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Research Article

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Effects of Exogenous Arachidonic, Eicosapentaenoic, and Docosahexaenoic Acids on the Generation of 5-Lipoxygenase Pathway Products by Ionophore-activated Human Neutrophils

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Abstract. Exogenous eicosapentaenoic acid (EPA) and docosahexaenoic acid (DCHA) have been compared with exogenous arachidonic acid for their capacity to modulate the oxidative metabolism of membrane-derived arachidonic acid by the 5-lipoxygenase pathway in ionophore-activated human neutrophils and for their suitability as parallel substrates in this pathway. The products from specific ^{14}C- or ^{3}H-labeled substrates were isolated by reverse phase high performance liquid chromatography (RP-HPLC) and were identified by elution of radiolabel at the retention times of the appropriate synthetic standards. Each product was also characterized by its ultraviolet (UV) absorption spectrum, and 7-hydroxy-DCHA was defined in addition by analysis of its mass spectrum. The metabolites, 5-hydroxyeicosatetraenoic acid, leukotriene B_4 (LTB_4), 6-*trans*-LTB_4 diastereoisomers, 5-hydroxyeicosapentaenoic acid, 6-*trans*-leukotriene B_5 diastereoisomers, leukotriene B_5 (LTB_5), and 7-hydroxy-DCHA were quantitated by integrated UV absorbance during resolution by RP-HPLC. LTB_4 and LTB_5 were also quantitated by radioimmunoassay of the eluate fractions, and leukotrienes C_4 and C_5 (LTC_4 and LTC_5, respectively) were quantitated by radioimmunoassay alone. None of the unlabeled exogenous fatty acids (5–40 μg/ml) altered the release of radioactivity from [^{14}C]arachidonic acid-labeled, ionophore-activated neutrophils. The metabolism of 5 and 10 μg/ml of exogenous EPA by ionophore-activated, [^{14}C]arachidonic acid-labeled neutrophils not only generated 5-hydroxyeicosapentaenoic acid, 6-*trans*-LTB_5, LTB_5, and LTC_5, but also stimulated the formation of 5-hydroxyeicosatetraenoic acid, 6-*trans*-LTB_4 diastereoisomers, and LTC_4 from membrane-derived arachidonic acid. In contrast, LTB_4 production was diminished throughout the EPA dose-response, beginning at 5 μg/ml EPA and reaching 50% suppression at 10 μg/ml and 84% suppression at 40 μg/ml. The selective decrease in extracellular LTB_4 concentrations in the presence of EPA was not due to a change in the kinetic appearance of LTB_4 or to an increase in conversion to its ω-oxidation metabolites. DCHA was metabolized to 7-hydroxy-DCHA, did not stimulate metabolism of membrane-derived arachidonic acid, did not appreciably inhibit LTB_4 formation, and was not a substrate for leukotriene formation. Incremental doses of exogenous arachidonic acid resulted in increased production of 5-hydroxyeicosatetraenoic acid and 6-*trans*-LTB_4 by ionophore-activated, [^{14}C]arachidonic acid-labeled neutrophils without any change in LTB_4 production. 5-hydroxyeicosapentaenoic acid and 7-hydroxy DCHA were inactive as chemotactic factors whereas 5-hydroxyeicosatetraenoic acid exhibited 2% of the potency of LTB_4. Thus, exogenous DCHA does not appreciably interfere with the metabolism of membrane-derived arachidonic acid by ionophore-activated, [^{14}C]ara-

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chidonic acid-labeled neutrophils and is converted only to a monohydroxy derivative. In contrast, exogenous EPA attenuates the generation of LTB_4 and is converted to LTB_5, which is a weak and partial agonist as compared with LTB_4.

Introduction

The oxidative metabolism of arachidonic acid (AA),[1] released from membrane phospholipids during cell activation, occurs by the cyclooxygenase pathway in many cell types (1) and by the 5-lipoxygenase pathway in cells involved in host inflammatory responses, such as human neutrophil polymorphonuclear leukocytes (PMNs) (2), eosinophils (3), monocytes (4), alveolar macrophages (5, 6), and mast cells (7, 8). 5-Lipoxygenase acts on AA to generate 5*S*-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid, which is reduced to 5*S*-hydroxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5-HETE) (9) or is converted to 5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid (leukotriene A_4, LTA_4) (10–12). LTA_4 is converted by an epoxide hydrolase to 5*S*,12*R*-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid (leukotriene B_4, LTB_4) (13) or by a glutathione-S-transferase to 5*S*-hydroxy-6*R*-S-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid (leukotriene C_4, LTC_4) (14). LTA_4 also undergoes nonenzymatic hydrolysis to the diastereoisomers 5*S*,12*R*- and 5*S*,12*S*-dihydroxy-6,8,10-*trans*-14-*cis*-eicosatetraenoic acid [(5*S*,12*R*)- and (5*S*,12*S*)-6-*trans*-LTB_4, respectively] and to minor products, the 5,6-dihydroxyeicosatetraenoic acid diastereoisomers (9).

Eicosapentaenoic acid (EPA) and its natural hydrocarbon chain extension and desaturation product, docosahexaenoic acid (DCHA), which are prominent in diets enriched with fish oil, competitively inhibit the conversion of AA to prostaglandins. In preparations of intact human platelets and of ram seminal vesicle microsomal-derived prostaglandin synthetase, both EPA and DCHA are poor substrates and compete with AA for conversion by cyclooxygenase (15–17). Furthermore, the prostaglandin endoperoxides and thromboxane A_3 derived from EPA by the cyclooxygenase pathway are substantially less active than their AA-derived counterparts for eliciting aggregation of human platelets (15). With respect to products of the 5-lipoxygenase pathway, leukotriene B_5 (LTB_5), the 5,12-dihydroxy derivative of EPA formed from leukotriene A_5 (LTA_5), is a weak and partial agonist as compared with LTB_4 (18–21) in eliciting chemotactic and aggregating responses of human PMNs. The sulfidopeptide pentaene leukotrienes are equiactive with their tetraene counterparts (20, 22, 23).

The effects of exogenous AA, EPA, and DCHA on ionophore-activated human PMNs have now been compared and EPA and DCHA are shown to differ in their capacity to regulate utilization of endogenous AA by the 5-lipoxygenase pathway. EPA metabolism is associated with preferential inhibition of LTB_4 generation from membrane-derived AA and with conversion to chemotactically inactive 5-hydroxyeicosapentaenoic acid (5-HEPE) or attenuated LTB_5. DCHA has only minimal effects on the metabolism of cell membrane-derived AA by the 5-lipoxygenase cascade and is itself converted only to 7-hydroxy-DCHA (7-HDCHA), which has no chemotactic activity for human neutrophils. Thus, exogenous EPA and DCHA inhibit the metabolism of AA by the cyclooxygenase pathway (15–17), and EPA also suppresses the generation of LTB_4 by the 5-lipoxygenase cascade. In addition, the major products of each alternative substrate have negligible or attenuated chemotactic activity.

