Comparison of human nasal mucosal secretion in vivo and in vitro

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The secretion of proteins from the human nasal mucosa induced by histamine, α-adrenergic, β-adrenergic, and cholinergic agonists was studied in vivo and in vitro. Glandular secretion of lactoferrin, lysozyme (in vivo only), and respiratory glycoconjugates (RGCs) was measured. Vascular permeability was determined in vivo by albumin secretion in relationship to the other proteins. Muscarinic stimulation by methacholine induced significant glandular secretion (lactoferrin, lysozyme and/or RCGs) both in vivo and in vitro, confirming that muscarinic receptors are stimulated directly. Histamine induced predominantly vascular permeability in vivo but caused some glandular secretion as well. However, in vitro, histamine had no effect on glandular secretion, suggesting that histamine acts predominantly on the nasal vascular bed and only affects glandular secretion through reflex actions. Phenylephrine, an α-adrenergic agonist, selectively stimulated lysozyme release in vivo, and both RGCs and lactoferrin release in vitro. Thus, α-adrenergic stimulation has some direct, albeit minimal, capacity to stimulate mucosal glands. β-Adrenergic agonists had no effect on glandular secretion or vascular permeability either in vivo or in vitro. Therefore, glandular secretion is directly stimulated by α-adrenergic and cholinergic agonists, but not by β-adrenergic agonists. The stimulation of glandular secretion by histamine is indirect and mediated through the action of neural reflexes.
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The identification of products secreted from vascular and glandular sources and the regulation of those secretory processes in the human nasal mucosa are currently being elucidated. Proteins secreted in vivo in large quantities include the plasma protein, albumin, and the submucosal gland serous cell products, lactoferrin and lysozyme[1-4] (Fig. 1). ^{3}H-glucosamine-labeled RGC ("mucus") are macromolecules that have been studied only in vitro and are a complex mixture of mucous glycoproteins, proteoglycans,[5, 6] and other glycoconjugates derived from epithelial goblet cells and submucosal-gland serous and mucous cells.[7-11] The contents of nasal secretion have been examined in vivo after nasal provocation[2-4, 12, 13] and in vitro,[7, 14, 15] but there are no studies that directly compare the effects of potential mediators and secretagogues both in vivo and in vitro on the secretion of macromolecules.

Abbreviations used
RGC: Respiratory glycoconjugate
HSA: Human serum albumin

A comparison was made of the effects of histamine, adrenergic agonists, and cholinergic agonists, both in human nasal provocation experiments in vivo and by exposing explant cultures of human inferior turbinate nasal mucosa to these agonists in vitro. The agonists examined included histamine, phenylephrine (α_1-ag-

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onist), isoproterenol (β-agonist), albuterol/salbutamol (β_2-agonists), and methacholine (muscarinic agonist). Each agent was examined in human volunteers in vivo to determine the degree of stimulation of vascular permeability (albumin secretion) and glandular secretion (lactoferrin and lysozyme), and in vitro to determine the effects on submucosal gland product release (lactoferrin and RGC). Vascular permeability cannot be studied in this model in vitro.

We reasoned that the response to these agents in vitro would reflect only direct actions (independent of neural reflexes), whereas the in vivo reactions might indicate the possible clinical relevance of the responses observed. These data therefore would help clarify both the mechanisms of secretion elicited and direct clinicians toward possible therapeutic approaches to counteract these responses.

MATERIAL AND METHODS

Material

Purchases from manufacturers were as follows: (1) **Nasal mucosa cultures:** L-15 medium, CMRL 1066 medium, and amphotericin B (Grand Island Biologicals, Grand Island, N.Y.); penicillin and streptomycin (Whittaker M. A. Bioproducts, Walkersville, Md.); ^{3}H-glucosamine (New England Nuclear, Boston, Mass.); L-phenylephrine hydrochloride, isoproterenol hydrochloride, salbutamol free base, methacholine chloride, histamine diphosphate, and atropine sulfate (Sigma Chemical Co., St. Louis, Mo.); (2) **ELISA:** rabbit antihuman lactoferrin (Dako Corp., Santa Barbara, Calif.); goat serum (GIBCO Laboratories, Grand Island, N.Y.); HSA, lysozyme standard, lactoferrin, and o-phenylenediamine dihydrochloride (Sigma Chemical Co.); peroxidase-conjugated rabbit antihuman lactoferrin (Pel-Freez, Rogers, Ark.); goat anti-HSA horseradish-peroxidase conjugated (Cappel Worthington Biochemicals, Malvern, Pa.).

In vivo nasal provocation

The nasal-challenge technique has been described previously.[2, 12] Soft 8F rubber catheters were inserted 4 cm into the nasal cavity and attached to suction. Nostrils were washed four times with 4 ml each of saline at 1-minute intervals with a hand-activated nasal nebulizer. A control challenge of 0.3 ml of saline was sprayed into each nasal cavity. Ten minutes later, each nostril was lavaged with 4 ml of saline.

Challenge solutions (0.3 ml each) were diluted in normal saline and delivered to the right nasal cavity. Ten minutes after each challenge, the right and left nasal cavities were lavaged with 4 ml of saline.

