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Arachidonic Acid Metabolism by Canine Tracheal Epithelial Cells

PRODUCT FORMATION AND RELATIONSHIP TO CHLORIDE SECRETION*

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Canine tracheal epithelial cells freshly isolated from mongrel dog trachea were used to study relationships between arachidonic acid metabolism and chloride ion movement. High performance liquid chromatography (HPLC) analysis of the cell incubation media after the addition of A23187 showed the presence of prostaglandin H synthase and lipoxygenase-derived metabolites. The major prostaglandin H synthase metabolite identified by HPLC, gas chromatography, and mass spectrometry was prostaglandin (PG) D_2. The major lipoxygenase metabolites were leukotriene (LT) C_4 and LTB_4. LTB_4 was identified by HPLC, UV spectroscopy, and gas chromatography. Straight phase HPLC of the methyl esters indicated only a minor formation of LTB_4 isomers. LTC_4 was identified by HPLC, UV spectroscopy, and conversion to LTD_4 by γ-glutamyl transpeptidase. Analysis by radioimmunoassays indicated approximately 1–2 ng of LTB_4 and peptide LT formed by 10^6 cells after A23187 stimulation. The addition of ionophore A23187 caused a rapid release of arachidonic acid metabolites which was completed within 5 min of stimulation. Cl^- secretion was measured in parallel studies of excised tracheas in Ussing chambers. Cl^- secretion occurred at 2–3 min after the addition of ionophore, and the most rapid change occurred with the highest PGD_2 concentrations. Indomethacin produced a concentration-dependent inhibition of PGD_2 formation and Cl^- movement. The addition of PGE_2, PGD_2, and PGH_2 effectively stimulated Cl^- secretion. LTC_4 also stimulated Cl^- secretion, but the stimulation was inhibited by indomethacin. These results indicate that canine tracheal epithelial cells metabolize arachidonic acid via both prostaglandin H synthase and lipoxygenase enzymes. It appears that endogenous PGD_2 formation is the important variable controlling the Cl^- ion movement in canine trachea.

Lung diseases are often characterized by derangements of the mucin and water content of airway secretions. The water content of airway secretions is modulated by active ion transport systems located in airway epithelia. The control of these transport processes in health or disease is not known (1–3). Previous work suggests that arachidonic acid metabolism has a role in controlling the rate of Cl^- secretion by canine trachea, a frequently used model for studying airway ion transport. The addition of PGE_2[1] or $PGF_{2\alpha}$ to excised canine tracheas stimulates Cl^- secretion while indomethacin and other prostaglandin H synthase inhibitors depress Cl^- secretion across the airway epithelia (4–6). The addition of Ca^{2+} ionophore, A23187, which stimulates the release of arachidonic acid and the subsequent metabolites also increased Cl^- secretion (4). Arachidonic acid metabolites are also implicated in the regulation of mucus secretion. Studies have shown that $PGF_{2\alpha}$ and more recently LTC_4 and LTD_4 stimulate mucus production in cat trachea (7), in dog trachea *in vivo* (8), and in human airways *in vitro* (9).

In the present study, we have sought to identify the source(s) of AA metabolites that may affect airway epithelial function. Preliminary reports have suggested that the epithelial cells themselves may form a spectrum of AA metabolites. Smith and co-workers (4) measured the release of a prostaglandin into the luminal bathing solution of canine tracheas mounted in conventional ion flux (Ussing) chambers. On the basis of radioimmunoassay data, they identified this compound as PGE_2. More recently, Holtzman *et al.* (10) reported that freshly isolated epithelial cells from dog tracheas synthesize LTB_4 from exogenous arachidonic acid. Therefore, in the present study we have evaluated the hypothesis that isolated airway epithelial cells metabolize endogenous arachidonic acid in significant quantities via both prostaglandin H synthase and lipoxygenases and that the metabolites formed play an important role in controlling the volume and composition of airway secretions. The experimental design was 1) to characterize the endogenous arachidonic acid metabolites produced by isolated airway epithelial cells after stimulation with the Ca^{2+} ionophore, A23187, and 2) in parallel studies to measure the changes in Cl^- secretion and endogenous arachidonic acid metabolism under various experimental conditions. The canine trachea was selected for these studies as a useful model system because of the availability of relatively large

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[1] The abbreviations used are: PGs, prostaglandins; LTA_4, leukotriene A_4, 5*S*,6*S*-oxido-7,9,11,14(E,E,Z,Z)-eicosatetraenoic acid; LTB_4, leukotriene B_4, 5*S*,12*R*-dihydroxy-6,8,10,14(Z,E,E,Z)-eicosatetraenoic acid; LTC_4, leukotriene C_4, 5*S*-hydroxy-6*R*-*S*-glutathionyl-7,9,11,14(E,E,Z,Z)-eicosatetraenoic acid; 5*S*,12*S*-diHETE, 5*S*,12*S*-dihydroxy-6,8,10,14(E,Z,E,Z)-eicosatetraenoic acid; 5-HETE, 5*S*-hydroxy-6,8,10,14(E,Z,Z,Z)-eicosatetraenoic acid; 12-HETE, 12*S*-hydroxy-5,8,10,14(Z,Z,E,Z)-eicosatetraenoic acid; 15-HETE, 15*S*-hydroxy-5,8,11,13(Z,Z,Z,E)-eicosatetraenoic acid; RP-HPLC, reverse-phase high performance liquid chromatography; HETES, hydroxyeicosatetraenoic acids; Isc, short circuit current; G, conductance, AA, arachidonic acid.

numbers of normal airway epithelial cells and the background information of Cl^- secretion in the tissue (4, 8, 9).

EXPERIMENTAL PROCEDURES

Materials—The [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid (83.8 Ci/mmol) and [1-^{14}C]arachidonic acid (56 Ci/mol), PGs, 5-HETE, 15-HETE, leukotriene C_4 (LTC_4), leukotriene D_4 (LTD_4), and radioimmunoassay kit for LTC_4 were obtained from New England Nuclear. [^{3}H]Leukotriene B_4 (LTB_4) and [^{14}C]$PGF_{2\alpha}$ were obtained from Amersham Corp. Unlabeled LTC_4, LTB_4, LTD_4, LTE_4, and LTF_4 were gifts of Dr. J. Rokach (Merck Frosst Laboratories, Quebec, Canada).

Dithiothreitol, γ-glutamyl transpeptidase, glutathione, indomethacin, ibuprofen, naproxen, methoxylamine HCl, diisopropyl ethylamine, and pyridine were obtained from Sigma. Trypan blue was obtained from Gibco Laboratories. The calcium ionophore, A23187, was purchased from Behring Diagnostics. Collagenase was obtained from Worthington. The pentobarbital was purchased from Barber Veterinary Supply (Fayetteville, NC). Glutaraldehyde, paraformaldehyde, osmium tetroxide, toluidine blue, and uranyl acetate were obtained from Fisher.

Pentafluorobenzyl bromide was purchased from Supelco, Inc. (Bellefonte, PA). Bis(trimethylsilyl)trifluoreoacetamide containing 1% trimethylchlorosilane was obtained from Bodman Chemical (Media, PA). Diethyl ether was purchased from Mallinckrodt Chemical Works. *N*-methyl-*N'*-nitro-*N*-nitrosoguanadine was purchased from Sigma.

HPLC grade methanol, HPLC grade acetonitrile, and HPLC grade hexane were obtained from Fisher while HPLC grade water was purchased from EM Industries Inc. (Gibbstown, NJ).

Pellicular C_{18} glass beads (37–53 μm) were used for the packing in the precolumn (Whatman). Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) was purchased from Aldrich. Atomlight (New England Nuclear) and Hydrofluor (National Diagnostics, Sommerville, NJ) were used as the scintillation mixtures.

