Characterization and Biologic Properties of 5,12-Dihydroxy Derivatives of Eicosapentaenoic Acid, Including Leukotriene B₅ and the Double Lipoxygenase Product*

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Leukotriene B₅ (LTB₅) and three stereoisomers were prepared biosynthetically from eicosapentaenoic acid and compared with the analogous derivatives of arachidonic acid for their chemotactic and aggregating effects on human neutrophilic polymorphonuclear leukocytes. Leukotriene B₅ (LTB₅), LTB₆, and the 6-trans-diastereoisomers of each were generated by activating polymorphonuclear leukocytes with the calcium ionophore A23187 in the presence of ¹⁴C-labeled and unlabelled arachidonic acid or ¹³C-labeled and unlabelled eicosapentaenoic acid, respectively. The double lipoxygenase products, (5S,12S)-6-trans-8-cis-LTB₄ and (5S,12S)-6-trans-8-cis-LTB₅, were generated from 5S-hydroxyicosatetraenoic acid and racemic 5-hydroxyeicosapentaenoic acid intermediates by incubation with platelet xonicates. The products of each reaction were isolated by reverse-phase-high performance liquid chromatography and identified by their detection times relative to the appropriate totally synthetic standards, ultraviolet absorption spectra, immunoreactivity in a radioimmunoassay for LTB₄, and, for all but the double lipoxygenase products, by incorporation of radiolabel from the specific polynaturated fatty acid source. When the concentration of LTB₄ eliciting maximum chemotactic response of human polymorphonuclear leukocytes, 50 ng/ml (1.5 x 10⁻⁷ M), and that eliciting a maximum aggregation response, 20 ng/ml (5.9 x 10⁻⁸ M), were compared with the interpolated values of LTB₄ eliciting comparable effects, the potency of LTB₅ relative to LTB₄ was approximately 1:8 as a chemotactic agent and about 1:20 as an aggregating agent. The double lipoxygenase products and the resolved 6-trans-diastereoisomers of the pentaene and tetraene series were about 2 logs less active as chemotactic factors than LTB₄ and only (5S,12S)-6-trans-8-cis-LTB₄ had even minimal aggregating activity.

Arachidonic acid released from membrane phospholipids during cell activation may be reacylated or oxidatively metabolized by the cyclooxygenase or lipoxygenase pathway (1, 2). 5-Lipoxygenase generates 5S-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid from arachidonic acid and this unstable intermediate is hydrolyzed to 5S-hydroxy-6-trans-8,11,14-cis-eicosatetraenoic acid (3) or is enzymatically converted to an unstable epoxide, 5,6-oxidio-7,9,11,14-trans-eicosatetraenoic acid (leukotriene A₄) (4-6). LTA₄ is converted by an epoxide hydrolase to 5S,12R-dihydroxy-6,14-cis,10-trans-eicosatetraenoic acid (leukotriene B₄) (7) or by a glutathione-S-transferase to 5S-hydroxy-6R-S-glutathionyl-7,9-trans-11,14-cis-eicosatetraenoic acid (leukotriene C₄) (8). LTA₄ also undergoes nonenzymic hydrolysis to 5S,12R- and 5S,12S-dihydroxy-6,8,10-trans-14-cis-eicosatetraenoic acid diastereoisomers ((5S,12R) and (5S,12S)-6-trans-LTB₄, respectively) and to the minor products 5,6-dihydroxyeicosatetraenoic acid diastereoisomers (9). Whereas authentic LTB₄ and the diastereoisomers of 6-trans-LTB₄ are products of the 5-lipoxygenase pathway of the human neutrophilic polymorphonuclear leukocyte, the double lipoxygenase product 5S,12S-dihydroxy-6,10-trans-8,14-cis-eicosatetraenoic acid ((5S,12S)-6-trans-8-cis-LTB₄), formed from 5-HETE requires a second cell such as the platelet to provide the 12-lipoxygenase (10).

