Immunologic and Neuropharmacologic Stimulation of Mucous Glycoprotein Release from Human Airways In Vitro

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ABSTRACT Human bronchial airways obtained after surgical resection were maintained in tissue culture for 24–48 h. Incorporation of [^{3}H]- or [^{14}C]-glucosamine, [^{14}C]threonine, or $Na_2[^{35}S]O_4$ to the culture media resulted in biosynthesis of two radiolabeled glycoproteins—one filtering in the exclusion volume of Sepharose 2B, and the other filtering with an approximate molecular weight of 400,000. Both fractions had similar elution patterns from DEAE-cellulose anion exchange chromatography. [^{3}H]Glucosamine was incorporated equally into the two fractions.

The effects of anaphylaxis, histamine, and several neurohormones upon the release of [^{3}H]glucosamine-labeled glycoproteins were analyzed, making no attempt to separate the two glycoprotein fractions. Three lines of evidence were found suggesting that mast-cell degranulation increases mucous release from cultured airways. (*a*) Supernatant fluids from anaphylaxed peripheral human lung that contained 200–400 ng/ml histamine and 400–1,000 U/ml slow-reacting substance of anaphylaxis (SRS-A) increased release by 40±18%. (*b*) The addition of antigen to IgE-sensitized airways led to the release of 26±7% of the total histamine and a 36±14% increase in mucous release. (*c*) Reversed anaphylaxis with anti-IgE antibodies induced a 36±6% release of histamine from the airways and an increase in the release of mucous glycoproteins of 25±9%.

Exogenous histamine added to airways increased mucous glycoprotein release, an effect prevented by cimetidine, an H-2 antagonist. Selective histamine H-2, but not H-1 agonists increased mucous glycoprotein release, suggesting the possibility that anaphylaxis of airways results in increased mucous glycoprotein release partly through histamine H-2 stimulation.

A cholinomimetic agonist, methacholine, increased mucous release; this response was prevented by atropine which alone had no effect. No response to β-adrenergic stimulation with either isoproterenol or epinephrine was noted. However, α-adrenergic stimulation with either norepinephrine combined with propranolol or phenylephrine alone resulted in dose-related increases in glycoprotein release. Both α-adrenergic and cholinergic stimulation of human tissues induce the formation of guanosine 3′,5′-phosphoric acid (cyclic GMP), and 8-bromo cyclic GMP added to the airways led to increased mucous secretion. Thus, it seems likely that neurohormones capable of stimulating cyclic GMP formation in human airways may lead to increased mucous glycoprotein release.

INTRODUCTION

Pathologic examinations of fatal cases of status asthmaticus almost always reveal diffuse secretions of mucus which appear to contribute significantly to the airways obstruction (1). In less severe asthmatic attacks, mucous secretion probably contributes to the bronchial obstruction but the degree varies. Several investigations in experimental animals in vivo and in vitro have demonstrated that cholinergic, α-adrenergic, and β-adrenergic stimulation can increase mucous secretion (2–6). Only a few in vitro studies of mucous secretion using human lung have been reported and these reports indicate, as analyzed by autoradiographical changes (7) or radiolabeled glycoprotein secretion (8), that muscarinic stimulation increases mucous synthesis and release. No investigation of the role allergic reactions may play in human or animal mucous secretion by lung tissue has previously been reported, although either immune complexes or anaphylaxis may stimulate rat intestinal goblet cell secretion (9–11).

To evaluate the effects of anaphylaxis on the release of mucus, we modified previously reported methods (8, 12) and maintained human bronchial airways in

culture for 24–48 h. Radiolabeled molecules were added to the culture media and became incorporated into newly synthesized molecules that were released into the supernatant fluid. The radiolabeled molecules were analyzed by size and charge, and the release of these molecules was taken as a reflection of airway mucous-glycoprotein release. Thereafter, the effects of anaphylaxis, histamine, adrenergic, and cholinergic agonists on the release of the radiolabeled glycoproteins were studied.

METHODS

Materials. [^{3}H]Glucosamine (30 Ci/mmol), $Na_2[^{35}S]O_4$ (100 mCi/mmol), L-[^{3}H]threonine (2 Ci/mmol), D-[^{14}C(U)]glucosamine hydrochloride (250–350 mCi/mmol), aquasol (New England Nuclear, Boston, Mass.); medium L-15, medium CMRL-1066, penicillin, streptomycin, bovine insulin (Grand Island Biological Co., Grand Island, N. Y.), amphotericin B (E. R. Squibb, Princeton, N. J.); gel foam (Upjohn Co., Kalamazoo, Mich.); rabbit antihuman IgE (Behring Diagnostics, American Hoechst Corp., Somerville, N. J.); ragweed antigen E (Research Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.); histamine diphosphate, methacholine, atropine, L-phenylephrine, norepinephrine, isoproterenol, epinephrine, propranolol, diphenhydramine, dibutyryl cyclic AMP, 8-bromo cyclic guanosine 5′-phosphoric acid GMP, and hydrocortisone (Sigma Chemical Co., St. Louis, Mo.); pyrilamine (Merck, Sharp, & Dohme, Canada Ltd., Montreal, Canada); Sepharose 2B (Pharmacia Fine Chemicals, Piscataway, N. J.); DEAE-52 microgranular cellulose (Whatman, Inc., Clifton, N. J.); and ultrapure urea (Bethesda Research Laboratories, Bethesda, Md.) were purchased from the manufacturers. Dimaprit, impromidine, cimetidine, and 2-methylhistamine were supplied by Dr. John Paul of the Smith Kline & French Co., Philadelphia, Pa.

