Effects of Arachidonic Acid, Monohydroxyeicosatetraenoic Acid and Prostaglandins on the Release of Mucous Glycoproteins from Human Airways In Vitro

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ABSTRACT Human lung explants maintained in culture for 7 d incorporate [^{3}H]glucosamine into mucous glycoproteins. Ethanol-precipitable, glucosamine-labeled mucous secretion was measured, and the effects of different pharmacologic agents upon this secretion were investigated. Anaphylaxed human lung generates prostaglandin (PG) synthesis and increased mucous release. Arachidonic acid (AA), PGA_2, PGD_2, and $PGF_{2\alpha}$ significantly increased mucous glycoprotein release, whereas PGE_2 significantly reduced release. Evidence which suggests that lipoxygenase products of AA augment mucous release includes the following: (*a*) Nonsteroidal anti-inflammatory drugs (NSAID: acetylsalicylic acid and indomethacin) increase mucous release while preventing prostaglandin formation. (*b*) The increase in mucous release induced by AA or NSAID is additive once the agents are combined. (*c*) Several nonspecific lipoxygenase inhibitors (eicosa-5,8,11,14-tetraynoic acid; vitamin E; nordihydroguaiaretic acid; and α-naphthol) inhibit mucous release. Three additional lines of evidence directly indicate that monohydroxyeicosatetraenoic acid (HETE) causes increased mucous release: (*a*) the addition of a mixture of synthetic HETE (24–600 nM) increases mucous release; (*b*) pure 12-HETE (1–100 nM) also increases mucous release; (*c*) mucous release is increased synergistically by the combination of HETE and NSIAD.

These data taken together demonstrate that HETE are capable of increasing mucous release and that conditions which may influence HETE production alter mucous release. Thus, although not directly demonstrating HETE production by human airways, the data strongly suggest that lipoxygenase products of AA in airways may profoundly influence mucous release; and it seems possible that lipoxygenase inhibitors may have a role in treating bronchorrhea.

INTRODUCTION

Hypersecretion of bronchial mucus is a major feature of many respiratory diseases and contributes to the morbidity of a large segment of the population. To study the controls of mucous synthesis and secretion, an in vitro model involving the culture of human airways (1) was developed (2). Human airways may be cultured for extended periods of time and newly synthesized mucous secretion may be analyzed either by autoradiography after pulse labeling (3, 4) or by incorporation of radiolabeled molecules into newly formed mucus (2, 5). Human airways incorporate sugars, amino sugars, amino acids, and sulfate equally well into glycoproteins. Two glycoproteins are synthesized: one with an apparent molecular weight $>7 \times 10^6$ and the other ~400,000 (2). Both of these molecules are rich in carbohydrates (80 and 60% of total composition, respectively), have similar charge characteristics of ion-exchange chromatography and by isoelectric focusing, and are formed in equal amounts by airway cultures. Similar molecules may be synthesized by airways of experimental animals (6). Anaphylaxis of human airways increases mucous release, partially due to histamine H-2 receptor stimulation (2). Muscarinic (2, 5) and alpha adrenergic (2) stimulation also increases mucous release, whereas beta adrenergic stimulation has no effect.

Prostaglandins (PG)[1] and their metabolites are widely distributed throughout the body and are thought

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[1] *Abbreviations used in this paper*: AA, arachidonic acid; ASA, acetylsalicyclic acid; ETYA, eicosa-5,8,11,14-tetraynoic acid; HETE, monohydroxyeicosatetraenoic acid; NDGA, nordihydroguaiaretic acid; NSAID, nonsteroidal anti-inflammatory drugs; PG, prostaglandin.

to contribute to the local regulation of many organ systems. Although the lung is one of the major sites of PG synthesis (7) and inactivation (8, 9), little is known about their role in mucous secretion. Inhalation of $PGF_{2\alpha}$ by normal humans induces mucous secretion (10). Similarly, the infusion of $PGF_{2\alpha}$ in dogs in vivo is associated with increased bronchosecretory activity (11). PG are generated during human lung anaphylaxis (12–14) and mucous release is enhanced in anaphylaxis (2); therefore, it seemed appropriate to analyze the effect of PG and other derivatives of arachidonic acid (AA) on mucous glycoprotein release.

