Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 5729–5733, September 1984
Cell Biology

Oxidation of leukotrienes at the ω end: Demonstration of a receptor for the 20-hydroxy derivative of leukotriene B_4 on human neutrophils and implications for the analysis of leukotriene receptors

(leukotriene B_4 receptor/chemotaxis/ω oxidation/autocoid)

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Communicated by Hans J. Müller-Eberhard, May 4, 1984

ABSTRACT **Leukotriene B_4 [LTB_4; (5*S*,12*R*)-5,12-dihydroxy-6,14-*cis*-8,10-*trans*-icosatetraenoic acid] and its 20-hydroxy derivative [20-OH-LTB_4; (5*S*,12*R*)-5,12,20-trihydroxy-6,14-*cis*-8,10-*trans*-icosatetraenoic acid] are principal metabolites produced when human neutrophils (hPMNs) are stimulated by the calcium ionophore A23187. These compounds were purified to homogeneity by Nucleosil C_{18} and silicic acid HPLC and identified by UV absorption and gas chromatographic/mass spectral analyses. 20-OH-LTB_4 is considerably more polar than LTB_4 and interacts weakly with the hydrophobic Nucleosil C_{18} resin, whereas LTB_4 interacts strongly, reflecting the hydrophobic C_{13}–C_{20} domain in LTB_4. Specific binding of highly purified [^{3}H]LTB_4 and [^{3}H]20-OH-LTB_4 to hPMNs was assessed. Binding of [^{3}H]20-OH-LTB_4 could be largely displaced by an excess of nonlabeled LTB_4 or 20-OH-LTB_4 but not by 15-hydroxyicosatetraenoic acid (15-HETE), (5*S*,12*S*)-5,12-dihydroxy-6,10-*trans*-8,14-*cis*-icosatetraenoic acid [(5*S*,12*S*)-diHETE], or the 6-*trans* stereoisomer of LTB_4 at 1 μM. In contrast, [^{3}H]LTB_4 displays a high level of nonspecific binding to human PMNs, which makes assessment of the K_d for LTB_4 binding unobtainable. Binding measurements for [^{3}H]LTB_4 were performed in a buffer containing bovine serum albumin, and under these conditions significantly less nonspecific binding was observed. The apparent K_d for high-affinity binding sites on human PMNs at 0°C was 31.3×10^{-9} M for LTB_4 and 14.3×10^{-9} M for 20-OH-LTB_4. In addition, we observed a saturable low-affinity receptor for 20-OH-LTB_4 with a K_d of approximately 100×10^{-9} M and 2×10^5 receptors per cell. The data from this study suggest that ω oxidation represents a major pathway for metabolism of LTB_4 as well as other arachidonate metabolites. LTB_4 and 20-OH-LTB_4 express similar functional activities and share common binding properties to hPMNs but differ significantly in their physical properties. It is the unique physical characteristics of 20-OH-LTB_4 that suggest that arachidonate metabolites oxidized at the ω position may be more important agents in inflammation than LTB_4.**

Chemotactic factors are produced as by-products of the immune response and presumably play an essential role in recruitment of human polymorphonuclear cells (hPMNs) at the site of inflammation (1–3). For example, during an immune response, arachidonic acid, which normally is esterified to phospholipid, is hydrolyzed to the free acid by specific lipases. The free arachidonate is metabolized by the 5-lipoxygenase pathway which transforms it to leukotriene B_4 [LTB_4; (5*S*,12*R*)-5,12-dihydroxy-6,14-*cis*-8,10-*trans*-icosatetraenoic acid]. LTB_4 is a potent chemoattractant for hPMNs, exhibiting measurable chemotactic activity at 1 nM with an ED_{50} of 10 nM (4–6). The LTB_4–hPMN interaction is highly stereospecific. For example, the isomer 6-*trans*-LTB_4, which differs structurally from LTB_4 only in the configuration at the C-6 double bond, is a weaker chemoattractant than LTB_4 by 3 orders of magnitude, and none of the other 5,12-dihydroxyicosatetraenoic acid (5,12-diHETE) isomers display significant chemotactic activity (6). Because LTB_4 is a potent and stereospecific chemoattractant, characterization of the LTB_4 receptor should be possible using direct ligand binding. Since LTB_4 may potentiate inflammatory responses in many acute and chronic human diseases, characterization of the LTB_4 receptor could be useful in designing antagonists of LTB_4.