Methods

Hanks' balanced salt solution (HBSS) (Microbiological Associates, Inc., Bethesda, MD); Ficoll-Hypaque and macromolecular dextran (Pharmacia Fine Chemicals, Uppsala, Sweden); *N*-2-hydroxyethyl-piperazine-*N'*-2-ethane sulfonic acid (Hepes) (Gibco Laboratories, Gibco Div., Grand Island, NY); calcium ionophore A23187 (Calbiochem-Behring Corp., La Jolla, CA); high performance liquid chromatography (HPLC)-grade methanol (Burdick & Jackson Laboratories, Inc., Hoffmann-LaRoche, Inc., Muskegon, MI); AA (Sigma Chemical Co., St. Louis, MO); and [^{14}C]AA (55 mCi/mmol), [^{14}C]EPA (55 mCi/mmol), [^{3}H]docosahexaenoic methylester (4.0 Ci/mmol), [^{3}H]LTB_4 (40 Ci/mmol), and [^{3}H]LTC_4 (36 Ci/mmol) (New England Nuclear, Boston, MA) were obtained as noted. Eicosapentaenoic acid and DCHA were prepared as described (17) and shown to be >99.8% pure by gas chromatography and 270-mHz proton nuclear magnetic resonance spectroscopy. Each synthetic fatty acid was stored under argon in benzene at $-70°C$ and a sample was evaporated to dryness and resuspended in HBSS before use. Synthetic 5-HETE, LTB_4, (5*S*,12*R*)- and (5*S*,12*S*)-6-*trans*-LTB_4, LTC_4, (5*R*,6*S*)LTC_4, leukotrienes D_4 and E_4 (LTD_4 and LTE_4, respectively), *rac*-5-HEPE (a racemic mixture of 5*S*-hydroxy-6-*trans*-8,11,14,17-*cis*-eicosapentaenoic acid and its diastereoisomer, 5*R*-hydroxy-6-*trans*-8,11,14,17-*cis*-eicosapentaenoic acid), LTB_5, (5*S*,12*R*)- and (5*S*,12*S*)-6-*trans*-LTB_5, and *rac*-LTC_5 (a racemic mixture of LTC_5 and its diastereoisomer, 5*R*-hydroxy-6*S*-glutathionyl-7,9-*trans*-11,14,17-*cis*-EPA), and 4-hydroxydocosahexaenoic acid were prepared as previously described (17, 24–30) and stored at $-70°C$ in 0.1 M phosphate buffer (pH 6.5) containing 20% (vol/vol) ethanol until use. A 0.25-nmol sample of [^{3}H]docosahexaenoic methylester (1 μCi) was hydrolyzed to its free acid by its addition to 200 μl of cold

1. *Abbreviations used in this paper:* λ_{max}, wavelength of maximum ultraviolet absorbance; A_{234} and A_{269}, absorbance at 234 and 269 nm, respectively; AA, arachidonic acid; DCHA, docosahexaenoic acid; 7-HDCHA, 7-hydroxy-DCHA; EPA, eicosapentaenoic acid; HBSS-BSA, HBSS-containing 30 mM Hepes and 0.1% bovine serum albumin; 5-HEPE, 5-hydroxyeicosapentaenoic acid; 5-HETE, 5-hydroxyeicosatetraenoic acid; hpf, high power fields; HPLC, high-performance liquid chromatography; ID_{50}, 50% inhibition of binding; LTA_4, LTA_5, LTB_4, LTB_5, LTC_4, LTC_5, LTD_4, LTD_5, LTE_4, and LTE_5, leukotrienes A_4, A_5, B_4, B_5, C_4, C_5, D_4, D_5, E_4, and E_5, respectively; *rac*-LTC_5, racemic leukotriene C_5; *rac*-5-HEPE, racemic 5-hydroxyeicosapentaenoic acid; RIA, radioimmunoassay; RP-HPLC, reverse phase-high-performance liquid chromatography; solvent I, 65% methanol/34.9% water/0.1% acetic acid (vol/vol, pH 5.6); solvent II, 75% methanol/24.9% water/0.1% acetic acid (vol/vol, pH 5.6); solvent III, 100% methanol; solvent IV, 58% methanol/41.9%/water/0.1% acetic acid (vol/vol, pH 5.6); UV, ultraviolet.

methanol/10 N NaOH (9:1, vol/vol) and standing at 4°C for 3 h (31); the solvent was evaporated under a stream of nitrogen, and the residue was resuspended in ethanol and stored at −70°C until use.

Preparation, [^{14}C]AA-labeling, and activation of PMNs. Human PMNs, obtained from citrate-anticoagulated blood, were purified to >95% by dextran sedimentation, centrifugation through Ficoll-Hypaque, and hypotonic lysis of erythrocytes (32). The PMNs were washed three times in HBSS and suspended at a concentration of 1×10^7/ml in HBSS containing 30 mM Hepes and 0.1% bovine serum albumin (HBSS-BSA). 1 ml of the PMN suspension was incubated with 0.1 μCi [^{14}C]AA (1.8 nmol) for 60 min at 37°C. A 50-μl sample of the PMN suspension was removed, and the remaining PMNs were washed three times in HBSS-BSA and resuspended at a concentration of 1×10^7/ml in HBSS-BSA. 50 μl of the initial PMN suspension in [^{14}C]AA, of a pool of the supernatants from the three washes, and of the washed, labeled PMN suspension were each added separately to 10 ml of scintillation fluid (Hydrofluor; National Diagnostics, Inc., Advanced Applications Institute Inc., Somerville, NJ), and the radioactivity was measured in a liquid β-scintillation counter (Mark III; Tracor Analytic Inc., Elk Grove Village, IL). More than 70% of the radiolabel was incorporated into the PMN preparations.

10×10^6 [^{14}C]AA-labeled PMNs in 500 μl HBSS-BSA were warmed to 37°C for 5 min, mixed with 250 μl of prewarmed, unlabeled AA, EPA, or DCHA, and activated by calcium ionophore A23187 dissolved in 250 μl of HBSS-BSA containing 0.4% dimethylsulfoxide; final concentrations were 0–40 μg/ml for fatty acid and 10 μM for ionophore. The mixtures were incubated at 37°C for 5 min, and the reactions were stopped by rapid cooling at 4°C and centrifugation at 10,000 *g* for 30 s. 50 μl of the supernatant from each sample was added to 10 ml scintillation fluid and the radioactivity was measured. The remainder of the supernatants were stored under argon at −20°C until resolution of reaction products by reverse phase-high performance liquid chromatography (RP-HPLC). Each cell pellet was suspended in 1 ml HBSS-BSA and sonicated at 4°C with a sonifier (setting 3, 40% pulse cycle, 20 pulses; Branson Sonic Power Co., Danbury, CT); a 50-μl sample of each sonicate was added to 10 ml scintillation fluid and the radioactivity was measured. The percent release of incorporated radiolabel from each reaction mixture was calculated as: [(counts per minute in supernatant)/(counts per minute in supernatant + counts per minute in companion cell pellet)] × 100. Cell viability in the absence or presence of 0–40 μg/ml AA, EPA, or DCHA was assessed by release of lactic dehydrogenase, which was consistently <3%.

Identification of PMN products by RP-HPLC. Products from each reaction mixture were applied to a 10-μm C_{18} Ultrasil-ODS column (4.6 × 250 mm) (Altex Scientific, Inc., Berkeley, CA). The column was eluted at a flow rate of 1 ml/min with the following three-solvent program which resolves dihydroxy leukotrienes and monohydroxy fatty acids: 65% methanol/34.9% water/0.1% acetic acid, vol/vol pH 5.6 (solvent I) for 30 min; then, after a step gradient over 0.2 min with 75% methanol/24.9% water/0.1% acetic acid, pH 5.6 (solvent II), isocratically to 55 min; and finally after a step gradient over 0.2 min with 100% methanol (solvent III), isocratically to 70 min. 1-ml fractions were collected, and absorbance was continuously monitored at 269 nm (A_{269}) during elution in solvent I, and at 234 nm (A_{234}) during elution in solvents II and III with an on-line spectrophotometer (model 100-40; Hitachi, Ltd., Tokyo) linked to a Hewlett-Packard Integrator (model 3380A; Hewlett-Packard Co., Avondale, PA). The RP-HPLC program was calibrated for the retention times of synthetic standards: (5*S*,12*R*)-6-*trans*-LTB_5 (12.7±0.33 min, mean±SD, *n* = 4); (5*S*,12*S*)-6-*trans*-LTB_5 (13.5±0.25 min, *n* = 4); LTB_5 (15.6±0.51 min, *n* = 4); (5*S*,12*R*)-6-*trans*-LTB_4 (19.3±0.67 min, *n* = 4); (5*S*,12*S*)-6-*trans*-LTB_4 (20.4±0.9 min, *n* = 4); LTB_4 (25±1.1 min, *n* = 4); *rac*-5-HEPE (44.1±2 min, *n* = 4); 5-HETE (50.8±2.9 min, *n* = 4); [^{14}C]EPA (60.5 min, mean, *n* = 2); [^{14}C]AA (62.1 min, mean, *n* = 2); and [^{3}H]DCHA (65.7 min, mean, *n* = 2).

