Formation of a 5-oxo metabolite of 5,8,11,14,17-eicosapentaenoic acid and its effects on human neutrophils and eosinophils

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Abstract We recently showed that human neutrophils convert arachidonic acid to its 5-oxo metabolite, 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE). 5-Oxo-ETE, which is synthesized by oxidation of 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) by a highly specific microsomal dehydrogenase, is a potent stimulator of human neutrophils and eosinophils. The objective of the current investigation was to determine whether neutrophils can convert 5,8,11,14,17-eicosapentaenoic acid (EPA) to its 5-oxo metabolite, 5-oxo-6,8,11,14,17-eicosapentaenoic acid (5-oxo-EPE) and, if so, to compare the biological activities of 5-oxo-EPE and 5-oxo-ETE. The two major eicosanoids formed by neutrophils incubated with EPA in the presence of A23187 were 5-hydroxy-6,8,11,14,17-eicosapentaenoic acid (5-HEPE) and 5-oxo-EPE. Smaller amounts of LTB5 and 20-hydroxy-LTB5 were also formed. Phorbol myristate acetate stimulated the formation of 5-oxo-EPE from both EPA and 5-HEPE. 5-HEPE and 5-HETE were equally good substrates for 5-hydroxyeicosanoid dehydrogenase (*K*m, ca. 0.85 μM; *V*max, ca. 1.4 pmol/min per μg protein). 5-Oxo-EPE mobilized calcium in neutrophils with an EC50 of 36 nM, about 10 times higher than that of 5-oxo-ETE. 5-Oxo-EPE was also about one-tenth as active as 5-oxo-ETE in stimulating the migration of both human neutrophils and human eosinophils. It is concluded that 5-oxo-EPE is readily formed from EPA via 5-HEPE. However, it is only about one-tenth as potent as 5-oxo-ETE in stimulating human neutrophils and eosinophils. These results support the contention that EPA can alleviate certain inflammatory diseases by reducing the contribution of arachidonate-derived eicosanoids.—**Powell, W. S., S. Gravel, and F. Gravelle.** Formation of a 5-oxo metabolite of 5,8,11,14,17-eicosapentaenoic acid and its effects on human neutrophils and eosinophils. *J. Lipid Res.* 1995. **36:** 2590–2598.

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Arachidonic acid (AA) and 5,8,11,14,17-eicosapentaenoic acid (EPA) are both metabolized by the 5-lipoxygenase pathway in neutrophils. The major initial stable metabolites of AA are leukotriene B4 (LTB4) and 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) (1), whereas EPA is converted to the corresponding ω-3 (n−3) compounds, LTB5 and 5-HEPE (2, 3). LTB4 is rapidly metabolized by two pathways in neutrophils: ω-oxidation by a specific cytochrome P450 (4–6) and oxidation of the 12-hydroxyl group by a 12-hydroxyeicosanoid dehydrogenase, followed by reduction of the 10,11-double bond (7, 8). In both cases, this results in substantial losses in biological activity (9, 10). LTB5 is a good substrate for ω-oxidation (11) and is also metabolized by the 12-hydroxyeicosanoid dehydrogenase/10,11-reductase pathway, although somewhat more slowly than LTB4 (12).

Like LTB4, 5-HETE is converted to its 20-hydroxy metabolite by LTB4 20-hydroxylase in neutrophils (13). In the presence of eosinophils or platelets, it can also be converted to 5S,15S-dihydroxy (14) or 5S,12S-dihydroxy (15) metabolites. We have recently discovered another pathway for the metabolism of 5-HETE in neutrophils (16) and eosinophils (17) that results in the oxidation of the 5-hydroxyl group by a highly specific NADP+-dependent dehydrogenase to give 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE). Metabolism of 5-HETE by this pathway is enhanced by phorbol 12-myristate 13-acetate (PMA) and zymosan which, by stimulating

Abbreviations: AA, arachidonic acid; EPA, 5,8,11,14,17-eicosapentaenoic acid; 5-HETE, 5-hydroxy-6,8,11,14-eicosatetraenoic acid; 5-HEPE, 5-hydroxy-6,8,11,14,17-eicosapentaenoic acid; 5-oxo-ETE, 5-oxo-6,8,11,14-eicosatetraenoic acid; 5-oxo-EPE; 5-oxo-6,8,11,14,17-eicosapentaenoic acid; 5,20-diHEPE, 5,20-dihydroxy-6,8,11,14,17-eicosapentaenoic acid; 5-oxo-20-hydroxy-EPE, 5-oxo-20-hydroxy-6,8,11,14,17-eicosapentaenoic acid; LT, leukotriene; PG, prostaglandin; PAF, platelet-activating factor; PMA, phorbol 12-myristate 13-acetate; RP-HPLC, reversed phase-high pressure liquid chromatography; PMNL, polymorphonuclear leukocytes; PBS, phosphate-buffered saline.