Culture of human airways. Macroscopically normal portions of human lungs were obtained at surgery for resection of carcinoma. The tumor-free portions of human lung were placed in L-15 carrier medium and transported as quickly as possible to the laboratory. Airways were identified (2–10 mm in diameter) and carefully dissected. Wherever visible, cartilage and alveolar tissue were cut away from the airway tissue. The usual explants were 2nd to 5th division bronchi. The airways were sectioned into 3 × 5-mm fragments. Two replicate fragments were placed on 5 × 10-mm gel foam pads which were 3.5 mm in height and had previously been affixed to 35-mm tissue culture dishes. The airway cultures were maintained in 2.0 ml of CMRL-1066 with bovine insulin (1 μg/ml), hydrocortisone hemisuccinate (0.1 μg/ml), penicillin (100 μg/ml), streptomycin (100 μg/ml), and amphotericin B (0.5 μg/ml). Cultures were maintained at 37°C in a controlled atmosphere chamber purged with 45% O_2, 50% N_2, and 5% CO_2 (12). Histologic analysis of airways maintained in culture for up to 96 h revealed a normal appearing mucosal surface including goblet cells. Submucous glands in these bronchial fragments were present in every section, outnumbered the goblet cells by a considerable margin, and remained histologically normal through at least 48 h.

Mucous glycoproteins were radiolabeled by adding [^{3}H]-glucosamine, [^{14}C]glucosamine, [^{3}H]threonine, or $Na_2[^{35}S]O_4$ (1 μCi/ml) into the culture medium. The airways were cultured in the presence of radiolabeled molecules for an initial period of 16 h (period I). Thereafter, the culture media were harvested, the airways washed twice with 1 ml of culture media, and the washings combined with the harvested supernatant fluid. Fresh culture media (without radiolabel) were added and the culture continued for an additional 4 h (period II). At the end of period II, the supernatant media were harvested, the cultures washed twice, and the washings added to the harvested supernatant fluids (8). Supernatant fluids were then dialyzed against 20 vol of 6 M urea in 0.005 M phosphate buffer, pH 6.8, for 96 h (four changes). After dialysis, supernatant fluids were brought to a 5-ml vol and an aliquot was taken for measurement of nondialyzable radioactivity in a Beckman 9100 Scintillation Counter (Beckman Instruments, Fullerton, Calif.).

In attempting to develop the most appropriate experimental controls for the analysis of modulation of mucous release, we analyzed the nondialyzable counts per minute per milliliter in period II in relation to airway weight, airway protein, total counts per minute incorporated into each fragment, and nondialyzable counts per minute incorporated into each airway fragment. None of the procedures resulted in consistent results, probably because the mucus-secreting cells constitute a variable portion of each airway fragment. We therefore elected, as have others (5, 8), to compare the nondialyzable radiolabeled glycoprotein released from each fragment with the nondialyzable radioactivity released in the control period. This analysis resulted in a consistent ratio from fragment to fragment within each experiment.

The effect of various manipulations on the release of labeled mucous glycoprotein was determined by adding agents to cultures at the beginning of period II. The ratio of the radiolabeled nondialyzable glycoprotein released in period II as compared to period I in these manipulated samples was contrasted with the ratio in matched control samples. Thus, each airway culture provided its own control period, as well as stimulated period, and the effect of each manipulation upon the ratios of period II/period I could be compared with identical, matched controls. All fragments were subjected to identical conditions in period I and period II except that stimulated fragments were incubated with pharmacologic or immunologic reagents during period II. In this manner, the matched control fragments provided a valid reference for statistical comparison. We used 24 individual lungs in all. The mean ratio of period II to period I in control samples was 0.70±0.08 (n = 24) with a range of 0.12 to 1.19. The mean nondialyzable counts per minute per milliliter of period I was 3,739±730 (n = 24) and of period II was 2,233±457.

Passive sensitization of airways. In experiments involving passive sensitization, the airway fragments were incubated in sera rich in IgE antibody directed against ragweed pollen for 2 h at 37°C (13). The airway preparations were thoroughly washed and placed in culture under the conditions described as period I. Antigen challenge was performed at the onset of period II by adding 0.5 μg/ml ragweed antigen E to the culture fluid. In the five experiments with passive anaphylaxis, 26±8% of the total airway histamine was released. The final concentration of histamine released into the culture media was 140±67 ng/ml.