Four protocols were used: (1) 25 mg of methacholine, (2) 1% phenylephrine, (3) 1 mg of histamine, and (4) 0.5% albuterol. The doses for histamine and methacholine were chosen based on the responses noted in previous studies.[2, 3] The doses for phenylephrine and albuterol were chosen based on the concentration used in therapy. Symptoms and signs were recorded throughout the study.

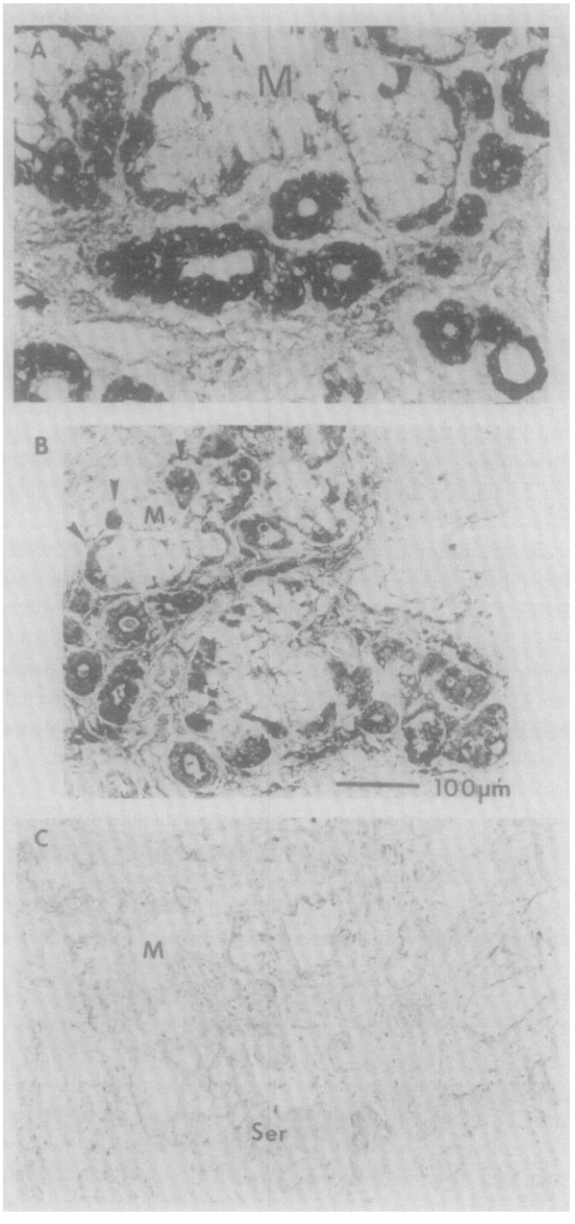

FIG. 1. Colocalization of **A,** lactoferrin and **B,** lysozyme in serous cells of human nasal mucosal submucosal glands, detected by indirect immunochemistry with immunogold technique as previously described,[9, 18] and appear as intense *black staining.* Mucous cells *(M)* did not contain immunoreactive material, indicating that lactoferrin or lysozyme was found only in serous cells of submucosal glands. **C,** Control slides, treated with nonimmune serum, revealed no specific staining.

Eight adult nonallergic healthy volunteers (mean age, 35.6 years) were studied in each phenylephrine and albuterol protocol after informed consent was obtained, which was approved by the Institution Review Board. Five and 14 additional volunteers were studied, respectively, for the methacholine and histamine protocols. All subjects were asymptomatic at the time of nasal challenge, and no med-

ication was allowed within 48 hours of nasal challenge. No subject was studied within 3 weeks after recovery from an upper respiratory tract infection.

In vitro human nasal mucosa culture

Human nasal inferior turbinate tissue was obtained after surgical resection from 13 patients with nasal airway obstruction. Sections from these tissues were analyzed and found histologically normal. The mucosa was separated from the bone and transported in L-15 medium at 4° C until placement in culture. Mucosal fragments were placed on gel foam in 10 by 35 mm Petri dishes with 2 ml of CMRL 1066 medium and added penicillin (100 μg/ml), streptomycin (100 μg/ml), and amphotericin (0.5 μg/ml). The atmosphere was controlled by purging the culture chamber for 5 minutes with 45% O_2, 50% N_2, and 5% CO_2 before sealing. The explants were incubated at 37° C.

^{3}H-glucosamine (1 μCi/ml) was added into the culture medium for 2 days to be incorporated into newly synthesized RCGs.[3,4,7] After this period of equilibration, the media were replaced, and turbinate mucosa explants were incubated with ^{3}H-glucosamine for a 4-hour baseline period (period 1). At the end of this period, the supernatants were collected, and the cultures were washed with 2 ml of CMRL media. Fresh media alone or fresh media plus agonists were then added for 1 hour (period 2). Supernatants and washes were collected. Two milliliters of each harvested supernatant was frozen at −70° C for lactoferrin quantitation, and 2 ml was used for RGC determination. The following protocols were used: (1) *methacholine:* The effect of methacholine was studied at different concentrations (0.1 to 100 μmol/L). Methacholine at 100 μmol/L, a concentration known as a potent secretagogue,[9] was used as a positive control in all experiments. (2) *atropine:* Plates were treated during period 2 with 100 μmol/L of methacholine or 100 μmol/L of atropine plus 100 μmol/L of methacholine. In the latter case, atropine was added 2 minutes before methacholine. (3) *other agonists:* Phenylephrine, isoproterenol, salbutamol, and histamine were added at 100 μmol/L. In preliminary experiments, lower doses of these agonists were ineffective.