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NADPH oxidase, raise intracellular levels of NADP+ (18). In contrast to LTB4, which loses much of its biological activity as a result of oxidation by the hydroxylase or dehydrogenase pathways, oxidation of 5-HETE by 5-hydroxyeicosanoid dehydrogenase results in a 100-fold enhancement in biological activity (19). 5-Oxo-ETE has been shown to be a potent stimulator of a variety of neutrophil responses, including mobilization of cytosolic calcium, migration, degranulation, and aggregation (19–21). It is also a potent stimulator of human eosinophil migration (17, 22) and calcium mobilization (22). 5-Oxo-ETE is more effective than platelet-activating factor (PAF) and LTB4 in stimulating migration of the latter cells, and appears to have a synergistic effect with PAF (17). 5-Oxo-ETE could thus be an important inflammatory mediator in diseases involving infiltration of granulocytes.

EPA can interfere with biological processes dependent on n-6-eicosanoids by two major mechanisms: *i)* inhibition of the formation of n-6-eicosanoids from arachidonic acid and *ii)* the production of n-3-eicosanoids with reduced biological potencies compared to the corresponding n-6 compounds. It would appear likely that these effects contribute to the beneficial effects of dietary fish oil in cardiovascular and inflammatory diseases (23). The objective of the current investigation was to determine whether EPA could be converted to the n-3 analogue of 5-oxo-ETE, 5-oxo-EPE, and, if so, to compare the biological activities of these two eicosanoids.

MATERIALS AND METHODS

Materials

5-HEPE (Cayman Chemicals, Ann Arbor, MI) and 5-oxo-ETE (Cascade Biochem Ltd., Reading, UK) were obtained from commercial sources. AA (Nu-Chek Prep Inc, Elysian, MN) and EPA (Cayman Chemicals) were purified by reversed-phase high pressure liquid chromatography (RP-HPLC) just prior to use (24). 19-Hydroxy-PGB1 was prepared as described in the literature (25). PMA and NADP+ were obtained from the Sigma Chemical Co, St. Louis, MO, whereas A23187 was obtained from Calbiochem, LaJolla, CA. Indo-1 was purchased from Molecular Probes, Eugene, OR.

Preparation of human polymorphonuclear leukocytes (PMNL)

PMNL were prepared by treatment of blood from healthy subjects with Dextran T-500 (Pharmacia), followed by centrifugation over Ficoll-Paque (Pharmacia) and removal of remaining red blood cells by hypotonic lysis (26). The cells were resuspended in Ca2+/Mg2+-free Dulbecco's phosphate-buffered saline (PBS) containing 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH2PO4, and 8.1 mM Na2HPO4 at a pH of 7.4. These cells were between 95 and 98% neutrophils.

Preparation of eosinophils

Eosinophils were purified from the PMNL fraction described above by negative selection using an immunomagnetic procedure as described in the literature (27). PMNL (2 × 108 cells in 200 µl PBS) were incubated with 200 µl of a suspension of a monoclonal antibody to CD16 conjugated to magnetic microbeads (Miltenyi Biotec Inc., Bergisch-Gladbach, Germany). After incubation for 30 min at 8°C, the cells were passed through a column containing a steel matrix placed in a permanent magnet (MACS; Miltenyi Biotec Inc). Magnetically labeled neutrophils were retained on the column, whereas eosinophils (91 ± 2% pure) were obtained in the pass-through fraction in a volume of 15 ml. After washing by centrifugation at 200·*g* for 10 min, the cells were suspended in either PBS or RPMI 1640 medium (Gibco BRL).

Preparation of microsomal fractions from PMNL

PMNL were sonicated in PBS containing phenylmethylsulfonyl fluoride (2 mM), dithiothreitol (1 mM), and EDTA (1 mM) as described previously (18). The post-nuclear supernatant, obtained by centrifugation at 1500 *g* at 4°C for 10 min, was centrifuged at 10,000 *g* at 4°C for 10 min and the supernatant was centrifuged at 200,000 *g* at 4°C for 60 min. The pellet was resuspended in PBS containing 1.8 mM CaCl2 and 1 mM MgCl2 at a concentration equivalent to 4 × 106 cells/ml.

Analysis of eicosanoids

Neutrophils (5 × 106 cells/ml) were preincubated with PMA (30 nM) for 6 min, followed by incubation with either 5-HEPE (2 µM) or EPA (30 µM) and A23187 (5 µM) for various times. Incubations were terminated by the addition of methanol (0.6 ml). The samples were diluted with water to give a final concentration of 15% MeOH and the internal standard (120 ng 19-hydroxy-PGB1) was added. Eicosanoids were quantitated by automated precolumn extraction/reversed-phase high pressure liquid chromatography (RP-HPLC) as previously described (24, 28). The stationary phase used for analysis of 5-HEPE metabolites was a column (150 × 3.9 mm) of Resolve C18 (Waters Ltd., Missisauga, Ontario), whereas a column (150 × 4.6 mm) of Ultracarb 5 µ ODS (Phenomenex, Torrance, CA) was used for the analysis of metabolites formed after incubation of neutrophils with EPA. The mobile phases were gradients between solvent A (water–acetonitrile–acetic acid 80:20:0.02) and solvent B (water–acetonitrile–methanol–acetic acid

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7.5:38.5:54:0.02) as follows: 5-HEPE metabolites (20 to 95% B over 45 min); EPA metabolites (35 to 95% B over 60 min). The flow rate was 1 ml/min.