Canine Tracheal Epithelium Isolation—Male mongrel dogs (15–25 kg) or male beagle dogs (Marshall Farms, N. Rose, NY) were put to death with intravenuous pentobarbital (120 mg/kg). The tracheas were rapidly excised through a midline incision and bathed in iced Krebs-Ringer bicarbonate solution which was gassed continuously with 95% O_2/5% CO_2. Excess connective tissue and the posterior membranous portion were removed by sharp dissection. The cartilaginous portion was cut into 3 × 3-cm segments. A solution of 0.5% dithiothreitol and 0.25% collagenase (150 units/mg) in Krebs-Ringer bicarbonate solution was injected submucosally with a 27-gauge needle. Care was taken to rid the submucosal space of blood via this maneuver. The segments were then floated on a 0.5% dithiothreitol solution in glass Petri dishes and incubated for 45 min in a 37 °C shaking incubator, gassed with 95% O_2/5% CO_2. The epithelium was then removed as sheets by gently scraping the mucosal surface with glass coverslips into Ca^{2+}-Mg^{2+}-free Krebs-Ringer bicarbonate solution and placed on ice. Cell yield was 0.7–3 × 10^8 cells/trachea, with viability >95% by trypan blue exclusion.

Morphology Studies—Epithelial cells were placed in buffered (pH 7.4) glutaraldehyde paraformaldehyde fixative and kept at 4 °C until further processed. The samples were washed in 0.2 M phosphate buffer, pH 7.4, and postfixed in phosphate-buffered 1% OsO_4 for 2 h, dehydrated in alcohol and propylene oxide, and embedded in Epon 812. Thick sections (0.5 μ) for light microscopy were cut with a Sorvall MT-I ultramicrotome and stained with toluidine blue. Thin sections, 400–600 Å by interference color, were stained with uranyl acetate and Reynold's lead citrate and examined with a Philips 400 electron microscope.

Incubation Conditions—The epithelial cells were suspended in Ca^{2+}-Mg^{2+}-free Ringer's solution at 37 °C. Cells were divided into several 2-ml aliquots containing 1–2 × 10^6 cells, the equivalent of 2.0–5.5 mg/ml protein as determined by a Lowry protein determination (16). [^{3}H]Arachidonic acid (5 μCi (87 Ci/mmol)) was added to the cells for 1 h prior to stimulation. Preliminary experiments had established the incorporation time of arachidonic acid into the phospholipid pools by measuring the disappearance of radioactivity from the media containing the isolated cells. Maximum incorporation occurred between 1 and 2 h as reflected by the loss of radioactivity from media. Incorporation of [^{3}H]arachidonic acid into the phospholipid pools was confirmed by extracting with chloroform:methanol (2:1) and analyzing the lipids by thin layer chromatography (17). TLC chromatograms indicated that between 50 and 60% of the [^{3}H] arachidonic acid added was incorporated into the phospholipid pools.

After the incorporation of [^{3}H]arachidonic acid, the cells were washed twice with Ca^{2+}-Mg^{2+}-free Ringer's solution at 4 °C. Ringer's solution containing 1 mM $CaCl_2$ and 1 μM A23187 at 37 °C was then added to the cells. The cells were incubated for 5 min at 37 °C and then placed immediately in ice and centrifuged at 9,000 × *g* at 10 °C for 10 min. An internal standard ([^{14}C]$PGF_{2\alpha}$; 10,000 dpm) was added to the supernatant. The supernatant was added to 8 ml of cold ethanol bringing the final solution to 80% ethanol. The supernatant was centrifuged at 10,000 × *g* for 20 min at 10 °C to precipitate the protein. The supernatant was removed, evaporated under N_2, and reconstituted in 1 ml of 30% MeOH:H_2O. Before injecting the sample on the reverse phase HPLC, the samples were centrifuged for 5 min in an Eppendorf centrifuge 5412 (Brinkmann Instruments). The cell pellet was dissolved in 1.0 ml of 0.1 N NaOH for protein determination.

In large scale incubations, the cells from 2–5 dogs were pooled and then were suspended in 6 ml of Ca^{2+}-Mg^{2+}-free Ringer's solution at 37 °C. [^{3}H]Arachidonic acid (15 μCi) was incorporated into the cellular phospholipids pools. Incubation, washing, and stimulation conditions are as described above. The internal standard of [^{14}C]$PGF_{2\alpha}$ (20,000 dpm) was added to the supernatant. The supernatant was added to 24 ml of cold ethanol bringing the final solution to 80% ethanol. The supernatant was prepared for HPLC analysis as described above. In other experiments, cells were incubated with [1-^{14}C] arachidonic acid, ethanol was added, and the supernatant prepared for HPLC analysis. For indomethacin studies, [^{3}H]arachidonic acid was incorporated for 1 h in Ca^{2+}-Mg^{2+}-free Ringer's and washed twice. Indomethacin was added for 2 min at 37 °C, following which 1 mM $CaCl_2$ and A23187, 1 μM, were added. The cells were incubated for 5 min at 37 °C, and the supernatant was prepared for HPLC analysis was described above.

Arachidonic acid metabolites produced from these large scale incubations were separated by reverse phase HPLC using solvent system 1 or 2. The individual metabolites were collected, pooled, the solvent removed and subjected to further analysis. UV spectra were measured using a Hewlett-Packard 8450 spectrophotometer with the sample dissolved in 30% MeOH:H_2O.

Separation of Metabolites—A Waters HPLC system consisting of a model U6K injector, two model 6000A solvent delivery pumps, Z module radial compression model, a model 720 system controller, and a model 441 absorbance detector set at 280 nm (Waters Associates, Milford, MA) was used for all HPLC methods. A Radiomatic radioactive flow detector was used to determine radioactivity in the eluant in 30-s fractions (Radiomatic Instruments and Chemical Co. Inc., Tampa, FL). Hydrofluor and Atomlight were used as the scintillation fluids. For some experiments, fractions were collected by a fraction collector, and the radioactivity was determined by a Packard 460C using quench curves and internal standardization. Radioactivity in the eluant was convereted to dpm/fraction.

Solvent systems 1 and 2 were used to separate prostaglandins, leukotrienes, and HETES (13). For solvent system 1 separation was achieved on a reverse phase Waters Radial-Pak cartridge C_{18} (5 μm; 8 mm, inner diameter) at 3 ml/min. Solvent system 2 used a reverse phase Altex C_{18} (5 μm; 4.6 × 250 mm, Beckman, Berkeley, CA) at 1 ml/min. A precolumn packed with Whatman 37–53-μm pellicular C_{18} beads was used in both systems.

Solvent system 1 used methanol and water buffered to pH 5.8 with NH_4OH and 0.01% acetic acid as the mobile phase. This system used a four-step gradient of 49, 57, 65, and 100% methanol in water over 60 min changing the steps at 15, 30, and 50 min. The solvent system 2 used a four-step gradient of 53, 60, 73, and 100% methanol in water over 100 min changing at 27, 52, and 74 min. The water was buffered to pH 5.05 with 0.02% glacial acetic acid and NH_4OH, and then methanol was added to bring the concentration of methanol to 10%.

Solvent system 3 was used to separate prostaglandins and HETES. Separation was achieved on a reverse phase Waters Radial-Pak cartridge C_{18} (10 μm; 5 mm, inner diameter) at 3 ml/min. Acetonitrile and water buffered with 1% acetic acid and NH_4OH to pH 3.5 was used as the mobile phase. Samples were eluted from the column with a three-step gradient of 26, 50, and 100% acetonitrile over 55 min changing at 25 and 45 min (14).

Solvent system 4 was used to separate LTB_4 methyl ester from its stereoisomer 5*S*,12*S*-diHETE. Separation was achieved with a Whatman straight phase column (25 cm × 4.6 mm, inner diameter; 10-μm particles) at 1 ml/min. Ethanol/hexane/acetic acid, 5:95:0.01 (v/v) was used as its mobile phase (15).

Metabolism of Peptide Leukotrienes by γ-Glutamyl Transpepti-

dase—The isolated peak 5 tentatively identified at LTC_4 was dissolved in 0.1 ml of 0.1 M Tris·HCl, pH 8.5, containing 0.01 M $MgCl_2$. γ-Glutamyl transpeptidase, 0.2 mg/ml, was added to the mixture and incubated for 30 min at 37 °C. Authentic LTC_4 was also incubated under the same conditions in parallel experiments. Methanol (0.1 ml) with 5.0% acetic acid was added to stop the reaction. The sample was injected directly onto a reverse phase HPLC column using solvent system 1 to determine the conversion to LTD_4 (18).