Comparative analysis has revealed natural and synthetic LTB₅ to be 100 times more potent than the double lipoxygenase product (5S,12S)-6-trans-8-cis-LTB₄ and the 6-trans-LTB₄ 12-S/R diastereoisomers in eliciting a comparable chemotactic response of human PMN (11). LTB₄ has addi*1

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1 The abbreviations used are LTA₄, leukotriene A₄; LTB₄, leukotriene B₄; LTC₄, leukotriene C₄; LTB₅, leukotriene B₅; λmax, maximum ultraviolet absorbance; Amax, absorbance at 266 nm; ACD, 0.8% citric acid, 2.2% trisodium citrate, and 2.45% dextrose (w/v); EPA, eicosapentaenoic acid; HBSS, Hank's balanced salt solution; Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; HBSS/Hepes, HBSS buffered with 20 mM Hepes; Hf, high power fields; HPLC, high performance liquid chromatography; PMN, neutrophilic polymorphonuclear leukocytes; rac-5-HEPE, racemic 5-hydroxyeicosapentaenoic acid; 5-HETE, 5-hydroxyeicosatetraenoic acid; RIA, radioimmunoassay; RP-HPLC, reverse-phase-high performance liquid chromatography; Solvent I, 65% methanol, 34.9% water, 0.1% acetic acid (v/v, pH 5.6); Solvent II, 59% methanol, 40.9% water, 0.1% acetic acid (v/v, pH 5.6); Tyrode's/Hepes/ACD, 9 volumes of Tyrode's solution with 5 mM Hepes:1 volume of ACD, pH 6.4.
tional biologic effects on human leukocytes which include aggregation of PMN (12), chemokinesis and chemotaxis for eosinophils and monocytes (13, 14), enhanced expression of C3b receptors on PMN and eosinophils (13), and release of lysosomal enzymes and stimulation of supernoxode anion generation by PMN (15). The injection of 1.6 nmol of LTB4 into human skin elicited an indurated lesion which, upon biopsy, revealed a predominant PMN infiltration (16).

Because EPA is incorporated into membrane phospholipids along with arachidonic acid and other polyunsaturated fatty acids in humans and animals on a fish-enriched diet, certain oxidative products of EPA generated via the cyclooxygenase pathway have been characterized (17-20). The only analysis of the dihydroxy products of EPA generated via the 5-lipoxy-gene pathway has utilized ionophore-induced activation in vitro of mouse mastocytoma cells obtained from tumor-bearing mice maintained on a diet enriched in EPA (21). Authentic LTBD and the diastereoisomers of 6-trans-LTBD were resolved by RP-HPLC and characterized by UV absorption spectra under a stream of nitrogen and the rac-5-HEPE salts were suspended in 900 µl of Tyrode's buffer, pH 7.4, with 1.3 mM calcium chloride and prewarmed to 37 °C for 5 min. One hundred µl of the platelet sonicate was added to each sample of substrate, incubated at 37 °C and the release of 5,12-dihydroxy derivatives within the platelet series or relative to the tetraenes. Accordingly, eachpaque and macromolecular dextran (Pharmacia, Uppsala, Sweden); lithium hydroxide (Fisher); N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Grand Island, Gibco, NY); calcium ionophore A23187 (Calbiochem); and [3]H-EPA (55 mCi/mmol), [3]C arachidonic acid (55 mCi/mmol), and [14]H-LTBD (40 Ci/mmol) (New England Nuclear) were obtained as noted. LTB4, LTB5, LTC4, and LTBD were assayed for reactivity in a radioimmunoassay for LTB4 (28).

