Hyperosmolar saline induces reflex nasal secretions, evincing neural hyperresponsiveness in allergic rhinitis

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Sanico, Alvin M., George Philip, Gordon K. Lai, and Alkis Togias. Hyperosmolar saline induces reflex nasal secretions, evincing neural hyperresponsiveness in allergic rhinitis. J. Appl. Physiol. 86(4): 1202–1210, 1999.—We investigated whether hyperosmolar saline (HS), applied via paper disk onto the septum of one nostril, induces a nasal secretory response. Furthermore, we examined whether this response is accentuated in patients with active allergic rhinitis (AR) compared with healthy volunteers. Unilateral HS produced significant nasal secretions both ipsilateral and contralateral to the site of challenge in the AR group and only ipsilaterally in the healthy group. The HS-induced nasal secretions were significantly greater in the AR vs. the healthy subjects. In a separate study, we ascertained that the nasal response to HS is neurally mediated and found that ipsilateral nerve blockade with lidocaine significantly attenuates the HS-induced secretions bilaterally. In another group of AR subjects, we determined whether nociceptive fibers were involved in this response and found that sensory nerve desensitization with repeated application of capsaicin attenuated the HS-induced nasal secretions. Finally, we determined whether the secretory hyperresponsiveness in AR is attributable to increased reactivity of submucosal glands rather than of nerves. We found that the dose response to methacholine, which directly stimulates the glands, was identical among AR and healthy subjects. We conclude that, in AR, nasal challenge with HS induces significantly greater reflex secretions involving capsaicin-sensitive nerve fibers, consistent with the notion of neural hyperresponsiveness in this disease. capsaicin; lidocaine; methacholine

ALLERGIC RHINITIS is characterized by increased nasal responsiveness to various stimuli such as cold dry air (28), and nerve involvement in its pathophysiology has been implicated by our previous investigations. We have shown that administration of cold dry air into one nostril induces nasal secretions not only ipsilaterally, but also contralaterally, to the site of challenge (18). The contralateral response, in particular, indicates the existence of a neurally mediated reflex (23). Other investigators have shown that contralateral secretory responses can be abolished by vidian nerve resection (13). Neural involvement is also indicated by our finding that the bilateral secretory response to unilateral cold dry air is attenuated by ipsilateral application of a local anesthetic (18).

We have further observed that cold dry air increases the osmolality of nasal secretions, only in those individuals who respond to this challenge (27). We and others have thus postulated that hyperosmolarity at the mucosal surface may be the factor that activates the airways response to cold dry air (1, 2). In the present set of studies, we initially determined whether nasal challenge with hyperosmolar saline can induce reflex secretions. To evaluate whether such a response is generated, we applied the challenge solutions onto one nostril in a localized manner and collected secretions both ipsilateral and contralateral to the site of challenge. In the first protocol, unilateral nasal challenge was performed using normal saline, followed by the same procedure using hyperosmolar saline. We hypothesized that, compared with the normal saline control, hyperosmolar saline will induce increased nasal symptoms and bilateral secretions, more so in subjects with allergic rhinitis than in healthy individuals. In the second protocol, we ascertained that the hyperosmolar saline-induced secretory reflex in the first protocol was reproducible and that it was not influenced by the preceding challenge with normal saline.

To further establish that the hyperosmolar saline-induced effects are neurally mediated, we determined whether these can be attenuated by pretreatment of the challenge site with a local anesthetic. In this third protocol, we hypothesized that topical administration of lidocaine will reduce the nasal secretory response to hyperosmolar saline.

In the fourth protocol, we sought to identify the specific nerve fibers that participate in this reflex. We have previously observed that both cold dry air and hyperosmolar saline induce a burning sensation in the nose (24, 26). This is reminiscent of the symptom induced by capsaicin (21), the pungent component of hot pepper that activates mostly unmyelinated C fibers and some myelinated A-δ fibers (12). The capsaicin receptor on these fibers, which has been recently cloned, is a nonselective cation channel that participates in heat sensation (6). We hypothesized that the secretory and symptomatic responses to hyperosmolar saline are mediated by capsaicin-sensitive nerve fibers and will thus be attenuated by repeated application of capsaicin that leads to desensitization. This type of intervention has been reported by several investigators to be effective in reducing various manifestations of rhinitis (5, 9, 15, 16, 25).

