Receptors for the 5-OxO Class of Eicosanoids in Neutrophils*

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5-Hydroxy- and 5-oxo-eicosatetraenoate (5-HETE and 5-oxoETE) activate polymorphonuclear neutrophils (PMNs) through a common, receptor-like recognition system. To define this system, we examined the interaction of these eicosanoids with human PMNs. PMNs esterified 5-[3H]HETE to glycerolipids at 37 and 4 °C. At 37 but not 4 °C, the cells also hydroxylated the label to 5,20-[3H]diHETE. The acyl-CoA synthetase blocker, triacsin C, inhibited esterification but also led to an increase in the hydroxylation of the label. PMNs processed 5-[3H]oxoETE through the same pathways but only or principally after reducing it to 5-[3H]HETE (37 or 4 °C). In the presence of these varying metabolic reactions, PMNs (37 or 4 °C; ± triacsin C) could not be shown to receptor bind either radiolabel.

Plasma membranes isolated from PMNs esterified but unlike whole cells did not reduce or hydroxylate 5-[3H]oxoETE. Triacsin C blocked esterification, thereby rendering the membranes unable to metabolize this radiolabel. Indeed, triacsin C-treated membranes bound (Kd = 3.8 nM) 5-[3H]oxoETE specifically and reversibly to 86 pmol of sites per 25 μg of membrane protein. 5-OxooETE, 5-HETE, and 5,15-diHETE displaced this binding at concentrations correlating with their potency in eliciting PMN Ca2⁺ transients. GTP and GTPγS, but not ATP or ATPγS, also reduced 5-[3H]oxoETE binding, whereas 15-HETE, leukotriene B₄, platelet-activating factor, IL-8, C5a, and N-formyl-Met-Leu-Phe lacked this effect. We conclude that PMNs and their plasma membranes use an acyl-CoA synthetase-dependent route to esterify 5-HETE and 5-oxoETE into lipids. Blockade of the synthetase uncovers cryptic plasmalemma sites that bind 5-oxoETE with exquisite specificity. These sites are aptly mediated responses to the 5-oxo class of eicosanoids and are likely members of the serpentine superfamily of G protein-linked receptors.

Cells respond to stimulation by converting storage arachidonic acid into products that signal their own or nearby cell responses (1, 2). Many such eicosanoids act by binding to receptors (3–5). Others have a less clear mechanism of action. 5-HETE<sup>1</sup> and LTB₄, for example, are companion metabolites formed by the attack of 5-lipoxygenase on arachidonic acid in, e.g. human PMNs (1, 2, 6). Although the two eicosanoids have similar stimulatory actions, 5-HETE has little affinity for LTB₄ receptors and is resistant to a LTB₄ receptor antagonist. In down-regulation assays, furthermore, 5-HETE desensitizes to itself but does not cross-desensitize with other chemotactic factors (LTB₄, FMLP, C5a, PAF, or IL-8) or eicosanoids (lipoxins A₄ and B₄ and prostaglandins D₂ and E₂) (7–20). The data imply that 5-HETE acts through a unique recognition system. However, evidence relating this system to receptor-like binding sites has proven difficult to obtain.