Peak 6 tentatively identified as LTD_4 was dissolved in 0.1 ml of 0.1 M Tris·HCl, pH 8.5, containing 0.01 M $MgCl_2$ and 8 mM glutathione. Cold authentic LTD_4, 3 μM, was added to the mixture. γ-Glutamyl transpeptidase, 0.2 mg/ml, was added, and the sample was incubated for 30 min at 37 °C. Methanol (0.1 ml) with 5.0% acetic acid was added to stop the reaction. The sample was injected onto a RP-HPLC column using solvent system 1 to determine the conversion to LTC_4 (18).

Separation of LTB_4 and (5S,12S)-diHETE Methyl Esters—The arachidonic acid metabolite tentatively identified as LTB_4 was separated by reverse phase HPLC using either solvent system 1 or 2. The LTB_4 peaks were pooled, evaporated under N_2, and treated with diazomethane in diethyl ether as described by Fels *et al.* (15). Authentic LTB_4 was also treated in the same manner. The resulting methyl esters were analyzed by normal phase HPLC using solvent system 3 as described previously.

Analysis by Gas Chromatography-Mass Spectrometry—The individual metabolite peaks were collected from columns using solvent system 1 or 2. In some cases the entire incubation mixture was used. After the removal of the solvent by evaporation, the metabolites were further purified by elution of C-18 Sep-Paks as described previously (11). The purified metabolites were esterified with pentafluorobenzyl bromide in the presence of diisopropyl ethylamine at room temperature. After the removal of the solvent, the esters were reacted with methoxylamine in pyridine, and the resulting derivatives were repurified on a silica Sep-Pak. The repurified metabolites were trimethylsilylated with bis(trimethylsilyl)trifluoroacetamide:trimethylchlorosilane, 99:1.

A Varian gas chromatograph modified for fused silica capillary columns (30 m × 0.25 mm, inner diameter; DB-5 fused silica (J. W. Scientific)) was used. The temperature of the injector was set at 250 °C, and the detector was set at 330 °C. The column was heated to 200 °C for 3 min, then increased to 300 °C at 5 °/min, and held at 300 °C for 15 min. The metabolites were detected by a constant current-pulse modulated electron capture detector. Negative ion chemical ionization-mass spectrometry with argon as the reagent gas was performed using a VG 7070E mass spectrometer equipped with a VG 2050 data system, a 30-m DB-4 fused silica capillary column, and split/splitless injector (10:1).

Radioimmunoassay for Leukotrienes—The quantity of LTB_4 and LTC_4 produced by dog tracheal epithelial cells was estimated by radioimmunoassay. Specific antisera to LTB_4 and peptide leukotrienes were used to quantitate LTB_4 and LTC_4 as described previously (19). The media from the cell incubations described above was assayed directly at 3 or more dilutions. The cross-reactivities and the method of analysis are as described previously (19). In all cases, the dilution curves for biosynthetic LTB_4 and LTC_4 were parallel to the standard curves. In other experiments, the chromatographic peak (see Fig. 2) which eluted with authentic LTC_4 was evaporated, redissolved in buffer, and analyzed for LTC_4 by radioimmunoassay. The dilution curve for biosynthetic LTC_4 was parallel to the standard curve.

Metabolism of LTC_4 by Epithelial Cells—Freshly isolated epithelial cells were suspended in Ca^{2+}-Mg^{2+}-free Ringer's solution and divided into 2-ml aliquots containing 2×10^6 cells. Cells were then sonicated for 30 s at a constant 4 °C. [^{3}H]LTC_4 (0.1 μCi) and sufficient unlabeled LTC_4 to achieve a final concentration of 0.1 μM were added to the sonicate. A23187, 1 μM, and $CaCl_2$, 1 mM, were then added, and the mixture was incubated for 5 min at 37 °C. The reaction mixtures were cooled to 0 °C, and the samples were prepared for HPLC analysis as described above.

Bioelectric Measurements and Solute Permeabilities—The posterior membranes of freshly excised dog tracheas were placed in chilled (4 °C) Krebs-Ringer bicarbonate solution, stripped of the trachealis muscle and the submucosal tissue by sharp dissection, and mounted in Lucite flux chambers (1.13 cm^2). The tissues were continuously exposed to warmed (37 °C) and gassed (95% O_2, 5% CO_2) Ringer's solution. The transepithelial electric potential difference and the DC current required to null the potential difference to zero (short circuit current (Isc)) were measured as previously described (3) and the conductance (G) calculated. In most instances, the basal bioelectric properties were allowed to stabilize for 60 min after mounting. The tissues assigned to protocols were matched on the basis of G (<0.5 ms·cm^{-2} difference) and Isc (<20 μA·cm^{-2} difference). For dose-response studies, A23187 was added cumulatively from 3×10^{-8} to 10^{-6} M, with Isc recorded continuously. These studies showed that the relationship between A23187 concentration and Cl^- secretion was steep, and 10^{-6} M ionophore induced the maximal effect. For the time course studies, a single maximal effective concentration (10^{-6} M) was added, and the Isc was measured continuously for 15 min. Ionophore additions were bilateral. The dose-effect relations between Isc and indomethacin were also explored. Indomethacin (10^{-6} to 3×10^{-5} M) dissolved in dimethyl sulfoxide was added to both mucosal and submucosal bathing solutions, and the Isc was recorded for 60 min. A23187 (10^{-6} M) was added to each chamber, and the Isc was recorded for an additional 30 min. To compare potencies of PGs, LTs, and HETEs, the following protocols were employed: 1) for PGs, the tissues were pretreated with indomethacin (10^{-6} M) for 60 min prior to PG addition to minimize basal PG generation and Isc; all additions were bilateral, cumulative, and ranged from 10^{-11} to 10^{-5} M; 2) for exposure to HETE and LTs, no pretreatment was performed; the additions were bilateral and cumulative. All effects were compared to vehicle-exposed time control tissues. Because the effect of LTC_4 on Isc in canine trachea has not been fully characterized, seven tissue pairs (G and Isc matched) were used to quantitate the effects of LTC_4 on unidirectional permeabilities of [^{22}Na]-, [^{36}Cl]$^-$, and [^{14}C]mannitol as previously described (3). Basal fluxes (60–120) were measured, and then the effects of LTC_4 on bioelectric properties and solute flows were measured over four 15-min intervals. Finally, to explore the interactions between LTC_4 effect and PG synthesis, tissues were either pretreated with indomethacin or other cyclooxygenase inhibitors (3×10^{-5} M) and then exposed to LTC_4 or exposed to indomethacin or other cyclooxygenase inhibitors (3×10^{-5} M) for 5 min after LTC_4 exposure.

RESULTS

Epithelial Cells

Sheets of cells isolated from dog trachea by enzymatic dissociation exhibited greater than 95% viability as determined by trypan blue exclusion. In addition, viewing grids prepared from seven separate preparations showed no (<0.1%) contamination of epithelial cells by leukocytes or macrophages (>1000 cells counted/specimen). Further examination of these grids did not indicate contamination by mast cells. Thick sections were stained with toluidine blue and examined by light microscopy. Numerous goblet cells staining blue were observed while mast cells which stain red or red blue were not observed. Examination of >1000 cells from four different dog preparations indicated no significant contamination by mast cells. Fig. 1 shows a typical electron micrograph of these canine epithelial cell preparations. One observes well preserved ultrastructure, with intracellular tight junctions intact, and normal appearing nuclei, mitochondria, and cilia. After A23187 stimulation, cell viability was not altered (trypan blue exclusion, 95%, $n = 7$).