Previous studies have indicated that LTB_4 receptors exist on hPMNs, and these studies reported a K_d of 10^{-8} M (7, 8). However, there were two major problems associated with the analysis. There was a high degree of nonspecific binding, which reflects the nonpolar character of LTB_4, and measurement of specific binding was represented by a small difference between two large (total and nonspecific) binding numbers. There also was an apparent lack of stereospecificity of [^{3}H]LTB_4 binding to the hPMN receptor because the 5,12-diHETE isomers of LTB_4, and 5-hydroxyicosatetraenoic acid (5-HETE) reportedly displaced [^{3}H]LTB_4.

Oxidation of LTB_4 at the ω position generates the 20-hydroxy derivative of LTB_4 (20-OH-LTB_4) (9), and this metabolic change confers new physical properties to the molecule. 20-OH-LTB_4 is a much more polar molecule than LTB_4, based on interactions of these molecules with the hydrophobic HPLC resin Nucleosil C_{18}. Therefore, 20-OH-LTB_4 is particularly amenable for use in analyzing the leukotriene receptor because nonspecific (nonpolar) membrane interactions are minimized. With respect to functional properties of the molecule, 20-OH-LTB_4 was found to be equally as potent as LTB_4 for PMN chemotaxis (6). Thus, the aliphatic ω end of the LTB_4 molecule had little influence on receptor interaction. Specific binding of [^{3}H]LTB_4 and [^{3}H]20-OH-LTB_4 to hPMNs was assessed. Our results show that 20-OH-LTB_4 exhibits considerably less nonspecific binding than does LTB_4 and that the interaction between 20-OH-LTB_4 and the LTB_4 receptor is stereospecific. Effective competition to [^{3}H]20-OH-LTB_4 binding was observed with the unlabeled compound and with LTB_4 but not with any of

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Abbreviations: diHETE, dihydroxyicosatetraenoic acid; 5-HETE, 5-hydroxyicosatetraenoic acid; LTB_4, leukotriene B_4 [(5*S*,12*R*)-5,12-dihydroxy-6,14-*cis*-8,10-*trans*-icosatetraenoic acid]; PMN, polymorphonuclear cell; 15-HETE, 15-hydroxyicosatetraenoic acid; 20-OH-LTB_4, (5*S*,12*R*)-5,12,20-trihydroxy-6,14-*cis*-8,10-*trans*-icosatetraenoic acid; (5*S*,12*S*)-diHETE, (5*S*,12*S*)-5,12-dihydroxy-6,10-*trans*-8,14-*cis*-icosatetraenoic acid; 6-*trans*-LTB_4, (5*S*,12*R*)-5,12-dihydroxy-6,8,10-*trans*-14-*cis*-icosatetraenoic acid; 5,12,20-triHETE, (5*S*,12*S*)-5,12,20-trihydroxyicosatetraenoic acid; GC/MS, gas chromatography/mass spectra.

the other diHETE isomers at concentrations up to 1 μM. Nearly identical K_ds in the range of 10 nM were observed for binding of LTB_4 and 20-OH-LTB_4 to the hPMN receptor. Therefore 20-OH-LTB_4 appears to be a useful tool in characterizing chemotaxis receptors for LTB_4.

Our results also show that 20-OH-LTB_4 is a principal metabolite when hPMNs are stimulated by the calcium ionophore A23187. Thus 20-OH-LTB_4 is a major lipoxygenase metabolite, exhibits less nonspecific hydrophobic interaction, and has a K_d identical to that of LTB_4. We conclude that 20-OH-LTB_4 may be the primary arachidonate-derived chemoattractant in inflammatory reactions.

METHODS

Preparation of Lipoxygenase Metabolites. Whole leukocytes or neutrophils (PMNs) were isolated from human venous blood as described (10) and resuspended in Dulbecco's phosphate-buffered saline (GIBCO) with 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 2 mM glucose at 2.5×10^7 cells per ml. Lipoxygenase metabolites were prepared when cells were given 10 μM calcium ionophore A23187 (Sigma) for 10 min at 37°C. In some experiments, cells were preincubated with 80 Ci (1 Ci = 37 GBq) of [^{3}H]arachidonic acid (New England Nuclear) per mmol for 5 min at 37°C prior to A23187 challenge. Arachidonate metabolites were extracted into ether and purified using silicic acid chromatography (11).

