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Predominant generation of 15-lipoxygenase metabolites of arachidonic acid by epithelial cells from human trachea

(leukotriene/inflammation/pulmonary airway)

J. A. HUNTER*, W. E. FINKBEINER*†, J. A. NADEL*‡§, E. J. GOETZL‡¶, AND M. J. HOLTZMAN*‡||

*Cardiovascular Research Institute, ¶Howard Hughes Medical Institute, and Departments of ‡Medicine, †Pathology, and §Physiology, University of California, San Francisco, CA 94143

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ABSTRACT **Epithelial cells of 99% purity and 92% viability were isolated from human tracheas obtained post mortem, and the cellular pathways for lipoxygenation of arachidonic acid were examined *in vitro*. The lipoxygenase metabolites were identified by comparison with synthetic standards during reversed-phase and straight-phase high-pressure liquid chromatography, UV spectroscopy, and gas chromatography/mass spectrometry. Epithelial cells incubated without arachidonic acid failed to generate detectable quantities of metabolites, while cells incubated with arachidonic acid at 1–50 μg/mf for 1–30 min invariably generated predominantly 15-lipoxygenase products, including 15-hydroxyicosatetraenoic acid (15-HETE), four isomers of 8,15-dihydroxyicosatetraenoic acid (two 8,15-diHETES and two 8,15-leukotrienes), at least one isomer of 14,15-dihydroxyicosatetraenoic acid, and smaller amounts of 12-HETE and 8-HETE, but little or no detectable 5-HETE or 5,12-diHETEs. The capacity of epithelial cells from human pulmonary airway to selectively generate 15-lipoxygenase metabolites of arachidonic acid suggests a potential role for the products as mediators of airway epithelial function.**

Epithelial cells on the surfaces of the skin, gastrointestinal tract, and pulmonary airways are exposed first to the highest concentrations of inflammatory stimuli in the environment. Consequently these cells may be primarily responsible for initiating and regulating the inflammatory response, in part by releasing mediators capable of recruiting and activating leukocytes. Among the most potent natural mediators of hypersensitivity and inflammation are the lipoxygenase metabolites of arachidonic acid, including the leukotrienes and other mono- and dihydroxyicosatetraenoic acids (mono- and diHETEs) (1). The possibility that surface epithelial cells are a source of diverse HETEs *in vivo* is supported, in fact, by the recent finding of the generation of 5-lipoxygenase products by canine airway epithelial cells *in vitro* (2).

The goal of the present study was to prepare human airway epithelial cells of a purity and viability sufficient to characterize the major enzymatic pathways for lipoxygenation of arachidonic acid in these cells. The present finding of the predominance of an active 15-lipoxygenase pathway in epithelial cells from human tracheas provides initial evidence that products of this pathway are potential mediators of human airway function.

MATERIALS AND METHODS

Isolation and Preparation of Epithelial Cells. Epithelial cells were isolated from human tracheas obtained 8–24 hr post mortem by using a modification of the method of enzymatic dissociation described by Widdicombe *et al.* (3). Tracheas were first rinsed with 200 ml of a balanced salt solution consisting of 118.5 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 24.9 mM $NaHCO_3$, 11.0 mM glucose, 10^5 units of penicillin per liter, 100 mg of streptomycin per liter, and 100 mg of gentamicin per liter, that was equilibrated with 95% O_2/5% CO_2 at 25°C. The washed tracheal epithelium was stripped off the underlying lamina propria and minced coarsely, and the fragments were suspended in a solution with the same composition, but also containing 0.02% type I collagenase and 5 mM dithiothreitol. The suspension was agitated at 37°C under 95% O_2/5% CO_2 for up to 60 min. At 30 and 60 min, the fluid phase was decanted into centrifuge tubes, and the dissociated cells were collected by centrifugation for 3 min at 150 × *g*. The cell pellets were temporarily resuspended in 25–50 ml of a mixture of 50% Dulbecco's modified Eagle's medium (1 g of glucose per liter) and 50% Ham's nutrient F12 medium containing the same antibiotics and then treated with 144 mM NH_4Cl buffered with 17 mM Tris·HCl, pH 7.2, for 2 min at 25°C to lyse erythrocytes (4). The epithelial cells were washed and resuspended at a concentration of 4×10^6/ml in Hanks' balanced salt solution containing 25 mM Hepes and the same antibiotics (pH 7.4).

