

In vivo human models for the study of anticholinergic drugs

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Background: *The parasympathetic nervous system plays an important role in the nasal response to external stimuli.*

Objective: *To review studies showing the role of the parasympathetic nervous system in the nasal response and the effect of topical, intranasal anticholinergic agents on that response.*

Method: *Literature review of in vivo human studies.*

Results: *Nasal provocation with methacholine leads to glandular stimulation. The response to methacholine provocation can be blocked by treatment with ipratropium bromide. Stimulation of one nostril with histamine, antigen, and cold, dry air leads to a bilateral secretory response that is partially inhibited by topical treatment with atropine.*

Conclusion: *Topical anticholinergic agents should be useful in the reduction of glandular hypersecretion regardless of the etiology. (J ALLERGY CLIN IMMUNOL 1995;95:1069-79.)*

Key words: *Atropine, ipratropium, antigen, methacholine, rhinitis, histamine, nasal provocation, cholinergic, parasympathetic*

The nervous system contributes to the pathophysiology of nasal disorders. Sneezing, an obvious example, shows how the nervous system can coordinate the response of the nose with other parts of the body. Sneezing also illustrates how a physiologic response can be associated with nasal disorders of different etiologies. This review focuses on the parasympathetic responses of the nose. To this end, we will describe nasal responses generated after stimulation of the nasal mucosa with different stimuli that generate activation of the parasympathetic nervous system. Furthermore, pretreatment with anticholinergic agents is used to help understand the relative contribution of the parasympathetic system to these responses. This in turn will shed light on the possible role of anticholinergic agents in the treatment of nasal disorders.

The presence of sympathetic and parasympathetic nerves and their transmitters in the nasal mucosa has been known for decades. Recently immunohistochemical evidence has established the presence of neuropeptides in the nasal mucosa,¹⁻⁵

Abbreviations used

CDA:	Cold, dry air
NAR:	Nasal airway resistance
PNU:	Protein nitrogen units

and animal studies have highlighted the importance of these peptides and the enzymes involved in their degradation.^{6,7} Their physiologic and pathophysiologic roles, like that of many mediators, have yet to be established. However, the fact that recent investigations have focused on neuropeptides and the nonadrenergic, noncholinergic, nervous system does not diminish the importance of the sympathetic and parasympathetic nervous systems in the pathophysiology of nasal disease.

The submucous glands, a major contributor to the volume of nasal secretions, have abundant innervation from the parasympathetic nervous system.⁸ Parasympathetic stimulation and the subsequent release of acetylcholine cause rhinorrhea. Furthermore, if the afferent nerves in one nostril are stimulated, there is an increase in nasal secretions in both nostrils (Fig. 1), and this neurally mediated response is termed the "nasonasal reflex." Atropine blocks the efferent arm of this reflex, which demonstrates the involvement of the parasympathetic nervous system. However, the most direct in vivo human evidence supporting the role of the nervous system in the nasonasal reflex is provided

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Supported in part by National Institutes of Health grant No. DC02714.

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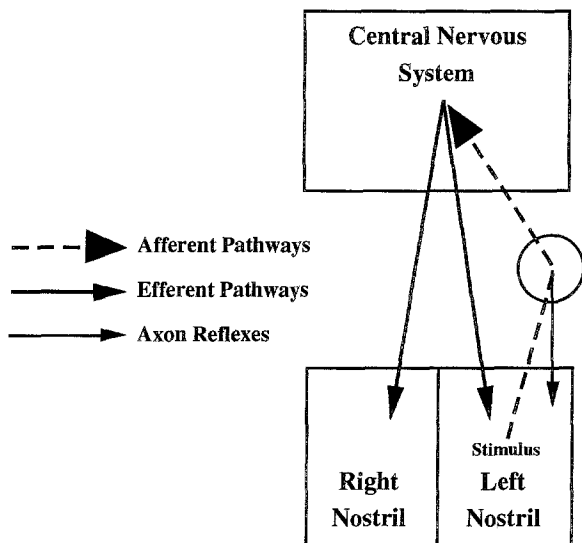


FIG. 1. Schematic drawing of nose and central nervous system. Stimulation of sensory nerves of nasal mucosa initiates a central neuronal reflex that affects both ipsilateral and contralateral nasal cavities via parasympathetic and sympathetic efferent pathways. Sensory stimulation can also lead to axonal reflexes, which affect ipsilateral nasal mucosa.

by surgical intervention. Konno and Togawa⁹ showed unequivocally the existence of reflex glandular response by abolishing it after vidian neurectomy, a surgical procedure that interrupts the parasympathetic and sympathetic innervation to the nose.

METHACHOLINE PROVOCATION

Methacholine, a stable synthetic derivative of acetylcholine, is a parasympathomimetic agent. It binds reversibly to the muscarinic receptors on the seromucous glands, thereby inducing rhinorrhea. It has been used routinely to stimulate glands and smooth muscles in the lower airway of man and animals. In the nose methacholine provocation stimulates glands directly and may have some effect on the vessels associated with the cavernous sinusoids.

Borum¹⁰ challenged the nose with methacholine and collected the draining secretions into a funnel for a fixed period of time. Using this technique he established the kinetics of the response, increased reactivity in women compared with men and in subjects with perennial rhinitis compared with normal subjects. He also studied the effect of treatment with ipratropium bromide, an anticholinergic agent, and demonstrated dose-dependent inhibition of methacholine challenge and estimated that the duration of action was at least 4 to 6 hours in normal human volunteers.