The nasal secretory pathway can be divided into neural and effector components. The former includes the afferent, central, and efferent nerves, whereas the latter is comprised of submucosal glands (23). Theoreti-
cally, upregulation of any combination of these elements can lead to increased secretions on sensory nerve stimulation. We hypothesized that the nasal secretory hyperresponsiveness in allergic rhinitis is largely attributable to the neural, instead of glandular, components. In the fifth protocol, we examined the possibility of increased gland reactivity by comparing the effects of methacholine in subjects with allergic rhinitis and in healthy volunteers. We expected that, by applying the same techniques as in the preceding protocols, gland hyperresponsiveness, if present, would be manifested by greater secretions on direct activation by this cholinergic agent.

In this report, we present the results of our series of protocols that collectively demonstrate that the effects of hyperosmolar saline nasal challenge are significantly greater in patients with allergic rhinitis and that this hyperresponsiveness is attributable to the neural elements of a secretory reflex.

METHODS

Subjects

A total of 54 volunteers (21 men and 33 women) with ages between 18 and 60 yr participated in these studies. Thirty-nine of the subjects had been diagnosed to have allergic rhinitis, with sensitivity to at least two aeroallergens confirmed by skin testing. They gave a history of experiencing typical symptoms of rhinorrhea, nasal congestion, and/or sneezing during the period when the studies were conducted. The remaining 15 volunteers had no rhinitis symptoms and had negative skin testing to common aeroallergens. The use of antihistamines or decongestants was avoided for at least 1 wk and of nasal steroids or cromolyn for at least 1 mo before study entry. All subjects gave informed consent, and the study was approved by the Institutional Review Board of the Johns Hopkins Bayview Medical Center.

Collection and Measurement of Nasal Secretions

Nasal secretions were collected by using paper disks as previously described (3, 18). Filter paper disks were punched from filter cards (no. 190005, Shandon, Pittsburgh, PA) by using an 8-mm hole puncher, sealed in 1.5-ml microtubes (Sarstedt, Newton, NC), and weighed (Mettler Analytical Balance AE240, Mettler Instruments, Hightstown, NJ). Under direct visualization with the aid of a surgical headlight, nasal speculum, and duckbill forceps, disks were placed one each on the right and left nasal septa, just beyond the mucocutaneous junction. For the methacholine challenges, however, placement of collection disks was done only ipsilateral to the challenge site, because previous work has shown that this agent does not induce contralateral secretions (3). The collection disks were left in place for 30 s, immediately resealed in the microtube on removal, and then reweighed. The weight of collected secretions was calculated by subtracting the precollection (dry) weight from the postcollection (wet) weight of each disk.

Nasal Disk Challenges

Challenges were performed by using either 50 µl of 0.15 M NaCl (normal saline), 50 µl of 5.13 M NaCl (hyperosmolar saline), 30 µl of 0.05 M methacholine (Spectrum Quality Products, Gardenia, CA), or 10 µl of diluent (normal saline) followed by 10 µl of methacholine in threefold incremental doses from $10^{-4}$ to 2.56 M. Challenge filter paper disks were prepared in a similar manner as for the collection disks. The challenge material was loaded on a disk, which was immediately applied onto one nasal septum for 1 min and then discarded.

Symptom Scores

Subjects recorded symptoms of local burning, rhinorrhea, and nasal congestion 3 min before and 1 min after challenge, by using 10-cm-long visual analog scales that yielded a continuous score from 0 to 10 ranging from “no sensation that you can notice” to “worst sensation that you could imagine.”

Protocol 1: Normal Saline Vs. Hyperosmolar Saline Nasal Challenge in Allergic Rhinitis and Healthy Subjects

Design. Twenty-three subjects with allergic rhinitis and ten healthy volunteers participated in this protocol. Secretions were collected bilaterally, before and after unilateral application of the challenge disk, which contained either hyperosmolar saline or the control normal saline. The subjects were blinded as to the order of challenges.

Application of collection and challenge disks. The subjects were asked to gently blow their nose, and the anterior nasal mucosa was visibly cleared of any residual secretions. Three minutes later, collection disks were applied bilaterally to obtain baseline secretions. After 2 min, a challenge disk containing normal saline was applied onto the right nasal septum for 1 min and then discarded. Secretions were collected bilaterally 1 and 5 min postchallenge. The subjects were then asked to blow their nose again, and the anterior nasal mucosa was cleared of any residual secretions. The same method was repeated, this time using a challenge disk containing hyperosmolar saline. Symptom scores were recorded 1 min after each challenge with normal saline and with hyperosmolar saline.