Aliquots of the cell suspension were used to assess yield and viability and to characterize morphology. Cell counts and viability were determined by using a hemocytometer and the vital dye erythrosin B. For examination by light microscopy, cells were sedimented onto glass slides by using a cytocentrifuge (Shandon, Sewickly, PA) and stained with Wright–Giemsa. For examination by electron microscopy, cell pellets were fixed with a solution of 2.5% (wt/vol) glutaraldehyde, 80 mM sodium cacodylate, 5 mM $CaCl_2$, and 1% sucrose (pH 7.4). After 12–24 hr, this fixative was replaced with 1.5% OsO_4 in 30 mM Veronal acetate, pH 7.4, for 2 hr. The fixed cell pellets were rinsed in 25 mM sodium hydrogen maleate (pH 6.0), stained *en bloc* with uranyl acetate (1.5% in 25 mM sodium hydrogen maleate, pH 5.2) for 90 min at 4°C in the dark, dehydrated in ethanol, and embedded in Epon 812. Thin sections with a silver interference color were cut, mounted on copper slot grids coated with Formvar resin (Fullam, Schenectady, NY), and then stained with uranyl acetate (2.5% in 40% methanol) and lead citrate before examination in a JEOL 100S electron microscope.

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Abbreviations: mono- and diHETEs, mono- and dihydroxyicosatetraenoic acids; 8,15-diHETEs, (8*R*,15*S*)- and (8*S*,15*S*)-dihydroxy-(5*Z*,9*E*,11*Z*,13*E*)-icosatetraenoic acid; 8,15-leukotrienes, (8*R*,15*S*)- and (8*S*,15*S*)-dihydroxy-(5*Z*,9*E*,11*E*,13*E*)-icosatetraenoic acid; 14,15-diHETES, (14*R*,15*S*)- and (14*S*,15*S*)-dihydroxy-(5*Z*,8*Z*,10*E*,12*E*)-icosatetraenoic acid; 15-HETE, (15*S*)-hydroxy-(5*Z*,8*Z*,11*Z*-13*E*)-icosatetraenoic acid; 8-HETE, (8*S*)-hydroxy-(5*Z*,9*E*,11*Z*,14*Z*)-icosatetraenoic acid; 12-HETE, (12*S*)-hydroxy-(5*Z*,8*Z*,10*E*,14*Z*)-icosatetraenoic acid; PGB_2, prostaglandin B_2; RP-HPLC, reversed-phase high-pressure liquid chromatography; SP-HPLC, straight-phase high-pressure liquid chromatography.

||To whom reprint requests should be addressed.

Replicate aliquots of the remaining cell suspension were incubated at 37°C with arachidonic acid at 0–50 μg/ml for 1–30 min to characterize both the time course and the substrate concentration dependence of metabolite generation. For all conditions, a volume of cell suspension was added to an equal volume of incubation medium containing arachidonic acid/ethanol to give a final concentration of 2×10^6 cells per ml and a level of 0.5% ethanol. Reagents were incubated in cell-free media under identical conditions to assess nonenzymatic generation of compounds by autooxidation.

Identification and Quantification of Arachidonic Acid Metabolites. Incubation mixtures were acidified to pH 3.5 with 2 M acetic acid, and arachidonic acid metabolites were extracted with 1 vol of diethyl ether. Prostaglandin B_2 (PGB_2) was added to each mixture to serve as an internal standard for recovery of arachidonic acid products. Preliminary experiments showed that recoveries of PGB_2 and mono- and diHETE standards were similar and ranged from 75% to 90%.

Extracts were dried under N_2 or reduced pressure and then reconstituted in chromatography solvent for analysis by reversed-phase high-pressure liquid chromatography (RP-HPLC). The analysis was carried out with a liquid chromatograph (model 1090, Hewlett Packard) fitted with a 5-μm-particle octadecylsilane precolumn (Altex, Berkeley, CA) and a 5-μm-particle octadecylsilane column (4.6 mm × 25 cm, Microsorb, Rainin Instruments, Emeryville, CA) that were developed at a flow rate of 1.0 ml/min with methanol/water/acetic acid (70:30:0.01, vol/vol) for 0–25 min and methanol/water/acetic acid (78:22:0.01, vol/vol) for 26–50 min. DiHETEs and monoHETEs were detected by their absorbance at 269 and 235 nm, respectively, using a diode-array detector fitted with a flow cell (model 1040A, Hewlett Packard). Metabolites in the effluent from RP-HPLC were collected separately in 1- to 2-ml aliquots and extracted with 1 vol of ethyl acetate after addition of 4 vol of 0.01% acetic acid. Recoveries of PGB_2 and mono- and diHETE standards from RP-HPLC were similar and ranged from 85% to 95%.