Preparation of PMN-derived 5,12-Dihydroxy Derivatives of Arachidonic Acid and EPA—Products from each reaction were initially resolved by RP-HPLC on a 10-µm C8 Ultrasil-ODS column (4.6 x 250 mm) (Altex, Rainin, Bainville, CA) at a flow rate of 1 ml/min with Solvent I. One-ml fractions were collected and absorbance at 269 nm (A269) was continuously monitored with an on-line spectrophotometer (Model 100-40; Hitachi, Tokyo) linked to a Hewlett-Packard Integrator (Model 3380A, Hewlett-Packard, Avondale, PA). The column was calibrated with synthetic standards for the retention times of LTB4 (13.0 ± 1.1 min, mean ± S.D., n = 4), LTBD (14.5 ± 0.9 min, n = 4), (5,12R)-6-trans-LTB4 (17.6 ± 0.1 min, n = 3), (5,12S)-6-trans-LTB4 (18.5 ± 0.3 min, n = 3), and LTBD (21.1 ± 0.1 min, n = 4). The UV absorption spectrum of each peak separately eluted from RP-HPLC was determined (Model 210 Varian spectrophotometer, Florham Park, NJ) and the concentration was calculated using the maximal extinction coefficient of 51,000 cm⁻¹ M⁻¹. In the experiments carried out in the presence of [3]H-arachidonic acid and [3]C-EPA, the column was eluted at the same flow rate in Solvent I for 30 min, then with a gradient to 100% methanol over 5 min, and isocratically in 100% methanol for 10 min more. In this solvent program, synthetic 5-HETE and arachidonic acid eluted at 37 and 39 min, respectively. One-µl samples of each fraction were added to 10 ml of scintillation fluid (Hydorfluor, National Diagnostics, Somerville, MA) and radioactivity was measured in a liquid scintillation counter (Model III, Tracer Analytic, Elk Grove, IL). In selected experiments, 5-µl samples of each fraction 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 a 100-µl sample of each was assessed for reactivity in a radiomunounassay for LTBD (28).

Fractions from several RP-HPLC procedures which eluted in peaks of A269 with the same retention time were pooled and the solvent was evaporated to dryness under reduced pressure. Each residue was dissolved in 1 ml of a mixture of 50% methanol, 40% water, and 10% acetic acid (v/v, pH 5.6) (Solvent II) and applied to a Sep-Pak Ultrasil-ODS column equilibrated in the same solvent. The column was eluted at a flow rate of 1 ml/min, UV absorbance was continuously monitored at 269 nm, and 1-ml fractions were collected. The fractions corresponding to a single product were combined, analyzed for UV absorption spectra and concentrations, and stored in Solvent II under argon at −20 °C until assessment of immunoactivity and biological function.

Immunoreactivity of the 5,12-Dihydroxy Derivatives of Arachidonic Acid and EPA—As determined by UV absorbance, defined amounts of the resolved natural products stored in Solvent II were dried under reduced pressure and resuspended in 250 µl of Isogel Tris buffer. The per cent inhibition of the binding of [14,15-3H]LTBD (5100-7325 cpm) to 15 µl of rabbit immune plasma by 0.06 µg of each of the natural products was assessed in duplicate in at least 3 separate
experiments with a previously described competition assay (28). Serial dilution of the unlabeled synthetic or natural 5,12-dihydroxy derivative of arachidonic acid and EPA, [14,15-3H]LTB4, and rabbit immune plasma were incubated in a final volume of 250 μl of Isogel Tris buffer for 60 min at 37 °C before the addition of 100 μl of goat anti-rabbit IgG plasma (previously titrated to antibody excess with respect to the rabbit IgG in the immune plasma and in the control nonimmune antiserum). The mixtures were shaken and the immune precipitates were formed overnight at 4 °C and sedimented at 1500 × g for 60 min at 4 °C. The supernatants were discarded and the immuno-precipitates were dissolved in 200 μl of 0.1 N NaOH and mixed with 2.5 ml of scintillation fluid for measurement of radioactivity. Synthetic LTB4 was detectable on the linear portion of its radioligand binding inhibition curve over a dose range from 0.05–1.0 ng.