Although PMNs process 5-HETE through various metabolic pathways (6–8, 12, 21–23), one route, esterification, has presented an obstacle for receptor studies. The reaction occurs in PMNs or their isolated plasma membranes at 4 or 37 °C and results in the acylation of 5-[3H]HETE to membrane glycerolipids: PMNs and plasmalemma accumulate 5-HETE almost exclusively in esterified form without evidence of receptor binding (10, 21). Because esterification is a ubiquitous means for processing fatty acids, other cell types are apt also to esterify 5-HETE and thereby obscure the receptor binding of the compound. Despite this difficulty, however, putative receptors for 5-HETE merit study. PMNs, eosinophils, and monocytes dehydrogenate 5-HETE to 5-oxoETE (6, 13–15, 25). 5-OxoETE is ~10-fold stronger than 5-HETE in stimulating PMNs and monocytes (9–13, 19). It is even more active on eosinophils (14–17), eliciting the chemotaxis response of this cell at concentrations 10,000-, 1000-, and >10-fold lower than LTB₄, 5-HETE, or other chemotactic factors, respectively (18). 5-OxoETE down-regulates these cells to 5-HETE but not to LTB₄ or other stimuli (7–19). Possibly, therefore, putative 5-HETE receptors and their preferred ligand, 5-oxoETE, participate in recruiting eosinophils to sites of allergic reactivity (18). These receptors might also mediate the remodeling of bone, growth of prostate cancer cells, contraction of uterus, and transmission of nerve impulses (26–29). We report here on studies identifying PMN membrane sites that bind 5-oxoETE with the specificity and other properties anticipated for receptors of the 5-oxo class of eicosanoids.

**EXPERIMENTAL PROCEDURES**

Buffers and Other Reagents—Cells and membranes were suspended in a modified Hanks’ balanced salt solution (9) containing 1.4 mM CaCl₂ and 0.7 mM MgCl₂ unless indicated otherwise. Stimuli, glycerolipids, and other reagents were obtained commercially (8, 9). Triacsin C was purchased from Biomol (Plymouth Meeting, PA).

Eicosanoids—We synthesized LTB₄, 5-HETE, 5,20-diHETE, rac-5-HETE, 5,15-diHETE, and 5-oxoETE (9, 21, 30, 31). Before use, 5,15-diHETE, 5(S),15(S)-dihydroxy-[E,Z,Z,Z]-6,8,11,13-ETE; 5,20-diHETE, 5(S),20-dihydroxy-[E,Z,Z,Z]-6,8,11,13-ETE; 15,15(S)-hydroxy-[E,Z,Z,Z]-5,8,11,13-ETE; LTB₄, leukotriene B₄; PAF, platelet-activating factor; FMLP, N-formyl-Met-Leu-Phe; PMN, polymorphonuclear neutrophil; HPLC, high pressure liquid chromatography; BSA, bovine serum albumin; IL, interleukin.
were incubated at 37 °C with label for 5, 10, 20, 40, or 60 min, as well as 80 min, by TLC, HPLC, and other systems revealed that PMNs contained glycerolipid-associated but virtually no intact 5-[3H]HETE (21). We next examined the effect of triacsin C with the expectation that this drug would block the charging of 5-HETE with CoA and thereby reduce its esterification while increasing its accumulation.

RESULTS

Effect of Triacsin C on 5-[3H]HETE Metabolism—PMN suspensions were treated with MeSO or the acyl-CoA synthetase inhibitor triacsin C (33) at 37 °C for 30 min, equilibrated to 4 °C or kept at 37 °C, incubated with 100 nmol/ml of 5-[3H]HETE, separated into cells and media, and analyzed by TLC. PMNs progressively incorporated the label to equilibrium levels over 40 min at 37 °C or 60 min at 4 °C. After 80 min at 37 °C, MeSO-treated PMNs had taken up >90% of the label. On TLC system I, cellular radioactivity migrated with triglyceride and to a small extent phospholipid (Fig. 1, top left panel), whereas medium radioactivity moved with 5,20-diHETE (Fig. 1, bottom left panel). In studies at 4 °C, in contrast, PMNs took up only ~25% of label, and most of this migrated with triglyceride. Media label migrated with 5-HETE (Fig. 2, left panels). The pattern of these results was not limited to 80-min incubations. Analyses of suspensions incubated at 37 or 4 °C with label for 5, 10, 20, 40, or 60 min, as well as 80 min, by TLC, HPLC, and other systems revealed that PMNs contained glycerolipid-associated but virtually no intact 5-[3H]HETE (21). We next examined the effect of triacsin C with the expectation that this drug would block the charging of 5-HETE with CoA and thereby reduce its esterification while increasing its accumulation.