In experiments in which the quantities of tetraene and pentaene sulfidopeptide leukotrienes were also measured, the reaction products were separately resolved isocratically with 58% methanol/41.9% water/0.1% acetic acid (vol/vol, pH 5.6) (solvent IV). 1-ml fractions were collected and absorbance was continuously monitored at 269 nm. The retention times of the synthetic standards in solvent IV were *rac*-LTC_5, 18.8 min; LTB_5, 30.1 min; LTC_4, 30.1 min; LTD_4, 49.0 min; LTB_4, 50.9 min; and LTE_4, 57 min.

ω-Oxidation products of LTB_4 were prepared for use as reference standards by incubation of [^{3}H]LTB_4 (1.7 pmol) with 1×10^7/ml PMNs in the presence of 10 μM calcium ionophore A23187 at 37°C for 15 min. The cells were sedimented at 10,000 *g* for 30 s at room temperature and the labeled products in the supernatants were resolved by RP-HPLC in solvent I. In two experiments a mean of 91.5% of the added counts was recovered after RP-HPLC, and of these, 93.8% eluted with an average retention time of 6.4 min, consistent with that for ω-oxidation products of LTB_4 as chromatographed in a comparable solvent system (33, 34); 4.4% of the recovered counts eluted at the retention time of synthetic LTB_4 (25 min). When [^{3}H]LTB_4 was incubated at 37°C for 15 min in HBSS without PMNs, a mean of 89% of the added counts was recovered after RP-HPLC, and of these, 91.5% eluted at the retention time of LTB_4 and <1% of the counts at the retention time of the ω-oxidation products.

Quantitation of products. Products were quantitated by integrated ultraviolet (UV) absorbance and by radioactive and immunochemical measurements in fractions of defined retention times on RP-HPLC. The quantities of each product recovered from RP-HPLC were calculated using the integrated UV absorbance value obtained with known quantities of the appropriate synthetic standard; for 7-HDCHA it was necessary to employ 5-HETE as the standard. After RP-HPLC of each sample from [^{14}C]AA-labeled PMNs, 10 ml of scintillation fluid was added to each fraction and the radioactivity was measured. More than 76% of the radioactive counts applied was recovered from RP-HPLC for each experiment.

In selected experiments 1×10^7 unlabeled PMNs were activated by 10 μM calcium ionophore A23187 at 37°C for 5 min in a final volume of 1 ml of HBSS-BSA in the presence of 0.1 μCi [^{14}C]EPA and 20 μg unlabeled EPA (66 μM), or of 0.1 μCi [^{3}H]DCHA and 20 μg unlabeled DCHA (61 μM). The reactions were stopped by rapid cooling to 4°C and centrifugation at 10,000 *g* for 30 s. Each supernatant was resolved by RP-HPLC and the products were quantitated by integrated UV absorbance and by radioactivity of the eluted fractions; >81% of the radioactive counts applied was recovered from RP-HPLC for each experiment.

For immunoassay of LTB_4 and LTB_5, 5-μl samples of the fractions were collected after RP-HPLC, and for immunoassay of the sulfidopeptide leukotrienes, 100-μl samples of the same fractions were evaporated to dryness under reduced pressure. The residues were resuspended in 500 μl of 10 mM Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl and 0.1% gelatin (Isogel Tris buffer), and 100 μl of each mixture was assessed by radioimmunoassay. The standard curve for the inhibition binding of [^{3}H]LTB_4 to anti-LTB_4 antibody (35) was linear over a dose range of 0.1–5 ng and 0.01–1.0 ng for synthetic LTB_4 and LTB_5, respectively; 50% inhibition of binding (ID_{50}) of LTB_4 and LTB_5 occurred at 0.18 ng and 0.06 ng, respectively (19). The standard curve

for the inhibition of binding of $[^3H]LTC_4$ to anti-LTC_4 antibody (36) was linear over the range of 0.01–5 ng for LTC_4, its diastereoisomer (5*R*,6*S*)LTC_4, LTD_4, LTE_4, and *rac*-LTC_5; the ID_{50} values for LTC_4, (5*R*,6*S*)LTC_4, LTD_4, LTE_4, and *rac*-LTC_5 were 0.20, 0.19, 0.66, 0.72, and 0.15 ng, respectively; the ID_{50} estimated for authentic LTC_5 was 0.15 ng based on the relative immunoreactivity of LTC_4 and its diastereoisomer. The ID_{50} values for leukotrienes D_5 and E_5 (LTD_5 and LTE_5, respectively) were derived from that of LTC_5 based on the observed ID_{50} ratios for the members of the tetraene series (20).

Characterization of the monohydroxy products of EPA and DCHA. 35×10^7 and 55×10^7 PMNs purified from the blood of two normal donors were suspended in HBSS-BSA at a concentration of 1×10^7 cells/ml and activated by 10 μM calcium ionophore for 5 min at 37°C in the presence of 20 μg/ml EPA. The reactions were stopped by the addition of 4 vol of cold ethanol and rapid cooling to 4°C. The mixtures were allowed to stand at 4°C for 1 h and the precipitates were removed by sedimentation at 1000 *g* for 15 min at room temperature. The supernatants were evaporated to dryness under reduced pressure and the residues were suspended in 2 ml of solvent I for resolution by RP-HPLC. The fractions eluting in a UV absorbance peak in solvent II at the retention time of *rac*-5-HEPE were combined and the UV absorption spectrum was determined on a spectrophotometer (model 210; Varian Associates, Inc., Palo Alto, CA).

37×10^7 and 40×10^7 PMNs purified from blood of two normal donors were suspended in HBSS-BSA at a concentration of 1×10^7 cells/ml and were activated by 10 μM calcium ionophore for 5 min at 37°C in the presence of 40 μg/ml DCHA. The reactions were stopped and the supernatants were processed as described for the EPA product. The RP-HPLC fractions corresponding to the UV absorbance peak of the single DCHA-derived product eluting in solvent II were combined and the UV absorption spectrum was determined. The product was sequentially converted to its methylester by a reaction with ethereal diazomethane and to its fully saturated trimethylsilylether derivative (31) for mass spectral analysis on a spectrometer (MS-50; Kratos Analytical Instruments, Ramsey, NJ) with a capillary gas chromatographic inlet after a 70 eV electron impact.

Chemotactic activities of 5-HETE, 5-HEPE, and 7-HDCHA. 5-HETE, 5-HEPE, and 7-HDCHA were obtained by incubating 20–45 × 10^7 PMNs at a concentration of 1×10^7 cells/ml with 20–40 μg/ml of AA, EPA, or DCHA, respectively, in the presence of 10 μM ionophore A23187 at 37°C for 5 min, as described above for characterization of the latter two products. The purity of the products resolved in solvent II after RP-HPLC was confirmed by straight-phase HPLC after the compounds were converted to their methylesters by a reaction with ethereal diazomethane (31). The esters were applied to a Zorbax Sil column (4.6 mm × 25 cm; DuPont Instruments, Wilmington, DE) in hexane/tetrahydrofuran (15:1, vol/vol) and eluted at a flow rate of 2 ml/min with continuous monitoring of absorbance at 235 nm. The methyl esters of 7-HDCHA, 5-HETE, and 5-HEPE eluted as single peaks at 17, 20, and 22 min, respectively; identical retention times were obtained with synthetic reference standards for the latter two compounds.