Protocol 2: Hyperosmolar Saline Nasal Challenge With Vs. Without Prior Normal Saline Challenge

Design. Six additional subjects with allergic rhinitis underwent unilateral nasal disk challenge with normal saline followed by hyperosmolar saline during the same visit, as in the first protocol. On another visit, they underwent nasal challenge with hyperosmolar saline alone. These visits for each subject were scheduled in a randomized, crossover manner, 1 day apart.

Application of collection and challenge disks. Collection and challenge disks were applied as described in the first protocol. Secretions were collected bilaterally before, as well as 1 and 5 min after, each challenge with hyperosmolar saline.

Protocol 3: Hyperosmolar Saline Nasal Challenge With Sham Vs. Lidocaine Pretreatment

Design. Nine subjects with allergic rhinitis who were included in the first protocol also participated in this study. On two separate visits, they received either lidocaine or sham pretreatment, followed by unilateral nasal disk challenge with hyperosmolar saline. The visits for each subject were scheduled in a randomized, crossover manner, at least 1 day apart. Secretions were collected bilaterally, before and after each challenge with hyperosmolar saline.

Lidocaine pretreatment. The nasal septal mucosa was anesthetized only at the side where the challenge disk was applied, by using previously described methods (18). The pretreatment agents were administered by using metered pump sprays that deliver ~75 µl/actuation. To prolong the
anesthetic effect of lidocaine, two sprays of oxymetazoline HCl (0.05% Nostrella, Boehringer Ingelheim, Ridgefield, CT), an α-adrenergic agonist and vasoconstrictor, were applied 2 min before the administration of lidocaine HCl (10% Sigma Chemical, St. Louis, MO). Lidocaine was given as two sprays every 2 min for a total of four sprays. Thereafter, lidocaine was applied locally onto the septal mucosa by using a soaked cotton-tipped swab until a complete anesthetic effect was achieved. Sham pretreatment, which served as control, included the oxymetazoline step followed by four sprays of normal saline.

Application of collection and challenge disks. Collection and challenge disks were applied as described in the first protocol. Secretions were collected bilaterally before, as well as 1 and 5 min after, each challenge with hyperosmolar saline.

Protocol 4: Hyperosmolar Saline and Methacholine Nasal Challenges Before Vs. After Capsaicin Desensitization

Design. Ten subjects with allergic rhinitis participated in this study. They underwent repeated application of capsaicin onto the right nasal septum during six to seven visits, over an average period of 16 days. Nasal disk challenges with hyperosmolar saline as well as with 0.05 M methacholine were performed on average 2 wk before, and 2 days after, the capsaicin desensitization protocol. The methacholine nasal challenge served as a negative control measure because it is known to directly activate submucosal glands, without any neural involvement (3).

Capsaicin desensitization. The subject's right nasal mucosa was first anesthetized before each application of capsaicin. As in the third protocol, subjects were administered two nasal sprays of oxymetazoline, followed by two sprays of lidocaine every 2 min for a total of two doses. Lidocaine was then locally applied by using soaked cotton-tipped swabs until an anesthetic effect was achieved. Capsaicin (0.001 M; Sigma Chemical) was subsequently swabbed onto the anterior one-third of the right nasal septum over a period of 1 min. This was repeated every 10–15 min, for a total of three applications per visit.

Application of collection and challenge disks. All challenge disks were applied onto the right nasal septum. Secretions were collected bilaterally before, as well as 1 and 5 min after, unilateral challenge with hyperosmolar saline. Collection of secretions before, as well as 1 and 5 min after, methacholine challenge were performed only at the ipsilateral side. Nasal challenges with hyperosmolar saline and methacholine were performed sequentially in this order, 15 min apart. Symptom scores were recorded 3 min before, and 1 min after, hyperosmolar saline challenge.

