using Wilcoxon signed-rank paired test. This was possible because we found no significant difference between the two treatment arms in their precapsaicin values. We also performed comparison of the various treatments by using the changes from prechallenge values that capsaicin or bradykinin induced in each protocol. The results of these comparisons obtained by using the changes were essentially identical to those obtained by using the actual postchallenge values, so we chose to present the latter for illustrative purposes. For analysis of the relationship between albumin and fibrinogen levels, the Spearman rank correlation coefficient was determined. A P value of <0.05 was considered significant.

**RESULTS**

**Capsaicin Nasal Challenge After Pretreatment With Atropine**

Volume of collected lavage fluids. The volume of collected nasal lavage fluids was significantly increased after capsaicin nasal challenge, with placebo pretreatment (Wilcoxon signed-rank paired test, pre- vs. postchallenge: P = 0.01). The capsaicin-induced increase in nasal secretions was completely inhibited by pretreatment with atropine (Wilcoxon signed-rank paired test, placebo vs. atropine postchallenge: P = 0.02; pre- vs. postchallenge with atropine pretreatment: P = 0.3; Fig. 1A). To avoid the confounding influence of the significant changes in the volume of collected samples on the concentration of albumin, fibrinogen, or lysozyme, the absolute amount of these components was calculated by multiplying their concentration by the corresponding volume of collected lavage fluids. These calculated
absolute amounts (in µg) were used in all subsequent comparative analyses.

Lysozyme. Capsaicin significantly increased the lysozyme content of nasal lavage fluids, with both placebo and atropine pretreatments (Wilcoxon signed-rank paired test, pre- vs. postchallenge: $P < 0.01$ for both placebo and atropine). Atropine diminished, but did not completely inhibit, the capsaicin-induced lysozyme increase (Wilcoxon signed-rank paired test, placebo vs. atropine postchallenge: $P < 0.05$; Fig. 1B).

Albumin. Capsaicin significantly increased the albumin content of nasal lavage fluids, with both placebo and atropine pretreatments (Wilcoxon signed-rank paired test, pre- vs. postchallenge: $P < 0.01$ for both placebo and atropine). Atropine pretreatment significantly augmented the capsaicin-induced albumin increase (Wilcoxon signed-rank paired test, placebo vs. atropine postchallenge: $P = 0.03$; Fig. 1C).

Fibrinogen. Capsaicin similarly significantly increased the fibrinogen content of nasal lavage fluids, with both placebo and atropine pretreatment (Wilcoxon signed-rank paired test, pre- vs. postchallenge: $P = 0.01$ for both placebo and atropine). Atropine pretreatment significantly augmented the capsaicin-induced fibrinogen increase (Wilcoxon signed-rank paired test, placebo vs. atropine postchallenge: $P = 0.02$; Fig. 1D).

Correlation between albumin and fibrinogen. There was significant correlation between the albumin and fibrinogen levels, by using pooled data from the placebo and atropine pretreatment protocols (Spearman rank correlation $r = 0.7$ and $P < 0.0001$).

Symptoms. Nasal challenge with 100 µg capsaicin induced acute and transient local burning sensation, rhinorrhea, and nasal congestion without any residual symptom or systemic effect (data not shown), consistent with our previous observations (37).

Capsaicin Nasal Challenge After Pretreatment With Lidocaine

Volume of collected lavage fluids. The volume of collected nasal lavage fluids was significantly increased after capsaicin nasal challenge, with placebo and lidocaine pretreatment (Wilcoxon signed-rank paired test, pre- vs. postchallenge: $P = 0.009$ for placebo and $P = 0.04$ for lidocaine). The capsaicin-induced increase in nasal secretions was significantly reduced by pretreatment with lidocaine (Wilcoxon signed-rank paired test, placebo vs. lidocaine postchallenge: $P = 0.01$; Fig. 2A).