Before straight-phase high-pressure liquid chromatography (SP-HPLC), isolated HETEs were converted to their methyl esters by treatment with ethereal diazomethane. The diazomethane was generated by the reaction of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine with NaOH in a sealed generator (5). The SP-HPLC was carried out on a silica gel column (3.9 mm × 30 cm, μPorasil, Waters Associates) developed at a flow rate of 2.0 ml/min with hexane/2-propanol (100:3, vol/vol) for diHETEs or hexane/2-propanol (100:0.5, vol/vol) for monoHETEs (6). Recovery of HETEs ranged from 65% to 75%.

Products collected separately from RP-HPLC and SP-HPLC were dissolved in methanol to determine UV absorption spectra in a scanning spectrophotometer (model 2600, Gilford). Molar extinction coefficients of 40,000 at 269 nm and 30,000 at 235 nm were used to quantify diHETEs and monoHETEs, respectively (7, 8).

For gas chromatography/mass spectrometry (GC/MS), the methyl esters of HETEs were converted to their trimethylsilyl ether derivatives by treatment with *N,O*-bis(trimethylsilyl)trifluoroacetamide and 1.0% trimethylchlorosilane at 65°C for 30 min. GC/MS was carried out with a Finnigan 4600 mass spectrometer interfaced with a gas chromatograph. Samples were injected onto a high-resolution capillary column (15 m × 0.32 mm, SE-54, Supelco, Bellefonte, PA) with helium as a carrier gas at 10 pounds/inch^2 (69 kPa). The column was heated to 150°C for 1 min and then up to 280°C by 25 min. Electron-impact mass spectra were obtained, using 70-eV ionizing voltage.

Materials. Arachidonic acid (containing <0.1% total monoHETEs and no detectable diHETEs; Supelco, Bellefonte, PA), type I collagenase (Sigma), dithiothreitol (Sigma), PGB_2 (Upjohn), methylating and silylating reagents (Pierce), and organic solvents (Burdick and Jackson, Muskegon, MI) were obtained from commercial sources. Authentic standards included the following: four isomers of 8,15-dihydroxyicosatetraenoic acid, (5*S*,12*S*)-dihydroxy-(6*Z*,8*E*,10*E*,14*Z*)-icosatetraenoic acid [(5*S*,12*S*)-diHETE], and (5*S*,12*R*)-dihydroxy-(6*Z*,8*E*,10*E*,14*Z*)-icosatetraenoic acid (leukotriene B_4) synthesized and generously provided by B. Fitzsimmons and J. Rokach (Merck Frosst Canada); two isomers of 14,15-dihydroxyicosatetraenoic acid biosynthesized from human platelets (9); (5*S*,12*R*)- and (5*S*,12*S*)-dihydroxy-(6*E*,8*E*,10*E*,14*Z*)-icosatetraenoic acid ("all-*trans*" isomers of leukotriene B_4) biosynthesized from human neutrophils (10); and (15*S*)-hydroxy-(5*Z*,8*Z*,11*Z*,13*E*)-icosatetraenoic acid (15-HETE), (8*S*)-hydroxy-(5*Z*,9*E*,11*Z*,14*Z*)-icosatetraenoic acid (8-HETE), (12*S*)-hydroxy(5*Z*,8*Z*,10*E*,14*Z*)-icosatetraenoic acid (12-HETE), and (5*S*)-hydroxy-(6*E*,8*Z*,11*Z*,14*Z*)-icosatetraenoic acid (5-HETE) obtained from Seragen (Boston, MA) or synthesized as described previously (11).