Reversed anaphylaxis of airways. No special preparation of lung tissue for reversed anaphylaxis was necessary (14). In the four experiments in which this procedure was followed, a dose response of rabbit antihuman IgE on autologous peripheral lung fragments was determined before challenge of the airway preparations. Optimal concentrations ranged from 1:40 to 1:100. The appropriate optimal concentration of anti-IgE was added at the onset of period II and resulted in 36±6% histamine release.

Preparation of mediator-rich peripheral-lung supernatant fluids. Peripheral-lung fragments were passively sensitized

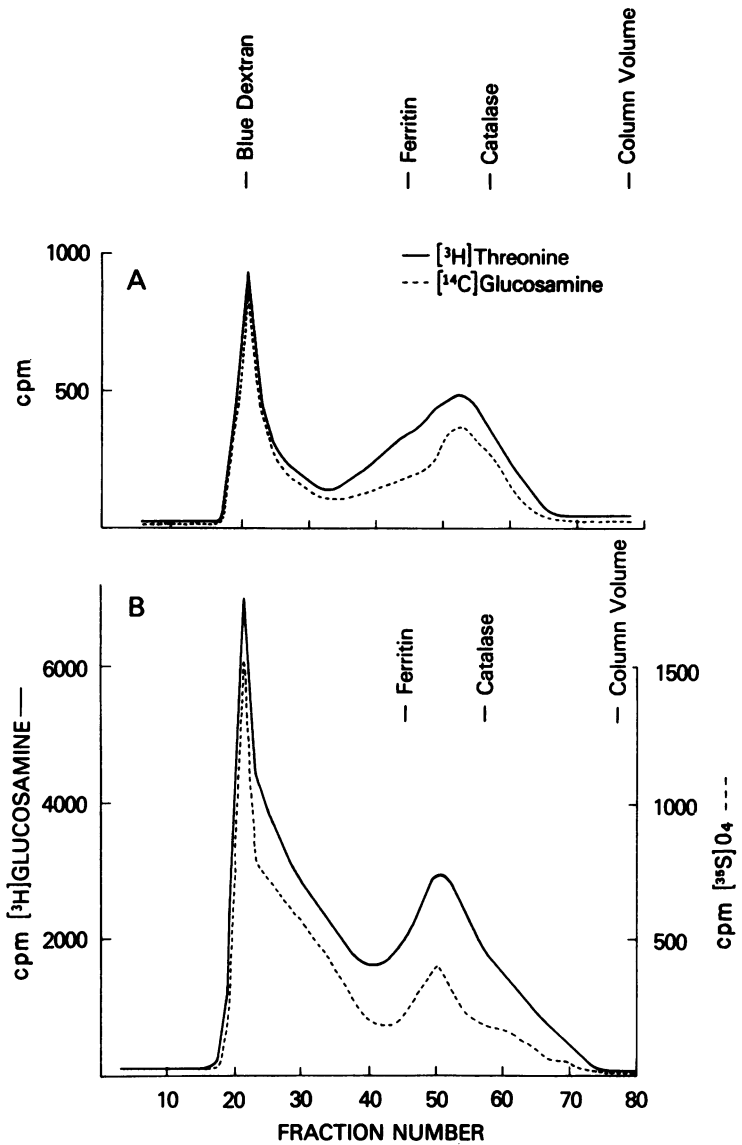

FIGURE 1 Comparison of the Sepharose 2B gel filtration pattern of human airway glycoproteins labeled with [^{14}C]glucosamine (– – –) or[^{3}H]threonine (——) (both upper panel), and [^{3}H]glucosamine (——) or $Na_2[^{35}S]O_4$ (– – –) (both lower panel). Human airways were co-cultured with either [^{3}H]threonine and [^{14}C]glucosamine or [^{3}H]glucosamine and $Na_2[^{35}S]O_4$ for 16 h. Supernatant fluids were dialyzed against 6 M urea in 0.005 M phosphate buffer, pH 6.8, and applied to a 1.5 × 100-cm Sepharose 2B column equilibrated in 6 M urea, phosphate buffer. Column was eluted by gravity flow at 1.5 ml/h and 1.4-ml fractions collected.

and challenged with specific antigen as described (13). Four separate lung supernatant fluids were prepared, each containing 200–400 ng/ml histamine and 400–1,000 U SRS-A/ml. These mediator-rich supernatant fluids (0.5 ml) were added to 1.5 ml of culture media at the onset of period II.