Measurement of cytosolic calcium

Neutrophils (107 cells/ml) were loaded in calcium- and magnesium-free PBS with the fluorescent dye indo-1 as previously described (19). Calcium measurements were performed at 37°C using a Hitachi Model F-4000 fluorescence spectrophotometer equipped with a magnetic stirrer. The excitation and emission wavelengths were set at 331 nm (3 nm slit width) and 410 nm (10 nm slit width), respectively. Prior to the addition of agonists, CaCl2 and MgCl2 were added to give final concentrations of 1 mM of each. Responses to 5-oxo-EPE and 5-oxo-ETE were measured after stabilization of the baseline fluorescence. *F*max was determined by adding digitonin (final concentration, 0.1%), whereas *F*min was calculated after determination of autofluorescence as described in the literature (29, 30).

Measurement of cell migration

Cell migration was measured by the modified Boyden technique using 48-well microchemotaxis chambers (Neuro Probe Inc., Cabin John, MD) and Sartorius cellulose nitrate filters (8 μm pore size; 140 μm thickness) (Neuro Probe Inc.) (31, 32). The filters were soaked in PBS containing calcium (1 mM), magnesium (1 mM), and 0.3% BSA before use. The substance to be tested was added to the bottom well in a volume of 30 μl PBS containing Ca2+, Mg2+, and 0.3% BSA, whereas neutrophils or eosinophils (150,000 cells in 55 μl PBS containing Ca2+, Mg2+, and 0.4% ovalbumin) were added to each of the top wells. Agonists were added in DMSO–water to give a final concentration of DMSO in the bottom chambers of 0.5% in all cases. The chambers were incubated for 2 h at 37°C in 5% CO2 and humidified air. The filters were then fixed by overnight immersion in a saturated solution of mercuric chloride (Eastman Kodak, Rochester, NY) in 50% aqueous ethanol, and the cells were stained using hematoxylin (Canada Wide Scientific) followed by chromotrope 2R (Sigma Chemical Co.) as described in the literature (33). The filters were mounted on slides with coverslips using Permount (Fisher Scientific Ltd., Ottawa, Ontario) and the numbers of cells on the bottom surfaces of the filters were counted in 10 different fields at a magnification of 400 × for each incubation, each of which was performed in triplicate. In cases in which there were large numbers of cells on the bottom surfaces of the filters, only quarter or half fields were counted and the results were corrected accordingly.

RESULTS

Neutrophils convert EPA to 5-oxo-EPE

Neutrophils were incubated for 20 min with EPA in the presence of A23187 (5 μM) and PMA (30 nM) and the products were analyzed by RP-HPLC (**Fig. 1**). Under these conditions EPA was converted to 5-HEPE, 5-oxo-EPE, LTB5, 6-*trans*-LTB5, 12-epi-6-*trans*-LTB5, and 20-hydroxy-LTB5. Due to the release of endogenous AA in response to A23187, the corresponding AA metabolites were also present. The amounts of the major metabolites of EPA formed by neutrophils in the presence of A23187 are shown in **Fig. 2A.** The most abundant metabolite of EPA was 5-HEPE, followed by 5-oxo-EPE. Smaller amounts of LTB5 and 20-hydroxy-LTB5 were formed. PMA stimulated the formation of 5-oxo-EPE but had no significant effects on the formation of any of the other metabolites of EPA. In contrast, PMA stimulated the formation of all of the corresponding metabolites of endogenous AA by neutrophils incubated with EPA and A23187 (Fig. 2B).

Consistent with the findings of others (3), exogenous EPA inhibited the formation of LTB4 from endogenous AA in A23187-stimulated neutrophils by 94 ± 6%. The synthesis of 5-oxo-ETE was also strongly inhibited by 72 ± 1% by EPA (data not shown). PMA slightly diminished the inhibitory effect of EPA on LTB4 formation by