The lipoxygenase metabolites were separated on a Nucleosil C_{18} (25 cm × 4.6 mm; Alltech, Deerfield, IL) reverse-phase HPLC, and the elution was monitored by absorbance as described (12). The 5,12-diHETE isomers and the (5*S*,12*S*)-5,12,20-trihydroxyicosatetraenoic acid (5,12,20-triHETE) isomers were methylated with diazomethane and purified on reverse-phase Nucleosil C_{18} HPLC using a mobile phase of methanol/water/acetic acid, 67:33:01 (vol/vol), followed by another purification on a Nucleosil 50 (25 cm × 4.6 mm; Alltech) straight-phase HPLC using a mobile phase of hexane/isopropanol/acetic acid (6). Finally, the methyl esters were hydrolyzed, and the free acid was separated on Nucleosil C_{18} reverse-phase HPLC using as mobile solvent the methanol/water/acetic acid mixture, and the metabolites were stored in methanol under argon.

Identification of Lipoxygenase Metabolites. Arachidonate metabolites were homogeneous as determined by their elution as a single peak in both straight-phase and reverse-phase HPLC, and the elution times of these metabolites were identical to previously published values (9, 11). The metabolites also were identified by UV spectroscopy with a Cary 217 spectrophotometer and by gas chromatographic/mass spectral (GC/MS) analysis with a HP 5981-A GC/MS spectrophotometer. 15-Hydroxyicosatetraenoic acid (15-HETE) was prepared from the reaction of soybean lipoxygenase (Sigma) and arachidonic acid (Nu Chek Prep) and had a UV spectrum with λ_{max} at 235 nm and a mass spectrum with fragment ions at m/z of 225, 316, and 335 (13). The 5,12-diHETE and 5,12,20-triHETEs each displayed a triplet UV peak and a mass spectrum with fragments at m/z of 167, 191, 203, 217, 229, 267, 293, and 383, which were consistent with previously published values (9, 11). LTB_4 and 20-OH-LTB_4 had identical UV spectra with peaks at 260.5, 270, and 281 nm as described (9).

Binding Assay. The specific activity of the biologically derived [^{3}H]LTB_4 and [^{3}H]20-OH-LTB_4 was 6.8 Ci/mmol. Synthetic LTB_4 was a generous gift from Eli Lilly (Indianapolis, IN).

PMNs were incubated in duplicate in 0.2 ml of Dulbecco's phosphate-buffered saline with 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 2 mM glucose at $2.5–5 \times 10^7$ cells per ml with [^{3}H]LTB_4 or [^{3}H]20-OH-LTB_4 in the presence or absence of 10 μM synthetic LTB_4. After incubation, the mixture was layered above 200 μl of 20 mM Hepes, pH 7.2/15% sucrose in a 400-μl polyethylene microtest tube (Brinkman Instruments, San Diego, CA). The tubes were then placed in a Microfuge and centrifuged for 15 sec. The supernatant and gradient were removed by aspiration. The pellet was solubilized with 0.2 ml of 1 M NaOH at 50°C for 2 hr. The solubilized pellet was neutralized with 0.2 ml of HCl. The cells could quantitatively be found in the pellet. The solubilized pellet was then placed in 10 ml of Insta Gel (United Tech Packard) scintillation fluid, and β emissions were monitored for 10 min in a Beckman model LS8100 scintillation counter with a counting efficiency for tritium of 10%. An incubation period of 45 min at 0°C was routinely used for the binding of [^{3}H]LTB_4 or [^{3}H]20-OH-LTB_4 to PMNs since equilibrium for total binding of radioactivity to the PMNs is reached within 30–60 min (8).

The amount of specific ligand bound was determined by subtracting from the total ligand bound the amount of ligand blocked from the receptor in the presence of 10 μM synthetic LTB_4. K_d values were determined from Scatchard analysis by the least-squares method. To illustrate the different classes of binding sites for various ligands used and to demonstrate saturability when the maximum number of receptors are occupied by ligand, we present data in a Bjerrum plot.