The chemotactic activities of 5-HETE, 5-HEPE, and 7-HDCHA were compared with each other and with that of synthetic LTB_4 over a concentration range from 1 to 1,000 nM by use of PMNs from three normal donors in a microchemotaxis assay (37). As determined by UV absorbance, defined amounts of each PMN-derived monohydroxy fatty acid and synthetic LTB_4 were evaporated to dryness under reduced pressure and suspended in HBSS containing 30 mM Hepes, pH 7.4. 25-μl samples of serial dilutions of each fatty acid were placed in the lower well of separate microchemotactic chambers, which were separated by a 3-μm pore-size nitrocellulose filter from the upper well containing 50 μl of 10^7 PMNs/ml HBSS with 30 mM Hepes, pH 7.4, with 0.4% ovalbumin. After incubation for 2 h at 37°C, the filters were fixed, stained with hematoxylin-chromatrope, and mounted on glass slides. The cells were counted at a filter depth corresponding to the maximal migration of cells exposed to control buffer alone. Results were expressed as the average number of PMNs/5 high power fields (hpf) after correction for background migration. The experiments were performed in duplicate and the intra-assay coefficient of variation for duplicate filters was <35%.

Results

Effects of exogenous AA, EPA, and DCHA on the percent release of incorporated radiolabel from [¹⁴C]AA-labeled PMNs. The effects of 40 μg/ml of exogenous AA, EPA, or DCHA on the time-dependent percent release of radiolabel from ionophore-activated $[^{14}C]$AA-labeled PMNs were compared with each other and with the percent release of radiolabel in the absence of any exogenous fatty acid. Less than 5% of the radioactivity was released without ionophore, in the presence and absence of AA, EPA, and DCHA by 5 min, and no further release occurred for incubation periods of up to 15 min. With ionophore activation the percent release of radiolabel increased to a plateau of ~15% 5–10 min after cell activation in the absence and presence of unlabeled exogenous AA, EPA, and DCHA (Fig. 1). The release of incorporated radiolabel from PMNs after a 5-min incubation with the ionophore was not influenced by any concentration of fatty acid and was not significantly different among the fatty acids. Cells not incubated with ionophore in the presence of exogenous fatty acid released <5% of incorporated radiolabel, regardless of the type of fatty acid or its concentration.

Identification and characterization of the PMN 5-lipoxygenase pathway products derived from exogenous EPA. The oxidative metabolism of EPA by ionophore-activated PMNs was assessed in parallel reactions in which one set of 1×10^7 cells/ml was unlabeled and activated in the presence of 0.1 μCi $[^{14}C]$EPA and 20 μg/ml unlabeled EPA, and the other set from the same donor was radiolabeled with $[^{14}C]$AA and activated in the presence of 20 μg/ml unlabeled EPA (Fig. 2). Thus, it was possible to identify unlabeled membrane-derived AA products and both $[^{14}C]$EPA-labeled and unlabeled products from exogenous EPA with the first set of cells (*B*), and $[^{14}C]$AA-labeled and unlabeled AA membrane-derived products and unlabeled products from exogenous EPA with the second set of cells (*C*). As detected by integrated A_{269} the products generated by both sets of cells eluted with the same retention times and thus only the pattern for the first set is depicted (*A*). Products eluted in solvent I as a single peak at 6.2 min distinct from the solvent front, as a doublet at 12.2 and 13.8 min, as a single peak at 15.8 min, as a doublet at 19.1 and 20.9 min, and as a single peak at 26.1 min. These retention times were identical to those of the reference standards for the ω-oxidation

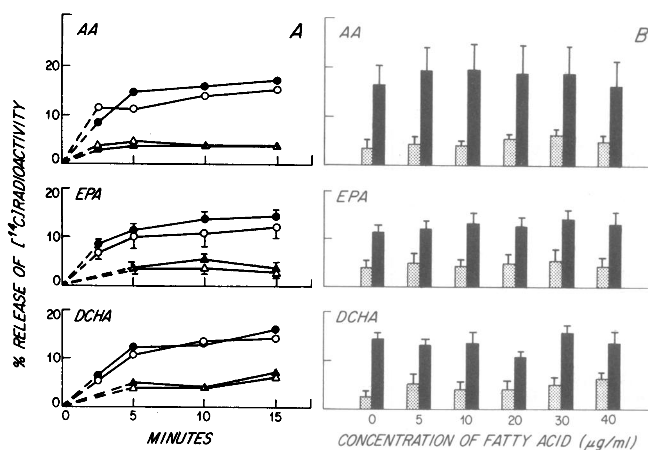

Figure 1. (*A*) The effects of exogenous AA, EPA, and DCHA (40 µg/ml) on the time-dependent release of radioactivity from [^{14}C]AA-labeled PMNs activated with ionophore A23187. PMNs (1×10^7/ml) were incubated in buffer alone (△), with each fatty acid alone (▲), with ionophore (10 µM) alone (○), and with both ionophore and fatty acid (●). The results for EPA are the mean±SEM of four experiments, and for AA and DCHA are the mean of two experiments. (*B*) Dose-response effects of AA, EPA, and DCHA on the release of radioactivity from [^{14}C]AA-labeled PMNs (1×10^7/ml) without (stippled bars) and with (solid bar) ionophore activation. The results are the mean±SEM of four experiments for AA and EPA and of three experiments for DCHA.

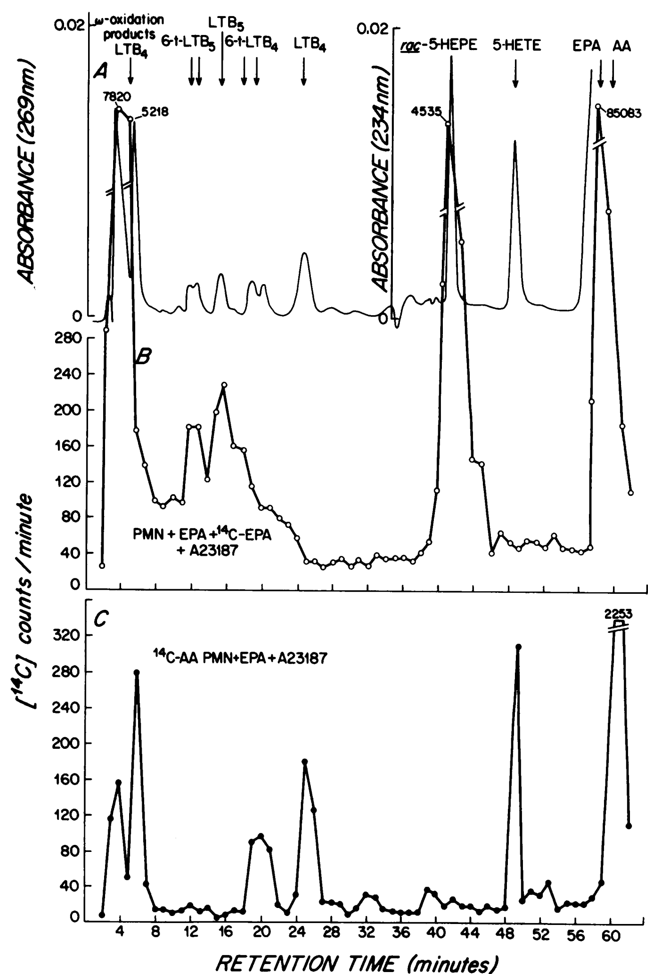

Figure 2. RP-HPLC of supernatants from PMNs (1×10^7 cells/ml) activated with ionophore in the presence of [^{14}C]EPA (0.1 µCi) and unlabeled EPA (20 µg/ml) analyzed by integrated UV absorbance (*A*) and counts per minute (○) (*B*), and of supernatants from [^{14}C]AA-labeled PMNs (1×10^7 cells/ml) activated with ionophore in the presence of unlabeled EPA (20 µg/ml) (●) (*C*). The retention times of reference standards are indicated by the arrows. The counts per minute values in *B* and *C* that greatly exceed the scale on the ordinate are inserted on the plot itself at the relevant peaks, with the discontinuity of scale indicated by the interrupted lines to and from those points.

products of LTB_4, 6-*trans*-LTB_5 diastereoisomers, LTB_5, 6-*trans*-LTB_4 diastereoisomers, and LTB_4, respectively. As monitored at A_{234}, two products eluted in solvent II at 41 and 50.2 min, the respective retention times of the *rac*-5-HEPE and 5-HETE standards. Products eluting with the retention time of 6-*trans*-LTB_5 diastereoisomers, LTB_5, and 5-HEPE incorporated radiolabel from [^{14}C]EPA only (Fig. 2, *B*), and products eluting with the retention time of 6-*trans*-LTB_4 diastereoisomers, LTB_4, and 5-HETE incorporated radiolabel from [^{14}C]AA only (Fig. 2, *C*).