Measurement of RGC

Two milliliter aliquots were added to 2 ml of 20% trichloroacetic acid and 2% phosphotungstic acid. After 16 hours at 4° C, the samples were centrifuged for 10 minutes at 2000 *g*, and the supernatant was decanted. The pellet was washed twice with 1 ml of 10% trichloroacetic acid and 1% phosphotungstic acid. The pellet was suspended in 1 ml of 0.1 N NaOH and kept at room temperature. After 16 hours, the suspensions were vortexed, and 0.5 ml aliquots were placed in 5 ml of scintillation fluid. Tritium decay was quantitated, and the secretory index was calculated (disintegrations per minute for period 2 per disintegrations per minute for period 1) for each plate. The means for each treatment and control plates were determined, and the percent change from control for each treatment was calculated.

Lactoferrin ELISA. Lactoferrin was measured by a modified noncompetitive ELISA as previously described.[4]

Albumin ELISA. HSA was measured by a specific, competitive, ELISA as described previously.[2,3,13]

Lysozyme turbidimetric assay. Lysozyme was measured by a turbidimetric assay based on the enzymatic hydrolysis of bacterial cell walls as previously described.[16]

Ratios. In the course of the experiments, it was found that total protein could not be measured accurately in each experiment by the Lowry protein method because of the intrinsic reactivities of phenylephrine and albuterol. The ratio of albumin to total protein has been extensively used as a measure of vascular permeability in previous studies,[2] and the lactoferrin/total protein and lysozyme/total protein ratios as measures of glandular secretion.[4] In the present study, since total protein could not be used to assess the relative contribution of secretions from glandular and vascular sources, lysozyme/albumin and lactoferrin/albumin ratios were determined instead. These ratios compared the secretion of glandular products in the numerator to albumin, a plasma protein secreted predominantly as a result of vascular permeability, in the denominator.

Indirect immunohistochemistry

Human nasal mucosa tissue was obtained as previously described[4] and fixed in formaldehyde. Paraffin-embedded sections were incubated in rabbit antisera to human lysozyme or lactoferrin, and their presence was detected by the immunogold method.[4] Sections were counterstained with alcian blue, which stains mucous cells. Control slides were treated with rabbit nonimmune serum.

Statistics

The statistical evaluation was performed on a microcomputer (Macintosh II, Apple Computer, Cupertino, Calif.) with a statistical software package (Statview 512+, Brainpower Inc., Calabasas, Calif.). Student's *t* test for paired-sample analysis was used for statistical comparisons. A $p < 0.05$ was considered statistically significant.

RESULTS

In vivo nasal provocation

Clinical symptoms. During methacholine challenges, subjects complained of nasal congestion, bitter taste, and occasionally of facial flushing. Histamine induced nasal congestion, itching, and sneezing, and reduced nasal patency. Saline, phenylephrine, and albuterol challenges did not cause symptoms.

Albumin secretion. After saline challenge, albumin concentrations ranged from 6.7 ± 1.3 μg/ml to 13.0 ± 5.9 μg/ml in different experiments (Fig. 2). This variability has been noted previously.[4] Methacholine (25 mg) significantly stimulated albumin secretion (39.9 ± 1.1 μg/ml; $p < 0.05$) compared to that of saline. However, histamine (1 mg) stimulated a far greater degree of increased albumin secretion (94.2 ± 15.2 μg/ml; $p < 0.001$). Phenylephrine (6.9 ± 1.8 μg/ml; $p < 0.2$) and albuterol (6.2 ±

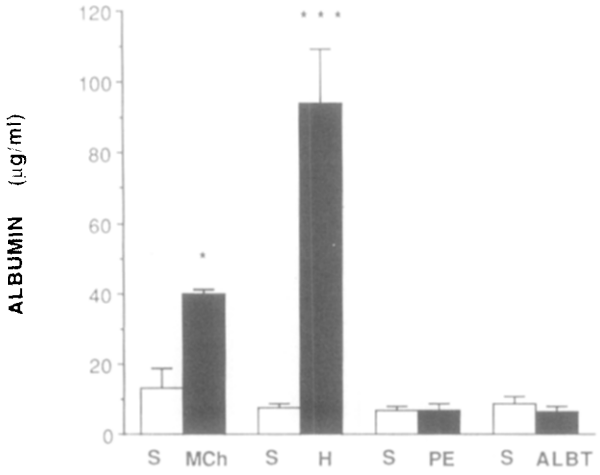

FIG. 2. Albumin secretion in vivo. In vivo effects of methacholine *(MCh)* (25 mg; n = 13), histamine *(H)* (1 mg; n = 22), phenylephrine *(PE)* (1% solution; n = 8), and albuterol *(ALBT)* (0.5% solution; n = 8) in nasal provocations (■) versus saline *(S)* (□) on albumin release are illustrated. *Each bar* presents the mean ± SEM. Paired Student's *t* test, $*p < 0.05$ and $***p < 0.001$.