Chromatography. Radiolabeled supernatant fluids were analyzed by gel filtration and ion-exchange chromatography. Aliquots of the lung supernatant fluid were concentrated (Amicon Corp., Lexington, Mass.), dialyzed against 6 M urea in 0.005 M phosphate, pH 6.8, and applied to a 1.5 × 100-cm Sepharose 2B column previously equilibrated in 6 M urea/0.005 M phosphate buffer, pH 6.8. The column was eluted by gravity with a flow rate of 1.5 ml/h. Elution of radiolabeled material (1.4 ml fractions) was monitored by liquid scintillation counting of portions of each fraction. The portions of the elution volume containing radioactively labeled molecules were pooled, concentrated to 1.0 ml, and applied to a 1.5 × 30-cm DEAE-cellulose column equilibrated in 6 M urea/0.005 M phosphate buffer, pH 6.8. The column was washed with 2-column vol of starting buffer followed by a linear salt gradient (4-column vol) from 0 to 1 M NaCl in the starting buffer. The column was run at 20 ml/h and 3.5-ml

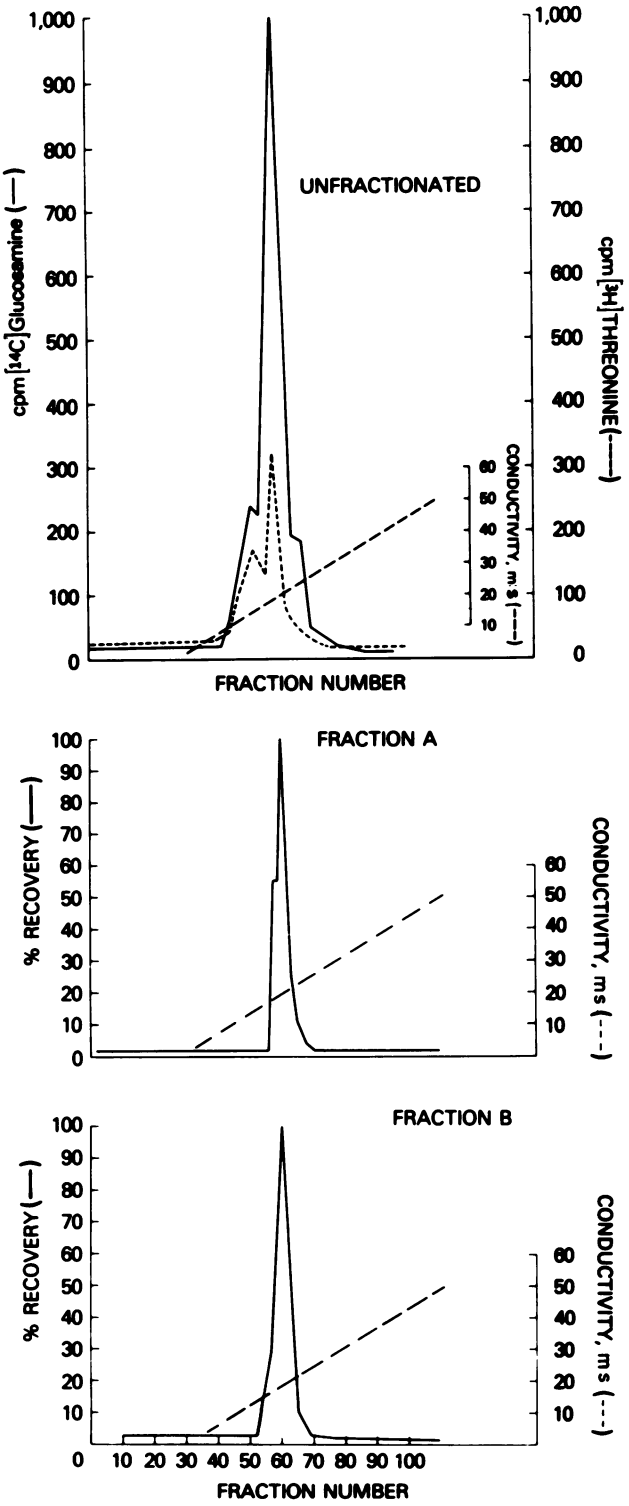

FIGURE 2 The elution pattern of human airway glycoproteins from DEAE-cellulose. DEAE-cellulose chromatography was performed on a 1.5 × 30-cm column equilibrated in 6 M urea, 0.005 M phosphate buffer, pH 6.8. Elution was conducted with a linear salt gradient from 0.0 to 1 M NaCl in the starting buffer; conductivity (- - -) is expressed as millisiemens. The sample applied in the upper panel was the supernatant fluids from a human airway cultured with [^{3}H]threonine (- - -) and [^{14}C]glucosamine (——). The samples used in the middle and lower panels were from a human airway cultured with [^{3}H]glucosamine. These latter supernatant fluids were fractionated over Sepharose 2B (see Fig. 1) and fractions A and B collected. Fractions A and B were applied separately to DEAE-cellulose. The elution pattern of fraction A is shown in the middle panel; fraction B is in the lower panel.

fractions were collected. Portions of each eluted fraction were analyzed for the presence of labeled glycoprotein by liquid scintillation counting.

Statistical analysis. The data were analyzed by the Student's t test with a P value of <0.05 considered statistically significant. The "n" in the tables indicates the number of experiments combined to generate the data unless otherwise indicated. In each experiment between four and eight samples were used to generate each experimental point. Data will be presented as the mean±SEM. Each experiment was repeated two or more times. Results will be presented in either of two forms: pooled data, or representative experiments, and this information is provided for each experiment presented. The asterisks in the figures indicate degrees of significance (one asterisk = $P < 0.05$, two asterisks = $P < 0.01$, and three asterisks = $P < 0.001$).