Using a different methacholine challenge system, Sjögren et al.¹¹ reported on the efficacy of increasing doses of ipratropium bromide in inhibiting methacholine-induced nasal secretions. In their trial, 40 and 100 μ g of ipratropium bromide inhibited methacholine-induced nasal secretions significantly compared with placebo, and 200 μ g further inhibited the response and was significantly more effective than the two lower doses. These trials support the fact that increasing doses of ipratropium bromide provide greater efficacy.

We¹² studied methacholine-induced nasal secretions using a localized nasal challenge system with objective quantification of nasal secretions. The application of methacholine and collection of secretions were performed with filter paper disks applied to the same area of the nasal mucosa under direct vision with the help of anterior rhinoscopy. All challenges were performed on the nasal septum because it is technically more accessible. The primary end point measured after each dose of methacholine was nasal secretion weight as calculated by the difference in the weight of the filter paper disk before and after the collection of secretions. This represents an objective measure of the response to methacholine challenge and is free of subject or physician bias. The protocol involved challenging with saline solution followed by 0.05, 0.15, 0.5, 1.5, and 5 mg of methacholine. Repeated saline challenges to control for nonspecific irritation produced no response. To study the nasonasal reflex, one side of the nose was stimulated by methacholine, and secretions were collected from both the ipsilateral and contralateral sides. The effect of 0.3 mg of atropine on the methacholine dose response was also studied. Methacholine induced a dose-dependent increase in secretion weight on the side of challenge with no response on the contralateral side. The response to methacholine was inhibited by atropine (59.2 ± 7.0 mg vs 19.9 ± 2.3 mg, $p < 0.05$), and repeated stimulation with 1.5 mg of methacholine did not result in tachyphylaxis. The mean of the coefficients of variation for three methacholine dose-response curves for each of nine subjects was 12.1%. Thus we established a reproducible technique for localized nasal challenge that demonstrates a dose-dependent increase in nasal secretions in response to methacholine and the absence of neuronal involvement in the response to this glandular stimulus as indicated by the absence of a nasonasal reflex and tachyphylaxis.

Using this model we studied the effect of pretreatment with ipratropium bromide on methacholine

line-induced nasal secretions. The results showed a dose-dependent inhibition of methacholine-induced nasal secretions by ipratropium bromide administered 1 hour before the challenges (Fig. 2). All drug doses were significantly better than placebo, and the highest dose (168 μ g) was significantly more effective than the three lower doses of the drug.¹³

To evaluate the onset and duration of action of this anticholinergic agent, we also performed a randomized, double-blind, placebo-controlled, crossover study with two different concentrations of ipratropium bromide nasal spray.¹⁴ Twenty-four subjects with perennial rhinitis participated in the trial. Fifteen minutes to 12 hours after administration of ipratropium bromide (42 or 168 μ g/nostril) or placebo nasal spray, methacholine challenges were performed and nasal secretion weights were measured. After placebo administration, the effect of methacholine remained unchanged over the 12-hour period (Fig. 3). Both the 42 and 168 μ g/nostril doses significantly inhibited the nasal hypersecretions induced by methacholine challenge within 15 minutes of treatment ($p < 0.05$). The 168 μ g dose of ipratropium bromide continued to significantly reduce secretion weights through 6 hours, but the effectiveness of the 42 μ g dose disappeared within 3 hours. In addition to having a longer duration, the 168 μ g/nostril dose produced approximately twice the inhibitory effect of the 42 μ g dose. One should keep in mind that repeated challenges with methacholine could displace ipratropium, a competitive inhibitor, from the receptor. This effect may be particularly important if the challenges are performed at the same site, such as in our model. Therefore our laboratory study may underestimate the clinical duration of drug action.

HISTAMINE PROVOCATION

Histamine, a naturally occurring autacoid contained in the granules of basophils and mast cells, is an important mediator of allergic reactions. It has direct effects on blood vessels and nerves. Using a model similar to that described above for methacholine, we studied the nasal response to histamine.¹² Whereas the application of methacholine was accompanied by a feeling of a runny nose, histamine also caused sneezing and nasal congestion. There was a dose-dependent increase in nasal secretion weights from 4.6 ± 0.6 mg after saline challenge to a maximum of 26.8 ± 2.3 mg after the highest dose of histamine (3 mg) ($p < 0.05$, $n = 11$). The reproducibility of the response after

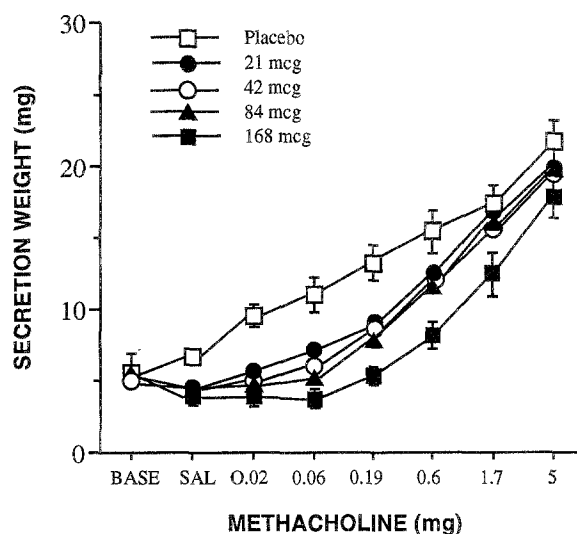


FIG. 2. Dose-response curves of secretion weight after placebo or different doses of ipratropium bromide. BASE, Baseline secretions; SAL, saline challenge; 0.02, 0.06, 0.19, 0.6, 1.7, and 5, doses of methacholine (mg) used for sequential challenges. Error bars are omitted for 21, 42, and 84 μ g doses for purposes of clarity. All doses of ipratropium were more effective in inhibiting secretory response to methacholine than was placebo, and 168 μ g dose was significantly more effective than other active treatments. (From Baroody FM, Majchel AM, Roecker MM, et al. Ipratropium bromide [Atrovent nasal spray] reduces the nasal response to methacholine. J ALLERGY CLIN IMMUNOL 1992;89:1065-75.)

histamine challenge was also tested by performing histamine dose-response curves separated by at least 1 week on each of eight patients. The coefficient of variation ranged from 1.3% to 42.8%, with a mean of 20.6%.