Protocol 5: Methacholine Nasal Challenge in Allergic Rhinitis and Healthy Subjects

Design. Eight subjects with allergic rhinitis and eight healthy volunteers participated in this protocol. To allow detection of any dose-dependent difference in the glandular responsiveness of these two groups, each subject underwent unilateral disk challenge with diluent followed by a total of 10 incremental doses of methacholine, every 30 min, during a single visit. The top dose of 5.0 mg (2.56 M) was chosen on the basis of a previous study applying similar methods (3).

Application of collection and challenge disks. All collection and challenge disks were applied onto the same nostril as described in the preceding protocols. Ipsilateral secretions were collected and symptom scores were recorded 1 min after each challenge.

Data Analysis

Nonparametric statistical methods were applied. Friedman's ANOVA was first used to detect significant differences in values obtained at different time points (prechallenge and 1 and 5 min postchallenge) within each nasal provocation in the first and second protocols. Provided that this analysis yielded significant results, individual time points were compared by using Wilcoxon signed-rank paired test (using StatView 4.5 software, Abacus Concepts, Berkeley, CA). Symptom scores obtained 1 min postchallenge were similarly compared by using Wilcoxon signed-rank paired test. To compare the nasal responsiveness in subjects with allergic rhinitis vs. healthy volunteers in the first protocol, the changes (Δ) in secretion weights from pre- to postchallenge with normal saline or hyperosmolar saline were calculated. These changes, as well as the symptom scores obtained after each challenge, were then compared by using the Mann-Whitney U-test. To evaluate the effects of lidocaine and of capsaicin desensitization on the response to hyperosmolar saline or to methacholine challenge, the changes in secretion weights from pre- to postchallenge were calculated and compared (sham vs. lidocaine pretreatment in the third protocol and pre- vs. postdesensitization in the fourth protocol) by using the Wilcoxon signed-rank paired test. To compare the nasal responsiveness in subjects with allergic rhinitis vs. healthy volunteers in the fifth protocol, the changes between postchallenge values obtained with diluent and with each of the 10 methacholine doses were calculated. The two groups were then compared with respect to the sum of all these changes in secretion weights and in symptom scores, as well as with respect to the changes at each individual dose, by using the Mann-Whitney U-test. The age and gender distributions of subjects in the two groups were compared in the first and fifth protocols by using the Mann-Whitney U-test and χ² test, respectively. Data are presented as integral values or as means ± SE. A P value of < 0.05 was considered significant.

RESULTS

Protocol 1: Normal Saline Vs. Hyperosmolar Saline Nasal Challenge

Secretion weights. In the group of subjects with allergic rhinitis, unilateral challenge with normal saline induced changes in secretion weights ipsilaterally but not contralaterally (Friedman's ANOVA: P = 0.004 and P = 0.1, respectively; Figs. 1 and 2). The increase in ipsilateral secretion weights was statistically significant at 1 min, but not at 5 min, after application of normal saline (Wilcoxon signed-rank paired test, prechallenge vs. postchallenge: P = 0.0003 and P = 0.08, respectively). Unilateral challenge with hyperosmolar saline induced significant changes in secretion weights both ipsilaterally and contralaterally (Friedman's ANOVA: P < 0.0001 for both). Hyperosmolar saline induced a significant increase in secretions collected ipsilaterally to the challenge site at 1 and 5 min (Wilcoxon signed-rank paired test, prechallenge vs. postchallenge: P < 0.0001 for both). Hyperosmolar saline similarly induced a significant increase in contralateral secretions, at 1 and 5 min (Wilcoxon signed-rank paired test, prechallenge vs. postchallenge: P < 0.0001 and P = 0.0002, respectively). Because there were significant increases in ipsilateral secretions at 1 min after normal saline, and at all time points after hyperosmo-
Fig. 1. Ipsilateral secretory effects of unilateral nasal challenge with normal saline vs. hyperosmolar saline in 23 subjects with active allergic rhinitis. Individual secretion weights obtained before (Pre) as well as 1 and 5 min after challenge are shown. Thick marks represent mean data from each time point. The 3 values within each data set were first compared by using Friedman's ANOVA. This was followed by comparison of pre- and postchallenge values by using Wilcoxon signed-rank paired test (P values shown). Evaluation of changes between pre- and postchallenge values showed significantly increased ipsilateral secretions induced by hyperosmolar saline, compared with normal saline (**P < 0.0001 and *P = 0.002).