METHODS

Tritiated $PGF_{2\alpha}$ (5,6,8,9,11,12,14,15-^{3}H[N], 100–500 Ci/mmol), [^{3}H]PGE_2 (5,6-^{3}H[N], 100–200 Ci/mmol), and Aquasol (New England Nuclear, Boston, Mass.); rabbit anti-$PGF_{2\alpha}$ (Clinical Assays, Cambridge, Mass.); rabbit anti-PGE (Accurate Chemical & Scientific Corp., Hicksville, N. Y.); medium L-15, medium CMRL-1066, penicillin, streptomycin, and bovine insulin (Grand Island Biological Co., Grand Island, N. Y.); gel-foam (Upjohn Co., Kalamazoo, Mich.); silica gel, linear K-6 plates for TLC (Whatman, Inc., Clifton, N. J.); Metricel membrane filters, 0.45 μm, 25 mm (Gelman Instrument Co., Ann Arbor, Mich.); methanol-nanograde and acetic acid, glacial (Mallinckrodt Inc., St. Louis, Mo.); sodium borohydride (Fisher Scientific Co., Pittsburgh, Pa.); arachidonic acid, 99%+ (Nu Chek Prep, Inc., Elysian, Minn.); ricinoleic acid, stearic acid, palmitic acid, and arachitic acid (Supelco, Inc., Bellefonte, Pa.); and acetylsalicyclic acid, indomethacin, DL-α-tocopherol (vitamin E), α-naphthol, nordihydroguaiaretic acid, and hydrocortisone (Sigma Chemical Co., St. Louis, Mo.) were all purchased from their respective manufacturers. Eicosa-5,8,11,14-tetraynoic acid was the gift of Dr. W. E. Scott, Hoffman-LaRoche, Inc., Nutley, N. J. Prostaglandins $F_{2\alpha}$, D_2, I_2, E_2, E_1, and thromboxane B_2 were a gift of the Upjohn Co. through the courtesy of Dr. J. E. Pike, and 12-hydroxyeicosatetraenoic acid was kindly provided by Dr. F. Sun of the Upjohn Co.

Preparation of human airways for culture. Tumor-free portions of human lung were obtained at surgery for resection of carcinoma. The specimens were maintained in L-15 carrier medium for transport to the laboratory. Airways, 2–10 mm in diameter, were dissected free of discernible alveolar tissue. Whenever possible cartilage was removed from the larger airways. The airways were fragmented into 3 × 5-mm (15–25 mg) replicates. Two fragments were placed on 5 × 10-mm gel foam pads previously affixed to 35-mm tissue culture dishes. The airway explants were maintained in 2.0 ml of CMRL-1066 medium with added 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), as described (1). The culture dishes were placed in a controlled atmosphere chamber and gassed at 10 pounds per square inch (psi) with 50% O_2, 45% N_2, and 5% CO_2 for 5 min. The chamber was placed on a rocker platform which rocked at 10 cycle/min, causing the media to flow intermittently over the surface of the explants. The explants were incubated at 37°C for an initial 16-h period.

Radiolabeling of mucous glycoprotein. Mucous glycoproteins may be radiolabeled by incorporating [^{3}H]glucosamine (1 μCi/ml) into the culture medium (2, 3, 5). After an initial 16-h incubation in the absence of [^{3}H]glucosamine, the cultures were washed twice with CMRL-1066 medium and incubated with [^{3}H]glucosamine for a 16–20-h base-line period (period I). Thereafter, the culture media were harvested, the airways washed twice with 1 ml culture media, and the washings were combined with the harvested supernate. Fresh culture media without [^{3}H]glucosamine were added to the cultures for an additional 4-h period (period II). At the end of period II, the supernatant media were harvested, the cultures washed twice, and the washings added to the harvested supernates (2, 5).

Airway cultures were subsequently recycled through 16-h base-line incubations in [^{3}H]glucosamine (period I), followed by 4-h (period II) incubations in the absence of [^{3}H]-glucosamine, for up to 7 d. The physicochemical characteristics of airways cultured for 3 or 14 d were compared by Sepharose 2B and DEAE-cellulose chromatography as described (2). The labeled glycoproteins were similar as assessed by both size and charge characteristics. Histologic analyses of airways maintained in culture for 7 d revealed a normal appearing mucosa including goblet cells. The submucous glands in these fragments were present in every section and appeared histologically normal throughout the 7-d period; they outnumbered the goblet cells by a considerable margin.