RESULTS

Characterization of Isolated Epithelial Cells. Overall yield averaged $7.4 \pm 1.2 \times 10^7$ cells per trachea (mean ± SEM) with a viability of 92 ± 2% ($n = 7$ tracheas). Examination of cell smears revealed 70–99% nonciliated epithelial cells, 1–24% ciliated epithelial cells, and 0–8% goblet cells, with fewer than 1% neutrophils and eosinophils. Ultrastructural features of the cells were similar to those described for basal epithelial cells, ciliated epithelial cells, and goblet cells isolated from canine trachea (3). Basal cells predominated in all preparations and possessed large nuclei that often had prominent nucleoli and relatively scant cytoplasm containing characteristic intermediate-type filaments surrounding the nucleus; ciliated cells contained oval nuclei located in the basal part of the cells and often a prominent apical "cap" of cilia and basal bodies; and goblet cells had oval nuclei located peripherally and a cytoplasm filled with mucus granules (Fig. 1).

Identification of Lipoxygenase Metabolites. No metabolites were detected after incubation of epithelial cells alone, but analysis of the ether extracts obtained from cells incubated with arachidonic acid at 1–50 μg/ml for 1–30 min consistently showed five prominent peaks of absorbance at 269 nm (diHETEs) and two prominent peaks of absorbance at 235 nm (monoHETEs) by RP-HPLC (Fig. 2). The retention times of the five peaks of absorbance at 269 nm corresponded exactly to the retention times of (8*S*,15*S*)- and (8*R*,15*S*)-dihydroxy-(5*Z*,9*E*,11*Z*,13*E*)-icosatetraenoic acid [(8*S*,15*S*)-and(8*R*,15*S*)-diHETEs], (8*R*,15*S*)- and (8*S*,15*S*)-dihydroxy-(5*Z*,9*E*,11*E*,13*E*)-icosatetraenoic acid [(8*R*,15*S*)- and (8*S*,15*S*)-leukotrienes], and (14*R*,15*S*)dihydroxy-(5*Z*,8*Z*,10*E*,12*E*)-icosatetraenoic acid [(14*R*,15*S*)-diHETE] (peaks 1–5, Fig. 2 and Table 1). The retention time of the major peak of absorbance at 235 nm corresponded exactly to the time of 15-HETE, while the retention time of the minor peak coincided with elution of 8-HETE or 12-HETE (peaks 6 and 7, Fig. 2 and Table 1).

Small peaks absorbing at 269 nm had retention times corresponding to those of (14*S*,15*S*)-dihydroxy-(5*Z*,8*Z*,10*E*,12*E*)-icosatetraenoic acid [(14*S*,15*S*)-diHETE] and of (5*S*,12*S*)-diHETE or leukotriene B_4, but the amounts were too small for further structural studies. No peaks were found corresponding to the retention time of the "all-*trans*" isomers of leukotriene B_4. Similarly, only a small peak absorbing at 235 nm had a retention time corresponding to that of 5-HETE (Table 1).

Methyl esters of the compounds contained in peaks 1–5 from RP-HPLC also chromatographed as single peaks on

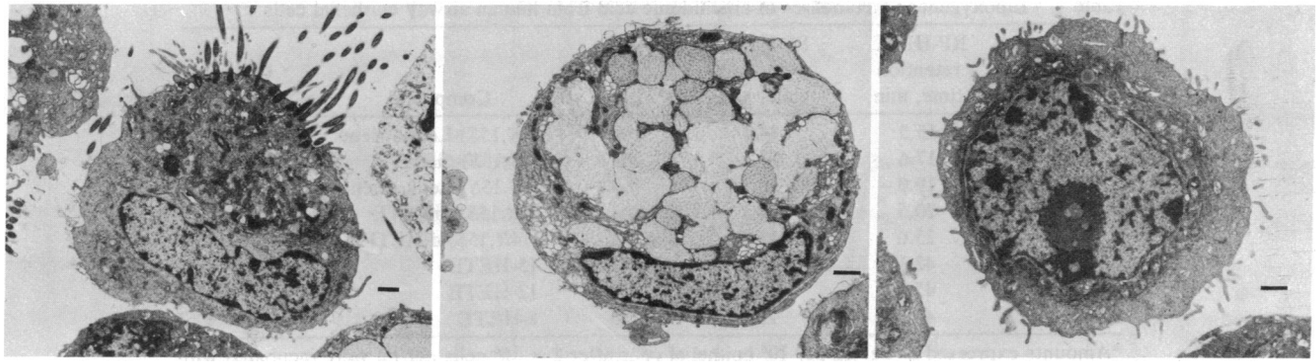

FIG. 1. Electron photomicrographs of epithelial cells isolated from human trachea. Ciliated cells (*Left*; ×2800), goblet cells (*Center*; ×2800), and basal cells (*Right*; ×3400) were identified and exhibited characteristic ultrastructure. (Horizontal bars = 1 μm.)