Fig. 2. Contralateral secretory effects of unilateral nasal challenge with normal saline vs. hyperosmolar saline in 23 subjects with active allergic rhinitis. The 3 values within each data set were first compared by using Friedman's ANOVA. Significant increases were noted only with hyperosmolar saline and not with normal saline (P values shown). Evaluation of changes between pre- and postchallenge values showed significantly increased contralateral secretions induced by hyperosmolar saline, compared with normal saline (**P < 0.0001 and *P = 0.002).
Symptom scores. The symptom scores for rhinorrhea, congestion, and burning sensation after normal saline challenge in subjects with allergic rhinitis, albeit minimal (average = 0.9 for each symptom), were greater compared with those reported by healthy volunteers (Mann-Whitney U-test, rhinitis vs. healthy subjects: \( P = 0.005 \), \( P = 0.0002 \), and \( P = 0.02 \), respectively). Hyperosmolar saline challenge induced significantly greater symptom scores for rhinorrhea and congestion, but not for burning sensation, in subjects with allergic rhinitis compared with healthy volunteers (Mann-Whitney U-test, rhinitis vs. healthy subjects: \( P = 0.005 \), \( P = 0.001 \), and \( P = 0.97 \), respectively). We also compared the two groups with respect to the differences between normal saline-induced and hyperosmolar saline-induced symptoms and found similarly greater differences in symptom scores for rhinorrhea and congestion, but not for burning sensation, in subjects with allergic rhinitis compared with healthy volunteers (Mann-Whitney U-test, rhinitis vs. healthy subjects: \( P = 0.02 \), \( P = 0.01 \), and \( P = 0.97 \), respectively; Fig. 4).

Protocol 2: Hyperosmolar Saline Nasal Challenge With Vs. Without Prior Normal Saline Challenge

In a separate group of subjects with allergic rhinitis, we found that unilateral challenge with hyperosmolar saline, after administration of normal saline as in the first protocol, again induced significant increases in secretion weights, both ipsilaterally and contralaterally (Friedman's ANOVA: \( P = 0.03 \) and \( P = 0.006 \), respectively). Unilateral challenge with hyperosmolar saline, without prior administration of normal saline, similarly induced significant ipsilateral and contralateral secretory responses (Friedman's ANOVA: \( P = 0.006 \) and \( P = 0.03 \), respectively). Before the application of hyperosmolar saline in both procedures, the prechallenge secretion weights were not significantly different, both ipsilaterally (Wilcoxon signed-rank paired test, pre-HS with vs. without prior NS: \( P = 0.5 \)) and contralaterally (Wilcoxon signed-rank paired test, pre-HS with vs. without prior NS: \( P = 0.8 \)). Evaluation of changes in secretion weights from pre- to postchallenge showed no significant difference in the effects induced by hyperosmolar saline given with or without prior administration of normal saline, both ipsilaterally (\( \Delta \) HS with vs. without prior NS at 1 min: \( 22.6 \pm 6.3 \) vs. \( 19.0 \pm 7.4 \) mg, \( P = 0.7 \); at 5 min: \( 13.2 \pm 5.1 \) vs. \( 16.6 \pm 7.5 \) mg, \( P = 0.9 \)) and contralaterally (\( \Delta \) HS with vs. without prior NS at 1 min: \( 12.2 \pm 3.1 \) vs. \( 10.1 \pm 3.7 \) mg, \( P = 0.5 \); at 5 min: \( 2.6 \pm 1.3 \) vs. \( 5.2 \pm 2.8 \) mg, \( P = 0.3 \)).

Protocol 3: Hyperosmolar Saline Nasal Challenge With Sham Vs. Lidocaine Pretreatment

Unilateral challenge with hyperosmolar saline after sham pretreatment in subjects with allergic rhinitis induced significant increases in secretion weights both ipsilaterally and contralaterally (Friedman's ANOVA: \( P = 0.002 \) and \( P = 0.004 \), respectively). On the other hand, hyperosmolar challenge with lidocaine pretreatment induced a significant increase in secretion weights ipsilaterally but not contralaterally (Friedman's ANOVA: \( P = 0.01 \) and \( P = 0.1 \), respectively). When the changes in secretion weights from pre- to postchallenge with hyperosmolar saline in the sham pretreatment arm were compared with those in the lidocaine pretreatment arm of the study, we found that the local anesthetic significantly attenuated the secretory response measured at 1 min but not at 5 min, both ipsilaterally (Wilcoxon signed-rank paired test, \( \Delta \) HS with sham vs. lidocaine: \( P = 0.008 \) at 1 min and \( P = 0.9 \) at 5 min) and contralaterally (Wilcoxon signed-rank paired test, \( \Delta \) HS with sham vs. lidocaine: \( P = 0.008 \) at 1 min and \( P = 0.4 \) at 5 min; Fig. 5).
Protocol 4: Hyperosmolar Saline and Methacholine Nasal Challenges Before Vs. After Capsaicin Desensitization