Separation of radiolabeled glycoproteins. The culture media and washes (4 ml, total) from periods II and I were stored at −70°C. 1-ml aliquots were added to 1 ml 95% ethanol, vortexed, and filtered through Metricel membrane filters. The filters were wetted with 1 ml 95% ethanol just before the addition of the culture media-ethanol mixture, and the retained filtrate was washed three times with 1 ml 95% ethanol. The filters were placed in scintillation vials, dried at 100°C for 20 min, and incubated at room temperature for 25 min before the addition of 10 ml Aquasol. The scintillation vials were vortexed and incubated for at least 1 h at room temperature before liquid scintillation counting in a Beckman 9100 scintillation counter (Beckman Instruments, Inc., Mountainside, N. J.).

Effect of pharmacologic agents upon the release of mucous glycoproteins. The effect of pharmacologic manipulations on the release of [^{3}H]glucosamine-labeled mucous glycoprotein was determined by adding agents to cultures at the beginning of period II. A ratio of the radiolabeled precipitated counts per minute of period II/period I for each sample was determined and was termed the secretory index. The actual counts per minute and secretory indices of control samples from eight separate lungs cultured for up to 7 d are provided in Table I. Due to the relatively high secretory index observed in day-1 cultures, no experiments were done during the initial cycle. Instead, all experiments were performed on lungs between days 2 and 7 in culture.

The effects of pharmacologic agents were determined by comparing the secretory indices of manipulated samples with matched, unmanipulated, control samples. Thus, each airway culture provided its own base-line period (period I), as well as stimulated period (period II), and the effects of each pharmacologic manipulation could be compared with matched controls. The example provided in Table I indicates the unmanipulated control data with which manipulated samples would be compared.

All unstable pharmacologic agents were prepared immediately before use. Indomethacin was dissolved in 0.1 N sodium carbonate. Eicosa-5,8,11,14-tetraynoic acid (ETYA) was dissolved in dimethylsulfoxide and all the antioxidants were dissolved in methanol. Aspirin was dissolved in ethanol. An aliquot of aspirin in ethanol was taken to dryness at 45°C by a stream of N_2, redissolved in medium, and immediately added to cultures (15). The PG were dissolved in 80% ethanol and maintained as 10-mg/ml stock solutions. All the chemicals were diluted to a final concentration with medium after going

TABLE I

*Counts per Minute of Period II and the Secretory Index (Period II/Period I) for Human Airways Cultured for up to 7 d**

	Days of culture						
	1	2	3	4	5	6	7
Period II, *cpm*, ($\bar{X}$±SEM)	1,154±138 n = 7‡	2,098±532 n = 6	1,344±254 n = 6	1,565±281 n = 5	2,018±429 n = 5	2,256±442 n = 4	1,785±678 n = 3
Secretory index (Period II/period I) (mean±SEM)	0.792±0.079	0.527±0.134	0.376±0.025 §	0.361±0.024 §	0.407±0.053 §	0.409±0.051 §	0.343±0.048 ‖

* Seven separate lungs were cycled from period I to period II over a 7-d period, as described. The actual mean cpm of period I from these lungs are provided. The secretory index was determined by dividing period II by period I for the same seven-lung cultures.

‡ The n in this and all tables and figures indicates the number of individual experiments on separate lungs which were combined to generate the data.

§ As compared with day 1; $P < 0.01$.

‖ As compared with day 1; $P < 0.001$.

into solution. The final dilution for each solvent was tested and found to have no modulating influence on mucous release.

Radioimmunoassay of prostaglandins. Another 1-ml aliquot of the combined culture media and washings from period II was assayed by radioimmunoassay after extraction for PGE and $PGF_{2\alpha}$ (13, 16). The aliquot was adjusted to pH 3.0 with 0.1 N HCl, extracted with 3 vol of ethyl acetate three times, and the ethyl acetate extractants evaporated to dryness under vacuum at 45°C (Buchler Rotary Evapo-Mix, Buchler Instruments, Div. of Searle Diagnostics, Inc., Fort Lee, N. J.). The dried samples were resuspended and assayed as described (12, 13).