SP-HPLC. Retention times of the five peaks on SP-HPLC corresponded again to the times of the respective synthetic diHETEs and leukotrienes (Table 1) and were similar to retention times published previously for the same five compounds chromatographed under identical conditions (6, 12). The methyl ester of the compound in peak 6 chromatographed as one major peak, while compounds in peak 7 resolved into two major peaks on SP-HPLC. Retention times of the peaks on SP-HPLC corresponded again to the respective synthetic monoHETEs (Table 1) and were equivalent to times reported previously for 15-, 12-, and 8-HETE studied under similar conditions (13).

UV absorption spectra of compounds in peaks 1–5 were characteristic for the conjugated triene of a diHETE or leukotriene, while spectra of compounds in peaks 6 and 7 were characteristic for the conjugated diene of a monoHETE. The spectrum for each compound was identical with that of its synthetic standard and exhibited wavelengths of maximal absorbance (Table 1) and for hypso- and bathochromic shoulders similar to those reported previously (12).

GC/MS analysis of the trimethylsilyl ether derivatives of the methyl esters of the compounds contained in peaks 1, 3, and 2 were consistent with their identities as 8,15-leukotrienes and 8,15-diHETE, respectively (6, 12, 14). Mass spectra showed characteristic ions (m/z) of 353 [M − 141, loss of $\cdot CH_2(CH)_2(CH_2)_3CO_2CH_3$], 263 [353 − 90, loss of Me_3SiOH], 243 [$Me_3SiO^+{=}CH(CH_2)(CH)_2(CH_2)_3CO_2CH_3$], and 173 [$Me_3SiO^+{=}CH(CH_2)_4CH_3$]. In each case, spectra were equivalent to those for the respective synthetic standard.

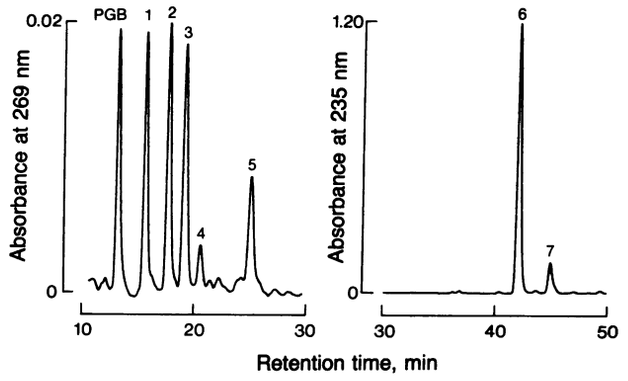

FIG. 2. Chromatogram from RP-HPLC of ether extract of 4 ml of epithelial cell suspension (2×10^6 cells per ml) incubated with arachidonic acid (25 μg/ml) for 5 min at 37°C. Absorbance was monitored at 269 nm from 10 to 30 min to detect diHETEs (left half of tracing) and at 235 nm from 30 to 50 min to detect monoHETEs (right half of tracing). The peak labeled PGB corresponds to PGB_2 added as an internal standard; other major peaks are numbered 1–7 and correspond to the numbers and compound identities listed in Table 1.

Although the profile of metabolite generation was constant under each incubation condition, the amounts of metabolites depended on incubation time and substrate concentration. The quantities of diHETEs were maximal within 1 min and were unchanged for up to 30 min, while amounts of monoHETEs approached a plateau only after 5 min and continued to increase slightly for up to 30 min (Fig. 3). The generation of each metabolite showed an equivalent dependence on substrate concentration (Fig. 3). A cytotoxic effect of arachidonic acid was also time and concentration dependent but was not required for product generation. Initial loss of viability occurred only after incubation with at least 25 μg of arachidonic acid per ml, yielding viabilities of 98 ± 8%, 89 ± 2%, 88 ± 11%, and 85 ± 7% of the control values after incubation for 1, 5, 15, and 30 min, respectively. Metabolite quantities for standard conditions are listed in Table 1.