RESULTS

Human PMNs produce LTB_4 when given the calcium ionophore A23187 (14). LTB_4 can undergo ω oxidation (9), and the metabolite exhibits markedly altered physical properties as shown in Table 1. LTB_4, a dihydroxyarachidonate metabolite, was readily separated from other mono-, di-, or trihydroxyarachidonate metabolites by HPLC chromatography on Nucleosil C_{18} and was eluted from this column with a retention time of 21 min. LTB_4 and 20-OH-LTB_4 are structurally identical from carbon atoms 1 to 19; however, 20-OH-LTB_4 was eluted with a retention time of only 6.0 min, reflecting a weak interaction with the resin. Furthermore, the methyl ester of 20-OH-LTB_4 was eluted with a retention time of 8.8 min compared with LTB_4, whose methyl ester was eluted at 38.1 min, indicating that the interaction of 20-OH-LTB_4 with the C_{18} resin is especially weak. These data confirm that 20-OH-LTB_4 is a more polar molecule than LTB_4. Interestingly, ω oxidation has no effect on the functional activity of the molecule, since 20-OH-LTB_4 and LTB_4 have identical functional characteristics with respect to PMN chemotaxis (6) and the cyclooxygenase-dependent contraction of lung tissue (9, 15).

The 20-OH-LTB_4 is a major metabolite of the 5-lipoxygenase pathway when hPMNs are stimulated by the calcium ionophore A23187. Supernatant fluids from the activated hPMNS were extracted as described in *Methods* and separated by Nucleosil HPLC chromatography as described in Table 1. This chromatographic procedure permits separation of the methyl esters of LTB_4, (5*S*,12*S*)-diHETE, 20-OH-LTB_4, and the 20-COOH derivative of LTB_4. When PMNs

Table 1. Retention times for various 20:4 arachidonate metabolites on a Nucleosil C_{18} HPLC column eluted with 67% methanol

Sample	Retention time, min	
	Free acid	Methyl ester
6-*trans*-LTB_4	17.9	31.0
(5*S*,12*S*)-DiHETE	21.0	35.7
LTB_4	21.0	38.1
20-OH-LTB_4	6.0	8.8
5,12,20-TriHETE	6.2	8.6

Arachidonate metabolites were purified to homogeneity as described in *Methods*. Methyl esters were formed by reaction with diazomethane (11). Elution of the arachidonate metabolites was monitored by absorption at 270 nm.

were treated with A23187, there was a rapid appearance of LTB_4 in the cellular supernatant, which reached a maximum of 450 pmol per 10^7 cells at 5 min (Fig. 1). There was no (5*S*,12*S*)-diHETE produced, consistent with the observation that this metabolite is produced only when platelets are present (unpublished data). The LTB_4 then rapidly disappeared to undetectable levels after 20 min. 20-OH-LTB_4 was also detectable in cellular supernatants immediately after ionophore challenge, and the quantity steadily increased to a value of 200 pmol per 10^7 cells over 20–30 min. In contrast, 20-COOH-LTB_4 was present at relatively low levels (<50 pmol per 10^7 cells) throughout the course of the stimulation by ionophore. 20-OH-LTB_4 was the major metabolite after just 10 min of ionophore stimulation. Therefore, it appears that ω oxidation is an active pathway that rapidly converts LTB_4 to 20-OH-LTB_4. Since 20-OH-LTB_4 apparently is a physiologically important metabolite, we undertook a study to demonstrate a receptor for 20-OH-LTB_4 on human neutrophils.

Reagents for the receptor study were prepared by incubating hPMNs with [^{3}H]arachidonic acid and calcium ionophore A23187 for 10 min at 37°C. Both [^{3}H]LTB_4 and [^{3}H]20-OH-LTB_4 were isolated from the reaction supernatant and purified to homogeneity by Nucleosil C_{18} and silicic acid HPLC. Products were identified by UV absorption and GC/MS.

The specific binding of highly purified [^{3}H]LTB_4 and [^{3}H]20-OH-LTB_4 to hPMNs was assessed as shown in Fig. 2. Binding of [^{3}H]LTB_4 to hPMNs was characterized by high levels of nonspecific binding. For example, when 10 μM LTB_4 was incubated with PMNs also exposed to 20 nM [^{3}H]LTB_4, only 40% of the total counts were displaced from the receptor (Fig. 2 *Upper*). In contrast, when 10 μM LTB_4 was incubated with PMNs exposed to 20 nM [^{3}H]20-OH-LTB_4, 90% of the total counts were displaced from the receptor (Fig. 2 *Lower*). Therefore, 20-OH-LTB_4 exhibits significantly less nonspecific binding than does LTB_4. Due to the high nonspecific binding, assignment of a K_d value for LTB_4 was unobtainable. The binding characteristics of 20-OH-LTB_4 are given on the Bjerrum plot in Fig. 3. The data in Fig. 3 are consistent with the presence of two saturable binding sites on PMNs for 20-OH-LTB_4. Scatchard analysis of the high-affinity site between ligand concentrations 0.05 and 30 nM had an apparent K_d of $14.3 \pm 4.0 \times 10^{-9}$ M ($n = 3$) with 4.2×10^4 receptors per cell, while the low-affinity site between 30 and 500 nM ligand concentration had an estimated K_d of 100×10^{-9} M with 2×10^5 receptor sites.