RESULTS

Chromatography: sepharose 2B. Supernatant fluids from lungs co-cultured with either labeled glucosamine and threonine or glucosamine and sulfate were analyzed chromatographically. The supernatant fluids were concentrated, dialyzed, and filtered on Sepharose 2B (Fig. 1). Two discrete peaks of radioactivity were observed in glucosamine, threonine, or sulfate-labeled material: about 50% of the radioactivity eluted in the void volume while the other half entered the column and eluted between ferritin and catalase. The void-volume fraction was designated fraction A and the included fraction was designated fraction B. The portion of radiolabeled glucosamine incorporated into fractions A and B in seven individual lung cultures was compared. Fraction A contained 52.1±3.2% of the radioactivity whereas fraction B contained 47.9±3.2%. Thus, amino sugars, amino acids, or sulfate were incorporated equally by human airways into two molecules that were synthesized in vitro by human airways.

DEAE-cellulose. Supernatant fluids from airways cultured in the presence of [^{14}C]glucosamine and [^{3}H]threonine, after dialysis, were applied to DEAE-cellulose (Fig. 2, top panel). Both the glucosamine and threonine-labeled molecules eluted in identical fractions, primarily at 0.2 M NaCl. To compare the anion exchange characteristics of fractions A and B, glucosamine-labeled supernatant fluids were filtered over Sepharose 2B and fractions A and B isolated, concen-

trated, and separately applied to DEAE-cellulose (Fig. 2, lower panels). Both fractions A and B eluted from the DEAE column in a single peak at 0.2 M NaCl, a pattern entirely concordant with the chromatography of the unfractionated airway supernatant fluid.

Modulation of mucous release. The mucous glycoprotein molecules were labeled equally well with glucosamine, threonine, or Na_2SO_4. For the remainder of the experiments, [^{3}H]glucosamine was used as the radiolabel. The rate of release of [^{3}H]glucosamine-labeled molecules was analyzed over a 48-h period after the initial 16-h (period I) labeling period. ^{3}H-Labeled, nondialyzable molecules were released continually (Table I). A 4-h incubation time for period II was selected because of convenience; the quantity of radiolabeled molecules released was adequate for accurate analysis, and previous investigators have employed a similar model thereby facilitating the comparison of results (5, 8). No attempt was made to separately analyze the release of the two glycoprotein molecules; instead the [^{3}H]glucosamine-labeled pools were analyzed solely by their retention after dialysis.

Anaphylaxis. The effects of the immunological release of the mediators of anaphylaxis upon the release of [^{3}H]glucosamine-labeled mucous glycoproteins were examined in three ways: direct passive anaphylaxis, reversed anaphylaxis, and by the addition of mediator-rich supernatant fluids (Table II). Increased mucous secretion was produced by each of these procedures.

Histamine. Antigen stimulation of IgE-sensitized human lung tissue results in the release and/or generation of a large number of biologically active mediators. One of the major mediators released is histamine.

TABLE I
*Analysis of Nondialyzable ^{3}H-Labeled Mucous Glycoprotein Released during Culture of Human Airways**

	Cumulative cpm/ml	Period II/period I
h		%
Period II		
0–1	363±29	41
0–2	719±82	81
0–4	915±99	104
0–24	2,284±264	262
0–48	3,020±311	349
Period I	883±93	

* Human lung airways were cultured for 16 h with [^{3}H]glucosamine and the supernatant fluid removed and dialyzed extensively. The nondialyzable counts in this sample are designated period I. The culture was continued for an additional 48 h in the absence of added [^{3}H]glucosamine. The culture media were changed at 1, 2, 4, 24, and 48 h and analyzed for nondialyzable counts.

TABLE II
Effects of the Mediators of Anaphylaxis upon the Release of Mucous Glycoproteins

	Increase above control
	%
Mediator-rich supernatant fluid	40±18 (n = 4)* $P < 0.05$
Passive anaphylaxis	36±14 (n = 5) $P < 0.025$
Reversed anaphylaxis	25±9 (n = 4) $P < 0.05$

* n indicates number of experiments combined to generate data.

Histamine may be capable of stimulating mucous secretion (15), although this has not been a universal finding (5, 7, 16). The capacity of histamine to induce the release of mucous glycoprotein was therefore investigated. Histamine (0.1–100 μM) was found to significantly increase the radiolabeled glycoprotein content of airway supernatant fluids at 100 μM concentration (Table III). The relative contribution of histamine H-1 and H-2 (17) stimulation on mucous glycoprotein secretion was analyzed using both receptor antagonists and selective agonists (Fig. 3). Histamine alone at 100 μM significantly increased mucous secretion. The H-2 receptor antagonist, cimetidine (100 μM), had no influence on mucous release when studied alone and prevented histamine-induced increases when added with histamine. We were unable to study the effect of H-1 receptor antagonists (either pyrilamine or diphenhydramine, both 100 μM) because they increased mucous secretion. The selective H-1 agonist, 2-methylhistamine (100 μM) (18), failed to significantly alter mucous glycoprotein release, whereas two separate H-2 agonists, dimaprit and impromidine (19),

TABLE III
Dose Response of Histamine upon Mucous Glycoprotein Release

Histamine	n*	Increase above control
μM		%
100	6	48.6±6 $P < 0.01$
10	3	15.3±8
1	3	14.3±7
0.1	2	0.5±8

* n indicates number of experiments combined to generate data.