Lysozyme. Capsaicin significantly increased the lysozyme content of nasal lavage fluids, with both placebo and lidocaine pretreatments (Wilcoxon signed-rank paired test, pre- vs. postchallenge: $P = 0.005$ for both placebo and lidocaine). Lidocaine significantly diminished the capsaicin-induced lysozyme increase (Wilcoxon signed-rank paired test, placebo vs. lidocaine postchallenge: $P = 0.03$; Fig. 2B).

Albumin. Capsaicin significantly increased the albumin content of nasal lavage fluids, with both placebo and lidocaine pretreatments (Wilcoxon signed-rank paired test, pre- vs. postchallenge: $P = 0.005$ for both placebo and lidocaine). Lidocaine significantly diminished the capsaicin-induced albumin increase (Wilcoxon signed-rank paired test, placebo vs. lidocaine postchallenge: $P = 0.03$; Fig. 2C).

Bradykinin Nasal Challenge After Pretreatment With Lidocaine

Volume of collected lavage fluids. There was no significant change in the volume of collected nasal lavage fluids after bradykinin challenge. There was no significant difference between the volume of collected...
nasal lavage fluids with placebo or lidocaine pretreatment (Fig. 3A).

Albumin. Bradykinin significantly increased the albumin content of nasal lavage fluids, with both placebo and lidocaine pretreatment (Wilcoxon signed-rank paired test, pre- vs. postchallenge: P = 0.03 for both placebo and lidocaine). Lidocaine pretreatment did not change the bradykinin-induced albumin increase (Wilcoxon signed-rank paired test, placebo vs. lidocaine postchallenge: P = 0.6; Fig. 3B).

DISCUSSION

Neurogenic inflammation, whereby nerve activation induces inflammatory changes such as plasma extravasation, has been well demonstrated in various animal models by using capsaicin (1, 7, 17–19, 39) and other stimuli (16, 20). The collective results of the present set of studies support the notion that this phenomenon can similarly occur in the human upper airway.

Capsaicin challenge induces a secretory response in both animal (21, 25) and human (12, 27) nasal mucosae. This is believed to involve two mechanisms: 1) glandular activation through a parasympathetically mediated central neuronal reflex and 2) direct stimulation of the glands by tachykinins released antidromically at the site of capsaicin application (22, 31). The capsaicin-induced secretory response is manifested by increases in the volume or weight of secretions. Also, glandular products such as lysozyme (32) would be expected to increase in the collected nasal fluids, as seen in the present study. Anticholinergic pretreatment significantly attenuated glandular activation. Compared with placebo, atropine eliminated the capsaicin-induced increase in the volume of nasal secretions. Given this significant change in the volume of collected lavage samples, we avoided any confounding influence on the relative concentration of measured proteins by using the calculated absolute amounts of these markers of glandular activation and plasma extravasation in our comparative analyses. In the first study, for example, if the concentration of lysozyme were to be considered, there would have been no significant difference between atropine and placebo (median levels postchallenge, respectively: 51.0 vs. 79.5 µg/ml, P = 0.3). This masking of the expected decrease in lysozyme is likely due to the significant decrease in the volume of collected lavage fluids with atropine vs. placebo (median: 4.8 vs. 5.9 ml, P = 0.02), thereby effectively increasing the relative concentration of this glandular product. Comparison of the calculated absolute amounts, however, reveals a significant decrease in the lysozyme content of nasal lavage fluids with atropine vs. placebo pretreatment (median: 263.4 vs. 505.6 µg, P < 0.05).