1.8 μg/ml; $p < 0.2$) were without effect on albumin secretion.

Lysozyme secretion. Lysozyme levels after saline were reproducible (range from 9.6 ± 1.5 to 11.0 ± 2.1 μg/ml) (Fig. 3, *A*). Both methacholine (35.8 ± 7.6 μg/ml; $p < 0.001$) and histamine (58.1 ± 12.7 μg/ml; $p < 0.001$) stimulated significant lysozyme secretion compared to that of saline. Phenylephrine induced a small but significant secretion (17.6 ± 4.5 μg/ml; $p < 0.01$), whereas albuterol had no effect (11.9 ± 1.6 μg/ml; $p < 0.2$).

Lysozyme/albumin ratio. After saline provocation, the lysozyme/albumin ratio ranged from 1.25 ± 0.51 to 2.0 ± 0.70 (Fig. 3, *B*). Methacholine and albuterol did not significantly affect the ratio (1.53 ± 0.38 and 3.21 ± 1.29 versus saline 1.25 ± 0.51 and 1.47 ± 0.31, respectively; $p < 0.2$). Histamine significantly reduced the lysozyme/albumin ratio (0.74 ± 0.14; $p < 0.001$) compared to that of saline (1.63 ± 0.26), indicating that histamine induced more albumin than lysozyme secretion. Phenylephrine induced an increase in the lysozyme/albumin ratio (3.71 ± 1.13) versus saline (2.01 ± 0.70) that was near significance ($p = 0.06$; n = 8). This result reflects increased glandular secretion of lysozyme relative to that of albumin after phenylephrine administration.

Lactoferrin secretion. Lactoferrin levels after saline provocation ranged from 0.49 ± 0.16 to 0.90 ± 0.21 μg/ml (Fig. 4, *A*). The concentration of lactoferrin was <10% of the concentration of lysozyme.

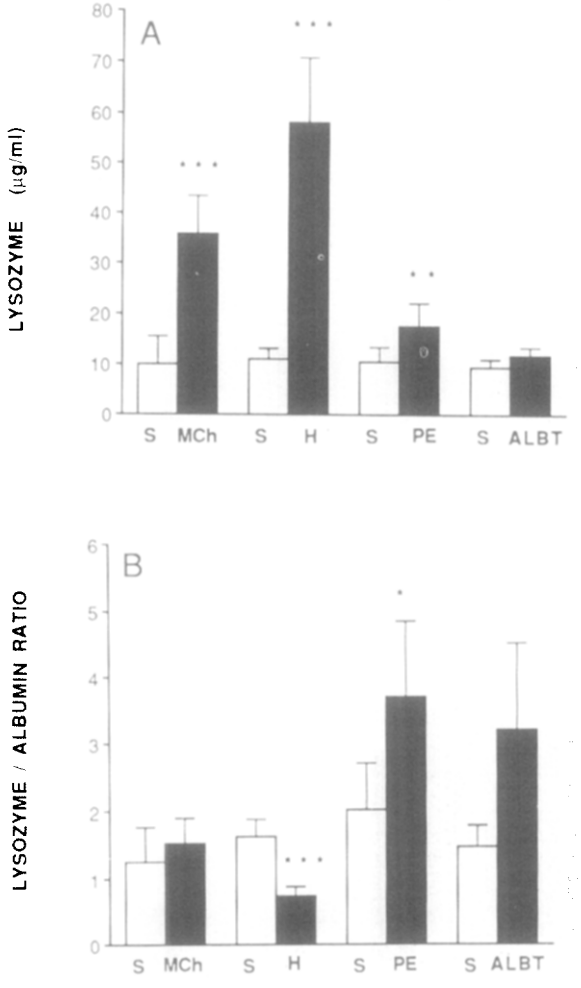

FIG. 3. Lysozyme secretion and lysozyme/albumin ratio in vivo. In vivo effects of methacholine *(MCh)* (25 mg; n = 13), histamine *(H)* (1 mg; n = 22), phenylephrine *(PE)* (1% solution; n = 8), and albuterol *(ALBT)* (0.5% solution; n = 8) nasal provocations (■) versus saline *(S)* (□). **A,** Effects on lysozyme release. **B,** Effects on lysozyme/albumin ratio. *Bars* present the mean ± SEM. Paired Student's *t* test, $***p < 0.001$, $**p < 0.01$, and $*p < 0.06$ (nonsignificant).

Both methacholine (10.63 ± 3.48 μg/ml; $p < 0.01$) and histamine (4.06 ± 1.25 μg/ml; $p < 0.01$) significantly stimulated lactoferrin secretion compared to that of saline. In contrast to lysozyme secretion, however, methacholine induced more lactoferrin secretion, whereas histamine favored lysozyme secretion. Phenylephrine and albuterol had no significant effect (0.83 ± 0.24 μg/ml and 0.95 ± 0.25 μg/ml; $p < 0.2$) on lactoferrin secretion.