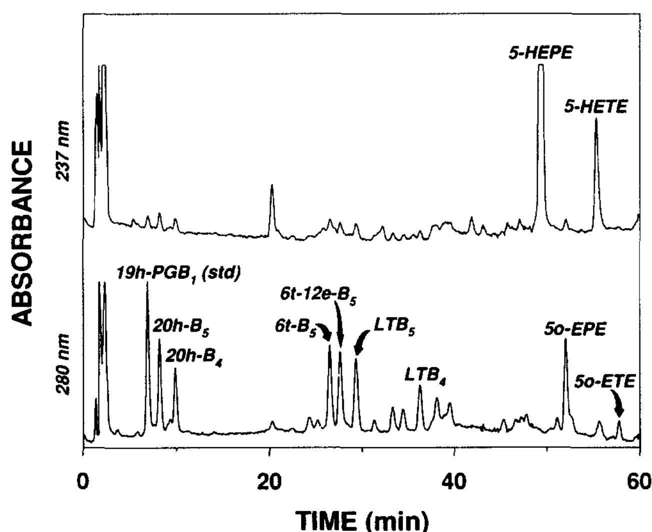

Fig. 1. Chromatogram of eicosanoids synthesized by neutrophils incubated with EPA in the presence of A23187 and PMA. Neutrophils (5×10^6 cells in 1 ml) were preincubated for 6 min with PMA (30 nM) and then incubated for a further 20 min with EPA (30 μM) and A23187 (5 μM). 19-Hydroxy-PGB1 (120 ng) was added as an internal standard and the products were analyzed by precolumn extraction/RP-HPLC using a column of Ultracarb ODS as described in Materials and Methods. UV absorbance was monitored at 237 nm (top tracing) and 280 nm (bottom tracing). Abbreviations: 19h-PGB1, 19-hydroxy-PGB1; 20h-B4, 20-hydroxy-LTB4; 20h-B5, 20-hydroxy-LTB5; 6t-B5, 6-*trans*-LTB5; 6t-12e-B5, 6-*trans*-12-epi-LTB5; 5o-EPE, 5-oxo-EPE; 5o-ETE, 5-oxo-ETE.

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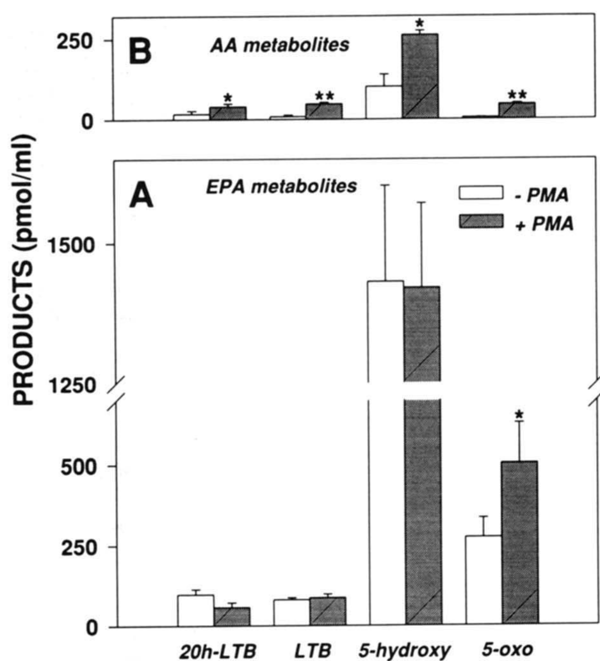

Fig. 2. Synthesis of eicosanoids by neutrophils incubated with EPA and A23187 in the presence or absence of PMA. Neutrophils (5×10^6 cells in 1 ml) were preincubated for 6 min in the presence of either vehicle (3 µl DMSO; open bars) or PMA (30 nM; shaded bars). EPA (30 µM) and A23187 (5 µM) were added and the cells were incubated for a further 20 min. The products were analyzed by precolumn extraction/RP-HPLC as described in Materials and Methods. A: Metabolites derived from EPA (i.e., 20-hydroxy-LTB_5, LTB_5, 5-HEPE, and 5-oxo-EPE). B: Metabolites derived from endogenous AA in the same incubations as described above (i.e., 20-hydroxy-LTB_4, LTB_4, 5-HETE, and 5-oxo-ETE).

A23187-stimulated neutrophils. Under these conditions EPA inhibited the formation of LTB4 by 71 ± 9%. In contrast, EPA had no effect on 5-oxo-ETE formation by neutrophils stimulated by A23187 in the presence of PMA (8 ± 22% inhibition; data not shown).

PMA stimulates the formation of 5-oxo-EPE from 5-HEPE

To look more specifically at the conversion of 5-HEPE to 5-oxo-EPE, the former substance was incubated with neutrophils for 40 min in the presence of PMA (30 nM) and the products were analyzed by RP-HPLC. As shown in **Fig. 3,** the major metabolite of 5-HEPE under these conditions was 5-oxo-EPE. Smaller amounts of the ω-oxidation products of 5-HEPE and 5-oxo-EPE, 5,20-diHEPE and 5-oxo-20-hydroxy-EPE were also formed. The peaks for the latter two metabolites were preceded by shoulders, which were presumably the corresponding ω-carboxy metabolites.