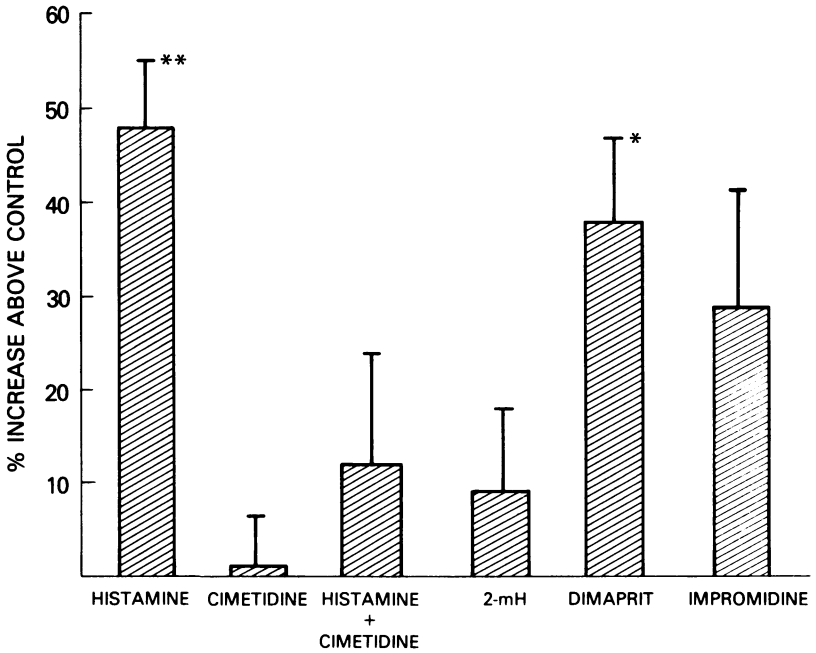

FIGURE 3 The effects of 100-μM concentrations of histamine, cimetidine, histamine plus cimetidine, 2-methylhistamine (2-mH), dimaprit, and impromidine on mucous secretion. Results are expressed as the percent increase above control cultures, and this figure presents the results from a representative experiment. Each bar presents the mean±SEM of quadruplicate samples. *, $P < 0.05$; **, $P < 0.01$.

induced increased secretion. Thus histamine, acting through stimulation of H-2 receptor sites in airways, increases mucous secretion.

Effects of neurohormones. Submucous glands are richly innervated with parasympathetic fibers (20). Other studies, including several of human lung airways in vitro (7, 8), have demonstrated that parasympathetic stimulation increases mucous glycoprotein secretion. The cholinergic agonist, methacholine (100 μM), added in period II caused a 62% increase ($n = 10, P < 0.001$) above control (Fig. 4). This increase was blocked by the addition of atropine (100 μM) before stimulation with methacholine. Atropine alone had no effect. Thus, muscarinic stimulation results in augmented mucous release.

The effects of adrenergic stimulation of human airways were also examined. The β-adrenergic agonists, isoproterenol (100 μM) and epinephrine (100 μM), added to airway cultures at the initiation of period II were without significant effect (% change = 3±3, $n = 2$, and 8±1, $n = 2$, respectively). By contrast, phenylephrine (1–100 μM) was found to induce a significant dose-related increase in mucous secretion (Table IV). Because these data suggest that α-adrenergic stimulation results in increased mucous secretion, the effects of norepinephrine, alone and in the presence of the β-adrenergic blocking agent propranolol, were examined (Fig. 5). Norepinephrine (1–100 μM) produced a nonsignificant reduction in mucous secretion. However, when norepinephrine was combined with propranolol (1 μM), a dose-related and statistically significant increase in mucous secre-

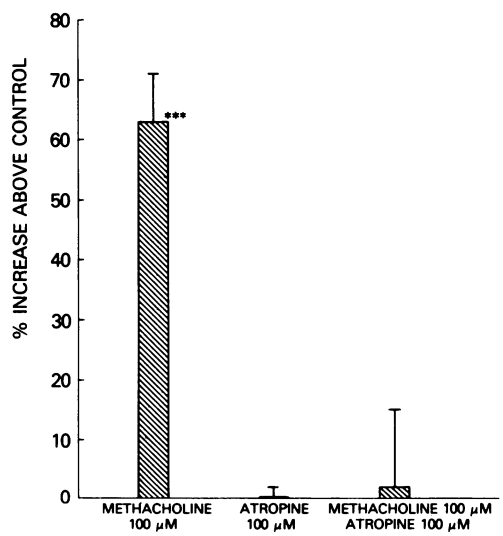

FIGURE 4 The effect of methacholine, atropine, and atropine plus methacholine (all 100 μM) on mucous secretion. The figure presents the results from a representative experiment and each bar represents the mean±SEM of quadruplicate samples. ***, $P < 0.001$.