DISCUSSION

Lipoxygenase enzymes catalyze the conversion of arachidonic acid to hydroperoxyicostatetraenoic acids (HPETEs) which, in turn, may be reduced to the corresponding monoHETEs or further metabolized to more complex diHETEs and leukotrienes. Each of the predominant mammalian lipoxygenases—i.e., the 5-, 12-, and 15-lipoxygenases—interacts specifically with arachidonic acid and, therefore, initiates the generation of a characteristic profile of metabolites (1). Consequently, initial evidence of a 15-lipoxygenase cascade in mammalian cells was obtained when it was shown that arachidonic acid was converted to 15-HETE and a series of 8,15- and 14,15-diHETEs and leukotrienes in human or porcine leukocytes (12, 14). Subsequent studies demonstrated that, among the various classes of blood leukocytes, the dominant source of 15-lipoxygenase metabolites was the eosinophil (6). The likelihood that the eosinophil also contained 5- and 12-lipoxygenase activities was suggested when even the purest preparations of eosinophils also produced 5-HETE, 12-HETE, and 5,12-diHETEs (6).

The present findings indicate that another type of mammalian cell contains a capacity for the selective generation of 15-lipoxygenase metabolites from arachidonic acid. By incubating suspensions of human tracheal epithelial cells with unesterified arachidonic acid and identifying the resultant metabolites by their chromatographic and spectral properties, we established that the cells can rapidly generate a complex but characteristic series of lipoxygenase products. In comparison to human eosinophils studied under similar conditions (6), epithelial cells generated approximately 10 times the amount of 15-HETE, 20 times the amounts of

Table 1. Lipoxygenase metabolites of arachidonic acid from human airway epithelial cells

RP-HPLC peak no.	RP-HPLC retention time, min	SP-HPLC retention time, min	UV λ_{max}, nm	Compound	Quantity, ng*
1	15.5	14.3	269	(8*R*,15*S*)-Leukotriene	13 ± 1
2	17.6	11.2	268	(8*S*,15*S*)-diHETE	12 ± 1
3	19.0	18.3	269	(8*S*,15*S*)-Leukotriene	13 ± 1
4	20.5	14.1	268	(8*R*,15*S*)-diHETE	4 ± 1
5	25.0	12.2	272	(14*R*,15*S*)-diHETE	8 ± 1
6	42.1	13.5	235	15-HETE	1434 ± 69
7	45.0	12.9	235	12-HETE	94 ± 1
7	45.3	22.0	235	8-HETE	45 ± 2

*Amounts expressed as net ng per 10^6 epithelial cells after 2 × 10^6 cells per ml were incubated with arachidonic acid at 50 μg/ml for 15 min at 37°C. Comparable amounts for 5,12-diHETEs or leukotrienes and for 5-HETE were <1 and 14 ± 1 ng per 10^6 cells, respectively. Values are corrected for recovery and for the trace amounts of monoHETEs generated nonenzymatically (amount in cell mixture minus amount in cell-free mixture) and represent the mean ± SEM for cells isolated from three tracheas.

8,15-leukotrienes, and 5–10 times the amounts of 8,15- and 14,15-diHETEs, but only relatively small amounts of 5-HETE, with little or no 5,12-diHETEs. The findings suggest that, if sufficient amounts of substrate were made available, the epithelial cells could generate 15-lipoxygenase products in the human airway. The mechanisms for regulating substrate concentrations in this cell are still uncertain.

The relative lack of 5-lipoxygenase activity in human tracheal epithelial cells contrasts with the prominent 5-lipoxygenase pathway in the same cell preparation obtained from dogs (2). Canine cells incubated with the same concentrations of arachidonic acid (up to 50 μg/ml) generate predominantly the 5-lipoxygenase products, leukotriene B_4, the two "all-*trans*" isomers of leukotriene B_4, and 5-HETE (2). Compared to the human cells studied under the same conditions, the canine cells generate much lower levels of 15-HETE (2) and no detectable 8,15- or 14,15-diHETEs (unpublished observation). Although the differences in lipoxygenase specificity between canine and human epithelial cells may be due to changes that occur in tissues post mortem, it is most likely due to genetic differences between species.