[^{3}H]Arachidonic Acid Metabolism

The dog tracheal epithelial cells rapidly incorporated [^{3}H] arachidonic acid into the cellular phospholipid. The maximum incorporation into the phospholipids was obtained after 1 h of incubation, and thus this incorporation time was used in all subsequent experiments. After washing the cells with buffer to remove unincorporated arachidonic acid and its metabolites, the addition of calcium-containing media and 1 μM A23187 induced a rapid release of arachidonic acid and its metabolites. Analyses of the media from the epithelial cells by RP-HPLC (Fig. 2) shows the presence of 10 metabolites in addition to arachidonic acid. However, most of these peaks were minor metabolites, and only four major metabolites were routinely observed. Three of the four major metabolites co-

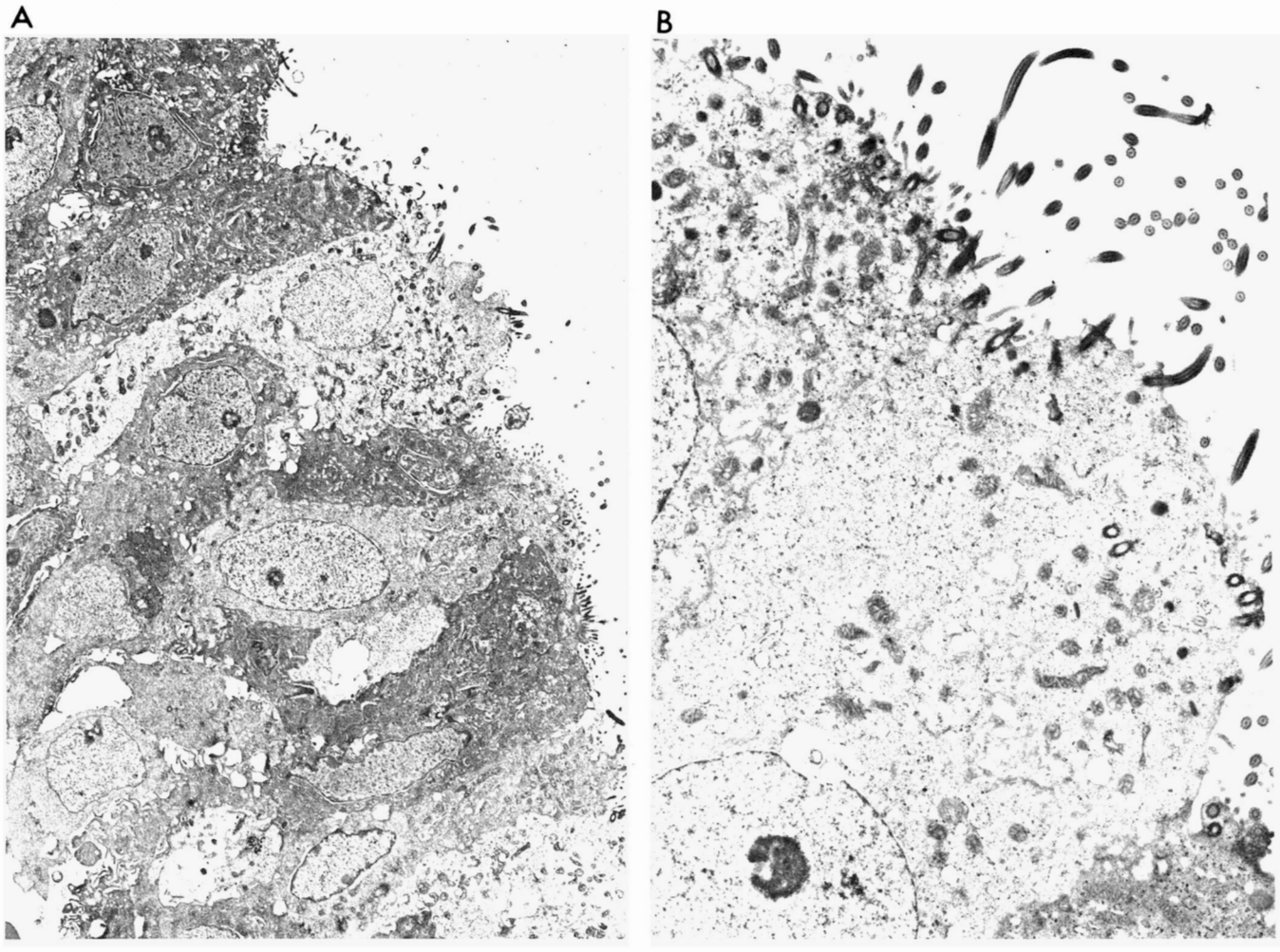

FIG. 1. *A*, transmission electron micrograph of an epithelial preparation. The integrity of the epithelium is maintained, and relatively normal appearing columnar ciliated and nonciliated cells are observed (× 2700). *B*, transmission electron micrograph of ciliated cells from an epithelium. The intercellular tight junctions remain intact. Although the endoplasmic reticulum is somewhat dilated, the nuclei, mitochondria, and cilia appear normal (× 5120).

eluted with authentic standards of PGE_2/PGD_2 (Peak 3), LTC_4 (Peak 5), and LTB_4 (Peak 7). The fourth metabolite, Peak 6, did not co-elute with any authentic standard available.

The analyses of the arachidonic acid metabolites by RP-HPLC showed variability in the metabolite profile between dog tracheal epithelial cell preparations. The tentative identification of the metabolites and the variability between experiments is shown in Table I. The minor metabolites, for the most part, were only occasionally observed and, therefore, not rigorously characterized. The tentative identification of these minor metabolites is based on co-elution with authentic standards (see Table I).

Identification of Major Metabolites

Peak 3—This metabolite co-eluted with authentic PGE_2 and PGD_2 on both solvent systems 1 and 2, and the formation of metabolite 3 by the dog tracheal epithelial cells was inhibited by the prior addition of indomethacin. The peak was collected and rechromatographed using solvent system 3 which separates PGE_2 and PGD_2. Peak 3 eluted with the same retention times as authentic PGD_2. As seen in Fig. 3, Peak 3 clearly separated from the authentic $[^3H]PGE_2$ which was added to the sample. Peak 3, isolated from RP-HPLC using solvent system 1, was derivatized to the pentafluorobenzyl estermethoxine-trimethylsilane ether and run on a gas chromatograph as described under "Experimental Procedures." Negative ion chemical ionization mass spectrometry, using argon as the reagent gas, produced fragmentation patterns and high molecular weight anions characteristic of derivatized PGD_2. Fig. 4 is a negative ion chemical ionization mass spectra as the pentafluorobenzylester-methoxine trimethylsilane derivatives of authentic PGD_2 (A) and the sample (B). The mass at m/z 524 corresponds to the carboxylate anion (M-181) resulting from the loss of the pentafluorobenzyl group. Losses of trimethylsilanol (m/z 90) from C9 and C15 appear at mass m/z 434 and 344. Derivatized authentic PGD_2 and peak 3 gave identical retention times on gas chromatography. Also authentic derivatized PGE_2 gave major ion at 524 (data not shown).

Peak 5—Peak 5 co-eluted on solvent systems 1 and 2 with an authentic standard of LTC_4. The formation of this metabolite was not inhibited by the addition of indomethacin to the incubation mixture. As seen in Fig. 2, peak 5 also absorbed at 280 nm suggesting a conjugated leukotriene. After the collection of peak 5, the solvent was removed, and the sample was dissolved in methanol. The characteristic UV spectra of peptide leukotrienes, with a peak at 280 nm and shoulders at 270 and 292 nm, were observed (20, 21). The metabolite of peak

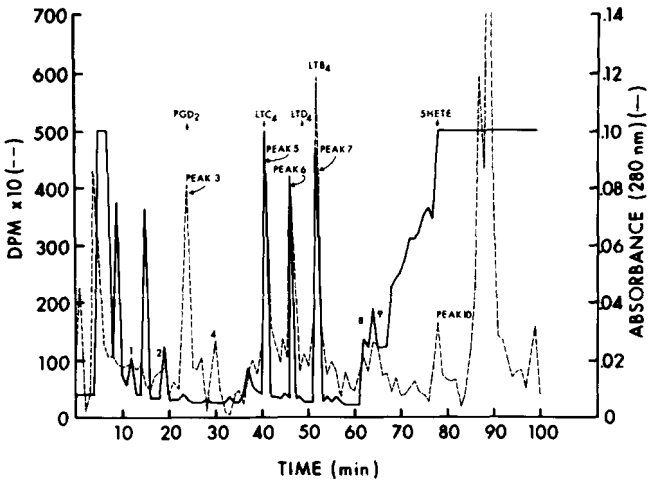

FIG. 2. **Reverse phase HPLC chromatographic profile for [^{3}H]arachidonic acid metabolites produced by dog tracheal epithelial cells.** [^{3}H]Arachidonic acid was incorporated into cellular phospholipid and released by the addition of ionophore, A23187. Metabolites were prepared for analysis as described under "Experimental Procedures" and chromatographed on solvent system 2. Elution of authentic standard is indicated. The individual metabolites were isolated and subjected to further analysis.