The more polar product obtained from 35 and 55×10^7 activated PMNs eluting at the retention time of synthetic *rac*-5-HEPE in solvent II had its UV λ_{max} at 234.3 nm (mean, $n = 2$) which was identical to that of synthetic *rac*-5-HEPE (234.2 nm, $n = 2$). The less polar product eluting at the retention time of synthetic 5-HETE had its λ_{max} at 234.8 nm (mean, $n = 2$), as compared with that of synthetic 5-HETE, 234.6 nm (mean, $n = 2$).

Effects of exogenous EPA on the generation of individual 5-lipoxygenase pathway metabolites from EPA and endogenous AA. The time-dependent generation of LTB_4 and LTB_5 from 1×10^7 [^{14}C]AA-labeled PMNs, activated by ionophore in the absence and presence of 40 µg/ml EPA, was measured by A_{269}, radioactive counts, and radioimmunoassay (RIA) and was maximal at 5 min for each product by each assay (Fig. 3). The generation of LTB_4 in the absence of EPA was maximal at 399±45 ng (mean±SEM, $n = 4$) by A_{269} and 480±30 ng (mean±SEM, $n = 3$) by RIA. In the presence of EPA the generation of LTB_4 and LTB_5 was 100±50 and 192±21 ng (mean±SEM, $n = 4$), respectively, by A_{269}, and 125 and 275 ng/10^7 PMNs, respectively, by RIA, 5 min after cell activation.

[^{14}C]AA-labeled PMNs (1×10^7/ml) were activated by calcium ionophore in the absence and presence of increasing exogenous concentrations of EPA, and the products were resolved by RP-HPLC and quantitated by integrated UV absorbance (Fig. 4). The quantity of 5-HETE generated in the

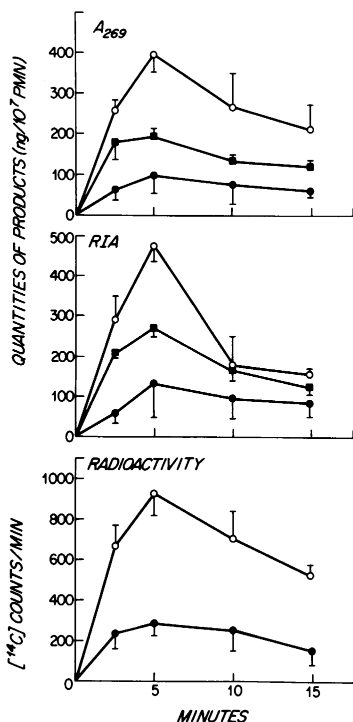

Figure 3. The time-dependent generation of LTB_4 (○) in the absence of EPA, and of LTB_4 (●) and LTB_5 (■) in the presence of 40 μg/ml EPA from [^{14}C]AA-labeled PMNs (1 × 10^7 cells/ml) activated by 10 μM ionophore. The results quantitated by A_{269} and radioactivity are the mean±SEM for four experiments, of which RIAs (mean±SEM) were performed in three.

absence of EPA, 334±226 ng (mean±SEM, *n* = 4), increased with 5 μg/ml exogenous EPA and reached a maximum of 590±209 ng (mean±SEM, *n* = 4) at 10 μg/ml EPA; 5-HETE production fell to 200±56 ng (mean±SEM, *n* = 4) and 200±55 ng (mean±SEM, *n* = 4) at 30 and 40 μg/ml EPA, respectively. The 6-*trans*-LTB_4 diastereoisomers, which totaled 80±53 ng (mean±SEM, *n* = 4) without EPA, rose to 131±36 ng (mean±SEM, *n* = 4) at 10 μg/ml EPA, and declined at higher EPA concentrations, such that only 61±8 ng (mean±SEM, *n* = 4) was generated in the presence of 40 μg/ml EPA. In contrast, there was no EPA-induced stimulation of LTB_4 generation but rather there was a dose-dependent decrement in the quantities of LTB_4 generated from 300±61 ng (mean±SEM, *n* = 4) in the absence of EPA to 49±12 ng LTB_4 in the presence of 40 μg/ml EPA. The quantities of 5-HEPE and the 6-*trans*-LTB_5 diastereoisomers increased in a dose-related manner with exogenous EPA to reach 311±73 ng (mean±SEM, *n* = 4) and 260±215 ng (mean±SEM, *n* = 4) at 40 μg/ml EPA, respectively. The quantities of LTB_5 increased to 102±35 ng (mean±SEM, *n* = 4) at 20 μg/ml EPA and then decreased to 70±24 ng (mean±SEM, *n* = 4) at 40 μg/ml EPA.

For comparison, 1 × 10^7 [^{14}C]AA-labeled PMNs (1 × 10^7/ml) were activated with ionophore in the absence and presence of increasing concentrations of exogenous AA (Fig. 5 *A*). Under these conditions, 5-HETE increased in a dose-related fashion from 157±76 ng (mean±SEM, *n* = 4) in the absence of exogenous AA to 950±225 ng (mean±SEM, *n* = 4) at 40 μg/ml AA and 6-*trans*-LTB_4 increased from 52±50 ng (mean±SEM, *n* = 4) to 400±105 ng (mean±SEM, *n* = 4) at 40 μg/ml AA. LTB_4 increased insignificantly from 183±44 ng (mean±SEM, *n* = 4) in the absence of exogenous AA to a plateau of 220±51 ng (mean±SEM, *n* = 4) at 5 μg/ml AA. In order to compare the dose-response effects of exogenous EPA

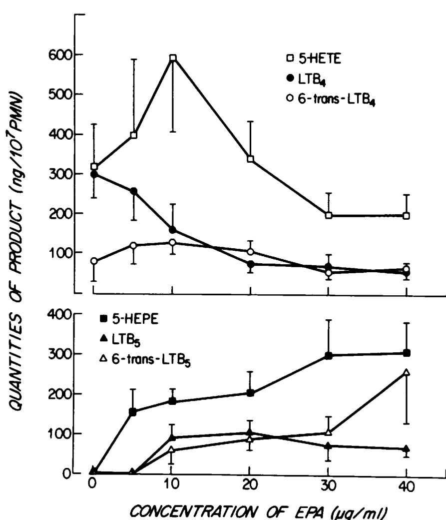

Figure 4. The effects of increasing concentrations of exogenous EPA on the generation by [^{14}C]AA-labeled PMNs (1 × 10^7 cells/ml) activated with ionophore of 5-lipoxygenase pathway metabolites from cell membrane-derived AA (*top*) and exogenous EPA (*bottom*). The results, quantitated by UV absorbance, are mean±SEM of four experiments.