Secretion weights. Before desensitization, unilateral disk challenge with hyperosmolar saline in subjects with allergic rhinitis induced significant increases in secretion weights, both ipsilaterally and contralaterally (Friedman’s ANOVA: \( P < 0.002 \) and \( P < 0.003 \), respectively). Methacholine similarly induced a significant increase in secretion weights ipsilaterally (Friedman’s ANOVA: \( P < 0.0005 \)).

After capsaicin desensitization, hyperosmolar saline induced a significant increase in secretion weights ipsilaterally but not contralaterally (Friedman’s ANOVA: \( P = 0.01 \) and \( P = 0.3 \), respectively). Prechallenge secretion weights were not affected by capsaicin treatment, both ipsilaterally and contralaterally (Wilcoxon signed-rank paired test, pre-HS before vs. after desensitization: \( P = 0.3 \) and \( P = 0.6 \), respectively). Comparison of the hyperosmolar saline-induced changes in secretion weights obtained before and after capsaicin desensitization showed attenuation of the secretory response at 1 min ipsilaterally (Wilcoxon signed-rank paired test, \( \Delta \) HS before vs. after desensitization: \( P < 0.05 \)) and, to a lesser extent, contralaterally (Wilcoxon signed-rank paired test, \( \Delta \) HS before vs. after desensitization: \( P = 0.07 \); Fig. 6A).

The hyperosmolar saline-induced secretory response at 5 min was not significantly attenuated by the capsaicin treatment, both ipsilaterally and contralaterally (Wilcoxon signed-rank paired test, \( \Delta \) HS before vs. after desensitization: \( P = 0.3 \) and \( P = 0.4 \), respectively).

Methacholine continued to induce a significant increase in secretion weights even after capsaicin desensitization (Friedman’s ANOVA: \( P = 0.0002 \)). The changes in secretion weights from pre- to postchallenge with methacholine were not reduced after repetitive application of capsaicin (Wilcoxon signed-rank paired test, \( \Delta \) methacholine before vs. after desensitization: \( P = 0.5 \) at 1 min and \( P = 0.3 \) at 5 min; Fig. 6B).

Symptom scores. After capsaicin desensitization, the pre-hyperosmolar saline challenge symptom scores were lower for burning but not for rhinorrhea or congestion (Wilcoxon signed-rank paired test, pre-HS before vs. after desensitization: \( P = 0.04 \), \( P = 0.7 \), and \( P = 0.8 \), respectively). Capsaicin desensitization significantly reduced the hyperosmolar saline-induced increases in symptom scores for burning and rhinorrhea but not for nasal congestion (Wilcoxon signed-rank paired test, \( P < 0.05 \) for burning and rhinorrhea, \( P = 0.5 \) for nasal congestion).

Fig. 5. Changes in ipsilateral and contralateral secretion weights from prechallenge to 1 min postchallenge with hyperosmolar saline in 9 subjects with active allergic rhinitis after sham or lidocaine pretreatment. Analysis using Wilcoxon signed-rank paired test showed significant attenuation of hyperosmolar saline-induced secretory response by nerve blockade with a local anesthetic, both ipsilaterally and contralaterally (\( P \) values shown).

Fig. 6. Changes in nasal secretion weights from pre- to 1 min postchallenge with hyperosmolar saline (A) or methacholine (B) before and after capsaicin desensitization in 10 subjects with active allergic rhinitis. Repeated application of capsaicin significantly attenuated hyperosmolar saline-induced secretory response ipsilaterally and, to a lesser extent, contralaterally. In contrast, nasal secretory response to direct gland activation by methacholine was not affected (\( P \) values shown).
Δ HS before vs. after desensitization: $P = 0.01$, $P = 0.02$, and $P = 0.07$, respectively; Fig. 7).