Time courses for the metabolism of 5-HEPE by neutrophils in the presence and absence of PMA are shown in **Fig. 4.** The values for 5,20-diHEPE include both this compound and its ω-carboxy metabolite, ω-carboxy-5-HEPE, as these two compounds were not well resolved by the HPLC conditions used. In the absence of PMA, 5,20-diHEPE was formed very rapidly, reaching a plateau at 40 min, and was the major product at all time points (Fig. 4). Only small amounts of 5-oxo-EPE were formed. The amounts of the latter substance reached a maximum after 20 min and then declined. PMA strongly stimulated the formation of 5-oxo-EPE and inhibited the formation of 5,20-diHEPE at all time points (Fig. 4).

5-HEPE and 5-HETE are equally good substrates for 5-hydroxyeicosanoid dehydrogenase

We examined the ability of microsomal fractions from neutrophils to oxidize 5-HEPE and 5-HETE to their 5-oxo metabolites. Various concentrations of these substrates were incubated with neutrophil microsomes for 10 min at 37°C in the presence of NADP+ (1 mM). Under these conditions the only major products were 5-oxo-EPE and 5-oxo-ETE. A low concentration of microsomes (ca. 25 µg protein/ml) was used so that not more than 20% of the substrate was metabolized. Lineweaver-Burk analysis indicated that 5-HEPE and 5-HETE are equally good substrates for 5-hydroxyeicosanoid dehydrogenase (**Fig. 5**). The *K*m values (n = 3) calculated for 5-HEPE and 5-HETE were 0.84 ± 0.05 and 0.89 ± 0.10 µM, respectively, whereas the *V*max values for these two substrates were 1.46 ± 0.08 and 1.37 ± 0.14 pmol/min per µg protein.

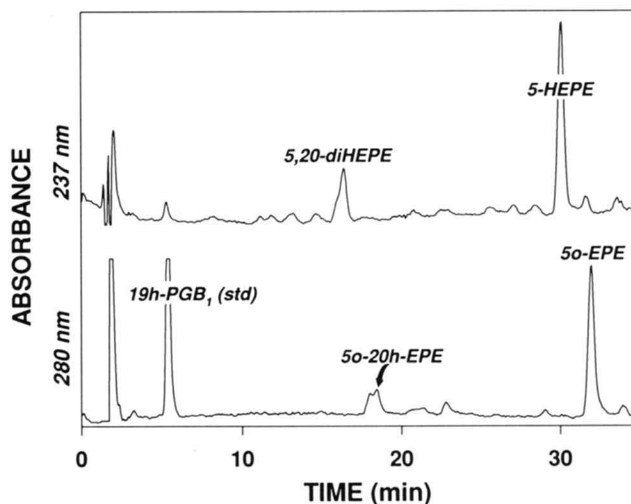

Fig. 3. Chromatogram of eicosanoids synthesized by neutrophils incubated with 5-HEPE in the presence of PMA. Neutrophils (2.5×10^6 cells in 0.5 ml) were preincubated for 6 min with PMA (30 nM) and then incubated for a further 40 min with 5-HEPE (2 µM). 19-Hydroxy-PGB_1 (120 ng) was added as an internal standard and the products were analyzed by precolumn extraction/RP-HPLC using a column of Resolve C_{18} as described in Materials and Methods. UV absorbance was monitored at 237 nm (top tracing) and 280 nm (bottom tracing). Abbreviations: 19h-PGB_1, 19-hydroxy-PGB_1; 5o-EPE, 5-oxo-EPE;

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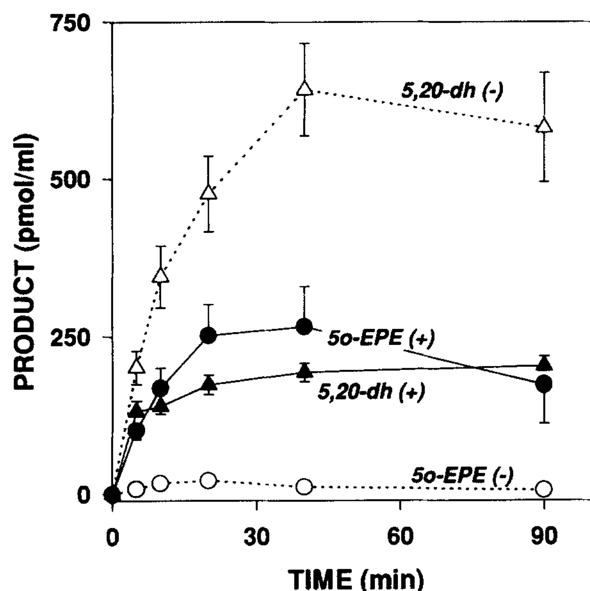

Fig. 4. Time courses for the formation of 5-oxo-EPE (●, ○; 5o-EPE) and 5,20-diHETE (▲, △; 5,20-dh) in the presence (closed symbols; +) and absence (open symbols; -) of PMA (30 nM). Neutrophils (2.5×10^6 cells in 0.5 ml) were preincubated for 6 min with PMA (30 nM) and then incubated for various times with 5-HEPE (2 μM). The products were analyzed by precolumn extraction/RP-HPLC using a column of Resolve C_{18} as described in Materials and Methods.