Challenging one side of the nose with increasing concentrations of histamine produced not only an ipsilateral increase in secretion weights but also a contralateral one. On the contralateral side, secretion weights increased from 3.9 ± 0.9 mg after saline challenge to 11.8 ± 1.8 mg after the highest dose of histamine (3 mg), and the contralateral secretion weights after 0.3, 1, and 3 mg of histamine were significantly increased over saline ($p < 0.05$). The contralateral reflex response was significantly less than the ipsilateral response ($p = 0.00001$).

To evaluate for tachyphylaxis, we challenged the nose with the same dose of histamine five consecutive times. Like methacholine, the secretion weights in response to all the ipsilateral histamine challenges were significantly higher than the response after saline challenge ($p < 0.05$). Unlike methacholine, however, the responses after the

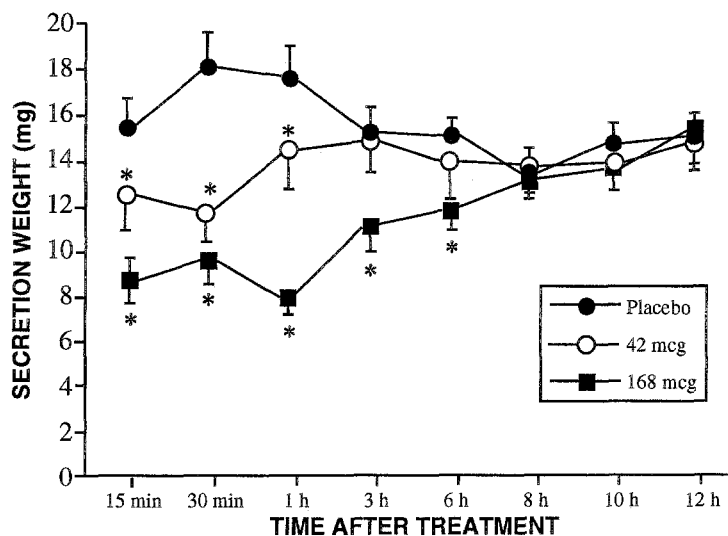


FIG. 3. Secretion weights after repetitive challenges with 0.19 mg of methacholine. Time after treatment of each challenge is displayed on abscissa. Data are presented as mean $\pm$ SEM of 24 subjects. * = $p < 0.05$ compared with placebo. The 42 μ g dose of ipratropium bromide significantly reduced secretion weights at 15-minute, 30-minute, and 1-hour time points ($p < 0.05$). The 168 μ g dose significantly decreased secretion weights from 15 minutes to 6 hours ($p < 0.01$). (From Wagenmann M, Baroody FM, Jankowski R, et al. Onset and duration of inhibition of ipratropium nasal spray on methacholine-induced nasal secretions. Clin Exp Allergy 1994;24: 288-90).

first two doses of histamine were significantly higher than after the last three doses ($p < 0.05$), which demonstrated tachyphylaxis after the fifth repeated dose. On the contralateral side, secretion weights increased from 1.6 ± 0.2 mg after saline to 8.5 ± 1.6 mg after the first dose of histamine. The response after the first dose of histamine was significantly higher than after the last five doses ($p < 0.05$), which demonstrated tachyphylaxis after the third repeated dose. Thus tachyphylaxis occurred earlier on the contralateral side than on the ipsilateral side.

To study the effect of atropine on histamine-induced nasal secretions, we sprayed either active drug or placebo on either one or both sides of the nasal cavity and challenged with increasing doses of histamine, collecting secretions from the ipsilateral and contralateral sides of stimulation. After applying atropine bilaterally on the nasal septum, there was a significant reduction in both the ipsilateral ($p = 0.003$) and contralateral responses ($p = 0.01$, $n = 10$ (Fig. 4). Looking at the total increase in secretion weights obtained after all challenges in the placebo group, the contralateral secretions were 42.9% of the ipsilateral side (39.0 ± 9.7 mg vs 91.0 ± 12.9 mg, $n = 10$). After atropine premedication, the ipsilateral response was reduced by 44% (51.3 ± 7.5 mg vs 91.0 ± 12.9

mg, $n = 10$) and the contralateral response by 77% (9.1 ± 2.3 mg vs 39.0 ± 9.7 mg) compared with that after placebo. Premedicating the nasal mucosa with atropine only on the side of stimulation ($n = 8$) produced partial inhibition of the ipsilateral response. In contrast, premedicating with atropine on the stimulated side alone led to no inhibition of secretions on the contralateral one ($p = 0.4$). Pretreatment with 60 mg of terfenadine by mouth 4 hours before challenge resulted in complete inhibition of histamine-induced ipsilateral and contralateral nasal secretions.