Lactoferrin/albumin ratio. The lactoferrin/albumin ratio after saline challenge ranged from 0.13 ± 0.02 to 0.40 ± 0.17 (Fig. 4, *B*). Methacholine, phenylephrine, and albuterol did not affect this ratio (0.44 ± 0.12, 0.16 ± 0.04, and 0.35 ± 0.17 compared to that of saline; $p < 0.2$), whereas hista-

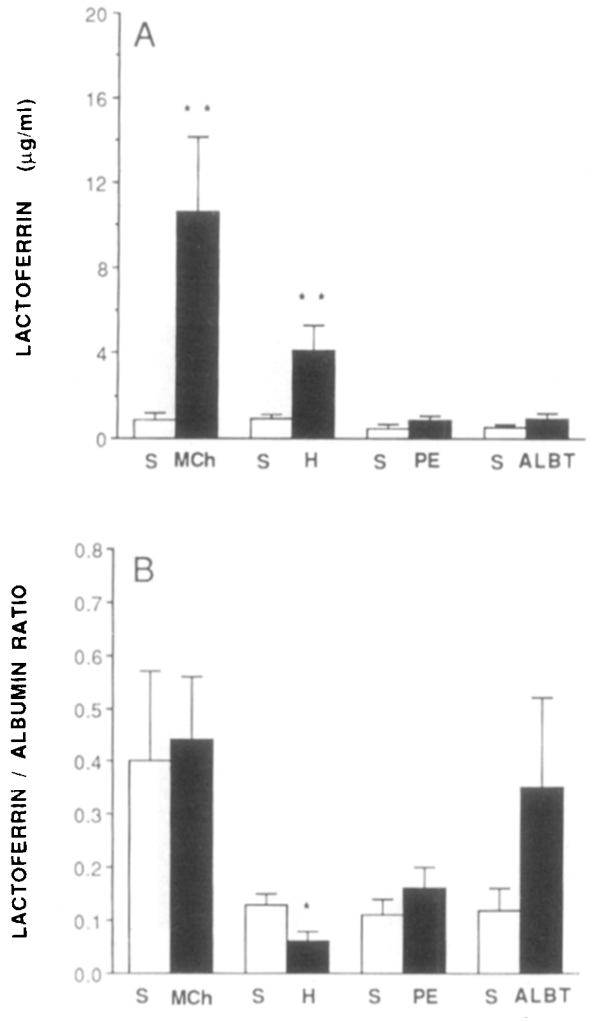

FIG. 4. Lactoferrin secretion and lactoferrin/albumin ratio in vivo. In vivo effects of methacholine *(MCh)* (25 mg; n = 13), histamine *(H)* (1 mg; n = 22), phenylephrine *(PE)* (1% solution; n = 8), and albuterol *(ALBT)* (0.5% solution; n = 8) nasal provocations (■) versus saline *(S)* (□). **A,** Effects on lactoferrin release. **B,** Effects on lactoferrin/albumin ratio. *Bars* present the mean ± SEM. Paired Student's *t* test, $**p < 0.01$ and $*p < 0.05$.

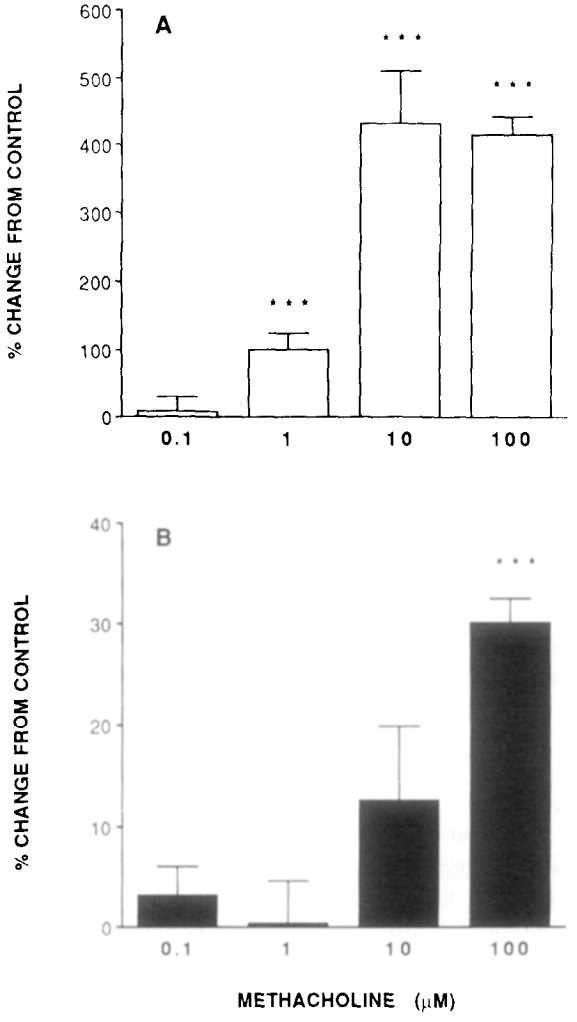

FIG. 5. Methacholine-induced secretion in vitro. Effect of methacholine (0.1 to 100 μmol/L) in secretion from human turbinates in vitro. **A,** Percent increase from controls of lactoferrin (□). **B,** RGC release (■). *Each bar* presents the mean ± SEM of at least five experiments. Paired Student's *t* test, $***p < 0.001$.

mine caused a significant reduction in the ratio (0.06 ± 0.02; $p < 0.05$) versus saline (0.13 ± 0.02). This change indicates that, although the lactoferrin concentration was increased after histamine stimulation, there was a proportionally greater increase in albumin.