Monohydroxyeicosatetraenoic acid (HETE) production. AA (100 mg) was coated on the surface of a 45-mm pyrex petri dish that had previously been thoroughly washed sequentially with distilled water, 95% ethanol, and ethyl acetate. The AA was irradiated with short-wave (average wave length = 253.7 mm) ultraviolet (UV) light, using a UVSL58 light source (Ultra-Violet Products, Inc., San Gabriel, Calif.) at a distance of 10 cm for 16 h at 22°C. The petri dish was washed twice with 1.5 ml methanol and 10 mg of sodium borohydride was added to the washings. After a 15-min incubation, the pH of the sample was adjusted to 3.0 with 0.1 N HCl. The acidified solution was heated to 45°C and dried with a stream of N_2 until the methanol was evaporated. The residual aqueous solution was extracted with 1 ml of ethyl acetate three times. The ethyl acetate extractant was evaporated to dryness under vacuum (Speed Vac Concentrator, Savant Instruments, Inc., Hicksville, N. Y.), resuspended in methanol, and applied to a thin-layer chromatography plate developed in ascending fashion in chloroform:methanol:acetic acid:water (90:8:1:0.8, vol/vol) (17). The material migrating with an R_f 0.66 was eluted with methanol. 15-HETE was used as a standard and migrated with the identical R_f. The 15-HETE was kindly provided by R. W. Bryant, Ph.D. (George Washington University School of Medicine, Washington, D.C.). The concentration of HETE synthesized was estimated by determining the absorbance at 235 nm (Gilford Spectrophotometer 250, Gilford Instrument Laboratories Inc., Oberlin, Ohio), using a molar extinction coefficient of 23,500 (18).

The specific identification of the mixture of the HETE contained in the eluate from the thin-layer chromatography was resolved by high-performance liquid chromatography employing a microporasil column (18). The mixture generated consisted of 5-, 8-, 11-, and 12-HETE. The identification of the HETE was kindly performed by Frank Sun, Ph.D., Upjohn Co.

Statistics. Data will be present as the mean±SEM. The n in the tables and figure legends indicates the number of experiments combined to generate the results. In each experiment, between four and eight samples were used to generate each experimental point. The data were analyzed both by Student's t test for unpaired samples and by paired sample t tests, with a P value of <0.05 considered statistically significant.

The results are expressed as percent change from control and represent the comparison of the secretory index (period II/period I) between pharmacologically manipulated samples and matched controls.

RESULTS

Effect of prostaglandins on mucous glycoprotein release. The addition of PG to airway cultures at the onset of period II was analyzed using concentrations of 0.0001–100 μM. A number of PG were examined (Table II). PGA_2, PGD_2, and $PGF_{2\alpha}$ significantly increased mucous glycoprotein release at 100-μM concentrations; $PGF_{1\alpha}$, PGI_2, and PGE_1 tended to increase mucous release, but the results were not statistically significant; and T × B_2 was inactive. PGE_2 significantly reduced mucous secretion with maximal activity at 1 μM.

Effect of arachidonic acid on mucous glycoprotein release. AA added to lung tissue is enzymatically converted to the specific PG ordinarily produced (19, 20). Therefore, the effects on mucous release of adding AA to airways were studied. As shown in Table III, AA from 1–100 μg/ml induced a dose-related increase in the release of mucus, suggesting that either AA itself or derivatives of AA increase mucous release. Arachitic acid and palmitic acid (both at 100 μg/ml) had no effect on mucous release.

Effect of nonsteroidal anti-inflammatory drugs (NSAID) on mucous glycoprotein release. To examine whether the inhibition of the endogenous generation of PG during period II might have an influence on the secretion of mucus, acetylsalicylic acid (ASA)

TABLE II
Effect of Prostaglandins on Mucous Release

Prostaglandin	Concentration	n	Mucous release
	μM		% Change
PGA_2	100	2	91.0±1.0*
PGD_2	100	7	28.0±10.1‡
	10	2	23.5±36.5
	1	2	33.5±86.5
$PGF_{1\alpha}$	100	3	28.0±10.0
$PGF_{2\alpha}$	100	9	25.7±9.1‡
	1	3	68.0±42.0
	0.01	3	8.3±11.9
	0.0001	2	5.0±11.0
TxB_2	100	2	5.0±3.0
PGI_2	100	5	49.8±50.6
	10	5	34.0±32.3
	1	4	72.8±55.1
	0.01	1	32
PGE_1	100	6	31.3±26.5
	10	4	51.8±24.6
	1	7	45.9±31.6
	0.01	4	6.5±6.5
	0.0001	2	−5.0±17.0
PGE_2	100	11	−6.4±8.9
	10	3	−17.0±10.5
	1	5	−57.0±13.3§
	0.01	4	−14.8±0.48*
	0.001	3	−15.3±4.41
	0.00001	1	−6

* $P < 0.001$ compared with control.
‡ $P < 0.05$ compared with control.
§ $P < 0.01$ compared with control.