TABLE IV
Dose-Response of Phenylephrine on Mucous Glycoprotein Release

Phenylephrine	n*	Increase above control
μM		%
100	4	60±14 $P < 0.001$
10	4	39±17 $P < 0.01$
1	4	24±7

* *n* indicates number of experiments combined to generate data.

tion resulted. Thus, α-adrenergic stimulation increases mucous glycoprotein release.

Effect of cyclic nucleotides. To investigate the potential mechanisms by which anaphylaxis, histamine, and neurohormones influence mucous secretion, we studied the effects of cyclic GMP and cyclic AMP, added at the onset of period II. Dibutyryl cyclic AMP (0.1–50 mM) had no effect on mucous release. However, 8-bromo cyclic GMP (0.01–100 μM) consistently increased the release of nondialyzable radiolabeled molecules (Fig. 6). In three separate experiments, the peak effect was noted at 1 μM with a mean increase above control of 34±5.5% ($P < 0.05$).

DISCUSSION

Human airways in short-term organ culture have been found to incorporate glucosamine, threonine, and sulfate into two macromolecules. By Sepharose 2B gel filtration analysis, these molecules filter with apparent molecular weights of >7,000,000 and ~400,000. These molecular weights are only approximate since glycoproteins are known to demonstrate anomalous behavior on gel filtration. Radiolabeled glucosamine was incorporated equally into each of these two fractions. Similar molecules have previously been found in expectorated human sputa (21, 22), as well as by analyses of experimental animal models of mucous secretion (23, 24). The two macromolecules demonstrated similar charge characteristics on DEAE-cellulose anion exchange chromatography. By nature of the incorporation of glucosamine, threonine, or sulfate into macromolecules synthesized by airway preparations, we conclude, as have others (8, 24), that the biosynthetically labeled molecules are mucous glycoproteins.

The bronchial airway preparations employed in these experiments contained both submucosal glands and goblet cells. The submucosal gland cells appeared to far outnumber the goblet cells but we cannot differentiate the cellular origin of the biosynthetically labeled molecules. Using an analogous culture system, Reid and co-workers (7, 25) have elegantly shown that radiolabeled sugars, amino sugars, amino acids, and sulfate may be autoradiographically demonstrated within submucosal acini of human airways. Similarly,

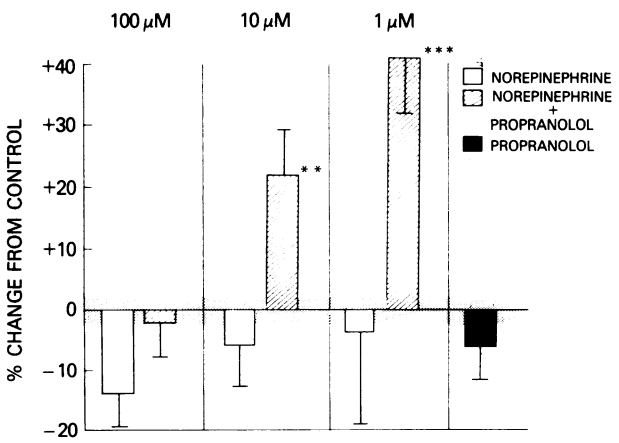

FIGURE 5 The effect of norepinephrine (1–100 μM, □), propranolol (1 μM, ■), and norepinephrine plus propranolol (1 μM, ▨) on mucous secretion. The results are from a representative experiment. The shaded area represents the mean±1 SEM of the control samples. Each bar presents the mean±SEM of quadruplicate experiments/sample. **, $P < 0.01$, ***, $P < 0.001$.

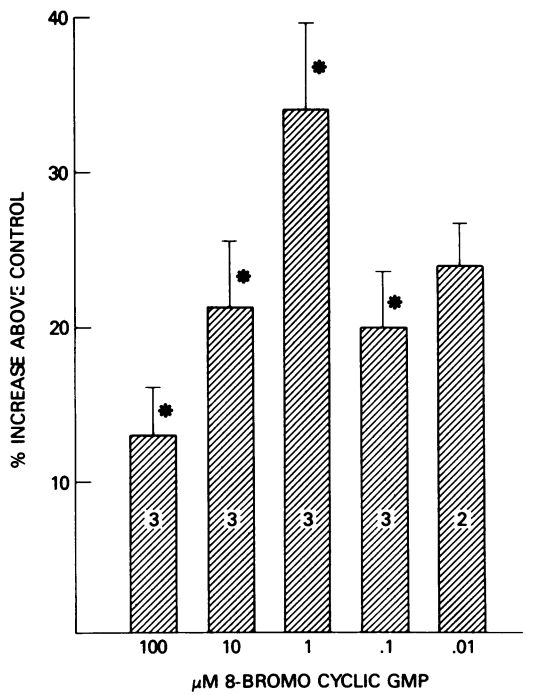

FIGURE 6 The effect of 8-bromo cyclic GMP (0.01–100 μM) on mucous secretion. The figure presents the mean percent increase above control of the pooled results from three individual lungs, *, $P < 0.05$.

radiolabeled sulfate and sugars may be demonstrated within goblet cells in canine airway epithelial cultures (26). Thus, radiolabeled molecules may appear from either goblet cells or submucosal glands.