Like methacholine, stimulation of the nasal mucosa with histamine produced an ipsilateral dose-dependent increase in nasal secretions. Unlike methacholine, however, histamine stimulation produced sneezing and nasal pruritus in the present studies as well as in those of other investigators. Again, in contrast to methacholine, histamine produced a reflex increase in secretions. This was also seen when one inferior turbinate was challenged with histamine, and secretions were collected from both inferior turbinates, suggesting that the contralateral response was not related to direct transfer of the stimulus across the septum. Tachyphylaxis was also observed ipsilaterally after the fifth repeated 1 mg dose of histamine and contralaterally after the third dose. These differences between

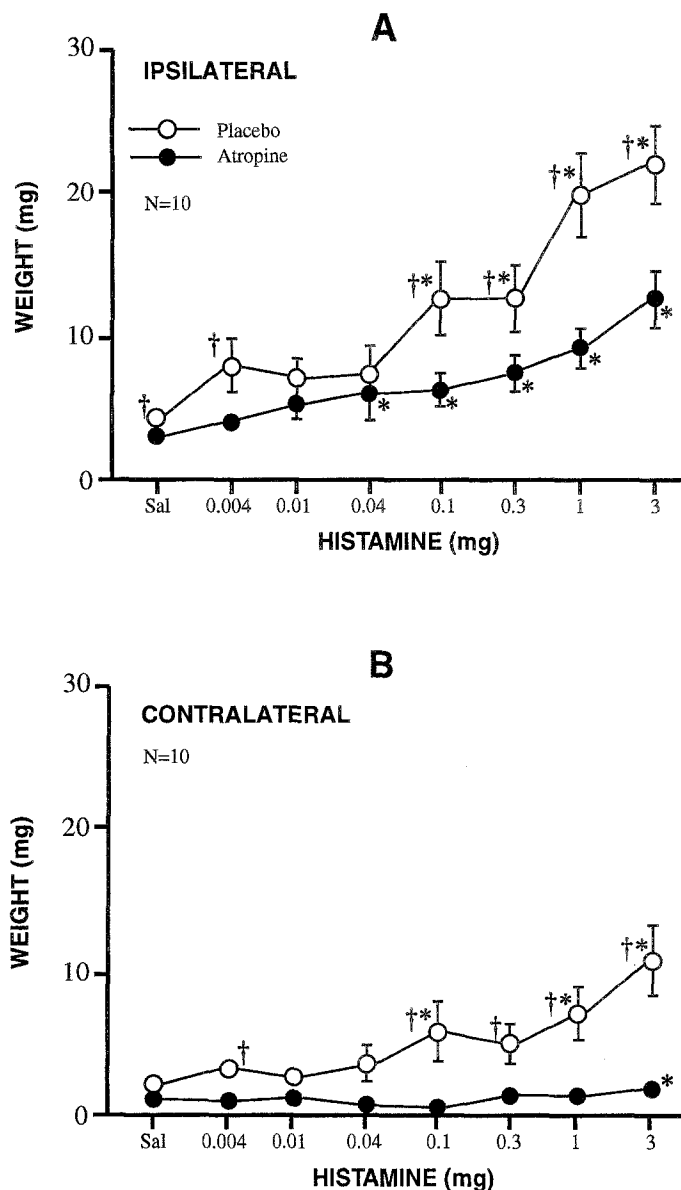


FIG. 4. Effect of atropine premedication of both sides of nose on ipsilateral (**panel A**) and contralateral (**panel B**) responses after stimulation of ipsilateral nasal cavity with histamine. In both panels abscissa represents challenge protocol with the initial saline challenge (*Sal*) followed by increasing doses of histamine in mg. * $p \leq 0.05$ compared with saline challenge within same curve and † $p \leq 0.05$ compared with atropine. (From Baroody FM, Wagenmann M, Naclerio RM. A comparison of the secretory response of the nasal mucosa to methacholine and histamine. *J Appl Physiol* 1993;74:2661-71.)

the two stimuli suggest different mechanisms of action. While methacholine acts exclusively through direct stimulation of nasal glands, histamine probably produces secretions by both direct effects on the end organs and indirectly by neural reflexes. The fact that tachyphylaxis and reflex secretions occur after histamine stimulation supports the involvement of nerves in the mediation of both the ipsilateral and contralateral responses.

When both sides of the nasal cavity were premedicated with atropine prior to histamine stimulation, there was partial inhibition of the ipsilateral response and almost complete inhibition of the contralateral one. This suggests a partial action of histamine on the side of application via atropine-sensitive cholinergic stimulation of the nasal glands. The lack of total inhibition of the ipsilateral response by atropine may be explained by the fact

that atropine-insensitive secretions originated either from nasal glands by a direct action of histamine on H_1 or H_2 receptors or from the nasal vasculature due to increased vascular permeability. This is supported by the work of Brofeldt et al.,¹⁵ who found that histamine provocation produces secretions from goblet cells and submucosal glands as well as marked transudation from blood vessels. A possible direct effect of histamine on nasal glands via H_2 receptors is supported by *in vitro* studies of airway tissues by Shelhamer et al.,¹⁶ who showed that histamine stimulation resulted in mucous glycoprotein secretions that were blocked by cimetidine (an H_2 receptor antagonist) and enhanced by pretreatment with dimaprit (an H_2 receptor agonist).