TABLE III
Effect of Arachidonic Acid on Mucous Release

	Concentration	n	Mucous release
	μg/ml		% increase above control
Arachidonic acid	1	2	11.5±4.5
	10	6	24.8±9.2*
	50	4	44.3±14.2*
	100	6	40.4±12.4*
Arachitic acid	100	2	3.0±1.2
Palmitic acid	100	2	2.3±1.4

Arachidonic acid (or another fatty acid) was added to the airway cultures at the onset of period II, and the secretory index of stimulated cultures compared with matched controls.
* $P < 0.01$ compared with control.

(100 μg/ml) and indomethacin (10 μg/ml) were studied. In each of four experiments in which both PG and mucous generation were measured, ASA and indomethacin significantly reduced the formation of $PGF_{2\alpha}$ and PGE by the airways in culture; however, mucous release was not inhibited but was enhanced (Table IV A). In a total of 10 experiments with ASA, the release of mucus was increased by 29.4±6.4% ($P < 0.01$) (Table IV B). In a total of 13 experiments indomethacin increased mucous release by 28.4±7.7% ($P < 0.01$). Therefore, inhibition of the cyclo-oxygenase enzyme system by NSAID results in an increase in mucous glycoprotein release. These results suggest either that reduction of the inhibitory influence of PGE_2 induces increased mucous release or that endogenous AA may become more available for metabolism by the lipoxygenase enzyme system once the cyclo-oxygenase system is impaired.

We reasoned that impairing cyclo-oxygenase enzymes and adding exogenous AA might result in further increases in the release of mucus if lipoxygenase enzyme products were responsible. Thus, experiments combining ASA or indomethacin with AA were conducted (Table V). In every instance, the increase in mucous release induced by each component of the experiment was increased in an additive fashion by the combination of agents. These experiments taken together suggest the possibility that lipoxygenase products of AA are capable of increasing the secretion of mucus from airways in culture.

Effect of lipoxygenase inhibitors on mucous glycoprotein release. To study the effects of lipoxygenase products upon mucous release more directly, the acetylenic derivative of AA, ETYA, was studied. ETYA was examined at concentrations from 1 to 100 μM (Fig. 1) and inhibited the release of mucus at all concentrations above 10 μM. In two experiments, the effects of ETYA (100 μM) upon both the release of mucous glycoprotein and the generation of $PGF_{2\alpha}$ and PGE were measured. ETYA significantly inhibited the release of mucus (−23.5±1.5%, $P < 0.05$) and the generation of $PGF_{2\alpha}$ (−53.5±38.5%) and PGE (−84.0 ±11.0%), directly indicating that this agent inhibits the cyclo-oxygenase pathway as well as putatively inhibiting the lipoxygenase pathway. Three additional, nonspecific inhibitors of the lipoxygenase enzyme system (21) were studied: nordihydroguaiaretic acid (NDGA), vitamin E, and α-naphthol (Table VI). All three antioxidants inhibited mucous release; NDGA, 4–400 μM, did so in a dose-related fashion. NDGA simultaneously suppressed $PGF_{2\alpha}$ and PGE generation as well.

Effect of HETE on mucous glycoprotein release. A mixture of HETE was prepared and examined to assess directly their effect on mucous release. Two separate preparations of HETE were prepared and studied on four individual lung cultures (Table VII). The addi-

TABLE IV

*Effects of NSAID on Mucous Glycoprotein Release and Prostaglandin Generation from Human Airways**

	n	Mucous release	$PGF_{2\alpha}$	PGE
			% Change	
A. NSAID				
ASA (100 μg/ml)	3	+28.33±8.21‡	−79±10.2‡	−80±3.0‡
Indomethacin (10 μg/ml)	4	+38.25±13.77	−76±9.0§	−69±9.7‡
B. NSAID				
ASA	10	+29.4±6.4§	—	—
Indomethacin	13	+28.9±7.7§	—	—