TABLE I

Arachidonic metabolites produced by dog epithelial cell preparations

Peaks	Retention time	Total metabolites ($\bar{x}$ ± S.D.; n = 7)	Designation
	min	%	
1	10	<1.0	6-Keto-$PGF_{1\alpha}$
2	18	2.6 ± 1.8	Thromboxane B_2
3	24	14.5 ± 4.5	PGD_2
4	30	3.3 ± 1.3	$PGF_{2\alpha}$
5	41	10.2 ± 2.5	LTC_4
6	47	9.3 ± 3.9	Unknown 1
7	52	12.8 ± 2.5	LTB_4
8	65	8.1 ± 3.0	Unknown 2
9	67		
10	78	4.5 ± 1.7	5-HETE

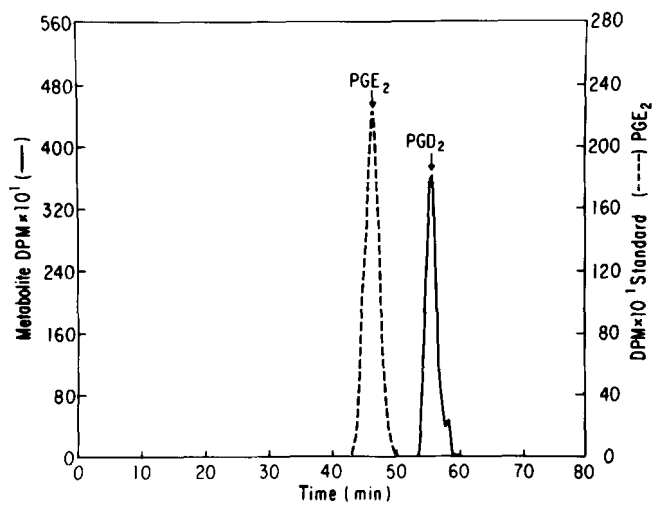

FIG. 3. **Reverse phase HPLC analysis of metabolite 3.** Metabolite 3 separated and isolated as described in Fig. 2 was chromatographed on solvent system 3. The *arrow* indicates the elution times of PGE_2 and PGD_2. The *solid line* is metabolite 3; the *dashed line* is an internal standard of [^{3}H]PGE_2.

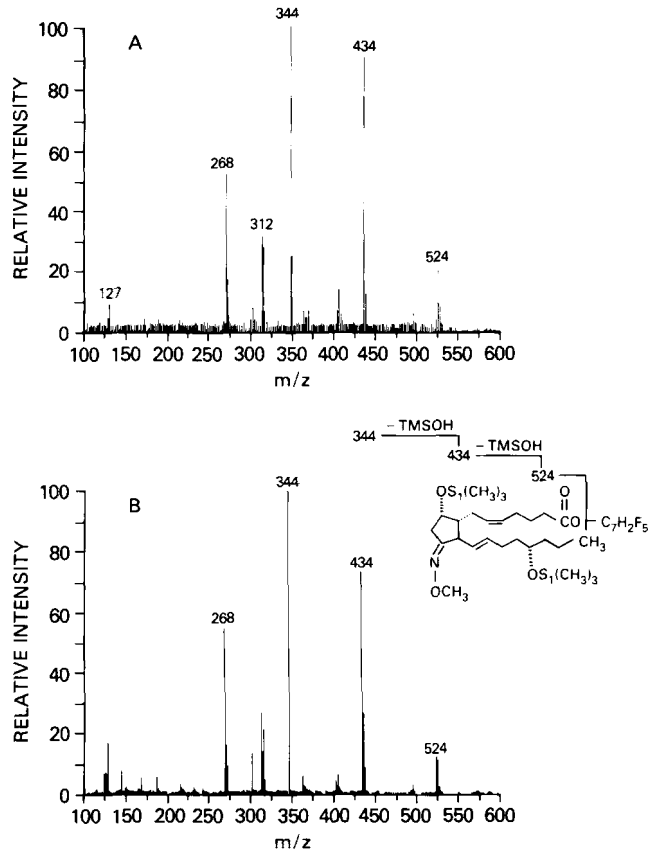

FIG. 4. **Characterization of metabolite 3 as PGD_2.** *A*, mass spectrum of authentic PGD_2; *B*, mass spectrum of metabolite 3.

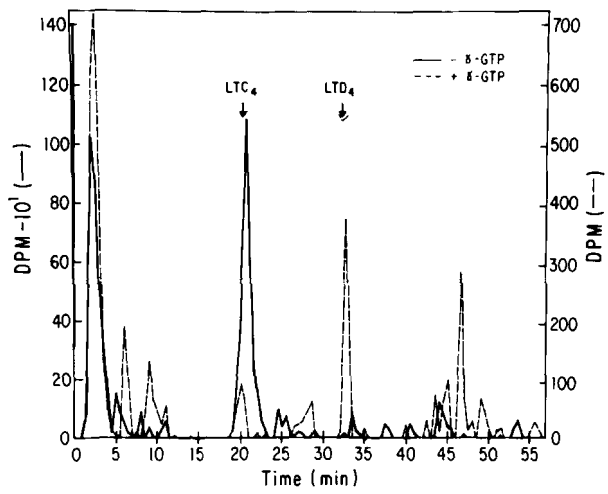

FIG. 5. **Metabolism of metabolite 5 to LTD_4 by γ-glutamyl transpeptidase.** Metabolite 5 was dissolved in 0.1 ml of 0.1 M Tris·HCl with 0.1 M $MgCl_2$, pH 8.5. 0.2 mg/ml γ-glutamyl transpeptidase was added and incubated for 30 min at 37 °C. 0.1 ml of MeOH + 5.0% acetic acid was added to stop the reaction, and an aliquot was injected onto the HPLC column. Authentic LTC_4 treated in an identical incubation was metabolized to LTD_4. The *arrow* indicates elution time of standards.

5 was further characterized by enzymatic digestion with γ-glutamyl transpeptidase (γ-GTP) which converts LTC_4 to LTD_4 (18). Peak 5 was incubated with γ-GTP as described under "Experimental Procedures." Authentic LTC_4 was converted to a metabolite which co-elutes with authentic LTD_4 (data not shown). Likewise, Peak 5 was also metabolized by γ-GTP to a product which co-eluted with authentic LTD_4

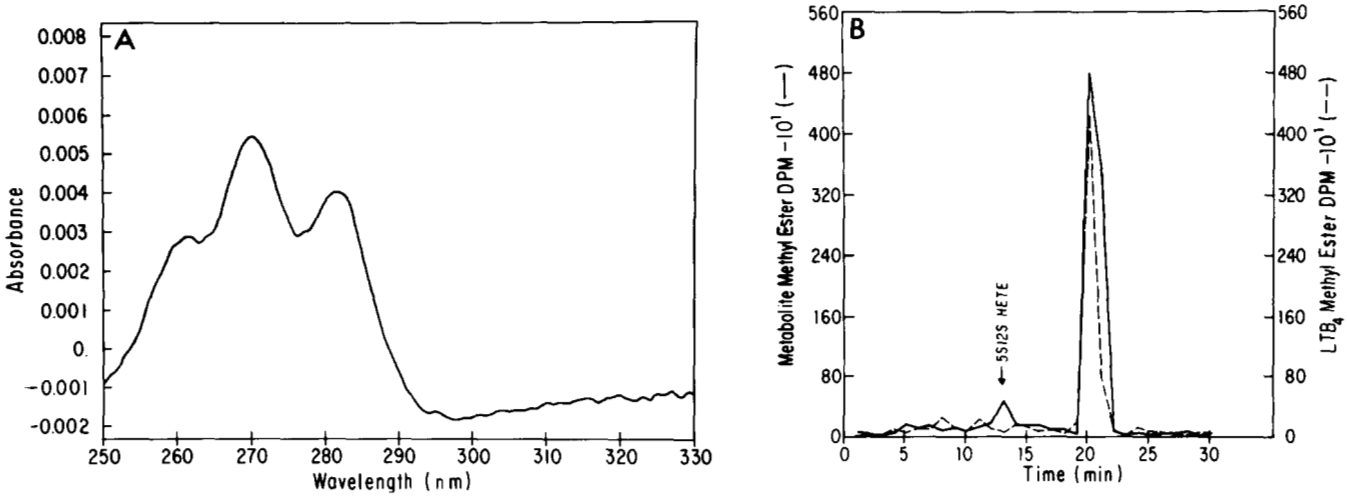

FIG. 6. **Characterization of metabolite 7 as LTB_4.** *A*, UV spectrum of metabolite 7 in methanol. *B*, straight phase HPLC separation of LTB_4 from 5*S*,12*S*-HETE. Metabolite 7 was converted to the methyl ester derivatives and chromatographed using solvent system 4.