Cell surface alkaline phosphatase (18) were pooled. Pools had <5% of total gradient markers for cytosol (lactate dehydrogenase), endoplasmic reticulum (NADPH-dependent cytochrome c reductase), primary granules (β-glucuronidase and myeloperoxidase), and secondary granules (lysosome- and vitamin B-12-binding protein) but ~50% of gradient activity for Golgi (UDP-galactose:N-acetylgalactosamine galactosyltransferase) and 100% of gradient activity for secretory vesicles (latent alkaline phosphatase (18)). Membranes were diluted with an equal volume of Hanks’ buffer (no CaCl2 or MgCl2) centrifuged (150,000 × g for 1 h at 4 °C), suspended in Hanks’ buffer (1.4 mM CaCl2, 0.7 mM MgCl2), and used immediately.

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Results are typical of studies on cells from four different donors

5,20-diHETE and to a lesser extent 5-HETE in TLC system I (Fig. 1, left panel), TLC system II (not shown), and HPLC (Fig. 3, top panel). In 4 °C experiments, cells had small amounts (<3%), and media had far larger amounts (>70%), of radioactivity that moved with 5-HETE in TLC system I (Fig. 2, top right panel), TLC system II (data not shown), and reversed-phase HPLC (data not shown). Analysis of triacsin C-treated PMNs exposed to 5-[3H]HETE (4 or 37 °C) for 20 min gave results paralleling those shown in Figs. 1 and 2. In particular, these PMNs never amassed more than 3% of total radioactivity as intact 5-[3H]HETE. We conclude that triacsin C blocks the esterification of 5-HETE into glycerolipids. It also leads to an increase in the oxidation of the fatty acid at 37 °C and promotes accumulation of intact 5-[3H]HETE with cells at 4 °C.

5-[3H]OxoETE Metabolism and Effects of Triacsin C—PMNs incubated with 100 pmol of 5-[3H]oxoETE, ± 20 μM triacsin C, for 80 min at 4 or 37 °C incorporated and processed the label in a fashion similar to their processing of 5-[3H]HETE. More particularly, cell label migrated with glycerolipids, media label migrated with 5,20-diHETE, and triacsin C reduced glycerolipid-co-migrating and increased 5,20-diHETE-co-migrating radioactivity (Figs. 4 and 5). However, the media from these incubations also had radioactivity that migrated with 5-HETE in TLC system I (Figs. 4 and 5, bottom panels), TLC system II (data not shown), reversed-phase HPLC (Fig. 3, bottom panel), and normal-phase HPLC (data not shown). Hence, PMNs readily reduce 5-[3H]oxoETE and might process the latter metabolite further. We accordingly examined the radioactivity migrating with lipids and 5,20-diHETE in greater detail. Cell extracts were digested with triglyceride lipase. The label recovered from these digests migrated with 5-HETE rather than 5-oxoETE on TLC (Fig. 6) and reversed-phase HPLC (not shown). Similarly, label in the media from these suspensions eluted on reversed-phase HPLC not only with 5-HETE but also with 5,20-diHETE (Fig. 3, bottom panel). We did not characterize the latter species because studies (12) indicate that it is composed of ω-oxidized metabolites of both 5-HETE and 5-oxoETE. In a final set of experiments, PMNs (± triacsin C; 37 or 4 °C) were exposed to 5-[3H]oxoETE for 20 rather than 80 min. These cells had radioactivity patterns similar to that seen in Figs. 4 and 5, e.g., cells from these shorter term incubations did not contain appreciable levels of intact 5-[3H]oxoETE. We conclude that (a) PMNs reduce 5-[3H]oxoETE to 5-[3H]HETE and esterify the product at 4 or 37 °C; (b) PMNs convert label to ω-oxidized species with-
typical of studies on cells from eight donors. Results are given as the percentage of total recovered radioactivity per 5-mm zone and are typical of studies on cells from eight donors.