* The effects of NSAID were studied in 13 separate lungs. The effects of NSAID on the release of mucous are presented in part B. Only four experiments were examined for PGE and $PGF_{2\alpha}$ formation as well, and the results for both PG and mucous release are provided in part A. The results are presented as percent change from control. Control $PGF_{2\alpha}$ was 3,772±455 pg/ml (n = 4) and for PGE was 2,389±731 pg/ml (n = 3).
‡ $P < 0.05$ compared with control.
§ $P < 0.01$ compared with control.

tion of 24–600-nM concentrations of HETE at the onset of period II increased mucous release by 8.0–15.7%. Two other hydroxy fatty acids, ricinoleic acid and 12-hydroxystearate in 100-μM concentrations had no effect on mucous release. In two experiments, the effects of 240 nM HETE upon PGE generation were determined; PGE release was inhibited by 54±4%. In two additional experiments, the effect of 600 nM HETE upon $PGF_{2\alpha}$ generation was examined; 73±16% inhibition was appreciated. Thus, the exogenous addition of a mixture of HETE may also suppress both PGE and $PGF_{2\alpha}$ generation by human airways.

The UV-irradiated preparation of HETE may contain sterioisomers which human airways in vivo may never encounter. Therefore, the effect of pure 12-HETE (1–100 nM) was examined. 12-HETE increased the release of mucus in a dose-related fashion: 1 nM increased mucous release by 22.7±6.8% ($P < 0.05$), 10 nM = +44.5±11.1% ($P < 0.05$), and 100 nM = +67.5 ±15.8% ($P < 0.05$). Thus, pure 12-HETE was even more potent than the UV-generated mixture of HETE.

Effect of combining NSAID and HETE upon mucous glycoprotein release. In three separate experiments, NSAID (either ASA [100 μg/ml] or indomethacin [10 μg/ml]) were studied alone or in combination with HETE (Table VIII). Each agent when studied alone enhanced the release of mucous glycoproteins. The combination of agents resulted in an additive effect in

TABLE V

Effect of NSAID Plus Arachidonic Acid on Mucous Release

	Concentration	Mucous release
	μg/ml	*% Change*
Experiment 1		
Arachidonic acid	50	+24.0±15.0*
Indomethacin	10	+43.0±7.0‡
AA + indomethacin	10	+54.0±8.0‡
Experiment 2		
Arachidonic acid	10	+20.0±7.0§
ASA	100	+38.0±3.0‖
AA + ASA	100	+58.0±11.0‖

* Results represent single representative experiments.
‡ $P < 0.01$ compared with control.
§ $P < 0.05$ compared with control.
‖ $P < 0.001$ compared with control.

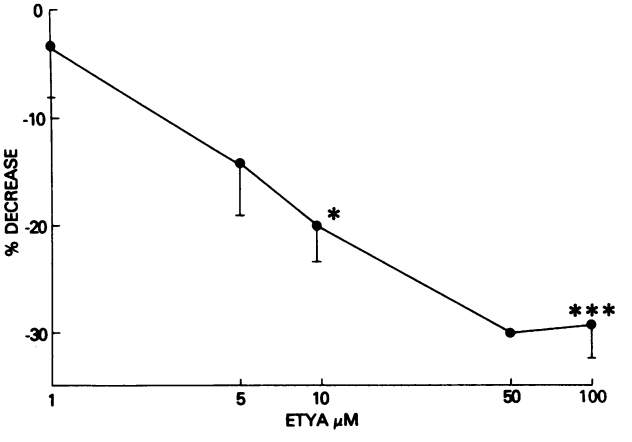

FIGURE 1 The effect of ETYA on the release of mucous glycoproteins. ETYA (1–100 μM) was examined in 13 individual lung cultures. The results represent the mean percent decrease below control. *, $P < 0.05$; ***, $P < 0.001$.

TABLE VI

*Effects of Antioxidants upon the Release of Mucous Glycoproteins and the Production of Prostaglandins**

Agent	Concentration	n	Mucous release	$PGF_{2\alpha}$	PGE
	μM		% Change		
NDGA	4	2	−7.0±1.0	−96‡	−94
	40	2	−17.0±5.0	−97	−90
	400	3	−23.3±4.4§	−96	−89
Vitamin E	500	1	−25.0	—	—
α-Naphthol	40	1	−10.0	—	—

* Antioxidants were added to airway cultures at the onset of period II, and the effects upon the release of [^{3}H]glucosamine-labeled glycoprotein were measured.