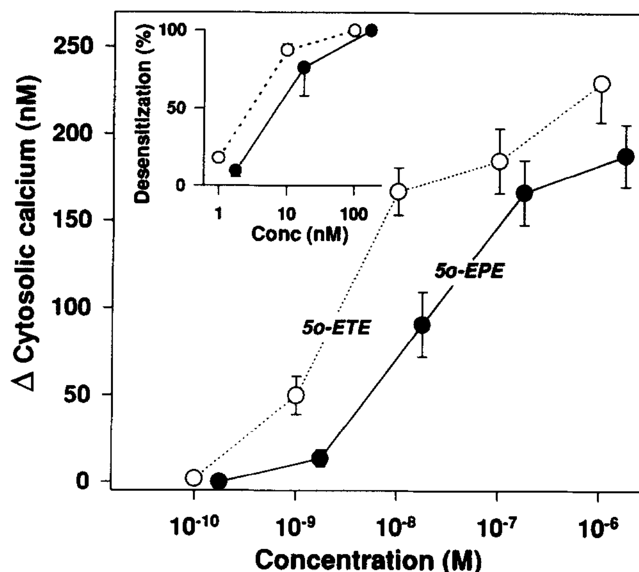

Fig. 6. Effects of 5-oxo-EPE and 5-oxo-ETE on cytosolic calcium levels in human neutrophils. Neutrophils loaded with the fluorescent dye Indo-1 were treated with either 5-oxo-EPE (●, 5o-EPE; n = 4) or 5-oxo-ETE (○, 5o-ETE; n = 4), and the changes in fluorescence were monitored. Changes in the levels of cytosolic calcium were determined as described in Materials and Methods. The results are expressed as increases above the basal levels of calcium in the cells. The inset shows the effects of various concentrations of 5-oxo-EPE (●; n = 4) and 5-oxo-ETE (○; n = 4) on calcium changes induced by the subsequent addition, 2.5 min later, of 5-oxo-ETE (100 nM). The results are expressed as % desensitization = 100 - $((\Delta Ca^{2+}_{after})/(\Delta Ca^{2+}_{before}) \times 100)$, where ΔCa^{2+}_{after} is the change in the level of calcium in response to 5-oxo-ETE after pretreatment with either 5-oxo-EPE or 5-oxo-ETE and ΔCa^{2+}_{before} is the change in the level of calcium in response to 5-oxo-ETE without pretreatment.

5-Oxo-EPE mobilizes cytosolic calcium in neutrophils but is less potent than 5-oxo-ETE

In agreement with previous work from our laboratory (19), 5-oxo-ETE was a potent stimulus of cytosolic calcium levels in human neutrophils (EC50, 3.5 nM). 5-Oxo-EPE also raised calcium levels in these cells, but had an EC50 value (36 nM) about 10 times higher than that of 5-oxo-ETE (**Fig. 6**). The maximal responses to the two agonists were similar. Pretreatment of neutrophils with 5-oxo-EPE (180 nM) completely blocked the response of these cells to 5-oxo-ETE (100 nM) (Fig. 6, inset). The IC50 for 5-oxo-EPE-induced desensitization to 5-oxo-ETE was 7.2 nM, compared to an IC50 of 3 nM for 5-oxo-ETE-induced desensitization to itself.

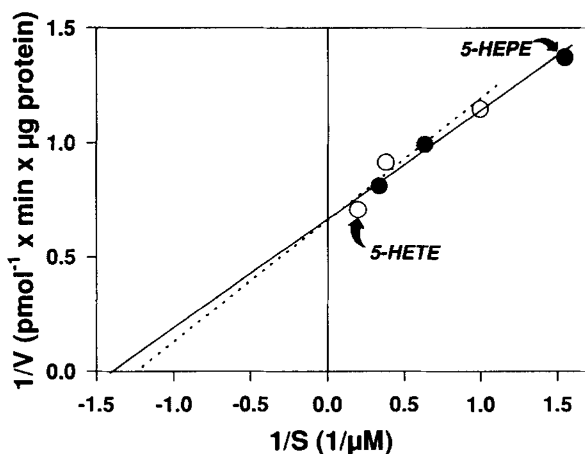

Fig. 5. Lineweaver-Burk plots for the metabolism of 5-HEPE and 5-HETE by a microsomal fraction from human neutrophils. PMNL microsomes (25 μg protein/ml; equivalent to 4×10^6 PMNL/ml) were incubated with different concentrations of 5-HEPE (●) or 5(S)-HETE (○) for 10 min and the products were quantitated by precolumn extraction/RP-HPLC as described in Materials and Methods. The results are means of incubations performed in duplicate and are representative of three such experiments.