Membrane Metabolism of Eicosanoids—PMN plasma membranes esterify 5-[^3H]HETE in lipids at both 37 and 4 °C with-likewise reflect partial digestion of triglyceride to monoglycerides and diglycerides (21). Higher triglyceride lipase concentrations decreased recovery of the latter species while increasing recovery of label migrating with 5-[^3H]HETE (right panels).

Membrane Binding of 5-[^3H]oxoETE—PMN plasma membranes were treated with 10 μM triacsin C, incubated with 25 fmol of 5-[^3H]oxoETE in a volume of 250 μl at 37 °C, and freed of soluble label by passage through GF/C filters. The membranes took up 5-[^3H]oxoETE to apparent equilibrium levels of ~1 fmo/25 μg of membrane protein over 20 min (Fig. 9, top panel, solid line). Binding fell by 80% when 25 pmol of 5-oxoETE was added simultaneously with, 20 min after, or 60 min after (Fig. 9, top panel, interrupted lines) 5-[^3H]oxoETE. 5-OxoETE binding thus was readily saturated, specific, and reversible. The parameter of specific binding rose linearly with membrane mass over a range of 0–40 μg/ml (Fig. 9, middle panel). Scatchard analysis revealed a single class of binding sites with an average $K_d$ of 3.8 nM and a $B_{max}$ of 86 pmol/25 μg of membrane protein (Fig. 9, bottom panel). To examine the specificity of these results, membranes were incubated with 5-[^3H]oxoETE in the presence of selected agents. 5-OxoETE, 5-HETE, and 5,15-diHETE displaced 5-[^3H]oxoETE (Fig. 10, top panel) with potencies that corresponded to their strength in eliciting PMN transient-stimulating (Fig. 10), or other actions (9, 11, 16, 31). 15-HETE had little radiolabel-displacing, Ca^{2+} transient-stimulating (Fig. 10), or other actions (9, 11, 16, 31). LTB₄, a ~30-fold stronger stimulus than 5-oxoETE, interfered minimally with 5-[^3H]oxoETE binding yet totally abrogated [^3H]LTB₄ binding. Conversely, 5-oxoETE did not alter [^3H]LTB₄ binding under conditions where it reduced 5-[^3H]oxoETE specific binding by 95% (Table I). FMLP, PAF, IL-8, and C5a did not displace binding (Table I). Finally, GTP and GTPγS, but not ATP or ATPγS, reduced 5-[^3H]oxoETE binding.

![Fig. 5. Effect of triacsin C on the metabolism of 5-oxoETE at 4 °C. PMN suspensions were treated with Me₆SO (left panels) or 20 μM triacsin C in Me₆SO (right panels), incubated with 100 pmol of 5-[^3H]oxoETE, separated into pellet (top panels) and supernatant (bottom panels) fractions, extracted, and analyzed as in Fig. 1. Results are given as the percentage of total recovered radioactivity per 5-mm zone and are typical of studies on cells from three donors. The radioactivity peaks in the bottom left panel likely reflect partial digestion of triglyceride to monoglycerides and diglycerides (21). Higher triglyceride lipase concentrations decreased recovery of the latter species while increasing recovery of label migrating with 5-[^3H]HETE (right panels).](image50x313)
PMNs presented with psi levels of 5-[^3H]HETE at 37 °C rapidly took up the label and, in pace with this, deposited it in triglyceride, principally triglyceride (Fig. 1, left panels). Our data agree with theirs but also indicate that PMNs ω-hydroxylate 5-[^3H]HETE and rapidly released the product. Triacsin C blocked this reaction and resulted in the accumulation of 5-[^3H]HETE in PMNs (Fig. 2, left panels). Triacsin C blocked this reaction and resulted in the accumulation of 5-[^3H]HETE in PMNs (Fig. 2, left panels). Triacsin C blocked this reaction and resulted in the accumulation of 5-[^3H]HETE in PMNs (Fig. 2, left panels).