On the other hand, the reflex action seems to be completely mediated by an efferent cholinergic stimulation of nasal glands and thus the virtually complete inhibition by atropine premedication of the contralateral response. This finding is supported by studies on the source of secretions after histamine stimulation performed by Raphael et al.,¹⁷ who assayed generated secretions for markers of glandular secretion and vascular transudation. Unilateral histamine challenge increased both the concentration and relative proportion of vascular markers (serum albumin and nonsecretory immunoglobulin A) on the same side of stimulation, whereas contralateral secretions were enriched with secretory immunoglobulin A, a glandular marker, with virtually no change in vascular markers. They did not study the effect of atropine on the contralateral reflex response and did not quantitate secretions in their studies. Raphael et al.¹⁸ also showed that reflex parasympathetic stimulation led to glandular secretion in their investigations of the phenomenon of gustatory rhinitis. Interestingly, in our studies the contralateral response to histamine was 42.9% of the ipsilateral response with patients receiving placebo, and atropine inhibited this ipsilateral response by 44%. This supports the assumption that the portion of the ipsilateral response inhibited by atropine is equivalent to the contralateral response, which is the result of cholinergic stimulation of nasal glands by a reflex mechanism.

Using techniques that allowed monitoring of the secretions generated on both sides of the nasal cavity separately, several investigators have shown that ipsilateral histamine stimulation produces bilateral secretions.^{9, 19} Konno et al.⁹ demonstrated the importance of neural reflexes in this response by observing responses on both sides of the nasal

cavity before and after vidian neurectomy in 10 subjects. After unilateral challenge with histamine before neurectomy, subjects with allergic rhinitis had bilateral nasal secretions, with the unstimulated side yielding less secretions. After right vidian neurectomy, stimulation of the right side led to minimal secretions, while a large amount of secretions was induced on the left intact side. The investigators concluded that nasal hypersecretion produced by histamine primarily stimulates sensory receptors and induces reflex stimulation of ipsilateral and contralateral nasal glands via afferent and efferent pathways. Our results led to similar conclusions but required dramatically less invasive techniques to establish.

When the ipsilateral nasal mucosa was premedicated with atropine before histamine challenge, there was partial inhibition of the response. In this case, however, the contralateral side, which was not premedicated, still demonstrated a reflex increase in secretions comparable with that seen after placebo. This finding implies that the afferent limb of the response is not cholinergically mediated and is similar to the findings of Raphael et al.,¹⁷ whereby pretreatment of the ipsilateral nasal cavity with atropine showed no significant alteration in the marker profile and no inhibition of glandular secretions on the contralateral side.

When terfenadine, 60 mg, was given orally prior to histamine challenge, it inhibited both the ipsilateral and contralateral secretory responses. This again suggests that the direct and indirect actions of histamine on glandular secretion are almost exclusively mediated by an H_1 receptor. The effect of terfenadine is not due to anticholinergic properties, because a similar dose of the drug did not affect the nasal secretory response to methacholine. Similar to our results, premedicating with topical chlorpheniramine (another H_1 antagonist) before histamine challenge, Raphael et al.¹⁷ showed no significant increase in the marker profile at the site of stimulation after chlorpheniramine premedication compared with control.

ANTIGEN PROVOCATION

We²⁰ also examined the effect of atropine on the nasal response to antigen challenge by enrolling nine allergic subjects in a double-blind study that compared the effects of premedication with 0.6 mg of atropine with placebo. The challenge consisted of unilaterally stimulating the nasal septum with diluent followed by three increasing doses of antigen (3, 33, and 333 protein nitrogen units [PNU]) and recording responses bilaterally. Ipsilateral se-