Biological Properties of the 5,12-Dihydroxy Derivatives of Arachidonic Acid and EPA—The chemotactic activity of each of the natural 5,12-dihydroxy derivatives was compared with synthetic LTB4 over a concentration range from 0.01–50 or 100 ng/ml using PMN from 3 normal donors in a modified Boyden assay. In two further experiments with different donors, the chemotactic activity of synthetic LTB4 was compared to synthetic LTB4. As determined by UV absorbance, known amounts of the synthetic or natural products stored in Solvent II were dried under reduced pressure and resuspended in HBSS containing 30 mM Hepes, pH 7.35 (HBSS/Hepes), at defined concentrations. Eight hundred-μl samples were placed into the lower wells of chemotactic chambers. One ml of 2 × 106 PMN in HBSS/Hepes buffer containing 0.4% ovalbumin (w/v) was added to the upper well of each chemotactic chamber; upper and lower wells were separated by an 8-μm pore size nitrocellulose filter (Sartorius, Gottingen, Germany). After incubation for 90 min at 37 °C, the filters were removed, fixed in ethanol, stained with hematoxylin/lithium carbonate and mounted bottom-side uppermost on glass slides. The cells which had migrated through the entire thickness of the filter were counted and the chemotactic activity was expressed as the number of PMN/10 high power fields after subtraction of the background migration in the chambers without a chemotactic stimulus (29). The experiments were performed in duplicate and the intra-assay coefficient of variation for duplicate filters was 19.5%.

Human PMN were aggregated in a Model 300 aggregometer (Payton Associates, Buffalo, NY) (30). One-half ml of a PMN suspension of 1 × 106 cells/ml of HBSS was added to a cuvette containing a stirring bar and the suspension was warmed for 2 min at 37 °C. Dose-aggregation response relationships were assessed by the addition of each natural or synthetic product in 1–40 μl of HBSS to a final concentration of 0.1–40 ng/ml. Addition of the agonist produced a rapid initial increase in light transmission, followed by a second more gradual increment. Since only the second phase increment in light transmission was associated with microscopic evidence of PMN aggregation, as also reported by ultrastructural analysis (30), this second phase response, recorded as millimeters deflection from baseline, was used to compare the response to each product with that to synthetic LTB4.

RESULTS

Generation and Initial Characterization of 5,12-Dihydroxy Derivatives of Arachidonic Acid and EPA from PMN—The products of ionophore activation of PMN in the presence of arachidonic acid from two separate experiments were resolved by RP-HPLC in Solvent I as depicted for one experiment in Fig. 1A. As assessed by A269 and incorporation of radiolabel from EPA, there were two peaks of products eluting before the diastereoisomers of 6-trans-LTB4 and LTB4. The most polar products eluting as a doublet with average retention times of 11.5 ± 0.2 min and 12.5 ± 0.4 min (mean ± S.D., n = 4) were presumed to be the diastereoisomers of 6-trans-LTB4 based on the incorporation of radiolabel from EPA and the RP-HPLC retention times reported for these products when eluted in similar solvent systems (21). The major peak eluting at 14.6 ± 0.3 min (mean ± S.D., n = 4) was presumed to be LTB4 based on prominent immunoreactivity, incorporation of radiolabel from EPA and co-elution with synthetic LTB4. In the four experiments, the average quantities of the putative diastereoisomers of 6-trans-LTB4, LTB4, 6-trans-LTB4 and LTB4 were 2.8 ± 1.2 μg (mean ± S.D., n = 4), 1.1 ± 0.6 μg, 0.8 ± 0.3 μg, and 0.3 ± 0.5 μg per 1 × 106 cells, respectively. In the presence of radiolabeled EPA, 4.8% of the recovered counts eluted in the position of the putative diastereoisomers of 6-trans-LTB4, 2.0% with LTB4, 8.5% in the solvent front, 37.5% with 5-HETE, and 39.8% with EPA.