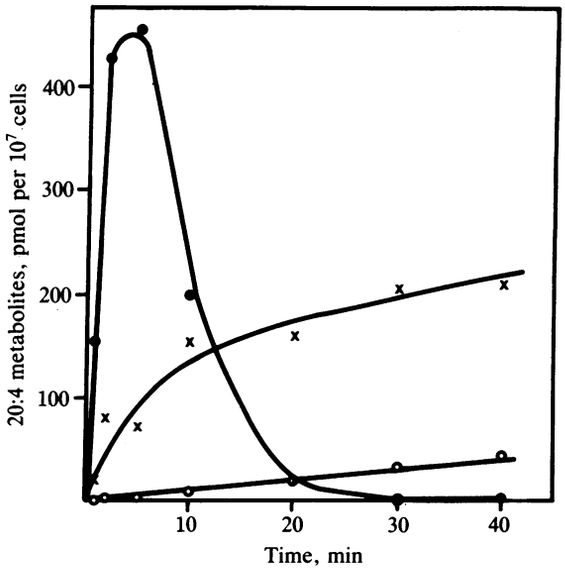

FIG. 1. Arachidonate metabolites recovered versus time from the cellular supernatant of 10^7 hPMNs stimulated with 10 μM calcium ionophore A-23187 at 37°C. Lipids were extracted from the reaction mixture as described and analyzed as methyl ester derivatives (see Table 1). ●, LTB_4; X, 20-OH-LTB_4; ○, 20-COOH-LTB_4.

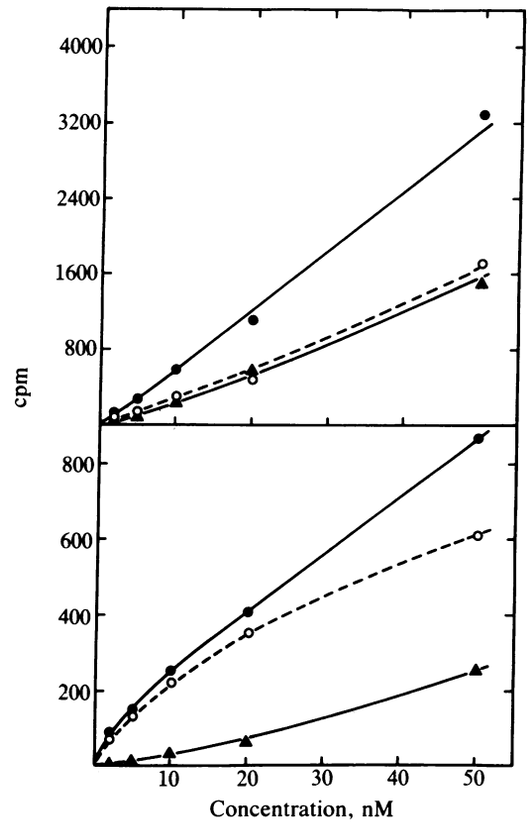

FIG. 2. The specific binding of highly purified [^{3}H]LTB_4 (*Upper*) and [^{3}H]20-OH-LTB_4 (*Lower*) to 10^7 hPMNs at 0°C for 45 min. Specific activity of each radiolabeled metabolite was 1500 cpm/pmol, and the binding assay was performed as described. The data are expressed as total binding (●), and nonspecific binding was assessed in the presence of 10 μM LTB_4 (▲). Specific counts (○) were determined by subtracting the nonspecific from total counts.