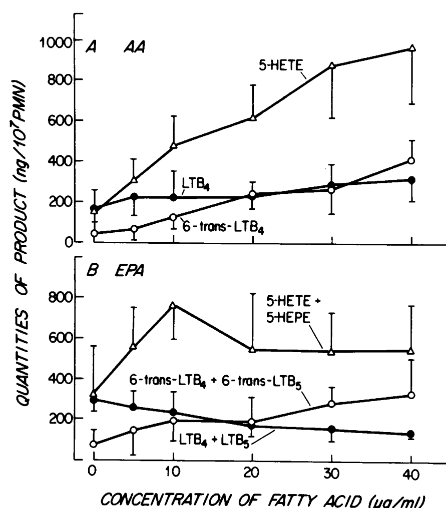

Figure 5. (*A*) The effects of increasing exogenous concentrations of AA on product generation by [^{14}C]AA-labeled PMNs (1 × 10^7 cells/ml) activated with ionophore. (*B*) Summation of the amounts of 5-HEPE and 5-HETE, LTB_5 and LTB_4, and 6-*trans*-LTB_5 and 6-*trans*-LTB_4 from the data shown in the top and bottom of Fig. 4.

with those of AA, the amounts of 5-HEPE and 5-HETE, LTB_5 and LTB_4, and 6-*trans*-LTB_5 and 6-*trans*-LTB_4 shown in Fig. 4 were summed at each dose of EPA (Fig. 5 *B*). The generation of the monohydroxy fatty acids (5-HEPE and 5-HETE) increased from 334±226 ng (mean±SEM, $n = 4$) in the absence of EPA to a peak of 760±155ng (mean±SEM, $n = 4$) at 10 μg/ml EPA and fell to a plateau of 550 ng at 20 μg/ml EPA. The quantities of 6-*trans*-LTB diastereoisomers (6-*trans*-LTB_5 and 6-*trans*-LTB_4) increased in a dose-dependent manner from 80±53 ng (mean±SEM, $n = 4$) without EPA to 321±176 ng (mean±SEM, $n = 4$) at 40 μg/ml EPA. The total quantities of LTB (LTB_5 and LTB_4) generated decreased in a dose-related fashion from 300±61 ng in the absence of EPA to 119±16 ng (mean±SEM, $n = 4$) at 40 μg/ml EPA.

The marked reductions in quantities of LTB_4 detected in the presence of increasing concentrations of EPA (Fig. 4) could have been due to decreased synthesis, increased ω-oxidation, or both. Therefore, the radioactive counts originating from membrane-derived [^{14}C]AA and eluting with the ω-oxidation products of LTB_4 during RP-HPLC were compared with those eluting with LTB_4. The sum of LTB_4 and its ω-metabolites increased to a plateau 5 min after cell activation, both in the absence and in the presence of EPA, with a continuing shift from LTB_4 to ω-metabolite(s) over the ensuing 10 min (Table I). As there was no significant difference in the percentage of total LTB_4 measured as ω-oxidation products at any time point in the presence and absence of EPA the reduced appearance of LTB_4 was attributed to decreased synthesis.

In one experiment the products generated by 1×10^7 PMNs activated by 10 μM ionophore for 5 min in the presence of increasing concentrations of EPA were resolved by RP-HPLC in solvent IV and quantitated by RIA and A_{269}

*Table I. Kinetic Analysis of LTB_4 and Its ω-Metabolites Produced by [^{14}C]AA-labeled PMNs (1×10^7 Cells/ml) Activated with 10 μM Calcium Ionophore A23187 in the Absence and in the Presence of 40 μg/ml EPA**

	No EPA		40 μg/ml EPA	
Length of incubation	Total LTB_4	% ω-metabolites§	Total LTB_4	% ω-metabolites
min	*cpm*‡		*cpm*	
2.5	781±165	23±5	258±66	20±3
5	1,033±395	34±12	404±44	30±12
10	917±325	55±22	407±34	50±22
15	1,010±282	61±12	376±40	59±19

* The LTB_4 and ω-metabolites of LTB_4 generated were resolved by RP-HPLC and quantitated in counts per minute; values are the mean±SEM of four experiments.

‡ LTB_4 and ω-metabolites of LTB_4.

§ Percentage of total LTB_4 as ω-metabolites.

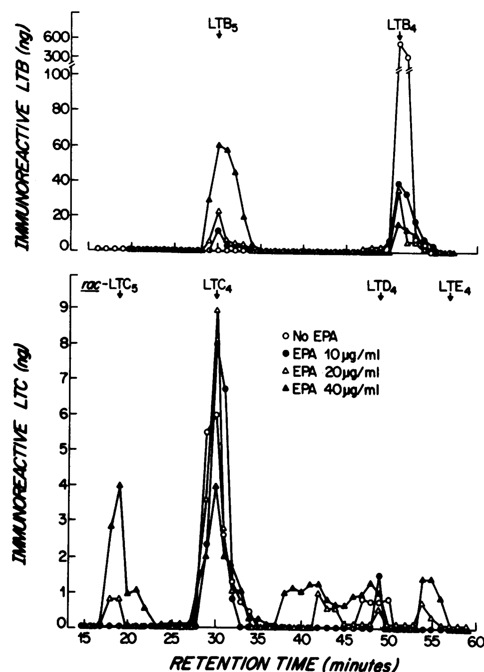

Figure 6. RP-HPLC of supernatants generated by PMNs (1×10^7 cells/ml) activated by ionophore in the absence and presence of increasing concentrations of EPA. The leukotriene concentrations of fractional eluates were measured by RIA. The retention times of synthetic reference standards have been indicated by the arrows.

(Fig. 6). In the absence of EPA, ionophore-activated PMNs produced 960 ng of LTB_4 by RIA and 925 ng by A_{269}, which eluted as one immunoreactive peak at the retention time of synthetic LTB_4 (51 min). With increasing concentrations of exogenous EPA, there was a dose-dependent decrease in the quantity of eluted immunoreactive LTB_4 to 44 ng by RIA and 55 ng by A_{269}, at 40 μg/ml EPA. LTB_5, which eluted as one immunoreactive peak at the retention time of synthetic LTB_5 (30 min) increased in a dose-dependent manner to a maximum of 218 ng by RIA and 196 ng by A_{269} at 40 μg/ml EPA. In the absence of EPA, there was a predominant immunoreactive peak eluting at the retention time of synthetic LTC_4 (30 min) and a minor peak eluting as LTD_4 (49 min). With 40 μg/ml EPA, three additional immunoreactive products eluted at peak retention times of 19, 41, and 55 min, respectively; the largest and most polar peak co-eluted with the *rac*-LTC_5 standard; the two smaller peaks eluting just before the LTD_4 and LTE_4 standards were presumed to be LTD_5 and LTE_5. In the absence of EPA a total of 18.1 ng of sulfidopeptide leukotrienes were detected, of which 15 ng was LTC_4 representing 1.8% of the quantity of LTB_4 measured in the same experiment. With increasing EPA concentrations, the quantities of LTC_4 rose to 18 ng at 10 μg EPA and fell to 11 ng with 40 μg EPA, whereas LTC_5 increased progressively to 9.3 ng at 40 μg/ml EPA. The

sum of LTC_4 and LTC_5 generated by 10^7 PMNs in the presence of increasing EPA concentrations exhibited an increment from 15 ng in the absence of EPA to 18.0, 17.6, and 20.3 ng at 10, 20, and 40 μg/ml EPA, respectively. The quantities of LTC_4 plus LTC_5 relative to LTB_4 and LTB_5 rose from 1.5% without EPA to 7.7% with 40 μg/ml EPA.

Because the quantities of tetraene sulfidopepide leukotrienes derived from LTC_4 were minimal, whereas the LTD_5 and LTE_5 were incompletely resolved by RP-HPLC and were estimated by an RIA without a specific standard, the quantitative effects of EPA on the sulfidopeptide leukotrienes are presented in terms of LTC_4 and LTC_5. The findings of a dose-related response of the pentaene sulfidopeptide leukotrienes to EPA would not be altered by the inclusion of the estimated values for the derivatives of LTC.

Identification and characterization of the PMN 5-lipoxygenase product derived from exogenous DCHA. The products of ionophore activation of 1×10^7 unlabeled PMNs, in the presence of 20 μg/ml exogenous unlabeled DCHA and 0.1 μCi/ml [^{3}H]DCHA/ml in two experiments were resolved by RP-HPLC, with the same results. The diastereoisomers of 6-*trans*-LTB_4 and LTB_4 eluted in solvent I at 19.0 min, 20.5, and 24.9 min, respectively. In solvent II, as assessed by A_{234}, a novel product incorporating radioactive counts from [^{3}H]DCHA eluted at 49.7 min, distinct from the elution of a 5-HETE standard at 51.4 min (Fig. 7). A minor peak of radioactivity eluted at 56 min, the retention time of the 4-hydroxy-DCHA standard. The products of the reaction with ~40 × 10^7 activated cells were resolved by RP-HPLC, and the major DCHA-derived product eluting in solvent II was shown to have a λ_{max} at 234.2 nm (mean, n = 2). The electron impact mass spectrum of the fully hydrogenated trimethylsilylether methyl ester derivative of this product yielded major mass ions at 313 (M^+-$C_7H_{13}O_2$) and 231 (M^+-$C_{15}H_{31}$), indicating that the underivatized compound was 7-HDCHA.