In vitro explant cultures

Methacholine. Lactoferrin release (Fig. 5, *A*) was dramatically and significantly stimulated in a dose-dependent fashion by different concentrations of methacholine: 100 μmol/L (+413% ± 29% change in secretory index compared to that of control cultures; n = 30; $p < 0.001$), 10 μmol/L (+431% ± 77%; n = 10; $p < 0.001$), and 1 μmol/L (+99.4% ± 23.5%; n = 10; $p < 0.001$). Lactoferrin release was not affected by 0.1 μmol/L of methacholine. A smaller, but highly significant, release of RGC also occurred after addition of 100 μmol/L of methacholine (+30% ± 2.4%; n = 32; $p < 0.001$) but not by lower concentrations (Fig. 5, *B*). Lysozyme was not measured in these experiments.

Atropine. The effect of atropine on methacholine-induced secretion was tested in six experiments (Fig. 6). Methacholine (100 μmol/L) significantly stimulated the release of ^{3}H-RGC (+22.2% ± 1.2%; $p < 0.001$ compared to that of controls by unpaired Student's *t* test) and lactoferrin (+713.2% ± 117.8%; $p < 0.01$). Atropine sulfate (100 μmol/L)

did not affect the baseline release of either ^{3}H-RGC (+1.6% ± 7.2%; $p < 0.2$) or lactoferrin (+20.5% ± 24%; $p < 0.2$). However, the combination of 100 μmol/L of methacholine plus 100 μmol/L of atropine sulfate blocked the release of ^{3}H-RGC (+3.7% ± 5.8%; $p < 0.05$) and lactoferrin (+3.7% ± 16.1%; $p < 0.01$) compared to that of methacholine (100 μmol/L) alone by paired Student's t test.

Phenylephrine. Phenylephrine (100 μmol/L) induced significant release of ^{3}H-RGC (+24.5% ± 4.4%; n = 9; $p < 0.001$) and lactoferrin (+24.5% ± 6.1%; n = 9; $p < 0.01$) (Fig. 7).

Isoproterenol. Isoproterenol (100 μmol/L) provoked changes of both ^{3}H-RGC (+23.8% ± 10.1%; n = 7; $p < 0.2$) and lactoferrin (+17.0% ± 9.1%; n = 7; $p < 0.2$) that were not significant (Fig. 7).

Salbutamol. Salbutamol (100 μmol/L) did not affect either ^{3}H-RGC (+1.9% ± 6.6%; n = 7; $p < 0.2$) or lactoferrin (−0.2% ± 14.1%; n = 7; $p < 0.2$) secretion (Fig. 7).

Histamine. Histamine (100 μmol/L) did not induce either ^{3}H-RGC (+5.9% ± 7.8%; n = 5; $p < 0.2$) or lactoferrin release (−5.4% ± 10.1%; n = 5; $p < 0.2$) (Fig. 7).

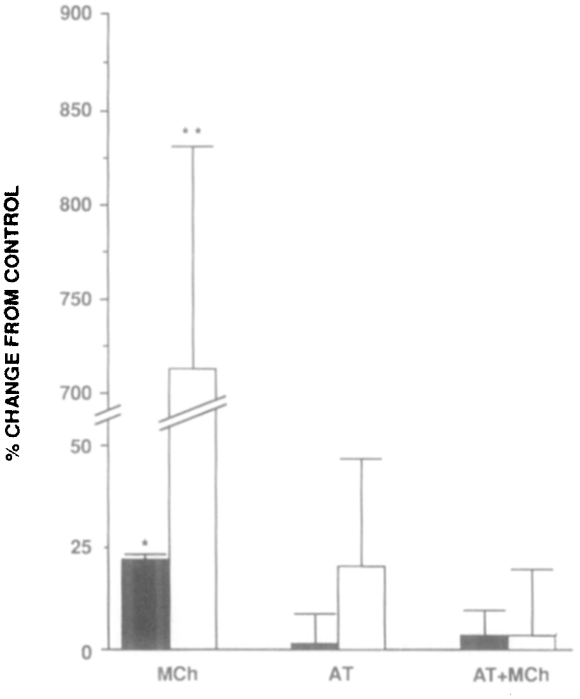

FIG. 6. Effect of atropine on methacholine-induced secretion in vitro. Effect of methacholine *(MCh)*, atropine *(AT)*, and atropine plus methacholine *(AT + MCh)*, all at 100 μmol/L, on RGC (■) and lactoferrin (□) release from human turbinate explants in vitro. Methacholine versus atropine plus methacholine data were compared by paired Student's t test (mean ± SEM; n = 6), $^{**}p < 0.01$ and $^{*}p < 0.05$.