‡ Two experiments were analyzed for $PGF_{2\alpha}$ and PGE release. Control $PGF_{2\alpha}$ was 1,660±410 pg/ml and PGE was 1,770±333 pg/ml.

§ $P < 0.001$ compared with control.

one experiment and marked synergism in the other two. Thus, the addition of HETE to airways with impaired cyclo-oxygenase enzymes results in further increases in release of mucous glycoproteins. The generation of PGE and $PGF_{2\alpha}$ was examined in one of the experiments in which indomethacin and HETE were combined; each agent reduced PG generation by a considerable degree, and the combination of agents was comparable to either agent alone (Table VIII).

DISCUSSION

Mucous secretion is an important feature of bronchial asthma as well as of other respiratory diseases. PG are formed during human lung anaphylaxis in vitro (13, 14, 22, 23) as well as in response to a variety of other stimuli (24, 25). Inhalation of $PGF_{2\alpha}$ by normal humans increases mucous secretion (10), whereas infusion of $PGF_{2\alpha}$ but not of PGE_2 increases bronchosecretory activity in dogs (11). To study the effects of exogenous PG upon mucous secretion by human airways in vitro, the release of glycoprotein molecules radiolabeled with glucosamine from airways cultured for up to 7 d was studied. Adding exogenous PG to the airway cultures generally increased the release of mucus, although statistically significant enhancement by $PGF_{2\alpha}$, PGD_2, and PGA_2 was seen only at 100 μM, a concentration somewhat above the physiologically conceivable range. PGI_2, PGD_2, and $PGF_{2\alpha}$ exhibited their peak enhancement at 1 μM, but the results were more variable and failed to achieve statistical significance. PGE_2, in contrast to all the other PG examined, consistently suppressed mucous release with a maximal effect at 1 μM. Indeed PGE_2, the predominant PG produced by airways (12, 14), was active at 0.01 μM (3.5 ng/ml), which is clearly within the physiologic range.

In most organ systems, including the lung, AA is the predominant precursor of the naturally occurring PG (19, 20). When added to airway tissue, AA is enzymatically converted to PG. AA induced a dose-related increase (1–100 μg/ml) in the release of mucus, indicating

TABLE VII

*Effect of HETE on Mucous Release**

Compound	Concentration	n	Mucous release
			% Increase above control
HETE, *nM*	24	2	8.0±2.0
	240	4	15.0±2.0‡
	600	3	15.7±0.33§
12-Hydroxy stearic acid, μM	100	1	0
Ricinoleic acid, μM	100	1	0

* Two separate preparations of HETE were produced and added to airway cultures for 4-h periods. The results for HETE represent the average of two to four individual lung experiments.

‡ $P < 0.001$ compared with control.

§ $P < 0.01$ compared with control.

TABLE VIII

*Effects of Combining HETE Plus NSAID upon the Release of Mucus and the Generation of Prostaglandins from Human Airways**

Agent	Concentration	Mucous release	$PGF_{2\alpha}$	PGE
			% Change	
Experiment 1				
HETE	600 nM	+15	—	—
ASA	100 μg/ml	+10	—	—
HETE + ASA	100 μg/ml	+75	—	—
Indomethacin	10 μg/ml	+23	—	—
HETE + indomethacin	10 μg/ml	+118	—	—
Experiment 2				
HETE	600 nM	+16	—	—
ASA	100 μg/ml	+32	—	—
HETE + ASA	100 μg/ml	+43	—	—
Indomethacin	10 μg/ml	+24	—	—
HETE + indomethacin	10 μg/ml	+41	—	—
Experiment 3				
HETE	240 nM	+17	−89‡	−57
Indomethacin	10 μg/ml	+7	−93	−75
HETE + indomethacin	10 μg/ml	+93	−94	−71

* HETE from two separate preparations were studied, and the results were obtained with three individual lung cultures.