Various arachidonate metabolites were assessed for their ability to inhibit the specific binding of [^{3}H]20-OH-LTB_4 to hPMN (Table 2). LTB_4 and 20-OH-LTB_4 were mutually effective for inhibiting specific binding, while (5*S*,12*S*)-diHETE, 15-HETE, and 6-*trans*-LTB_4 at concentrations of 1 μM were ineffective at inhibiting the specific uptake of 20-OH-LTB_4. The IC_{50} for LTB_4 inhibition of specific 20-OH-LTB_4 binding was 30 nM.

A direct comparison of the binding parameters for LTB_4 and 20-OH-LTB_4 was possible when measurements were carried out in the presence of bovine serum albumin. When hPMNs were incubated with [^{3}H]LTB_4 in bovine serum albumin, specific binding was observed. There was identical specific ligand bound for LTB_4 and 20-OH-LTB_4 between ligand concentrations of 1 and 50 nM (Fig. 4). At concentrations of 20-OH-LTB_4 above 50 nM, there was a low-affinity binding site indicated by Scatchard analysis (data not shown). From Scatchard analysis we estimate a K_d of 31.3×10^{-9} M, and the corresponding experiment with 20-OH-LTB_4 exhibited a K_d of 17×10^{-9} M. Therefore, LTB_4 and 20-OH-LTB_4 have similar ED_{50} values for chemotaxis as well as similar affinity constants for receptor binding on hPMNs.

DISCUSSION

ω oxidation to 20-OH-LTB_4 is a major pathway for the metabolism of LTB_4. For example, we observed that LTB_4 produced when PMNs were stimulated by calcium ionophore A23187 was rapidly metabolized to 20-OH-LTB_4. After 10

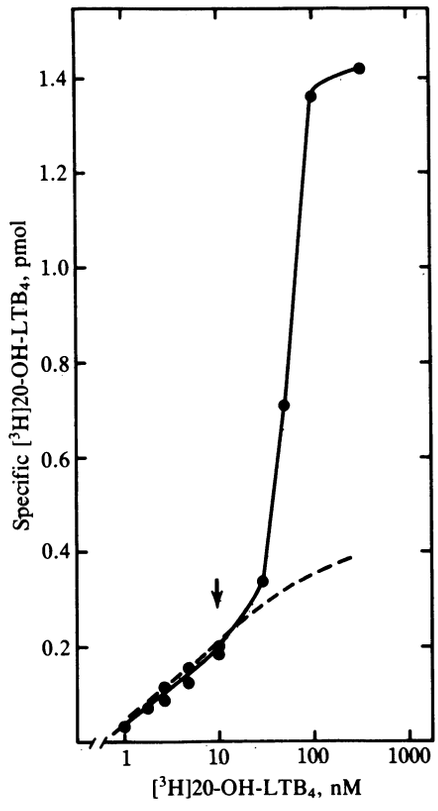

FIG. 3. A plot of specific ligand bound versus the ligand concentration is given for the binding of [^{3}H]20-OH-LTB$_4$ to 6 × 10^6 hPMNs after 45 min at 0°C. The data indicate two receptor populations are present. The dashed line indicates the predicted binding profile for the high-affinity 20-OH-LTB$_4$ binding site. The K_d for the high-affinity site was determined from the range of 0.5–30.0 nM 20-OH-LTB$_4$. The arrow indicates the ED$_{50}$ for hPMN chemotaxis by 20-OH-LTB$_4$ (6).

min 20-OH-LTB$_4$ was the major arachidonate metabolite (Fig. 1). hPMNs metabolize exogenous arachidonic acid when stimulated by the human complement anaphylatoxin C5a (12), and 20-OH-LTB$_4$ is also a major metabolite of this reaction (unpublished observation). Palmblad postulated that ω oxidation of LTB$_4$ is a catabolic pathway that inactivates this mediator (16, 17). However, this appears not to be the case because 20-OH-LTB$_4$ and LTB$_4$ have nearly identical ED$_{50}$ values for inducing cyclooxygenase-dependent contraction of lung tissue and for promoting hPMN chemotaxis (6, 9, 15). Therefore, one can legitimately question the true effect and purpose of ω oxidation on the metabolic fate of LTB$_4$.

Table 2. Competition of 20-OH-LTB$_4$ binding to hPMNs by arachidonate metabolites

Metabolite added	Concentration, nM	% uptake
None	—	100
20-OH-LTB$_4$	1,000	36
(5*S*,12*S*)-DiHETE	100	97
	1,000	98
15-HETE	1,000	108
6-*trans*-LTB$_4$	100	101
	1,000	87
LTB$_4$	1	107
	3	100
	10	80
	30	68
	100	43
	10,000	13

hPMNs (10^7 cells) were exposed 45 min at 0°C to 5 nM [^{3}H]20-OH-LTB$_4$ in the absence and presence of various arachidonate metabolites. Radiolabel associated with the cells was measured as described in *Methods*.