DISCUSSION

The effects of parasympathomimetics on human nasal mucosal secretion in vivo are well documented.[2, 4, 12, 17, 18] In our studies, methacholine induced a significant secretion in vivo of both lysozyme and lactoferrin, two products of submucosal-gland serous cells (Fig. 1). Albumin secretion was also increased, but to a lesser degree. This finding may be due to passive movement of plasma proteins in glandular secretions, possibly to an increase in vascular permeability, or to an increase in the flux of albumin across the tight junctions between acinar cells (glandular permeability) or epithelial cells (epithelial permeability).[2, 12] Each of these processes would facilitate the transport of albumin from the intravascular space into the interstitium, and thence into secretions.

The consistent stimulation of albumin (and other vascular proteins, such as IgG[19] and nonsecretory IgA) with glandular secretions has favored the hypotheses that these changes represent the passive movement of plasma proteins mixed with glandular secretions.[2, 12] In the current study, the ratios of lysozyme/albumin and lactoferrin/albumin were not significantly affected by methacholine. This observation suggests that methacholine stimulates glandular secretion (lysozyme and lactoferrin secretion) and albumin secretion (vascular permeability and/or glandular permeability) to an equal extent. The albumin/total protein ratio, a ratio of the total amount of plasma extravasation relative to the total protein derived from plasma proteins plus glandular proteins, is typically unchanged after methacholine challenge.[2] Thus, it appears likely that methacholine-stimulated glandular secretions contain constant proportions of both specific glandular products and albumin.

Previous in vitro studies in humans[9, 14, 15] and in ferrets[20-22] have demonstrated that parasympathomimetic stimulation increases secretion of submucosal-gland serous and mucous cell products and that this effect was blocked by atropine.[9, 14, 15, 20-22] In comparison, our results of human nasal mucosa explant cultures demonstrated a relatively small increase of RGC and an exceptional augmentation of lactoferrin release from serous cells. The increases in both RGC and lactoferrin by methacholine were blocked by atropine. Thus, muscarinic stimulation in vivo and in vitro results in augmented mucous and serous cell secretion.

Previous studies in vivo have demonstrated that aerosolized, inhaled histamine induced sputum production from human bronchi.[17] In the nasal mucosa, histamine provokes secretion predominantly of albu-

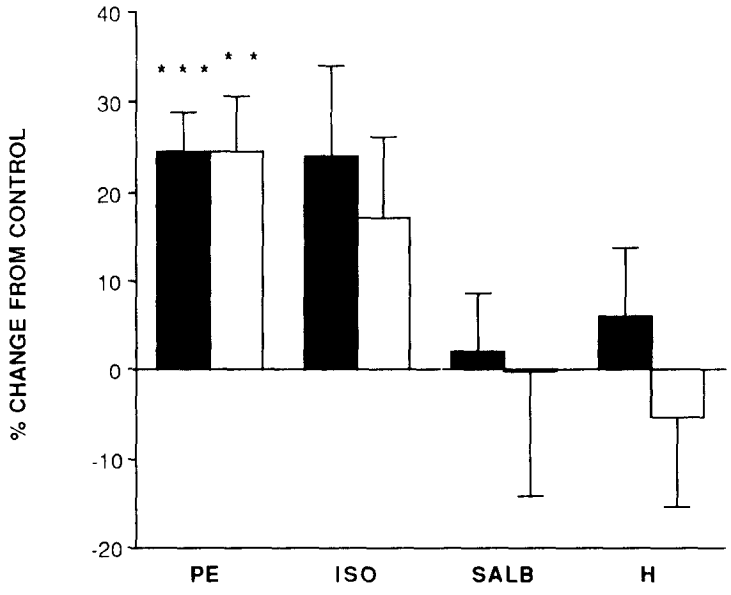

FIG. 7. Agonist-induced secretion in vitro. Effects of 100 μmol/L concentrations of phenylephrine *(PE)* (n = 9), isoproterenol *(ISO)* (n = 7), salbutamol *(SALB)* (n = 7), and histamine *(H)* (n = 5) on RGC (■) and lactoferrin (□) release in human turbinates in vitro. Results are expressed as the percent increase above control cultures. *Each bar* presents mean ± SEM. Paired Student's *t* test, $***p < 0.001$ and $**p < 0.01$.

min-rich fluid accompanied by a small amount of glanduar products. However, in the contralateral, unchallenged nostril, glandular secretion is induced through a histamine-stimulated reflex.[3, 4] Therefore, in vivo, histamine acts as a neural stimulant, indirectly causing glandular secretions by stimulating neural reflexes. In the present in vivo study, histamine induced a much larger increase in albumin than did methacholine. Lysozyme and lactoferrin release were also stimulated. However, the lysozyme/albumin and lactoferrin/albumin ratios were decreased, indicating that the increases in lysozyme and lactoferrin were relatively smaller than the impressive increase in albumin secretion. Therefore, the vascular component of the histamine response was relatively more important than the glandular component.