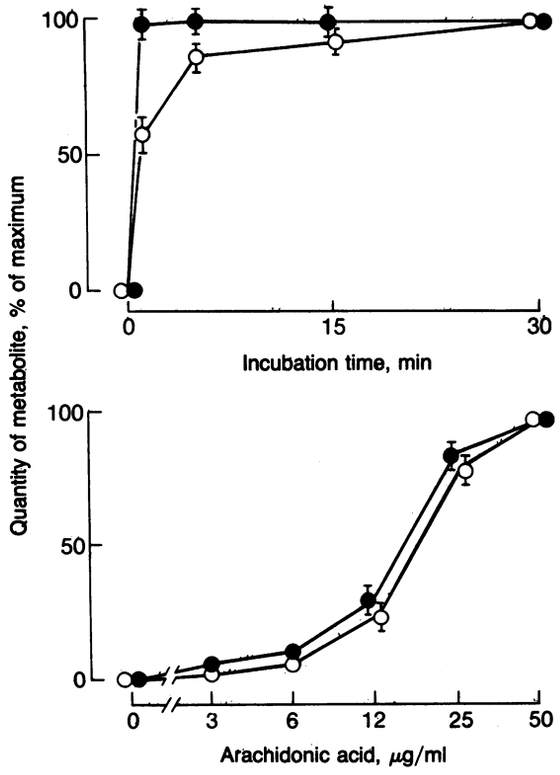

FIG. 3. Time dependence (*Upper*) and substrate concentration dependence (*Lower*) of the generation of mono- and diHETEs by tracheal epithelial cells. 15-HETE (○) and (8*S*,15*S*)-diHETE (●) were predominant metabolites as quantified by UV absorbance after 2 × 10^6 cells per ml were incubated with arachidonic acid at 25 μg/ml for 1–30 min at 37°C (*Upper*; n = 6 values from three tracheas) or at 0–50 μg/ml for 15 min at 37°C (*Lower*; n = 3 values from one trachea). Maximal values for 15-HETE and (8*S*,15*S*)-diHETE for these experiments were 1492 ± 55 and 12 ± 1 ng per 10^6 cells; mean values for all products under standard conditions are listed in Table 1.

For both human and canine epithelial cell preparations, the isolation procedure results in a heterogeneous suspension of cells containing basal cells, ciliated cells, and goblet cells. The absence of ciliated cells and goblet cells in some preparations of human cells did not affect the generation of lipoxygenase products, suggesting that the 15-lipoxygenase activity is contained in basal cells. Whether ciliated cells and goblet cells also contain lipoxygenase activity is uncertain. It is possible that 15-lipoxygenase activity changes during cell differentiation and even that the enzyme may play a role in cell maturation, a role suggested previously for the enzyme in reticulocyte maturation (15).

Lipoxygenase activity in other types of epithelial cells has been only partially characterized. One study showed that epidermis from patients with psoriasis exhibited 12-lipoxygenase activity, but no diHETEs were detected, and the cell source of the 12-HETE was uncertain (16). Similarly, 12-HETE was generated by pancreatic islet cells isolated from rats, but no diHETEs were detected (17). Interestingly, the epithelial cells isolated from human trachea also generate 12-HETE. Although the significance of 12-lipoxygenase activity is uncertain, the activity has been proposed as a mechanism for the formation of an epoxide intermediate leading to the generation of 8,15-leukotrienes (9). Presumably, the same activity may also account for the generation of 8-HETE as another derivative originating from hydrogen abstraction at carbon-10.

The functional significance of 15-lipoxygenase activity contained in human tracheal epithelial cells is also uncertain. One possibility comes from the observation that 15-HETE (as well as 8-HETE and 12-HETE) in concentrations as low as 0.3 μg/ml stimulate mucus glycoprotein release from cultured human airways (18). Quantitative comparisons are indirect, but the similarity of the concentrations causing mucus release with those generated by isolated epithelial cells suggests at least a potential role for the HETEs in regulating airway secretion.

Another possibility comes from the proposal that airway epithelial cells modulate the inflammatory response to inhaled stimuli. One concept is that the 15-lipoxygenase products have direct inflammatory effects and is supported by the recent finding that (8*S*,15*S*)-diHETE at concentrations as low as 5 ng/ml directly stimulates human neutrophil chemotaxis *in vitro* (19). An alternative concept is that the 15-lipoxygenase products influence inflammation indirectly by modulating arachidonic acid metabolism in leukocytes. For example, 15-HETE stimulates 5-lipoxygenase activity in murine and canine mast cells (20, 21) and inhibits it in rabbit neutrophils (22). Whether direct or indirect effects predominate *in vivo* or whether either may provide a mechanism for epithelial cell regulation of leukocyte activity is uncertain. Our present findings provide only the initial evidence that human tracheal epithelial cells have the capacity for releasing 15-lipoxygenase products *in vitro*.

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