(Fig. 5). Peak 5 prepared using [1-^{14}C]arachidonic acid was analyzed LTC_4 by radioimmunoassay. Peak 5 contained immunoreactive material with a dilution curve parallel to the standard. Thus, the arachidonic acid metabolite in peak 5 is LTC_4.

Peak 6—The formation of the arachidonic acid metabolite(s) present in peak 6 was not inhibited by the addition of indomethacin to the incubation mixture. The metabolite(s) eluted at approximately 47 min in solvent system 2, which did not co-elute with any authentic leukotriene available. LTF_4 and LTE_4 eluted at 43 and 55 min, respectively, while the 11-trans isomers of LTC_4 and LTD_4 elute approximately 1 min before the corresponding parent leukotrienes. Peak 6 isolated and repurified by HPLC had a characteristic UV spectra with maximum absorbance at 280 nm and shoulders at 272 and 293 nm (data not shown). The metabolite(s) was apparently not a sulfoxide leukotriene which has a maximum absorbance at 284 nm (22) nor a sulfone which absorbs at 280 nm but elutes 1–2 min earlier than the corresponding leukotriene (22, 23) on reverse phased HPLC.

LTD_4 is converted to LTC_4 by γ-glutamyl transpeptidase in the presence of reduced glutathione (18). Authentic LTD_4 and peak 6, repurified by reverse phase HPLC, were treated with γ-glutamyl transpeptidase and GSH. The authentic LTD_4 added to the incubation mixture was converted to LTC_4 while the metabolite, peak 6, was not metabolized to LTC_4. These data suggest that peak 6 is not LTD_4. Furthermore, LTC_4, LTD_4, or LTE_4 incubated in the presence of ionophore with cells was not converted to peak 6 or other products (data not shown). Identification of this arachidonic acid metabolite was complicated by the variability of the biosynthesis in the experiments which may be a reflection of the variability among mongrel dogs.

Peak 7–The formation of the arachidonic acid metabolite in peak 7 was not inhibited by the addition of indomethacin to the incubation mixture. Peak 7 co-eluted in systems 1 and 2 with authentic LTB_4, and UV absorption at 280 nm was detected. The isolated peak showed a characteristic leukotriene spectrum similar to authentic LTB_4 (Fig. 6*A*) with a maximum absorbance at 270 nm with shoulders at 262 and 281 nm. Since our RP-HPLC does not separate LTB_4 from its isomers, (12, 15) the purified metabolite was converted to a methyl ester and analyzed by straight phase HPLC. As seen in Fig. 6*B* the metabolite, peak 7, co-eluted with authentic LTB_4 methyl ester and showed only a minor contamination with the 5*S*,12*S*-diHETE. After conversion to pentafluorobenzyl-trimethylsilane derivative, the metabolite in peak 7 co-eluted on gas chromatography with authentic LTB_4. The metabolite in peak 7 is LTB_4 with only a minor contamination from the 5*S*,12*S*-diHETE isomer.

Peak 10—Peak 10 co-eluted on reverse phase HPLC with authentic 5-HETE using solvent systems 2 and 3 described under "Experimental Procedures." The formation of peak 10 was not inhibited by the addition of indomethacin to the incubation mixture. Peak 10 is tentatively identified as 5-HETE.

Quantitation of Leukotriene Formation

Radioimmunoassays using specific antisera for LTB_4 and peptide leukotrienes were used to estimate the formation of these lipids by dog tracheal epithelial cells. A direct assay of the incubation media with a series of dilutions indicated a parallelism of the sample dilutions and the standard curve. Leukotriene formation varied with individual dog preparations, but the ratio of LTC_4 to LTB_4 was approximately 1:1. Epithelial cells produced approximately 1 ng each of LTC_4 and $LTB_4/10^6$ cells (Table II).

Effect of Exogenous Arachidonic Acid Metabolites of Isc (Cl^- Secretion)

To estimate the relative potencies of arachidonic acid metabolites in inducing Cl^- secretion across excised canine tracheal epithelium, cumulative additions of authentic compounds were performed. As shown in Fig. 7*A*, all PG products increased Cl^- secretion. However, PGH_2, PGD_2, and PGE_2 were clearly the most potent and effective metabolites. As shown in Fig. 7*B*, LTC_4 was the most potent and effective leukotriene tested. LTB_4 exerted only minor effects, and 5-HETE was ineffective. Because the effect of LTC_4 on canine trachea ion transport has not been reported, isotope flux measurements were performed to confirm that the increased Isc reflected increased Cl^- secretion. The results are shown in Table III. In six tissue pairs, net Cl^- secretion rose from -1.03 ± 0.42 μeq cm^{-2} h^{-1} prior to stimulation of 1.80 ± 0.59 μeq cm^{-2} h^{-1} for the 45-min interval after the addition of LTC_4 ($\Delta_{net} = 0.94 \pm 0.28$ μeq cm^{-2} h^{-1}). The change in Cl^- secretion accounted for the change in Isc. The increase in net flux primarily reflected an increase in the unidirectional flux

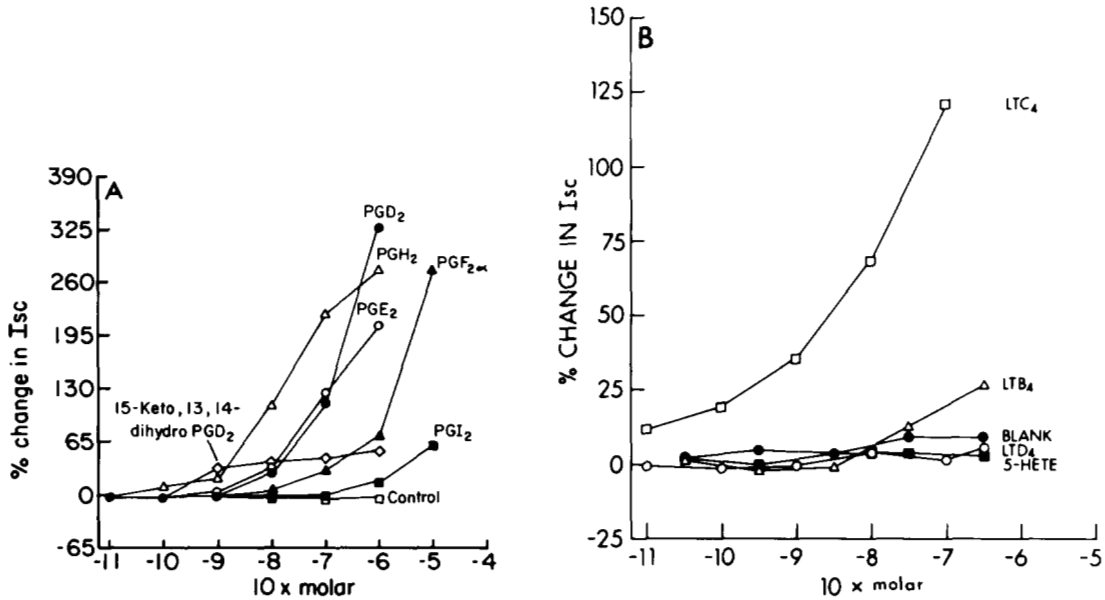

FIG. 7. **Relationships between short-circuit current and exogenous AA metabolites for excised canine trachea.** In *panel A*, the relationship between Isc for indomethacin-pretreated (10^{-6} M) excised tracheas and cyclooxygenase product additions are shown. The average Isc immediately prior to agonist addition was 27 $\mu A \cdot cm^{-2}$ and did not significantly differ between groups. In *panel B*, the relationship between basal Isc and lipoxygenase product additions is shown. The average Isc prior to addition was 42 $\mu A \cdot cm^{-2}$ and did not differ between groups. Each *point* represents mean for 5 or more tissues. S.E. are less than 10% of mean.