Generation and Initial Characterization of Tetraene and Pentaene Double Lipoxygenase Products from Platelet Sonicates—Borgeat et al. (10, 31) previously demonstrated that platelets converted 5-HETE to a single product which was characterized as (5S,12S)-6,10-trans-8,14-cis-eicosatetraenoic acid. In each of two similar experiments, 5S-HETE and rac-5-HETE were separately incubated with sonicates of 1 × 106
platelets and the products were resolved on RP-HPLC in Solvent I (Fig. 2, A and B). As assessed by $A_{269}$ as well as by immunoreactivity, a single major product from 5-HETE eluted at a retention time of 22.7 min and a single major product from rac-5-HEPE eluted at 15.1 min. Additional experiments in which 2.75 $\mu$g of 5-HETE and 2.75 $\mu$g of rac-5-HEPE were separately incubated with sonicates of 22, 64, and 75 $\times$ 10$^8$ platelets from three separate donors yielded separate major products from each substrate which eluted from RP-HPLC as a single peak at 22.5 ± 0.5 min (mean ± S.D., $n = 3$), respectively. The quantity of putative (5S,12S)-6-trans-8-cis-LTB$_5$ (67.5 ± 29.4 ng/10$^8$ platelets (mean ± S.D., $n = 4$) exceeded the production of (5S,12S)-6-trans-8-cis-LTB$_4$ (25.8 ± 20.5 ng/10$^8$ platelets) in these experiments.

Additional Purification of Each Pentaene and Tetraene Product with Analysis of Its UV Spectrum—In order to obtain adequate quantities of homogeneous products for further characterization, comparable fractions from samples resolved by replicate RP-HPLC in Solvent I were chromatographed in Solvent II. In this isocratic solvent system, synthetic LTCA and LTB$_6$ had average retention times of 27.5 and 59 min, respectively, and the natural 5,12-dihydroxy derivatives of arachidonic acid and EPA eluted as discrete peaks with the retention times noted in Table I. LTB$_4$ and LTB$_5$, which eluted as single peaks in Solvent I, also eluted as single peaks when chromatographed in Solvent II. By applying only the ascending and descending portions of the doublet representing the diastereoisomers of 6-trans-LTB$_6$, and analogous portions of the doublet representing the diastereoisomers of 6-trans-LTB$_5$, it was possible to resolve each diastereoisomeric pair into homogeneous peaks whose mean retention times were separated by 6.2 and 2.5 min, respectively. In the case of the 6-trans-LTB$_6$ diastereoisomers, the initial component of the doublet was presumed to be the 5S,12R-isomer by analogy to the relative retention times of the 6-trans-LTB$_4$ diastereoisomers. (5S,12S)-6-trans-8-cis-LTB$_5$ resolved as a distinct peak in Solvent I and again in Solvent II. The product formed by the interaction of the rac-5-HEPE and platelet sonicates was resolved as 2 unequal peaks in Solvent II with retention times separated by 2 min (Fig. 2B, inset); the fractions from the descending portion of the major and less polar peak were pooled and presumed to represent (5S,12S)-6-trans-8-cis-LTB$_5$. Both authentic LTB$_4$ and LTB$_5$ gave UV absorption maxima ($\lambda_{max}$) greater than 269 nm, whereas the $\lambda_{max}$ of the other products was less than 268 nm (Table I); for each product the UV spectrum exhibited shoulders at $\lambda_{max} \pm 10$ nm.

**Immunoreactivity of the 5,12-Dihydroxy Derivatives of Arachidonic Acid and EPA**—Inhibition of [14,15-3H]LTB$_4$ binding by 5,12-dihydroxy derivatives of arachidonic acid and EPA was assessed in three separate dose-response experiments (Fig. 3). The rank order of potency expressed as the quantity of material required to achieve 50% inhibition of binding ($ID_{50}$) was 0.06 ng of LTB$_4$, 0.18 ng of synthetic LTB$_6$, 0.23 ng of (5S,12S)-6-trans-8-cis-LTB$_5$, 0.8 ng of (5S,12S)-6-trans-LTB$_5$, 3 ng of (5S,12S)-6-trans-8-cis-LTB$_4$, 4 ng of (5S,12S)-6-trans-LTB$_6$, and 4.5 ng of (5S,12R)-6-trans-LTB$_5$. (5S,12R)-6-trans-LTB$_6$ did not have adequate immunoreactivity to assign an ID$_{50}$. When the immunoreactivity of synthetic LTB$_5$ was compared to that of synthetic LTB$_6$ in another 3 experiments, the ID$_{50}$ was 0.05 ng for LTB$_5$, as compared to 0.2 ng for LTB$_6$.