In addition to the secretory response, capsaicin also induces plasma leakage in rodent airways (1, 7, 10, 11, 17–19, 29, 39). This effect is believed to be mediated through the release of neuropeptides (7, 22, 26). Previous investigations have suggested that albumin in nasal secretions can be derived from submucosal glands (23, 33). We, therefore, set out to perform these studies to confirm 1) capsaicin-induced plasma extravasation and 2) the neurogenic nature of this phenomenon. In the present study, we found a significant increase in the albumin content of nasal lavage fluids after capsaicin administration, consistent with our previous study results. To further evaluate this finding, we also measured the levels of fibrinogen, which is a larger serum protein with a molecular weight of 400,000 compared with albumin with a molecular weight of 69,000 (8). We found that capsaicin challenge similarly and significantly increased the fibrinogen content of nasal lavage fluids. Furthermore, the levels of fibrinogen significantly correlated with those of albumin, attesting to their common intravascular origin. The demonstration of fibrinogen leakage provides further evidence for capsaicin-induced plasma extravasation. The same increases in albumin and fibrinogen are evident even if we use their measured concentrations, instead of their calculated amounts (for albumin, median levels pre- vs. postchallenge, with placebo pretreatment: 20.0 vs. 84.7 µg/ml, P = 0.01, and with atropine pretreatment: 25.3 vs. 305.1 µg/ml, P = 0.01; for fibrinogen, median levels pre- vs. postchallenge, with placebo pretreatment: 467.9 vs. 962.5 ng/ml, P = 0.01, and with atropine pretreatment: 485.2 vs. 2,674.5 ng/ml, P = 0.05).

If the measured albumin in nasal lavage fluids after capsaicin challenge is derived from submucosal glands, its level should decrease in parallel with lysozyme after atropine pretreatment. In the present study, we found
that this did not occur, thus making the notion of significant glandular origin of albumin untenable. In agreement with our results, Lundberg and Saria (18) have demonstrated that, in the rat trachea, atropine did not reduce the capsaicin-induced extravasation of Evans blue dye. In contrast, Asakura et al. (1) reported a partial reduction in the dye leakage response with atropine pretreatment in the guinea pig nasal mucosa, although no clear explanation was provided.

In the present study, anticholinergic pretreatment did not attenuate, but rather significantly augmented, the capsaicin-induced increase in albumin and fibrinogen. This result was unexpected. One possible explanation for this finding is that atropine pretreatment produced drying of the mucosal surface, reducing the protective diluting and cleansing effect of secretions, thus resulting in a higher effective concentration of capsaicin. This may be in keeping with our previous finding that the albumin increase after capsaicin challenge is dose dependent. Another possible interpretation could be that acetylcholine provides a protective effect on blood vessels against the increased permeability induced by capsaicin. However, we can offer no precedent from the literature for such concept.

That capsaicin-induced plasma extravasation is a dose-dependent phenomenon may explain the absence of albumin leakage in previous investigations (5, 13), where the dose delivered was <100 µg. Our use of a higher capsaicin dose in the present study may raise the possibility that the observed plasma leakage could be due to a nonspecific, even toxic, effect and not necessarily to neuronal activation. To address this concern, we performed our second study in which we pretreated the nasal mucosa with lidocaine before capsaicin challenge in a group of subjects with allergic rhinitis. Lidocaine inhibits cell membrane sodium channels, thereby preventing the generation and conduction of nerve impulses (14).

Lidocaine pretreatment significantly attenuated the secretory response to capsaicin challenge. This indicates that capsaicin-induced glandular activation is neurally mediated. Indeed, the application of this local anesthetic does not alter the secretory response elicited by methacholine (28), an agent that directly acts on glands. Lidocaine in the present study similarly attenuated the plasma leakage induced by capsaicin. This result correlates well with the findings of Lundberg and Saria (18) in the rat trachea and of Asakura et al. (1) in the guinea pig nasal mucosa that lidocaine pretreatment significantly attenuates the dye leakage after capsaicin challenge. These observations again indicate that the effects of capsaicin are neurally mediated. However, some studies have suggested that lidocaine may have protective effects against plasma extravasation through endothelial stabilization (40) and not necessarily through nerve blockade. To address this possibility, we determined whether lidocaine would attenuate the plasma extravasation induced by bradykinin. Because bradykinin induces this effect directly through vascular receptors (2), we hypothesized that the albumin leakage would not be decreased by lidoca-
Neurogenic Plasma Extravasation in the Nose