TABLE II

Estimation of leukotriene formation by dog tracheal epithelial cells using radioimmunoassays

Animal	LTC_4 ($n = 3$)	LTB_4 ($n = 3$)
	ng/10^6 cells ± S.D.	*ng/10^6 cells ± S.D.*
A	1.16 ± 0.25	1.26 ± 0.25
B	0.76 ± 0.22	0.90 ± 0.2
C	1.50 ± 0.25	1.36 ± 0.15

TABLE III

Effect of exogeneous LTC_4 (10^{-7} M) on bioelectric properties and solute fluxes across excised and short-circuited canine tracheal epithelia

	Control	±LTC_4
Bioelectric properties		
PD[a] (mV)	27.2[b] ± 1.7	32.6[c] ± 2.1
Isc ($\mu A \cdot cm^{-2}$)	75.8 ± 7.9	102.9[c] ± 11.6
G ($ms \cdot cm^{-2}$)	2.9 ± 0.3	3.2[c] ± 0.3
Ion fluxes ($\mu eq\ cm^{-2}\ h^{-1}$)		
$\vec{J}$, MS[d] Na^+	2.49 ± 0.54	2.36 ± 0.43
$\vec{J}$, SM[e] Na^+	1.23 ± 0.31	1.41 ± 0.39
J, net Na^+	1.26[f] ± 0.43	0.95 ± 0.48
$\vec{J}$, MS Cl^-	2.05 ± 0.29	2.27 ± 0.39
$\vec{J}$, SM Cl^-	3.08 ± 0.41	4.06[c] ± 0.52
J, net Cl^-	−1.03[f] ± 0.42	−1.80[f] ± 0.57
Mannitol permeability ($\times 10^{-7}\ cm\ s^{-1}$)	9.1 ± 1.3	10.3 ± 1.8

[a] PD, potential difference.
[b] Values are the mean ± S.E. ($n = 6$).
[c] Different than control value ($p < 0.05$).
[d] MS, mucosal to serosal
[e] SM, serosal to mucosal
[f] Different than zero ($p < 0.05$)—$\vec{J}$, net = secretion.

in the secretory direction (3.08 ± 0.41 eq $cm^{-2}\ h^{-1}$ for control; 4.06 ± 0.52 $\mu eq\ cm^2\ h^{-1}$ after LTC_4). No changes were observed in unidirectional Na^+ fluxes or mannitol permeability.

To test whether LTC_4 exerted its effect on Cl^- secretion directly, or via an action that required the generation of PGs, indomethacin and other cyclooxygenase inhibitors were added to determine whether it prevented LTC_4-induced Cl^- secretion. As shown in Fig. 8, the LTC_4 effect was abolished by indomethacin regardless of whether the drug was administered before or after LTC_4 addition. To test whether the indomethacin action was nonspecific or toxic, tissue response was measured by the addition of $PGF_{2\alpha}$. All tissues showed large increases in Isc in response to $PGF_{2\alpha}$ addition. Ibuprofen and naproxen (3×10^{-5} M) also inhibited LTC_4-induced Cl^- secretion (data not shown). In six tissue pairs, Isc for control conditions was 81.8 $\mu A \cdot cm^{-2}$ which increased to 140 ± 47 $\mu A \cdot cm^{-2}$ after addition of 10^{-7} M LTC_4. In the presence of 3×10^{-5} M ibuprofen the response to LTC_4 was 94.2 ± 30 $\mu A \cdot cm^{-2}$. Ibuprofen as control gave an Isc of 87.5 ± 24 $\mu A \cdot cm^{-2}$.

We have attempted to directly test for the stimulation of PGD_2 by LTC_4 after incorporation of [^{3}H]arachidonic acid into cellular phospholipid. However, the results were so variable that we could not unequivocally show a PGD_2 increase. The variable was observed primarily with the control samples which suggests that the manipulation that occurs with the

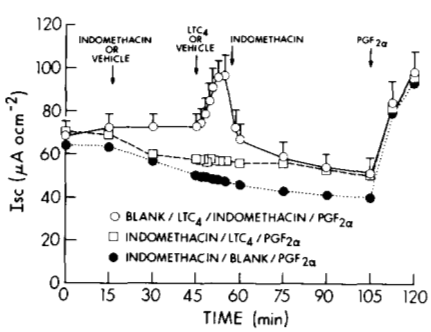

FIG. 8. **Effect of cumulative additions of indomethacin and LTC_4 on Isc of excised canine tracheas.** In one group of tissues, indomethacin (3×10^{-5} M) preceded LTC_4 (10^{-9} M) addition (□). In another group, indomethacin was added at the peak of the LTC_4 response (○). The trend group represents the vehicle (dimethyl sulfoxide)/time control (7). Each group represents 5–7 tissues. Mean ± S.E. shown. S.E. was equal to or less than 10% of mean.

technique stimulated arachidonic acid release. Thus, the LTC_4 effect on Cl^- secretion appears to require prostaglandin H synthase activity.

Time Course of Formation of Arachidonic Acid Metabolites and Stimulation of Cl^- Flux after A23187

We attempted to identify the endogenous compounds that stimulate Cl^- secretion by comparing the time course of product generation with the time course of increased Cl^- secretion in the same cell preparation. The addition of Ca^{2+} and A23187 (1 μM) to the dog tracheal epithelial cells induced a rapid release of incorporated [^{3}H]arachidonic acid and metabolism to prostaglandins and leukotrienes. As shown in Fig. 9, the metabolism of arachidonic acid was essentially complete by 5 min of incubation. All the observed arachidonic acid metabolites appeared to be formed within the same time frame and preceded the ionophore-induced increase in Cl^- secretion. The initiation in the Cl^- flux occurred as the concentration of arachidonic acid metabolites approached a maximum. These results indicate a temporal relationship between the formation of prostaglandins and leukotrienes and the Cl^- ion flux in dog tracheal epithelial cells and suggest that PGD_2 formation precedes Cl^- ion secretion.

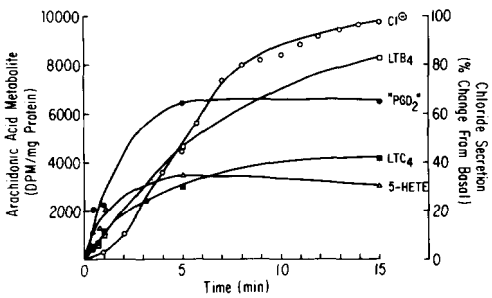

FIG. 9. **Time course of chloride secretion and arachidonic acid metabolism by canine tracheal epithelial cells after ionophore stimulation.** Canine tracheal epithelial cells were prepared as described under "Experimental Procedures." An intact section of canine trachea was used to study chloride ion flux while the remaining trachea was used to provide disaggregated cells that were used to examine metabolism. [^{3}H]Arachidonic acid was incorporated into the phospholipid pool and released by the addition of 1 μM A23187. Metabolites were isolated and separated as described under "Experimental Procedures" using solvent system 1. Values are average of 2–3 determinations for metabolism and 5–7 determinations for Isc (S.E. are less than 10% of mean).

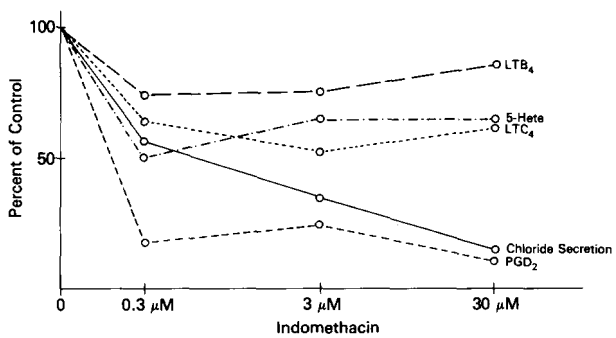

FIG. 10. **Effect of indomethacin on arachidonic acid metabolism and chloride ion secretion.** Chloride ion flux (Isc) and arachidonic acid metabolism were carried out as described in the text. Cells were first preincubated for 10 min with indomethacin before the addition of 1 μM A23187. Cl^- ion secretion was calculated as an increase over basal Isc for each set of chamber. Values are the average of 2–3 determinations for metabolism and 6–8 determinations for ion secretion. S.E. are less than 10% of mean.