DISCUSSION

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Our findings agree with this notion in that PMNs converted 5-oxoETE to 5-HETE (Fig. 4) but had no propensity to conduct the reverse reaction (Fig. 1). Moreover, 5-oxoETE reduction neither reduced nor reversed with excess 5-HETE and therefore could not be ascribed unambiguously to a receptor binding event. The data indicate that either PMNs lack discrete 5-HETE receptors or 5-[^3H]HETE is unsuited to detect these receptors in whole PMNs even at 4 °C and in the presence of triacsin C.

Powell et al. (6, 34) found that the endoplasmic reticulum of PMNs has both NADPH-dependent reductase and NADP⁺-dependent dehydrogenase activities that, in the presence of the appropriate cofactor, convert 5-HETE to 5-oxoETE and 5-oxoETE to 5-HETE, respectively, in vitro. Whole PMNs, however, reduce 5-oxoETE but do not dehydrogenate 5-HETE, perhaps because the cells maintain a high ratio of NADPH to NADP⁺ (6). Our findings agree with this notion in that PMNs converted 5-oxoETE to 5-HETE (Fig. 4) but had no propensity to conduct the reverse reaction (Fig. 1). Moreover, 5-oxoETE reduction occurred at both 4 and 37 °C and appeared to dominate esterification. That is, PMNs incubated with 5-[^3H]oxoETE deposited the label in triglyceride-co-migrating material. Triglyceride lipase digestion of the extracts from these cells recovered...
5-[^3]H[HETE but not 5-[^3]H[oxoETE (Fig. 6). Because plasmalemma can esterify 5-[^3]H[oxoETE to glycerolipids (Fig. 7, top panel), whole PMNs likely have a latent capacity to esterify 5-oxoETE. Failure to observe this esterification may be due to the enzymatic reduction of substrate as it enters the cell, an esterification pathway that prefers 5-HETE to 5-oxoETE, or an NADPH-dependent dehydrogenase that rapidly esterifies 5-oxoETE.

At 37 °C, PMNs converted 5-[^3]H[oxoETE to a species (Figs. 4 and 5, bottom panels) that moved as free fatty acid on TLC system II (data not shown) and eluted with 5,20-diHETE on reversed-phase HPLC (Fig. 3). The latter label likely contains system II (data not shown) and eluted with 5,20-diHETE on the bottom panel. In any event, the combined operation of the various metabolic pathways left no residual 5-[^3]H[oxoETE in PMNs at 37 °C (Fig. 4). At 4 °C, PMNs showed no evidence of ω-oxidizing activity yet efficiently reduced 5-[^3]H[oxoETE and esterified product 5-[^3]H[HETE into lipid. Triacsin C inhibited this reaction. Nonetheless, cells treated with the drug reduced 5-[^3]H[oxoETE and thereafter efficiently released the product (Fig. 5). Triacsin C-treated PMNs did not accumulate 5-[^3]H[oxoETE. The latter label thus seems as unsuited as 5-[^3]H[HETE for whole cell receptor binding studies.

Plasmalemma from PMNs readily takes up 5-[^3]H[HETE but then deposes virtually all of the incorporated label in membrane lipid (8). Fig. 7 shows that membranes also metabolize 5-[^3]H[oxoETE, converting it to a triglyceride lipase-sensitive species. This finding indicates that the membranes, unlike whole PMNs, readily esterify 5-oxoETE. Triacsin C inhibited this reaction (Fig. 8, top panel). Because our membrane isolates have almost no endoplasmic reticulum marker enzymes or LTB_4 ω-hydroxylase activity (36), it is unlikely they would have endoplasmic reticulum-associated (6) 5-oxoETE reductase or ω-hydroxylase activities. This notion was confirmed here: triacsin C-treated membranes failed to metabolize 5-[^3]H[oxoETE to any measurable extent. The last finding also defined an experimental condition for detecting 5-oxoETE receptors.