Protocol 5: Methacholine Nasal Challenge in Allergic Rhinitis and Healthy Subjects

Age and gender distribution. There was no significant difference between the two groups with respect to age (rhinitis vs. healthy subjects: $35.1 \pm 3.1$ vs. $32.4 \pm 2.2$ yr, $P = 0.6$) and gender distribution (men:women, rhinitis vs. healthy subjects, 1:7 vs. 1:7).

Secretion weights. Methacholine produced dose-dependent increases in ipsilateral secretion weights in subjects with allergic rhinitis and in healthy volunteers (Fig. 8). In both groups, the secretion weights were significantly increased after a dose of at least $0.01$ M (Wilcoxon signed-rank paired test, diluent vs. methacholine).

DISCUSSION

The present study demonstrates that hyperosmolar saline induces significantly greater nasal secretions in subjects with allergic rhinitis compared with healthy individuals. We and others have observed similar findings of secretory hyperresponsiveness among patients with this disease by using other stimuli (19, 22, 28).

The experimental protocols sequentially presented in this report were designed to examine the mechanism(s) by which hyperosmolar saline effects changes in the nasal mucosa of individuals with active allergic rhinitis. In this group of subjects, we found that hyperosmolar saline significantly increases nasal secretions, both at the site of challenge and, more interestingly, at the contralateral side. This bilateral response to a unilateral stimulus is concordant with the secretory reflex that we previously observed by using cold dry air (18), thus providing support to the concept that cold dry air may act through an increase in the osmolarity of the epithelial lining fluid (27).

To ensure that the effect of hyperosmolar saline was not confounded by the preceding challenge with normal saline, we repeated the initial protocol in a separate group of subjects with allergic rhinitis and compared the results with those obtained by administering the hyperosmolar saline alone. We found that the prehyperosmolar saline challenge secretion weights, with and without a preceding normal saline control, were comparable. The significant increases in bilateral secretions induced by hyperosmolar saline, in both procedures, were also identical. These findings confirm that the observed nasal reflex is specifically attributable to the hyperosmolar saline, without any influence from a preceding control challenge with normal saline. Furthermore, these data demonstrate that the hyperosmolar saline-induced responses are reproducible.

The increases in secretion weights after unilateral hyperosmolar saline challenge were significant both
ipsilaterally and contralaterally in subjects with allergic rhinitis and only ipsilaterally in healthy subjects. This significant difference between the two groups, particularly in the contralateral effect of hyperosmolar saline, implicates a variance in neural responsiveness. To further confirm the activation by this stimulus of a nerve-mediated reflex, we determined whether pretreatment of the ipsilateral side with lidocaine could attenuate the secretory response. This topical anesthetic inhibits membrane sodium channels, thereby preventing the generation and conduction of nerve impulses (11). We found that sensory nerve blockade significantly reduced the secretory effects induced by hyperosmolar saline. Both the ipsilateral and contralateral responses were attenuated by anesthetizing only the site of challenge, confirming the essential role of the afferent sensory arm of the reflex. These results are similar to our earlier findings using unilateral cold dry air challenge and lidocaine pretreatment (18).

The effect of lidocaine was observed only in the analysis of secretions collected at the 1-min, and not at the 5-min, time point. This may suggest that the activity of the local anesthetic was transient, leading to restitution of the responses to hyperosmolar saline. However, results of the subsequent protocol employing capsaicin desensitization revealed similar findings. Taken together, these observations provide an explanation that the apparent lack of reduction in secretion weights at 5 min may be due to the fact that the effects measured at this time point were quite small, thus rendering the demonstration of a statistically significant diminution more difficult. Indeed, compared with the changes in secretion weights at the 1-min time point, the values obtained 5 min postchallenge were significantly less, both ipsilaterally and contralaterally. Retrospective analysis shows that at least 45 subjects would be needed to provide 80% power in detecting a significant reduction of the 5-min secretory response. Alternatively, the secretions at 5 min may represent activation of nerve fibers that are impervious to lidocaine or to capsaicin desensitization. It is known, however, that the vast majority of nerve endings in the human nasal mucosa are composed of unmyelinated thin filaments (7), most compatible with capsaicin-sensitive fibers.