Effect of Indomethacin on AA Metabolism and Cl^- Secretion

The addition of indomethacin to the epithelial cells produced a concentration-dependent inhibition of A23187-induced prostaglandin formation. At a concentration of 0.3 μM, approximately 70% inhibition of PGD_2 formation was observed (Fig. 10). At 30 μM indomethacin, approximately 90% inhibition of PGD_2 formation was observed. Chloride ion flux (Isc) was also inhibited by the addition of indomethacin to the canine tracheal epithelial cells. The inhibition of the Cl^- flux and PGD_2 formation appears to occur at similar indomethacin concentrations. Indomethacin also appears to inhibit leukotriene formation, particularly at the higher concentrations used in this study. However, the formation of the metabolites was too variable to be able to determine the significance of any apparent effect.

DISCUSSION

Freshly isolated canine tracheal epithelial cells incorporate [^{3}H]arachidonic acid, release arachidonic acid upon exposure to the calcium ionophore, A23187, and metabolize arachidonic acid via both prostaglandin H synthase and lipoxygenase pathways. The major prostaglandin H synthase metabolite produced by the tracheal cells was PGD_2. The identification of PGD_2 as the major prostaglandin H synthase product differs from previous reports which by radioimmunoassay criteria suggested that PGE_2 was the major prostaglandin H synthase product (4). Several criteria indicate that canine tracheal cells in this study generated PGD_2: 1) solvent system 3 produced good separation of PGD_2 and PGE_2, and peak 3 co-eluted with authentic PGD_2 rather than PGE_2 (Fig. 3); 2) the derivatized product co-eluted with authentic derivatized PGD_2 on gas chromatographs; 3) mass spectrometry of the biosynthetic sample was identical to authentic PGD_2.

The major lipoxygenase metabolites formed by tracheal epithelial cells were LTC_4 and LTB_4. LTB_4 was identified by HPLC analysis, gas chromatography, and UV spectrophotometry. LTC_4 was characterized by UV spectroscopy, characteristic elution of HPLC, radioimmunoassay, and conversion to LTD_4 by γ-glutamyl transpeptidase. LTB_4 and LTC_4 were synthesized in approximately equal quantities after A23187 stimulation.

We also observed occasionally an unidentified peptide leukotriene which did not appear to be LTE_4, LTF_4, or a leukotriene sulfone or sulfoxide. This metabolite may be a metabolite or degradation product of LTC_4 since the biosyntheses of LTC_4 and the unidentified metabolite were usually inversely related. However, authentic LTC_4 incubated with the epithelial cells was not converted to the metabolite. The variability of the cells' biosynthetic activity with respect to this chemical made the identification of this metabolite difficult.

Arachidonic acid metabolism by dog tracheal epithelial cells in this study differs somewhat with the data of Holtzman and co-workers (10) and Smith *et al.* (4). Like Holtzman *et al.*, we observed the synthesis of LTB_4. The criteria for identification of LTB_4 in the two studies (HPLC and spectral characteristics) are similar. In contrast to Holtzman *et al.* (10) we observed a significant production of LTC_4 by dog tracheal epithelial cells. The most obvious difference in the two studies is the stimulation employed, *e.g.* exogenous arachidonic acid *versus* A23187, but the exact reasons for this discrepancy are not known. Also, we found that PGD_2 is the major prostaglandin H synthase metabolite rather than PGE_2 as reported by Smith *et al.* (4) who used the antiserum for PGE_2. We have good evidence for the formation of PGD_2 by our cell preparations and can only speculate as to the difference between

our findings and that reported by Smith *et al.* (4).

Because several of the products identified as metabolites of our epithelial preparations are also produced by inflammatory cells (mast cells and/or polymorphonuclear cells), we evaluated the possible contamination of our epithelial preparation by inflammatory cells by three approaches. First, we performed a series of morphological evaluations on our cell preparations. An evaluation of >7000 cells (7 random preparations) by electron microscopy did not reveal mast cells or leukocytes. Histologic evaluation of the preparations, employing stains for inflammatory cells, also failed to reveal contamination of the epithelial preparation by inflammatory cells. Second, epithelial preparations from disease-free purebred beagles gave identical patterns of arachidonic acid metabolic products as did our routine preparations from standard laboratory animals. Third, the arachidonic acid metabolic products of the dog leukocytes were not known and consequently were partially characterized.[2] Dog leukocytes metabolize arachidonic acid to the 8,15-diHETE- and monooxygenase-catalyzed metabolites indicating that leukocyte contamination would not contribute to the pattern of arachidonic acid products we have identified. Dog mastocytoma cells in response to A23187 oxidize arachidonic acid to a variety of lipoxygenase products. The major metabolites were 15-HETE, 12-HETE, and 5-HETE. LTB_4, LTD_4, and LTC_4 were detected but in significantly lower amounts (24). We did not observe LTD_4, 12-HETE, or 15-HETE with our canine epithelial preparations. Assuming mast cells and mastocytoma cells are similar also suggests that mast cells did not contribute to the arachidonic acid metabolism by our epithelial cell preparation. The data from these three approaches strongly suggests that the arachidonic acid metabolites identified in our preparations after A23187 reflect products of epithelial cells and not contaminating inflammatory cells. Our preparation represents all cells from the epithelium, *i.e.* basal, mucus, and ciliated cells. Therefore, we cannot assign a role in arachidonic acid metabolism to specific epithelial cell types.

Our findings suggest that the epithelia, through the production of these mediators, may play an important role in modulating functional activities that control the volume and composition of airway secretions. Several lines of evidence indicate that the activation of the epithelial Cl^- path (which may control the rate of liquid secretion toward the airway lumen) is controlled by the rate of the prostaglandin H synthase-dependent PGD_2 formation. First, the A23187 induced an increase in Cl^- secretion and PGD_2 formation. These were inhibited by the indomethacin pretreatment indicating a requirement for prostaglandin H synthase activity. Second, most prostaglandin H synthase metabolites were effective in raising Cl^- secretion. None were inhibitory, suggesting that the rate of product formation rather than the class of prostaglandin formed is rate limiting for Cl^- secretion. While certain lipoxygenase products were effective in raising Cl^- secretion (LTC_4 most potently), this effect appeared to be indirect and involve cyclooxygenase activity since the LTC_4-induced activation was blocked by cyclooxygenase inhibitors. Consistent with this notion is the observation that the indomethacin-induced inhibition of Cl^- secretion was correlated with the inhibition of prostaglandin H synthase but not lipoxygenase. Third, the temporal relationship between the prostaglandin H synthase product formation and the Cl^- path activation indicates that Cl^- secretion toward the canine tracheal lumen is controlled by PGD_2 concentration.

Our data unfortunately does not allow us to determine the site(s) that control the basal rate of Cl^- secretion in canine trachea. Most studies were performed in epithelia stimulated with A23187. More importantly, even though LTC_4 (or other lipoxygenase products) production may exert its actions indirectly, the rate of formation of LTC_4 still may exert an important role in modulating the basal Cl^- secretory state. Our data indicates the need to focus on prostaglandin synthase products in exploring the relationship between arachidonic acid metabolism and Cl^- secretion.

Our observation that airway epithelia metabolize arachidonic acid to LTC_4 and LTB_4, as well as prostaglandin H synthase products, has two other implications for airway physiology. First, LTC_4 appears to be an important inducer of mucin secretion (9). Thus, the rate of mucin addition to airway secretions by the airway mucosa may in part be determined by "local" stimulation of the epithelium to form mucin secretagogues. Second, because LTB_4 is an important chemotactic agent, the production of LTB_4 by airway epithelia may assign an important role in the development of the airway inflammatory response to the epithelium itself.

In summary, airway epithelia can metabolize arachidonic acid to a variety of products that modulate the flow of salt and water, mucin, and migratory cells toward the airway lumen. This suggests that studies of the control of arachidonic acid metabolism in airway epithelia may yield important insights into the control of mucociliary clearance in health and the pathophysiology of certain diseases that reflect abnormal airway secretion, such as cystic fibrosis or asthma.

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[2] T. E. Eling, unpublished observations.