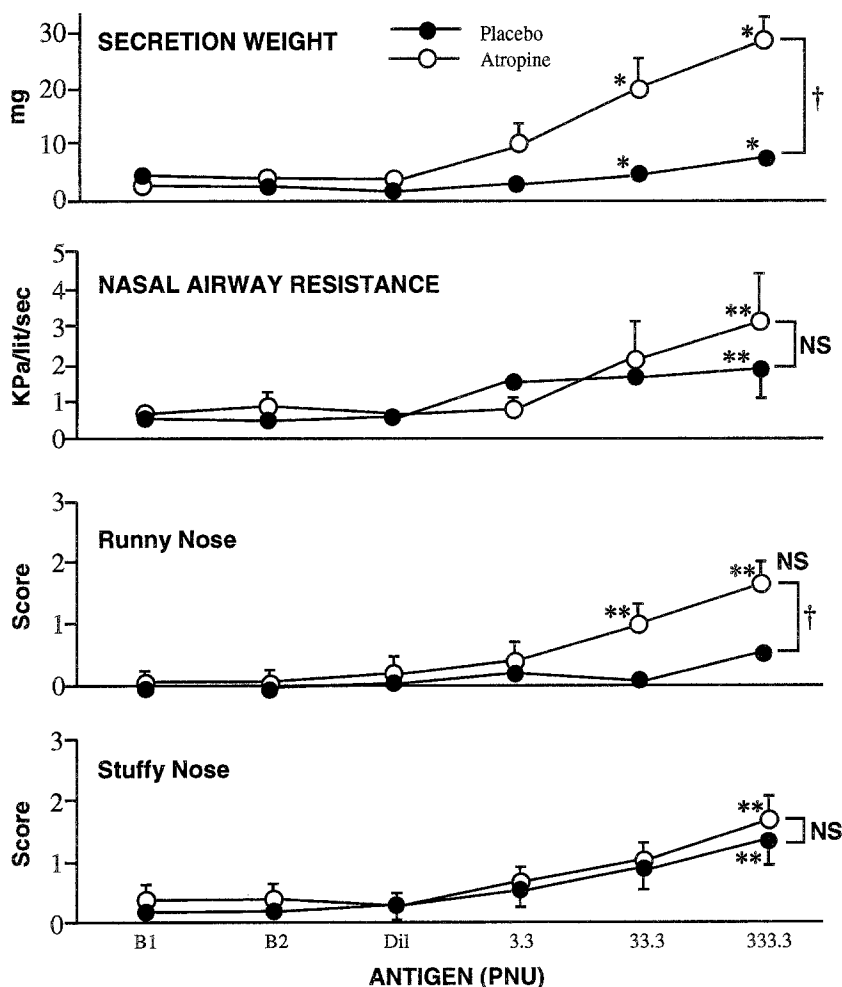


FIG. 5. Effect of topical atropine premedication on ipsilateral changes after antigen challenge. Challenge protocol is shown on abscissa: B, Baseline; Dil, diluent challenge; 3.3, 33.3, and 333.3, doses of antigen used in PNU. Data represent mean $\pm$ SEM of nine volunteers. * $p \leq 0.01$, ** $p \leq 0.05$ compared with diluent challenge. † $p < 0.05$ placebo vs atropine. (From Baroody FM, Ford S, Lichtenstein LM, et al. Physiologic responses and histamine release after nasal antigen challenge: effect of atropine. *Am J Respir Crit Care Med* 1994;149:1457-65.)

cretion weights increased significantly with subjects receiving placebo. After atropine, secretion weights on the ipsilateral side increased from 2.0 ± 0.3 after diluent to only 3.6 ± 1.5 , 4.6 ± 1.2 , and 8.1 ± 1.9 mg after antigen doses ($p < 0.01$ diluent vs 33 and 333 PNU) (Fig. 5). Examining total change from diluent, atropine had a significant inhibitory effect on ipsilateral nasal secretions compared with placebo (10.3 ± 4.37 vs 48.0 ± 10.3 mg, $p < 0.01$). Ipsilateral nasal airway resistance (NAR) increased significantly after antigen challenge with subjects receiving placebo ($p < 0.05$) and atropine (Fig. 5). Comparing total change from diluent for ipsilateral NAR, there was no difference between treatments (3.9 ± 1.9 vs 3.3 ± 2.6 KPa/L/sec, placebo vs atropine, $p = 0.9$).

Symptom scores were analyzed separately for "runny" and "stuffy" nose scores. Ipsilateral rhinorrhea scores increased significantly from 0.2 ± 0.2 after diluent to 0.4 ± 0.2 , 1.0 ± 0.2 , and 1.7 ± 0.3 after the three antigen doses for subjects receiving placebo ($p < 0.05$ diluent vs 33 and 333 PNU). After premedication with atropine, rhinorrhea scores increased from 0.1 ± 0.1 after diluent to 0.2 ± 0.2 , 0.1 ± 0.1 , and 0.6 ± 0.2 , but none of these changes were significant compared with diluent (Fig. 5). Total change from diluent for ipsilateral rhinorrhea scores decreased from 2.4 ± 0.7 after placebo to 0.6 ± 0.4 after pretreatment with atropine ($p < 0.01$). Congestion scores increased significantly on the ipsilateral side of challenge for subjects receiving both placebo and atropine ($p <$

0.05 diluent vs 333 PNU) (Fig. 5). Total change from diluent for ipsilateral congestion scores decreased from 2.3 ± 0.8 after placebo to 1.8 ± 0.7 after atropine, but the change was not statistically significant ($p = 0.1$).

On the contralateral side of challenge, there was a modest increase in secretion weights after atropine, from 1.2 ± 0.2 after diluent to 4.2 ± 1.8 mg after the highest dose of antigen ($p < 0.01$ diluent vs 333 PNU). Total change from diluent for secretion weights decreased significantly, from 30.3 ± 8.1 mg after placebo to 4.3 ± 3.1 mg after atropine premedication ($p = 0.008$). Rhinorrhea scores did not increase after atropine premedication ($p = 0.2$). Total change from diluent challenge for contralateral rhinorrhea scores decreased from 1.6 ± 0.6 after placebo to 0.3 ± 0.4 after atropine premedication ($p = 0.01$). Contralateral congestion scores increased significantly after both placebo and atropine premedications, with no difference between treatments ($p = 0.001$ for both). The data suggest that the volume of glandular secretions in response to nasal antigen stimulation is primarily controlled by parasympathetic stimulation in view of the marked inhibition of both ipsilateral and contralateral secretion weights by atropine.

Our findings are consistent with those of Malmberg et al.,²¹ who used a challenge protocol with only one dose of antigen that was known to provoke a significant reaction on previous screening and those of Raphael et al.,²² who also challenged one side of the nose and collected secretions bilaterally using a suction lavage technique. The ipsilateral but not contralateral increase in histamine, which was observed in our experiments and those of Malmberg et al.,²¹ and in prostaglandin D₂, which was observed by Raphael et al.,²² suggests that mast cell degranulation occurs at the site of antigen application. The lack of a reflex increase in mast cell mediator levels supports this notion and negates the idea that the increase in the volume of secretions is responsible for increases in histamine levels.