‡ Control $PGF_{2\alpha}$, 3,850 pg/ml and PGE, 3,748 pg/ml.

that derivatives of AA are secretagogues. NSAID inhibit the enzyme cyclo-oxygenase, thereby preventing the formation of PG and thromboxane (26). We anticipated that NSAID added to airway cultures might inhibit mucous release. Aspirin at 100 μg/ml and indomethacin at 10 μg/ml significantly reduced the formation of $PGF_{2\alpha}$ and PGE by the airways in culture; mucous release, however, was not inhibited but was instead consistently enhanced. There are two possible explanations for the enhancement of mucous secretion induced by NSAID: either the NSAID ablation of PGE_2-induced inhibition is the predominant action or impairment of the cyclo-oxygenase enzyme system facilitates the generation of lipoxygenase products of AA with enhancing activity.

We therefore added AA in the presence of NSAID; each agent increased the release of mucus, and the combination of agents acted in an additive fashion. The capacity of AA in the presence of NSAID to increase mucous release indicates that either AA itself or perhaps lipoxygenase derivatives are responsible. Furthermore, the additive relationship between AA and NSAID suggests that each agent is acting on the same pathway in the same fashion; thereby strongly suggesting that lipoxygenase products of AA are the responsible factors.

The actual existence of lipoxygenase enzymes in human airways has not been directly demonstrated as yet. However, slow-reacting substance is generated by human lung preparations (27–29) undergoing anaphylaxis. Slow-reacting substance is a mixture of leukotrienes derived from 5-hydroxy-arachidonic acid (30, 31), indicating that human lung preparations contain lipoxygenase enzymes. ETYA inhibits fatty acid lipoxygenase and, in higher concentrations, cyclo-oxygenase as well (32–35). ETYA inhibited mucous release at concentrations above 10 μM. In two experiments, 100 μM ETYA also inhibited both PGE and $PGF_{2\alpha}$ formation. The decrease in mucous secretion produced by ETYA strengthened the suggestion that lipoxygenase products were responsible for increased mucous release. To further substantiate this suggestion, three additional inhibitors of the lipoxygenase enzyme system (NDGA, vitamin E, and α-naphthol; (21) were studied. All three antioxidants inhibited mucous release, NDGA did so in a dose-related fashion. NDGA also inhibited $PGF_{2\alpha}$ and PGE synthesis.

A portion of AA added to human tissues is converted by specific lipoxygenases to unstable hydroperoxyeicosatetraenoic acids, which are transformed to HETE. The type and quantity of the HETE produced are specific to the cellular source. For example, human polymorphonuclear leukocytes synthesize 11-HETE, 8-HETE, and 5-D-HETE (18), whereas platelets produce 12-HETE. Although the full spectrum of biological activities for these HETE has not been fully elucidated, 12-HETE is chemotactic for human polymorphonuclear leukocytes (36) and modulates other human neutrophil functions (37). A role for lipoxygenase products in secretory reactions has been suggested by the inhibitory action of ETYA on mast cell secretion (15) as well as the capacity of several HETE to induce neutrophil degranulation under experimental conditions (38). To examine the effect of HETE upon mucous release, a mixture of HETE was synthesized by UV irradiation of AA. The HETE synthesized included 5-, 8-, 11-, and 12-HETE although additional products were probably formed as well. The addition of nanomolar concentrations of this mixture of HETE to airway cultures increased mucous release. As the mixture of HETE synthesized might have included steriosomers to which airways may never be exposed, we also examined the effects of 12-HETE (1–100 nM). 12-HETE was even more potent than the mixture of HETE, having a significant action at 1 nM. We further tested the effect of adding HETE to NSAID-treated airway cultures. Both NSAID, which may act to increase endogenous HETE formation, and the mixture of exogenously added HETE increased mucous release alone; in two out of three experiments, however, the combination of these agents acted in a synergistic fashion to increase mucous release. These data indicate that the addition of exogenous HETE increases mucous release and that pharmacologic manipulations resulting in alterations in HETE production may influence the release of mucus.

The clinical implications of these observations relate both to therapeutic approaches to states of excessive mucous production and to possible abnormalities in patients with aspirin-induced asthma. If the influence of HETE production noted in vitro applies to mucous production in vivo, antioxidants might present useful therapeutic agents for the treatment of bronchorrhea. In much the same way, aspirin challenge of aspirin-sensitive asthmatic patients usually induces bronchorrhea and rhinorrhea which contribute to the airways' obstruction; it seems possible that aspirin-induced shifts of AA metabolism to the lipoxygenase pathway might be partly responsible for the bronchosecretory stimulus. It seems appropriate to study the modulation of HETE formation in vivo in humans in order to determine the effects of the agents on mucous secretion and their possible clinical implications.

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