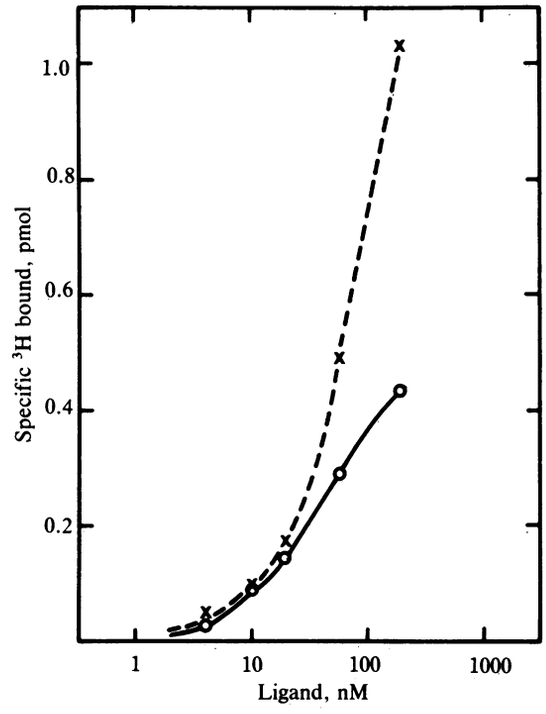

FIG. 4. A plot of specific ligand bound versus the ligand concentration is given for the binding of [^{3}H]20-OH-LTB$_4$ (X) and [^{3}H]LTB$_4$ (○) to 4 × 10^6 hPMNs after 45 min at 0°C. The reaction conditions were identical to those described in Fig. 3 except that the reaction buffer contained 1% bovine serum albumin.

One effect of ω hydroxylation on LTB$_4$ was to alter the physical property of the molecule, which in turn lowers nonspecific cellular binding (Fig. 2). Thus 20-OH-LTB$_4$ has greater potential as an extracellular effector and could be regarded more accurately as a hormone than LTB$_4$.

ω oxidation represents a major pathway for the metabolism of arachidonate metabolites other than LTB$_4$. When PMNs are given (5*S*,12*S*)-diHETE, the products 5,12,20-triHETE and (5*S*,12*S*)-20-carboxy-diHETE are found in the supernatant (unpublished observation). Also Marcus and coworkers found 12,20-diHETE in PMN supernatants when cells were given 12-HETE (18, 19). Several prostaglandins can also undergo ω oxidization (20). Consequently, both cyclooxygenase and lipoxygenase products may serve as substrates for ω oxidation. One also can envision how leukotrienes or prostaglandins from one cell type may provide substrate for ω oxidation in another cell type, providing a means for one cell type to communicate with a second cell type. The end product of such intracellular metabolism is then an "autocoid" that may stimulate a third cell type, etc.

Bioactive arachidonate metabolites such as leukotrienes and prostaglandins exhibit marked nonpolar characteristics, and the receptor studies performed to date have not adequately considered that nonspecific hydrophobic partitioning of these hydroxyhydrocarbons is considerable. Because there are large numbers of hydrophobic binding sites in a membrane, nonspecific hydrophobic partitioning is not adequately displaced by competition in a membrane. This explains why the data in Fig. 2 *Upper* show such a high degree of nonspecific binding. We believe that even in the "specific" bound ligand component (Fig. 2 *Upper*), a considerable fraction of ligand represents nonspecific hydrophobic parti-

tioning that cannot be displaced by competition from the membrane. The only valid method to measure receptors for arachidonate metabolites may be to use a ligand oxidized at the ω position or otherwise modified that has sufficient polarity to prevent nonspecific membrane partitioning. Powell has shown that many arachidonate metabolites can undergo ω oxidation and that the pathway is active in the PMN (21).