NAR did not increase on the side contralateral to stimulation in our experiments and those of other investigators,^{20, 21} suggesting the lack of an effect of antigen challenge on the nasal vasculature on the side contralateral to challenge. On the other hand secretion weights and symptom scores, increased directly and on the contralateral side after antigen stimulation as previously reported by Malmberg et al.²¹ and Konno and Togawa.⁹ Surprisingly, subjects reported increased nasal congestion on the contralateral side after both placebo and atropine premedication. This discrepancy be-

tween the subjective sensation of congestion and the objective measure of NAR has been reported previously.²³

Atropine treatment at the dose given did not affect NAR or congestion scores, which is consistent with prior observations that the main regulators of nasal congestion and changes in NAR are nasal blood vessels, which are predominantly under the control of the sympathetic nervous system.²⁴ Atropine had no effect on sneezing after antigen challenge, which suggests that acetylcholine has no influence on the neuronal components affecting this reflex.

Bilateral atropine treatment almost completely reduced the volume of nasal secretions bilaterally after unilateral nasal challenge with antigen, which strongly supports the role of parasympathetic stimulation of glands in the nasal secretory response. Nasal secretions are derived from several sources, which include parasympathetically innervated submucosal seromucous glands, anterior serous glands, goblet cells, lacrimal glands, and transudation from blood vessels.^{25, 26} Raphael et al.²² examined the protein content of ipsilateral and reflex secretions after unilateral antigen challenge. They concluded that the predominant source of ipsilateral secretions was increased vascular permeability, while the virtually exclusive source of contralateral secretions was nasal glands. Our experiments did not involve the measurement of markers of glandular secretion and vascular permeability but rather investigated the volume of nasal secretions. The results are not discrepant and illustrate the importance of the manner in which data are interpreted. The source of nasal secretions appears to be almost exclusively glandular on the contralateral side but a mixture of vascular and glandular components on the ipsilateral side. The volume of secretions originating from the nasal glands on the ipsilateral side is mixed with a minimal volume originating from plasma due to vascular leakage. Because the concentration of albumin in plasma is high, the small amount of this fluid that admixes with glandular secretions leads to the marked elevations in albumin observed by Raphael et al. However, this does not necessarily imply that the primary source of ipsilateral secretion volume after antigen challenge is secondary to increased plasma influx due to vascular permeability. On the contrary, it seems to be secondary to glandular secretions, as evidenced by marked inhibition by atropine.

COLD, DRY AIR PROVOCATION

Cold, dry air (CDA) causes rhinorrhea and nasal congestion in most people. This response can be