5-Oxo-EPE is less potent than 5-oxo-ETE in stimulating granulocyte migration

We previously showed that 5-oxo-ETE stimulates the migration of neutrophils (19) and eosinophils (17) across a filter in a Boyden chamber. 5-Oxo-EPE also stimulates migration of both neutrophils (**Fig. 7A**) and eosinophils (Fig. 7B), but, as was observed for calcium mobilization, the concentration–response curve was shifted to the right by nearly one order of magnitude. It is not clear whether the maximal response was attained to either 5-oxo-ETE or 5-oxo-EPE at the highest concentrations used (1 μM and 1.8 μM, respectively), so it is not possible to determine the EC50 values for this effect.

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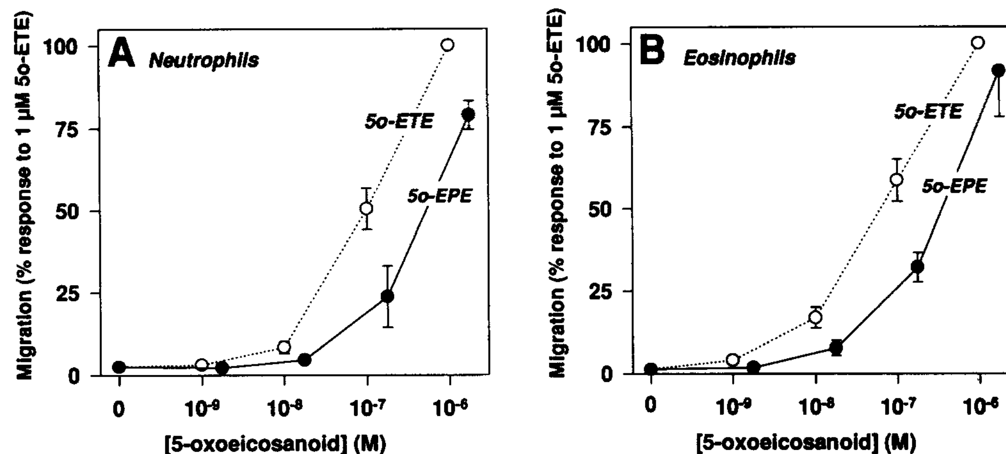

Fig. 7. Effects of 5-oxo-EPE and 5-oxo-ETE on the migration of neutrophils (A) and eosinophils (B). Effects on cell migration were determined using modified Boyden chambers as described in Materials and Methods. The bottom wells contained various concentrations of either 5-oxo-EPE (●) or 5-oxo-ETE (○), whereas the top chambers contained neutrophils or eosinophils (150,000 cells in 55 μl medium). After incubation for 2 h at 37°C, the filters were stained and the numbers of cells that had migrated completely through to the bottom of the filter were counted under a microscope. The numbers of cells in 10 high power (× 400) fields were counted for each incubation. The values are the means ± SE of determinations on neutrophils from four different individuals and are expressed as a percent of the response to 1 μM 5-oxo-ETE. The mean values obtained for 5-oxo-ETE (1 μM) and control (diluent) for neutrophils were 1239 ± 235 and 30 ± 5 cells per 10 high power fields, respectively. For eosinophils, the corresponding values were 3029 ± 252 and 39 ± 9 cells per 10 high power fields, respectively.

Unfortunately, we were able to prepare only limited amounts of 5-oxo-EPE for biological testing.

DISCUSSION

EPA appears to be a substrate for all of the pathways of metabolism of AA. In agreement with others (3) we found that neutrophils convert EPA to 5-HEPE and LTB5, as well as to 6-*trans* isomers and ω-oxidation products of the latter substance. However, in addition to these products, substantial amounts of 5-oxo-EPE were also detected. PMA selectively stimulated the formation of 5-oxo-EPE from exogenous EPA, having little effect on the formation of other EPA metabolites. This is presumably due to the activation of NADPH oxidase, the enzyme responsible for the respiratory burst in neutrophils. This would result in conversion of NADPH to NADP+, the cofactor required by 5-hydroxyeicosanoid dehydrogenase, which is the enzyme responsible for the formation of 5-oxo-EPE. In agreement with this, PMA strongly stimulated the conversion of 5-HEPE to 5-oxo-EPE by neutrophils, and inhibited the formation of 5,20-diHEPE, which would require NADPH.

In contrast to its limited effects on the formation of metabolites of exogenous EPA, PMA stimulated the formation of all the metabolites of endogenous AA that were measured (5-oxo-ETE, 5-HETE, LTB4, and 20-hydroxy-LTB4) in neutrophils incubated with EPA and A23187. This is presumably due to the protein kinase C-dependent activation of the cytosolic form of phospholipase A2 (34), which would enhance the release of endogenous AA from neutrophils.