We have used 20-OH-LTB_4 successfully to characterize the LTB_4 receptor for chemotaxis. The high-affinity site [K_d of $14.3 \pm 4.0 \times 10^{-9}$ M ($n = 3$)] for [^{3}H]20-OH-LTB_4 on PMNs was approximately equal to the concentration of 20-OH-LTB_4 and LTB_4 that elicits a half maximal chemotactic response *in vitro*. This correlation suggests that the receptors involved in binding may also function in leukocyte chemotaxis (Fig. 3). In addition, we observed a saturable low-affinity receptor for 20-OH-LTB_4 with a K_d of approximately 100×10^{-9} M. There were an estimated 4.2×10^4 high-affinity receptors and 2×10^5 low-affinity receptors.

The utility of 20-OH-LTB_4 as a LTB_4 receptor probe was demonstrated further by the competition experiments. Specificity is maintained by 20-OH-LTB_4 as demonstrated by the fact that 15-HETE, (5*S*,12*S*)-diHETE, and 6-*trans*-LTB_4, which are not chemoattractants for PMNs at 1 μM, did not inhibit the binding of [^{3}H]20-OH-LTB_4 to the high-affinity receptor (Table 2). Only LTB_4 competitively inhibits [^{3}H]20-OH-LTB_4 binding with an IC_{50} of 30 nM. Thus [^{3}H]20-OH-LTB_4 promises to be particularly valuable for studies directed at the LTB_4 receptor or at identifying antagonists of LTB_4 receptor interaction.

We were unable to obtain meaningful measurements for [^{3}H]LTB_4 binding in the absence of bovine serum albumin (Fig. 2 *Upper*). However, in the presence of the albumin, we obtained specific binding for [^{3}H]LTB_4 with a K_d of 31.3×10^{-9} M (Fig. 4). It appears that [^{3}H]LTB_4 bound to albumin (unpublished observation) competes only with the high-affinity LTB_4 chemotaxis receptor. Therefore, albumin or other plasma proteins may act as physiologic vehicles for delivering LTB_4 to the high-affinity receptor of hPMN. Direct comparison of the binding measurements performed for LTB_4 and 20-OH-LTB_4 in the presence of bovine serum albumin resulted in identical specific ligand uptake at concentrations of 1 nM to 50 nM. The specific binding at these low concentrations is probably more meaningful than K_d estimates obtained by Scatchard's analysis, which cannot be applied rigorously for the data shown in Figs. 3 and 4. Titration at low concentration ranges probably corresponds to the same interactions required to induce chemotaxis—e.g., chemotaxis induced by *N*-formylated peptides is initiated by occupancy of a small number of receptors (22, 23).

Information provided in this study should be useful in designing potential receptor probes. Since we have proven that the aliphatic ω end of the LTB_4 molecule has little effect on receptor interaction, analogues of LTB_4 can be synthetically prepared that make receptor detection and quantitation more readily obtainable. For example, one could synthesize an LTB_4 analogue with a fluorescent moiety on the distal end of the molecule. If one assumes that this analogue retains its PMN chemotactic activity and ability to displace [^{3}H]20-OH-LTB_4 in a competition binding assay, it would be possible to perform extensive binding studies, taking advantage of both the sensitivity and potential for obtaining rapid kinetics through fluorescence spectroscopy (24). Another experimental approach could be to synthesize ligands that can be coupled to affinity support or used as haptens in generating antibodies for an RIA assay.

Metabolism of arachidonate products by ω oxidation results in a previously unreported class of metabolites designated "ω-oxidation autocoids." The oxidation of carbon-20 radically alters the physical properties of the molecule. For example, 20-OH-LTB_4 is measurably more polar than LTB_4, based on its interaction with the hydrophobic HPLC resin Nucleosil C_{18}. However, oxidation of carbon-20 does not alter the functional properties of the molecule. 20-OH-LTB_4 and LTB_4 had identical ED_{50} values for hPMN chemotaxis and similar K_ds for the high-affinity receptor of hPMNs.

We have shown that 20-OH-LTB_4 is a primary arachidonate metabolite for PMNs when these cells are stimulated by A23187 or the humoral mediator C5a. Therefore, we conclude that 20-OH-LTB_4 and possibly other yet undetected ω-oxidation autocoids are major effector metabolites of arachidonate metabolism.

R.M.C. is supported by a National Research Service Individual Postdoctoral Fellowship (HL06692); T.E.H. is supported by Public Health Service Grants HL23584, AI17354, and HL16411; and C.A.D. is supported in part by the Swiss National Science Foundation. This is publication number 3429-IMM from Scripps Clinic and Research Foundation, La Jolla, CA.

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