**Biological Activities of the 5,12-Dihydroxy Derivatives of Arachidonic Acid and EPA**—The chemotactic activities of each of the natural 5,12-dihydroxy derivatives of arachidonic acid and EPA were compared to each other and to synthetic

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**TABLE I**

| Compounda | Retention time | $\lambda_{max}$ |
|-----------|---------------|----------------|
|           | min           | nm             |
| Synthetic LTB$_4$ | 59 (n = 2) | 269.7 ± 0.5 (n = 3) |
| Synthetic LTB$_5$ | 31.7 (n = 2) | 269.3 ± 0.2 (n = 3) |
| Natural LTB$_4$ | 58.9 (n = 2) | 268.6 ± 0.4 (n = 4) |
| Natural LTB$_5$ | 31.6 ± 2.1 (n = 4) | 269.2 ± 0.5 (n = 7) |
| (5S,12R)-6-trans-LTB$_4$ | 40.7 (n = 2) | 267.8 ± 0.3 (n = 5) |
| (5S,12S)-6-trans-LTB$_4$ | 46.9 (n = 2) | 267.8 ± 0.5 (n = 5) |
| (5S,12S)-6-trans-LTB$_5$ | 21.9 ± 1.8 (n = 4) | 267.8 ± 0.4 (n = 5) |
| (5S,12R)-6-trans-LTB$_5$ | 24.4 ± 1.7 (n = 4) | 267.7 ± 0.3 (n = 5) |
| (5S,12S)-6-trans-8-cis-LTB$_4$ | 62.3 ± 1.5 (n = 3) | 267.8 ± 0.1 (n = 3) |
| (5S,12S)-6-trans-8-cis-LTB$_5$ | 39.6 ± 1.1 (n = 3) | 267.8 ± 0.1 (n = 3) |

* Each of the products was resolved by RP-HPLC with an isocratic solvent of 59% methanol, 40.9% water, and 0.1% acetic acid, pH 5.6 (Solvent II).
Leukotriene B₅ and Analogs

LTB₅ with PMN from 3 different donors. Natural LTB₅ elicited a dose-dependent chemotactic response curve which was superimposable on that of synthetic LTB₅. The response to natural LTB₅ reached 365 ± 80 PMN/10 hpf (mean ± S.E.) at 50 ng/ml as compared to 497 ± 48 PMN/10 hpf at 50 ng/ml of natural LTB₅. By interpolation, 6 ng of LTB₅ would attract the same number of cells as 50 ng of LTB₅, thereby indicating that LTB₅ was approximately 1/3 as potent (Fig. 4A). When the dose-related chemotactic activity of synthetic LTB₅ was compared to that of synthetic LTB₅ using 2 additional PMN donors, LTB₅ and LTB₅ each reached a plateau response at 50 ng/ml even though the maximum dose tested was 100 ng/ml; the maximum response to LTB₅ was only 1/3 that of LTB₅. By interpolation, 8 ng of LTB₅ would attract the same number of cells as 50 ng of LTB₅ indicating that synthetic LTB₅ was approximately 1/6 as potent. Each of the double lipoxygenase products, (5S,12S)-6-trans-8-cis-LTB₄ and (5S,12S)-6-trans-8-cis-LTB₅, was about 1/4 as potent as LTB₅. Each of the diastereoisomers of 6-trans-LTB₅, was somewhat more chemotactically active than the 6-trans-LTB₄ diastereoisomers and, for each series, the 6-trans-diastereoisomers were similar or slightly less active than the double lipoxygenase products (Fig. 4, A and B).