The purpose of this investigation was to analyze the capacity of a variety of stimuli to modulate the release of mucous glycoproteins to assess the possible mechanisms underlying mucous secretion in asthma. Despite meticulous attention to airway dissection and replication, preliminary experiments failed to uncover an experimental model in which stimulated secretory periods could be compared between parallel cultures. Therefore, we, as have others (5, 8), chose to compare each culture with its own control period. The ratio of stimulated to control period corrects each culture for the variable number of mucous-secreting cells contained therein and permits valid comparisons of the effects of various procedures.

Allergic rhinitis and allergic asthma are frequently associated with excessive mucous secretion, although the mechanisms involved are unclear. No previous analysis of the effects of anaphylaxis on human respiratory mucous secretion has been reported, although anaphylaxis of rat intestinal preparations has recently been noted to increase ^{35}S-labeled goblet cell secretions (11). Therefore, we analyzed the responses of human airway cultures to allergic reactions. Three lines of evidence have been uncovered which indicate that mast-cell degranulation increases mucous secretion from cultured human airways. (*a*) Supernatant fluids from peripheral human lung tissue passively sensitized with human IgE and challenged with specific antigen contain a mixture of the mediators of anaphylaxis; these mediator-rich supernatant solutions significantly increased mucous secretion. (*b*) Cultured airways themselves may be passively sensitized with IgE and challenged with a specific antigen; the resulting immunologic reaction induced the release of both histamine and increased quantities of mucus. (*c*) Antibodies directed against human IgE may interact with IgE molecules present on airway mast cells and cause mediator release; this reaction also caused increased mucous secretion. Thus, the mediators of anaphylaxis as well as reversed and direct anaphylaxis of airways induces mucous secretion.

These data indicate that a product derived from degranulated lung mast cells is capable of stimulating mucous secretion. Histamine has previously been suggested as a mucous secretagogue (15), although other in vitro experiments have been inconsistent with this conclusion (5, 7, 16). Histamine (1–100 μM) increased mucous secretion maximally at 100 μM. Peripheral human lung contains 10 μg of histamine/g wet wt (27). It might be anticipated that the immunologic release of as little as 20% of total histamine (the mean percent histamine released by allergen-IgE interactions in human lung in vitro) would generate histamine concentrations in the interstitial space around mast cells exceeding 100–1,000 μM. The effects of exogenous histamine on mucous secretion could be prevented by H-2 receptor antagonists and reproduced by H-2 agonists. H-1 receptor antagonists could not be studied as they appeared to be stimulatory; however, 2-methylhistamine, an H-1 agonist, was not effective. These data indicate that histamine stimulates mucous secretion by interacting with H-2 receptors.

Asthmatic patients appear to have an array of abnormalities of autonomic responsiveness including reduced β-adrenergic responses (28, 29) and heightened α-adrenergic (30) and cholinergic responses (31, 32). It was therefore of interest to examine the effects of the neurohormones which mediate these neurologic functions. The selective β-adrenergic agonist, isoproterenol, which has previously been shown to have no effect on human airway mucous glycoprotein synthesis (7) and secretion (8), was also found to be ineffective in the current experiments. By contrast, isoproterenol has been demonstrated to increase tracheal mucous secretion in the cat in vivo (2). Epinephrine, like isoproterenol, had no effect in our experiments. Norepinephrine alone appeared to produce no significant effect on mucous secretion. However, when the β-adrenergic actions of norepinephrine were blocked by exposing the airways to propranolol, a dose-related increase in mucous secretion was observed. The possibility that α-adrenergic stimulation might increase mucous secretion was confirmed when the effects of the relatively pure α-adrenergic agonist phenylephrine were examined. Phenylephrine induced a dose-related increase in mucous secretion. Therefore, α-adrenergic stimulation of human mucous glands causes increased secretions much as seen in the cat (3). Cholinergic stimulation of muscarinic receptor sites was also found to increase mucous release, as has been previousy reported (5, 7, 8). Both cholinergic stimulation of human lung in vitro (13) and α-adrenergic agonist infusions into humans in vivo (33) are associated with increased cyclic GMP. Therefore, the observation that exogenously administered cyclic GMP augments mucous secretion in vitro suggests the possibility that elevations in this molecule might be a common pathway for cholinergically and α-adrenergically mediated stimulation of secretion.

Evidence has been presented suggesting that several factors relevant to allergic reaction of human airways may contribute to increased mucous glycoprotein secretion. These factors include the mediators of anaphylaxis generated by human lung undergoing allergic reactions as well as several neurohormones to which asthmatics have been shown to be hyperreac-

tive (30–32). Therefore, it seems likely that allergic reactions in human lung initiate responses leading to heightened mucous secretion.

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