We further established specific nerve involvement in effecting the changes induced by hyperosmolar saline by determining whether these responses could be attenuated by capsaicin desensitization. Capsaicin activates mostly unmyelinated C fibers and some Aδ fibers (12) via specific receptors that have been recently identified (6). Repetitive application of this agent can decrease neural responsiveness in a reversible manner (8, 10, 25). Possible mechanisms involved in this process include decreased afferent transmission (4), as well as depletion of neuropeptides as suggested by previous studies in human (15) and rodent nasal mucosa (17, 31). This knowledge has been successfully applied in the treatment of various disease states, such as diabetic neuropathy and arthritis (32). In rhinitis, repeated application of capsaicin has been reported to effectively reduce nasal symptoms (5, 9, 15, 16, 25).

In the present study, we determined whether capsaicin desensitization could affect a physiological response that can be objectively measured, namely, the secretory reflex. To ameliorate the discomfort associated with capsaicin, we anesthetized the nasal mucosa before each application of this agent. Others have shown that pretreatment with a local anesthetic neither disrupts the effects of capsaicin desensitization (8) nor improves the nasal condition by itself (5). We found that capsaicin desensitization significantly attenuates the hyperosmolar saline-induced secretory response ipsilaterally and, to a lesser extent, contralaterally. This result supports the hypothesis that the effects of hyperosmolar saline are, at least in part, mediated by capsaicin-sensitive nerves. As a negative control measure, we assessed the nasal secretory response to methacholine, which is known to activate submucosal glands directly, without any neural involvement. This is based on previous findings that it does not induce any reflex contralateral secretion and is not affected by lidocaine (3). As expected, capsaicin desensitization did not attenuate the methacholine-induced increase in nasal secretions.

The fact that methacholine directly activates glandular elements without neural involvement also allowed us to test the possibility that the greater secretory effects seen in allergic rhinitis are due to increased reactivity of the effector glands, rather than of the nerves. In the final protocol, we found that the secretory response to methacholine disk challenge was not different among subjects with allergic rhinitis and healthy volunteers. This is in contrast to the observations reported by other investigators that suggested increased secretory responsiveness to methacholine in subjects with allergic rhinitis (29, 30). The conflicting results may be due to differences in methodology because methacholine was delivered by nasal spray and secretions were collected by using syringe-equipped funnels (29) or suction devices (30) in these earlier studies. It is conceivable that these techniques are more prone to extraneous factors, particularly in the assessment of nasal secretory reflexes. In the present study, we applied the same method of localized nasal challenge and collection of secretions that has proved useful in demonstrating group differences in nasal responsiveness to hyperosmolar saline and other stimuli (19, 22). Despite the fact that we used a wide dose range of 10 methacholine provocations to the point of inducing extranasal effects, we were unable to detect any difference in the secretory responsiveness to direct gland stimulation among allergic rhinitis and healthy subjects. This allows us to conclude that the observed secretory hyperresponsiveness in allergic rhinitis is, indeed, secondary to upregulation of the neural reflex arc.

We also measured subjective symptom scores by using visual analog scales and found that hyperosmolar saline induces symptoms of local burning, rhinorrhea, and congestion, as previously reported (14, 26). The hyperosmolar saline-induced symptoms of rhinorrhea and nasal congestion, but not of a burning sensation, were significantly greater in the allergic rhinitis subjects compared with the healthy group. Repeated appli-
cation of capsaicin significantly reduced the hyperosmolar saline-induced symptoms of local burning and rhinorrhea but not of nasal congestion. That capsaicin desensitization can attenuate hyperosmolar saline-induced nasal symptoms is concordant with earlier findings (20) and again supports the notion of mediation by capsaicin-sensitive nerves.

In summary, unilateral nasal challenge with hyperosmolar saline induces bilateral nasal secretions that are significantly greater in subjects with allergic rhinitis compared with healthy individuals. This secretory reflex can be attenuated by ipsilateral pretreatment with a local anesthetic and also by capsaicin desensitization. Similar nasal challenge with methacholine induces dose-dependent secretions that are comparable among these two groups of subjects. We conclude that hyperosmolar saline induces a reflex secretory response in the human nose that is mediated, at least in part, by capsaicin-sensitive nerve fibers. These findings collectively support the notion of neural hyperresponsiveness in the setting of allergic rhinitis.

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