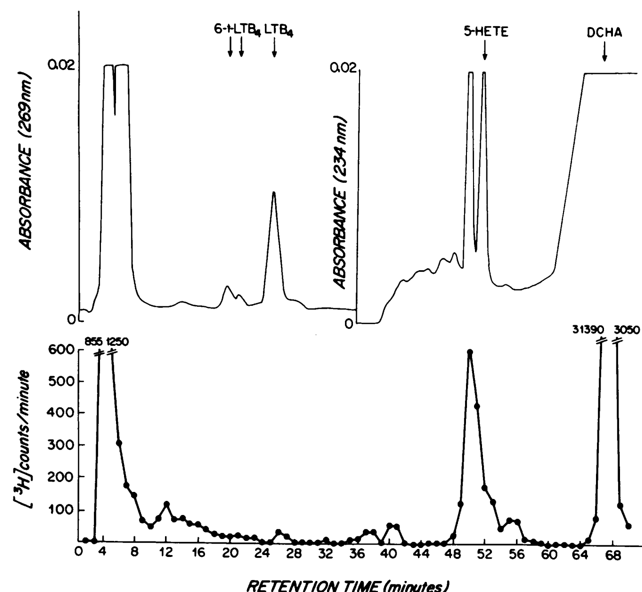

Figure 7. RP-HPLC of the supernatants from PMNs (1 × 10^7 cells/ml) activated by ionophore in the presence of 20 μg/ml DCHA and 0.1 μCi/ml [^{3}H]DCHA analyzed by integrated UV absorbance and radioactivity.

Effects of exogenous DCHA on the generation of individual 5-lipoxygenase pathway metabolites from DCHA and endogenous arachidonic acid. [^{14}C]AA-labeled PMNs (1 × 10^7 cells/ml) were activated by calcium ionophore in the absence and presence of increasing concentrations of DCHA and the products were resolved by RP-HPLC and quantitated by integrated UV absorbance (Fig. 8). The quantities of 5-HETE generated in the absence of DCHA, 303±66 ng (mean±SEM, n = 3) were not consistently altered with increasing concentrations of DCHA, being 347±63 ng at 5 μg/ml DCHA and 291±45 ng at 40 μg/ml DCHA. In contrast, there was a dose-related increase in the generation of 7-HDCHA to 250±111 ng (mean±SEM, n = 3) at 40 μg/ml DCHA (Fig. 8 *A*) such that the total production of monohydroxy products, as indicated by the sum of 5-HETE and 7-HDCA, increased from 303±66 to 541±165 ng at 40 μg/ml DCHA (Fig. 8 *B*). The production of 6-*trans*-LTB_4 was unaffected by incremental concentrations of exogenous DCHA, whereas the generation of LTB_4 was minimally reduced from 250±51 ng in the absence of DCHA to 148±35 ng at 40 μg/ml DCHA (Fig. 8 *B*).

Chemotactic activities of 5-HETE, 5-HEPE, and 7-HDCHA. The chemotactic activities of 5-HETE, 5-HEPE, and 7-HDCHA for human PMNs were compared with each other and with

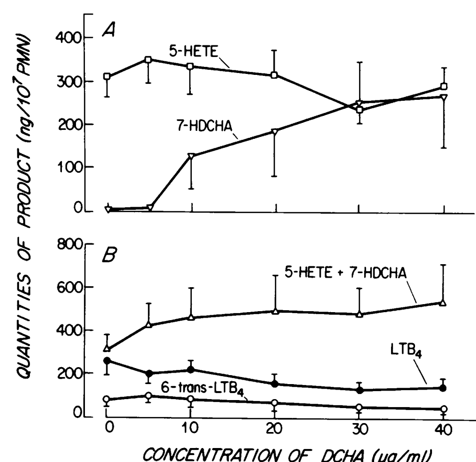

Figure 8. The effects of increasing concentrations of exogenous DCHA on the generation by ionophore-activated [^{14}C]AA-labeled PMNs of 5-HETE and 7-HDCHA (*A*) and of LTB_4 and 6-*trans*-LTB_4 (*B*); the sum of 5-HETE and 7-HDCHA is also depicted in *B*. The results are the mean±SEM of three experiments.

synthetic LTB_4 in three separate assays (Fig. 9). LTB_4 elicited a dose-related chemotactic response from 161±55 PMNs/5 hpf (mean±SEM, $n = 3$) at 1 nM to a plateau of ~600 PMNs/5 hpf at 0.1 μM. 5-HETE was inactive at 1 nM, attracted 131 PMNs/5 hpf at 50 nM, and elicited a maximum response of 216±50 PMNs/5 hpf (mean±SEM, $n = 3$) at 100 nM. By interpolation, 5-HETE at a concentration between 50 and 100 nM would elicit a response equal to 1 nM LTB_4, indicating that 5-HETE was 1–2% as potent as LTB_4. Furthermore, the maximum response to 5-HETE was only one-third that to LTB_4. 5-HEPE and 7-HDCHA were inactive as chemotactic agents at all the dosages tested.

Discussion

EPA and DCHA, the most prominent of the fatty acids in a fish oil-enriched diet (38), are shown to differ not only in their suitability as exogenous substrates for the enzymes of the 5-lipoxygenase pathway of ionophore-activated human PMNs but also in their capacity to modulate the utilization of endogenous AA by the same pathway. Because the instability of human PMNs in culture does not permit the incorporation of substantial amounts of EPA and DCHA into the cell membrane, these alternative substrates were introduced exogenously in order to assess their effects on the metabolism of endogenous membrane-derived AA by ionophore-activated PMNs. The reactions were carried out in the presence of BSA to prevent re-esterification of the released products (39), and the fatty acid substrates were trace radiolabeled in the initial experiments to allow definition of products by their retention times relative to synthetic standards during RP-HPLC (Figs. 2 and 7). The products resulting from the oxidative metabolism of exogenous EPA and DCHA and endogenous AA by ionophore-activated cells were then quantitated by integrated UV absorbance during resolution of products by RP-HPLC and, in addition, for the tetraene and pentaene leukotrienes, by RIA of the eluate fractions. The profile of 5-lipoxygenase pathway products included: 5-HETE, 6-*trans*-LTB_4, LTB_4, and the sulfidopeptide tetraene leukotrienes derived from endogenous AA; 5-HEPE, 6-*trans*-LTB_5, LTB_5, and the sulfidopeptide leukotrienes derived from EPA; and the predominant monohydroxy product, 7-HDCHA, derived from DCHA. The effect of the exogenous fatty acids on the generation of 5-lipoxygenase pathway products by activated human PMNs was quantitated for the eicosanoids of the tetraene series alone, for the sum of the tetraene and the pentaene eicosanoids, and for the sum of the tetraene and hexaene products. This approach permitted comparison of the two exogenous alternative fatty acids for their effects on the quantitative metabolism of endogenous AA by the 5-lipoxygenase pathway and for their associated suitability as simultaneous exogenous substrates.

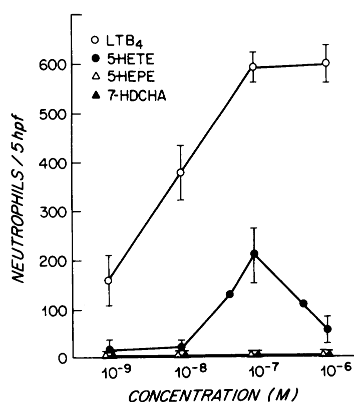

Figure 9. The chemotactic activities of synthetic LTB_4 (○) and PMN-derived 5-HETE (●), 5-HEPE (△), and 7-HDCHA (▲). Each point represents the mean±SEM of three separate assays with human PMNs from different donors performed in duplicate, except for 50 nM and 500 nM 5-HETE, which were the mean of two assays.