In agreement with the work of others (3), we found that EPA inhibits the formation of LTB4 by A23187-stimulated neutrophils, both in the presence and absence of PMA. EPA also inhibited the formation of 5-oxo-ETE by neutrophils stimulated by A23187. However, this inhibitory effect was not observed in the presence of PMA. This may be due to the stimulatory effect of PMA on the release of endogenous AA from neutrophils as noted above. Furthermore, PMA could increase the capacity of 5-hydroxyeicosanoid dehydrogenase by increasing the levels of intracellular NADP+. EPA has also been reported to inhibit the formation of prostanoids (35–37), although this is not true for all eicosanoids. At a concentration similar to the one used in the present study, EPA was shown to stimulate the formation of LTC4 by A23187-stimulated human eosinophils (38).

The proportion of LTA4 converted to LTB4 by neutrophils incubated with EPA in the presence of PMA and A23187 appeared to be greater than the proportion of LTA5 converted to LTB5, based on the ratios of the amounts LTB4 and LTB5 to their respective 6-*trans* isomers (Fig. 1). Moreover, in stimulated neutrophils, the amount of LTB4 formed from endogenous AA was nearly half the amount of LTB5 formed from a presumably much greater amount of exogenous substrate (Fig. 2). This is probably due to the fact that LTA4 is a better

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substrate than LTA5 for LTA hydrolase (39). In contrast, the ratio of 5-oxo-EPE to 5-HEPE (0.36 ± 0.08) was higher than the ratio of 5-oxo-ETE to 5-HETE (0.18 ± 0.02) ($P < 0.05$). This difference may be due, at least in part, to the fact that the exogenous EPA was immediately available for metabolism, whereas there would be a delay in the release of endogenous AA. It is also possible that 5-oxo-ETE could be metabolized more rapidly than 5-oxo-EPE under these conditions. The amount of 5-oxo-ETE formed from endogenous AA was less than one-tenth the amount of 5-oxo-EPE formed from exogenous EPA. Thus, although AA appears to be a better substrate than EPA for the formation of B leukotrienes, this does not appear to be the case for the formation of 5-oxoeicosapolyenoic acids. This is supported by the finding that 5-HETE and 5-HEPE are equally good substrates for oxidation to their 5-oxo metabolites by neutrophil microsomes (Fig. 5).

5-Oxo-EPE is approximately one-tenth as potent as 5-oxo-ETE in raising cytosolic calcium levels in neutrophils and in inducing migration of both neutrophils and eosinophils. This is somewhat surprising, in view of the substantial distance between the Δ17-double bond and the 5-oxo group, which is presumably the most important determinant of the biological activity of these compounds. This suggests that the ω-end of the molecule may be important for its biological activity and might be involved in the interaction of 5-oxo-ETE with its receptor, if indeed such a receptor exists. In agreement with this, recent work from our laboratory indicates that addition of a hydroxyl group to the 20-position of 5-oxo-ETE results in an even greater reduction in biological activity (unpublished results). 5-Oxo-EPE appeared to be somewhat more active in desensitizing neutrophils to 5-oxo-ETE than in directly stimulating calcium levels in these cells, as the IC50 for desensitization is only about 2.5 times greater than that of 5-oxo-ETE. This raises the possibility that analogues of 5-oxo-ETE in which the ω-end of the molecule has been altered could possibly act as antagonists.

The lower biological activity of 5-oxo-EPE is consistent with other studies indicating that certain other n-3-eicosanoids also have lower potencies than their n-6 counterparts. LTB5 has been shown to be about 10–30 times less potent than LTB4 in stimulating chemotaxis (40, 41) and calcium mobilization (42) in neutrophils. In our hands, LTB5 (EC50, 12 nM) was 26 times less potent than LTB4 (EC50, 0.5 nM) in mobilizing intracellular calcium (data not shown). In contrast, the presence of a Δ17-double bond appears to have little effect on the biological activities of cysteinyl-LTs (43, 44).

Our findings on the biosynthesis and biological effects of 5-oxo-EPE lend further support to the concept that dietary fish oil could have a beneficial effect in inflammatory diseases in which AA metabolites are involved. 5-HEPE is a major product of EPA metabolism by neutrophils and can be converted to 5-oxo-EPE by 5-hydroxyeicosanoid dehydrogenase. 5-Oxo-EPE has only about one-tenth the potency of 5-oxo-ETE in stimulating neutrophils and eosinophils. Thus substitution of EPA for AA in tissue lipids could attenuate the activation of these cells in conditions in which 5-oxo-ETE may play a role. It is too early to know exactly what the biological function of 5-oxo-ETE is, but it may be important in conditions in which eosinophilia is prominent as it is the most effective of all the lipid mediators we have tested in stimulating the migration of human eosinophils (17). Further studies to determine the levels of 5-oxo-ETE in biological fluids from inflammatory sites will be necessary to define more clearly the biological role of this substance.

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