The aggregating response of human PMN to natural LTB₅ was analyzed in 2 experiments, to synthetic LTB₅ in 3 experiments, and to the other natural compounds in 6 experiments, with synthetic LTB₅, used as the standard in each. Natural LTB₅ elicited a dose-dependent second phase aggregation curve which was virtually superimposable on that elicited by synthetic LTB₅ (Fig. 5). The PMN aggregation in response to natural LTB₅ reached a plateau at 20 ng/ml as compared to the response plateau reached at 5 ng/ml of synthetic or natural LTB₅. Further, the maximal aggregating response elicited by LTB₅ at 20 ng/ml was 1/3 that obtained with LTB₅ at 5 ng/ml. By interpolation, 1 ng/ml of LTB₅ would elicit the same response as 20 ng/ml of LTB₄ indicating that LTB₅ was approximately 1/20 as potent. When the dose-related PMN aggregating activity of synthetic LTB₄ was compared to that of synthetic LTB₅, LTB₅ was about 1/3 as potent as LTB₄. Of the other natural 5,12-dihydroxy derivatives of arachidonic acid and EPA, only (5S,12S)-6-trans-8-cis-LTB₄ had PMN aggregating activity and that was maximal at 20 ng/ml, but achieved only 1/3 the maximal response evoked by 5 ng/ml of LTB₄. Thus, both LTB₄ and (5S,12S)-6-trans-8-cis-LTB₄ performed as weak and partial agonists.

DISCUSSION

EPA, which is incorporated from dietary sources into membrane phospholipids along with arachidonic acid (17-21, 32) and other polyunsaturated fatty acids (33), has been documented to yield pentaene-derived products via the cyclooxygenase pathway. Two of these, thromboxane A₂ and prostaglandin I₂, are markedly less potent than the analogous arach-
Leukotriene B₅ and Analogs

FIG. 5. Aggregation of human PMN induced by natural 5,12-di-hydroxy derivatives of EPA and arachidonic acid as compared to synthetic LTB₄. Results represent the mean of 2 experiments for native LTB₄ and the mean ± S.E. of 6 experiments for the other products with PMN from different donors.

idonic acid derivatives (19, 34). The limited information available on the sulfidopeptide pentaene products of the lipoxygenase pathway reveals little or no difference in potency of their spasmodicnic activities on ileal smooth muscle (35). Because of the likely fundamental importance of leukocyte chemotaxis and aggregation in response to LTB, in inflammatory processes and the complete lack of comparative biologic studies with the pentaene analogues, the natural 5,12-dihydroxy derivatives of both arachidonic acid and EPA were compared within each series and between the two, with the same source of human cells being used to generate the eight natural products under study.

Human PMN were interacted with ¹⁴C-labeled and unlabeled arachidonic acid to generate authentic LTB₄ and the 6-trans-LTB₄ diastereoisomers and with ¹⁴C-labeled and unlabeled EPA to produce the additional products LTB₅ and the 6-trans-LTB₅ diastereoisomers. After resolution by RP-HPLC (Fig. 1, A and B), the products were tentatively identified by their elution times relative to standard markers, the incorporation of radiolabel which defined the polyunsaturated fatty acid source of the product, and their competition in a RIA for LTB₄, which revealed whether a product expressed immunochemically detectable determinants. Synthetic 5S-HETE, which corresponded to a natural 5-lipoxygenase pathway product, and rac-5-HEPE were interacted with sonicates of human platelets to yield the double lipoxygenase products (5S,12S)-6-trans-8-cis-LTB₄ and (5S,12S)-6-trans-8-cis-LTB₅, respectively. These products were tentatively identified by their elution as single reaction products approximately 2 min after synthetic LTB₄ and LTB₅ standards and by their immunoreactivity (Fig. 2).