Histamine has previously been suggested as a mucus secretagogue, since histamine stimulation increases radiolabeled RGC secretion from human bronchi in vitro.[9] In contrast, the present studies in nasal mucosa in vitro demonstrate that neither RGC nor lactoferrin release was stimulated by histamine. In earlier, unpublished studies of histamine-stimulated nasal turbinate RGC release, histamine also failed to cause increased secretions. That histamine is capable of causing glandular secretion in vivo but fails to do so in vitro suggests several possibilities. Histamine might act by induction of other mediators (such as prostaglandins[23]) and these in turn might act as secretagogues. Alternatively, histamine could stimulate sensory nerves and induce gland secretion by axon responses or central parasympathetic reflexes. Pretreatment of the nasal mucosa in vivo with atropine reduces the secretory response to histamine, confirming that reflexes contribute to the secretory response to histamine.[2] Thus, in the nose, histamine acts directly on blood vessels to cause vascular permeability and, indirectly through nerves, to stimulate mucous secretion.

Phenylephrine is an α-agonist active on postsynaptic α_1-receptors. Previous in vivo studies in cat[24] and dog[25] have demonstrated α-adrenergic stimulation of submucosal-gland secretion. In the present in vivo study, phenylephrine was used in a standard dose (0.3 ml of 1% solution, 3 mg) that clinically reduces nasal blood flow.[26] Administration of phenylephrine did not affect albumin secretion. Therefore, vasoconstriction of nasal vessels did not stimulate the baseline extravasation of albumin. Phenylephrine did stimulate lysozyme levels and a minimal increase in lactoferrin secretion, although the increases were only significant for lysozyme ($p < 0.01$). There was a tendency for the lysozyme/albumin and lactoferrin/albumin ratios to increase, suggesting some selective glandular secretion. Therefore, α-receptor stimulation had minimal stimulatory effects on serous cell secretion in vivo.

In vitro, α-adrenergic agonists have been demon-

strated to induce significant increases in RGC secretion from human airways and nasal mucosa[9, 15, 27, 28] and from cat airways.[29] Alpha stimulation selectively augments the release of serous cell products[20-22] from ferret trachea. In vitro, phenylephrine increased both RGC and lactoferrin release from the nasal mucosa. The enhanced activity of phenylephrine in vitro relative to in vivo might be due to the capacity of α-agonists to contact the glands in culture without having to cross the mucosa, which is necessary in vito. Since we cannot stimulate endogenous α-adrenergic release in vivo, we cannot test this hypothesis but are disturbed by the discrepancy between in vivo and in vitro α-adrenergic activity. However, these data suggest that topical α-adrenergic agonists may selectively stimulate glandular secretion in vivo.

Albuterol, a β_2-agonist, did not cause albumin, lysozyme, or lactoferrin secretion in vivo in nasal provocation studies. This observation suggests that β_2-receptor stimulation did not directly stimulate vascular permeability or gland secretion. The dose of albuterol used (0.3 ml of 0.5% solution, 1.5 mg) is known to induce clinical relief of bronchospasm in subjects with asthma. Since β-adrenergic agonists cause vasodilation and increase blood flow, these negative data indicate that increases in blood flow alone are insufficient to cause plasma protein secretion.

Neither isoproterenol (a β-adrenergic nonselective agonist) nor albuterol/salbutamol (β_2-adrenergic selective agonists) manifested any significant effect on RGC or lactoferrin release from turbinate explants in vitro. Although this observation confirms previous studies in which β-adrenergic agonists, added to human airway cultures, were without significant effect on mucus secretion in human[9, 14, 15] and ferret preparations,[20, 21] other studies have demonstrated in vitro glandular mucus secretion in human,[27, 28] cat,[29] and ferret[22] airways, whereas one study in cats was equivocal.[30] Therefore, the absence of a human respiratory response to β-agonists in vivo supports the conclusion that β-agonists do not stimulate nasal airway glandular secretion.

In these experiments, total protein was not measured because both phenylephrine and isoproterenol interfere with the assays used; therefore, ratios of glandular products to albumin were used instead. Although these ratios are less useful than the usual ratios with total protein as the denominator, they did provide insights into the sources of proteins secreted in vivo. Methacholine did not alter the ratio of lactoferrin or lysozyme, histamine decreased these measurements (suggesting increased albumin secretion), and phenylephrine increased the ratio (suggesting enriched glandular secretions).

Overall, there was a close correlation between secretory responses in vivo and in vitro. We can conclude that cholinergic stimulation causes glandular (both mucous cell and serous cell) secretion. Histamine acts predominantly on the vascular bed to induce permeability and indirectly through neural reflexes to cause some glandular secretion. α-Adrenergic stimulation causes minimal glandular secretion, predominantly of lysozyme, whereas β-adrenergic stimulation has no effect on secretions. These data confirm that the in vitro studies largely reflect the results of in vivo testing but suggest that both experiments are necessary for the complete understanding of the physiologic controls of nasal secretion.

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