Neither of the exogenous alternative substrate fatty acids nor exogenous AA in concentrations of 5–40 μg/ml altered the release of endogenous [^{14}C]AA from ionophore-activated human PMNs (Fig. 1). The dose-response effects of exogenous EPA on the oxidative metabolism of endogenous AA were different for 5-HETE and 6-*trans*-LTB_4 than for LTB_4. There was a twofold increase in 5-HETE with 10 μg/ml EPA along with an ~50% increase in 6-*trans*-LTB_4; the generation of both products was suppressed with increasing doses of EPA, with 5-HETE production falling below base-line concentrations at 20 and 40 μg/ml EPA (Fig. 4 *A*). In contrast, the generation of LTB_4 was suppressed in a dose-related fashion, falling to half base-line values at 10 μg/ml EPA and being maximally suppressed at 20–40 μg/ml. The stimulation of the 5-lipoxygenase by low dose EPA could reflect activation by the formation of a hydroperoxy intermediate, as has been reported for activation of the cyclooxygenase by its prostaglandin endoperoxide (1). This could be due to 5-hydroperoxy eicosapentaenoic acid formed either because EPA is the preferred substrate for the 5-lipoxygenase (40), or, less likely, because EPA is oxidized nonenzymatically by the generation of superoxide from the respiratory burst stimulated by the presence of exogenous fatty acids (41). The selective suppression of LTB_4 formation was not due to a change in the time course of appearance of maximal amounts of LTB_4 in the presence of EPA (Fig. 3) and was not due to an increase in the conversion of LTB_4 to its ω-oxidation metabolite (Table I). Presumably, an intermediate of EPA metabolism, such as LTA_5, down regulates preferentially the conversion of LTA_4 to LTB_4 by the epoxide hydrolase.

The incubation of 5 μg/ml of exogenous EPA with ionophore-activated PMNs generated 5-HEPE, whereas a concentration of 10 μg/ml was required for the appearance of 6-*trans*-LTB_5 and LTB_5. There was a further increase in 5-HEPE and 6-*trans*-LTB_5 with incremental amounts of EPA, while the generation of LTB_5 did not change significantly (Fig. 4 *B*). The dose-response effects of exogenous EPA on the composite utilization of EPA and membrane-derived AA (Fig. 5 *B*) again revealed a quantitative difference between the formation of the monohydroxy and the 6-*trans*-LTB_4 products, as compared with the sum of LTB_4 and LTB_5. The additive

generation of 5-HEPE and 5-HETE was maximal at 10 μg/ml and plateaued at twice the base-line concentrations from 20–40 μg/ml. There was also an increase in the concentrations of 6-*trans*-LTB_4 and 6-*trans*-LTB_5 diastereoisomers, which was progressive throughout the dose range studied. In contrast, the additive generation of LTB_4 and LTB_5 decreased in a dose-related fashion to half the cumulative base-line levels at 30 μg/ml EPA. Thus, the metabolism of exogenous EPA facilitated the oxidative metabolism of both EPA and endogenous AA to the monohydroxy and 6-*trans*-LTB products while decreasing the generation of the epoxide hydrolase products, LTB_4 and LTB_5. The selective inhibition of the utilization of the intermediate epoxides by the epoxide hydrolase contrasts with their conversion by glutathionyl-*S*-transferase to the sulfidopeptide leukotrienes. Although the conversion of LTA_4 to LTC_4 in human PMNs is modest as compared with the formation of LTB_4 (3), incremental concentrations of exogenous EPA permitted increased formation of LTC_5, so that the sum of LTC_5 and LTC_4 from membrane-derived AA was greater than the formation of LTC_4 in the absence of EPA (Fig. 6). That the inhibition of epoxide hydrolase activity is apparently due to a specific effect of exogenous fatty acid, especially EPA, is indicated by the dose-dependent effects of exogenous AA. Incremental concentrations of exogenous AA resulted in an increased production of 5-HETE and 6-*trans*-LTB_4 by ionophore-activated human PMNs without a significant change in LTB_4 generation (Fig. 5 *A*). The progressive increase in the generation of 5-HETE by ionophore-activated PMNs in response to increasing exogenous AA concentrations contrasts with the generation of 5-HETE and 5-HEPE derived from membrane AA and exogenous EPA, respectively, in response to increasing concentrations of exogenous EPA (Fig. 5, *A* and *B*). The sum of the 5-HETE and 5-HEPE generated was maximal at 10 μg/ml EPA and then plateaued from 20 to 40 μg/ml EPA, suggesting that increasing concentrations of EPA limited the activity of the 5-lipoxygenase enzyme. There may well be two types of effects of exogenous EPA on the oxidative metabolism of endogenous AA by the 5-lipoxygenase pathway: dose-dependent stimulation and subsequent inactivation of 5-lipoxygenase by 5-HEPE, and selectively consistent inhibition of LTA_4 epoxide hydrolase by LTA_5.

The dose-response effect of exogenous DCHA on ionophore-activated human PMNs was characterized by the predominant generation of 7-HDCHA and possibly minor quantities of 4-HDCHA (42). 7-HDCHA production began at 10 μg/ml DCHA and increased in amount so as to equal the concomitant formation of 5-HETE, which was unchanged by the presence of this exogenous alternative fatty acid (Fig. 8). Thus, in the presence of exogenous DCHA, the production of monohydroxy products increased to approximately two times base-line values at 40 μg/ml. The production of 6-*trans*-LTB_4 was unaffected by the incremental inputs of DCHA, whereas the generation of LTB_4 was minimally reduced at 30 and 40 μg/ml. Thus, as compared with EPA, DCHA did not stimulate membrane-derived AA metabolism at a low dosage, did not appreciably inhibit LTB_4 formation, and was not a substrate suitable for the generation of leukotrienes. The apparent lack of competition by exogenous DCHA for utilization of endogenous AA may relate to its poor substrate function as compared with AA or EPA (40). Neither of the monohydroxy fatty acids of the alternative substrate fatty acids, 5-HEPE and 7-HDCHA, was biologically active as a chemotactic factor in an assay in which 5-HETE exhibited ~2% of the potency of LTB_4 (Fig. 9).

The respective capacities of EPA and DCHA to attenuate the oxidative metabolism of endogenous AA to pro-inflammatory products are distinctly different with regard to their action on the 5-lipoxygenase pathway, whereas both fatty acids are poor substrates and competitive inhibitors of the cyclooxygenase cascade (15–17). DCHA does not appreciably interfere with the metabolism of membrane-derived AA by ionophore-activated PMNs and is not converted to leukotrienes, as was also observed in subcellular systems (17). The capacity of a fish oil enriched diet to attenuate the ex vivo production of LTB_4 by ionophore-activated human neutrophils has been observed with an intake of 7 to 12 g EPA/d, combined with a restriction in dietary vegetable and animal fats in four volunteers for 3 wk; the neutrophils obtained after EPA consumption elaborated 25% less LTB_4, as compared with that generated by neutrophils obtained in the pre-diet period. Production of LTB_5 was increased so that total generation of LTB compounds was not reduced (43). In contrast, in six volunteers receiving 4 g EPA for 4 wk without additional alteration of their usual diets there was no suppression of LTB_4 production; the ionophore-activated cells generated LTB_4 and LTB_5 in a ratio that corresponded to the representation of AA and EPA in the membrane phospholipids, suggesting that dosage may be an important determinant of the effects of EPA on the 5-lipoxygenase pathway (44). The conditions used in the present in vitro studies maximized the effects of EPA as an alternative fatty acid and demonstrated the dose-related suppression of LTB_4 production.

Acknowledgments

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