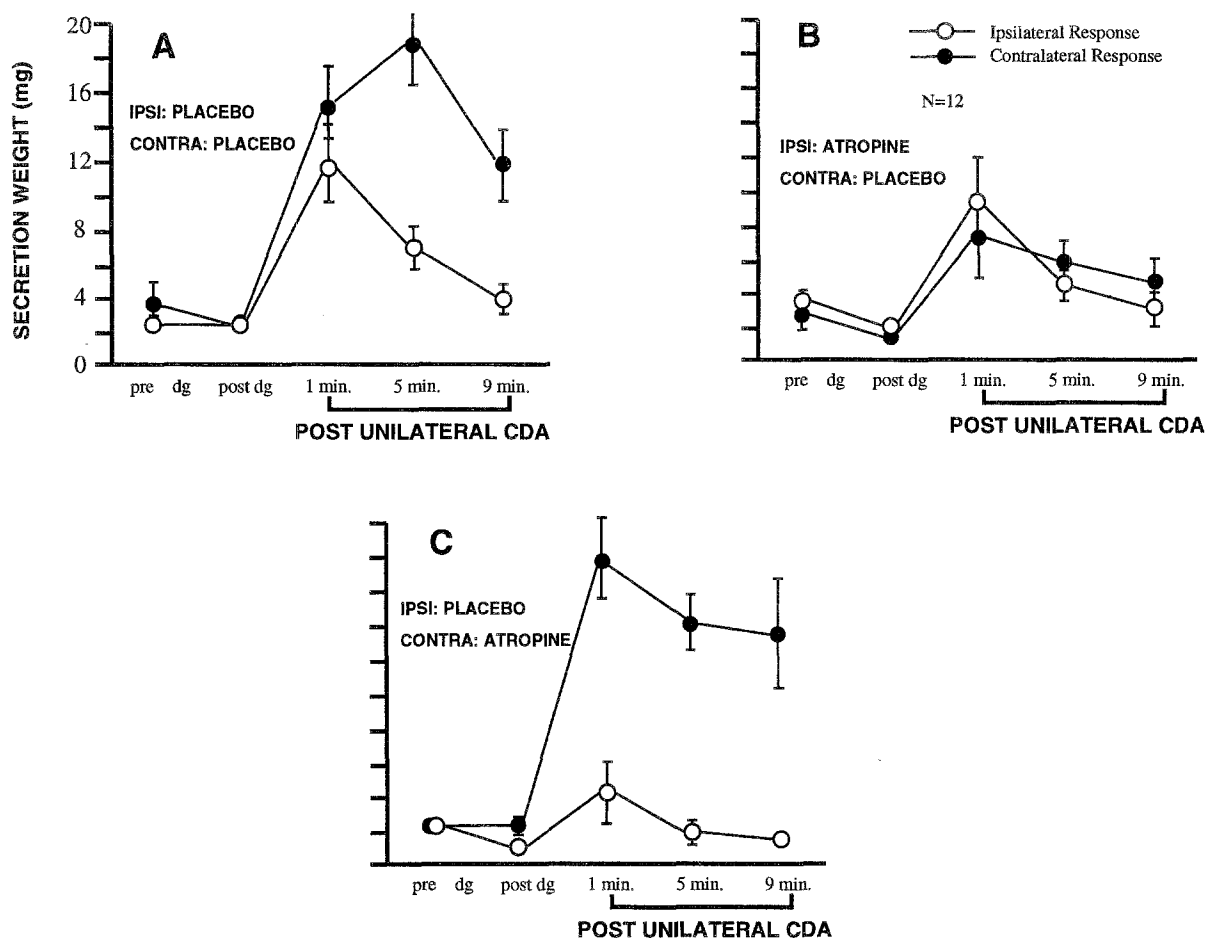


FIG. 6. Bilateral secretory response after unilateral CDA nasal challenge. **A**, Both sides treated with placebo; **B**, atropine ipsilateral and placebo contralateral to challenge; **C**, placebo ipsilateral and atropine contralateral to challenge. *ipsi*, Ipsilateral to CDA challenge; *contra*, contralateral to CDA challenge; *pre dg*, pre drug; *post dg*, post drug. (From Jankowski R, Philip G, Togias A, Naclerio R. Demonstration of bilateral cholinergic secretory response after unilateral nasal cold, dry air challenge. *Rhinology* 1993;31:97-100.)

TABLE I. Summary of rhinorrhea response to unilateral nasal challenge

Stimulant	Ipsilateral	Inhibited by anticholinergics	Contralateral	Inhibited by anticholinergics
Methacholine	++	+	—	NA
Histamine	++	+	+	++
Antigen	++	+	+	++
CDA	++	+	+	++

NA, Not applicable

mimicked in the laboratory by exposing susceptible individuals to CDA nasal breathing. One of the characteristics of this response is that nasal secretions are produced by both nostrils after a unilateral challenge. We evaluated the role of cholinergic innervation on the ipsilateral and contralateral responses to unilateral CDA challenge.²⁷ Twelve subjects participated in a randomized, double-blind, placebo-controlled, three-way crossover

study in which local atropine and placebo were alternated ipsilateral and contralateral to the CDA challenge. The reproducibility of the model, which was assessed by the response after pretreatment with placebo, was excellent. After placebo, the ipsilateral response was double the size of the contralateral response. Regardless of the side of application, atropine significantly reduced the secretory response to CDA by 60% to 70% (Fig. 6).

However, significant secretions were still induced by CDA, even after atropine treatment. These results suggest that although cholinergically mediated neuronal pathways play a major role in the nasal secretory response to CDA, additional neuronal pathways are also involved.

The mechanism by which CDA challenge triggers the secretory response is not known. In previous experiments²⁸ we found that CDA responders have significant increments in nasal fluid osmolality, which correlate with the release of inflammatory mediators such as histamine and N- α -tosyl-L-arginine methyl ester-esterase enzymatic activity. Because this and our previous studies indicate that nerves are activated, it would be interesting to examine what triggers the neuronal component. A possible explanation is that hyperosmolality, or cooling, affects sensory nerve endings triggering the afferent limb of a reflex, of which the efferent limb is at least partially cholinergically mediated.

On the other hand, it appears that the effector neuronal pathway is not only cholinergic since a high dose of atropine failed to totally inhibit not only the ipsilateral but also the contralateral secretory response. Although one can argue that a higher atropine dose would have been more effective, alternative neuronal pathways may exist. Neuropeptides are prime candidates as mediators for these pathways. For example, vasoactive intestinal peptide is colocalized with acetylcholine in efferent nerve endings and may be released concurrently with the cholinergic mediator. This peptide may be responsible for the atropine-resistant contralateral secretory response. Ipsilateral to the challenge, additional neuronal pathways may be involved. Activation of sensory nerves may lead not only to central but also to axon reflexes that can antidromically stimulate other sensory arborizations to release proinflammatory neuropeptides such as substance P and calcitonin-gene-related peptide. Both substances are present in the human nasal mucosa²⁹ and both have potent vasodilatory properties. Substance P also activates human submucosal glands.²⁹

DISCUSSION

By challenging one nostril and measuring the response bilaterally, one can study the effects of centrally mediated neurogenic reflexes. The above data provide convincing evidence that within minutes of histamine, antigen, and CDA provocation, a contralateral response occurs (Table I). Other stimulants such as capsaicin, rhinovirus, hyper- and hyposmolar solutions, bradykinin, and smoke and

irritants, which involve neuronal activation, would be expected to have similar mechanisms contributing to their pathophysiology. The effector arm of the response primarily involves parasympathetically mediated glandular activation. The ability to inhibit these secretory responses with anticholinergic agents provides a rationale for treating the clinical disease correlates of these models with anticholinergic agents (Table I). The models strongly suggest that the overwhelming effect will be on the volume of secretions generated and not on other physiologic changes such as NAR or mediator release.

Many interesting questions are also raised by these studies. Does allergic inflammation in the nasal cavity stimulate sensory nerves that, through centrally mediated and axonal reflexes, affect nasal mucosal function, thereby amplifying the allergic response? For example, do central neuronal reflexes stimulate cytokine (interleukins 6 and 8 and granulocyte-macrophage colony-stimulating factor) generation by epithelial cells, and does epithelial activation result from the direct effects of antigen stimulation or through neuronal reflexes? Neuropeptides, like vasoactive intestinal peptide, have the potential to stimulate the epithelium and activate lymphocytes, and therefore this mechanism might contribute to an amplification of nasal responses mediated by neuropeptides. Clearly, more research is required to elucidate the contribution of the nervous system and its various components (cholinergic and noncholinergic and adrenergic and nonadrenergic) to the pathophysiology of various nasal disorders.

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