The eight natural products were resolved by RP-HPLC in a second solvent and each was further characterized by its immunoreactivity, UV absorption spectrum, and biologic activity. Authentic LTB₄ and LTB₅ resolved as single peaks in both Solvents I and II at the elution times of the appropriate synthetic markers and yielded characteristic UV absorption spectra with λmax at 269.6 nm and 269.2 nm, respectively, and shoulders at λmax ± 10 nm. The resolution of the diastereoisomers of 6-trans-LTB₄ and of 6-trans-LTB₅ required that each doublet obtained in Solvent I be divided into fractions composed only of the ascending and descending limbs. These fractions were then fully resolved in Solvent II with peak retention times that differed by 6.2 min for (5S,12R)- and (5S,12S)-6-trans-LTB₄ and by 2.5 min for (5S,12R)- and (5S,12S)-6-trans-LTB₅. The order of elution for the diastereoisomers of 6-trans-LTB₄ had been previously established with synthetic standards (36, 37) and was assumed also to represent the order of elution for the 6-trans-LTB₅ diastereoisomers. The UV absorption spectra of the 6-trans-diastereoisomers of the tetaene and pentaene series yielded a λmax of 267.8 nm (Table I), with shoulders at λmax ± 10 nm. The 1.8 nm difference for λmax between LTB₄ and each of its isomers is consistent with the observations of Borgeat and Samuelsson (7) in which the difference in the λmax was 2 nm. In contrast to the present study, the only previous UV absorption data on 5,12-diol of the pentaene series failed to recognize a different λmax for authentic LTB₄ and its 6-trans-LTB₅ diastereoisomers (21). Whereas (5S,12S)-6-trans-8-cis-LTB₄ resolved as a single peak in both Solvent I and Solvent II, the products formed from the rac-5-HEPE resolved in Solvent II as two unequal peaks whose mean retention times were separated by 2 min. The descending portion of the less polar peak (Fig. 2B, inset) contained only the predominant product and exhibited a λmax of 267.8 nm with shoulders at λmax ± 10 nm, identical with that of (5S,12S)-6-trans-8-cis-LTB₄ (Table I) (10).

Competitive inhibition for binding of [³H]LTB₄ to immune rabbit plasma by the natural products served as an additional parameter for identifying the degree of structural relationship between pairs of pentaene and tetaene products. The rank order of cross-reactivity was similar for both the pentaene and tetaene series: LTB₄/LTB₄ > (5S,12S)-6-trans-8-cis-LTB₄/LTB₄ > (5S,12S)-6-trans-LTB₅/LTB₄ > (5S,12R)-6-trans-LTB₅/LTB₄ > (5S,12S)-6-trans-LTB₅/LTB₄. The resolution of the compounds in the pentaene series was more immunoreactive on a weight basis than the analogous compounds in the tetaene series may have resulted from preparing the immunogen by coupling
the carrier to LTB₃ through the 12-oxo function. The resulting proximity of the C-17 double bond to the coupling site may contribute to active site specificity by the greater rigidity of the pentaene. The definition of the degree of cross-reactivity complex biologic fluids following their resolution. for each moiety provides the possibility of measurement in human PMN were approximately l/4 and l/6 to l/8 as potent, respectively, as those of LTB₄ (Figs. 4 and 5). The impaired chemotactic activity of LTB₄ has been reported in a preliminary statement (38). The assessment of potency was made by comparing the concentrations of natural and synthetic LTB₅ respectively, the maximum effect achieved by LTB₃, was less than that of nary statement (38). The assessment of potency was made by comparing the concentrations of natural and synthetic LTB₅ giving a comparable response. Further, the maximum effect achieved by LTB₄ was less than that of LTB₅, as well as those of 6-trans-LTB₄ and synthetic LTB₅, giving a comparable response. Thus, LTB₅, as well as those of 6-trans-LTB₄, did not elicit aggregation at the maximal doses studied and each set of these pathway (17-21, 32, 34, 39).

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