In the presence of triacsin C, PMN plasma membranes bound 5-[^3]H[oxoETE. Binding was time-dependent, proportional to membrane mass, and dispersed as well as reversed by excess 5-oxoETE (Fig. 9). The ligand bound to 86 fmol per 25 μg of membrane protein of sites with an average dissociation affinity of 3.8 nM. PMN plasma membranes treated with triacsin C thus express a finite number of sites that reversibly bind 5-oxoETE. To establish the relationship of these sites to 5-HETE receptors, we performed three sets of studies. First, 5-oxoETE, 5-HETE, and 5,15-diHETE use the same recognition system to stimulate PMNs (31, 37). The eicosanoids elicited Ca^{2+} transients with ED_50 potencies of 10, 200, and 7000 nM, respectively (Fig. 10, bottom panel). Their respective ID_{50} (37) values for reducing 5-[^3]H[oxoETE binding were 70, 50, and 5000 nM. In addition, 15-HETE failed to stimulate PMNs and only marginally (ID_{50} >10,000 nM) altered 5-[^3]H[oxoETE binding (Fig. 10). The correlation between these two actions of the eicosanoids was 0.99 (p < 0.05), a correspondence implying that the binding sites transmit the Ca^{2+}-mobilizing action of the analogs. There is a similarly high correlation between the binding displacement of the analogs and PMN degranulating (9), chemotactic (16), and other effects (9, 12, 13, 16). Second, binding sites for 5-oxoETE and LTB_4 were essentially non-

Fig. 10. The effect of selected eicosanoids on the membrane binding of 5-[^3]H[oxoETE (top panel) or PMN Ca^{2+} transients (bottom panel). In the top panel, membranes (20–45 μg of protein) were treated with 10 μM triacsin C for 30 min, incubated with 100 μM 5-[^3]H[oxoETE plus the indicated concentration of eicosanoid for 60 min at 37 °C, and passed through GF/C filters. Test eicosanoids were 5-oxoETE (1), 5-HETE (2), 5,15-diHETE (3), and 15-HETE (4). Data are corrected for the amount of radioactivity adherent to filters from experiments incubating label without membranes. Results are the mean percentages of radioactivity trapped by filters ± S.E. for 3–6 experiments. In the bottom panel, fura2-treated PMNs were challenged with an eicosanoid for 1 min and assayed for the concentration of cytosolic Ca^{2+} for four experiments (Ca^{2+} was ~45 nM in resting and BSA-stimulated PMNs).

TABLE I

| Agent    | Label         | μM  | 5-[^3]H[oxo  | 5-[^3]H[LTB_4 | μM  | 5-[^3]H[oxo | 5-[^3]H[LTB_4 |
|----------|---------------|-----|--------------|--------------|-----|--------------|--------------|
| BSA      | 4.9 ± 1.2a    | 4.9 ± 0.5    | 30.5 ± 3.2   | BSA          | 5.4 ± 0.4    | 28.8 ± 2.1   |
| 5-oxo, 1 | 0.4 ± 0.5     | 0.4 ± 0.5    | 29.2 ± 6.6   | GTP_8, 1     | 0.8 ± 0.4a   | 11.8 ± 0.3a  |
| 5-oxo, 0.1| 0.6 ± 0.2     | 0.6 ± 0.2    | 31.5 ± 8.8   | GTP_8, 0.1   | 1.8 ± 0.9a   | 18.4 ± 2.1a  |
| 5-oxo, 0.01| 1.6 ± 0.6     | 1.6 ± 0.6    | 27.8 ± 5.2   | GTP, 10      | 2.4 ± 0.4a   | 17.9 ± 0.6a  |
| LTB_4, 1 | 3.8 ± 0.4a    | 3.8 ± 0.4a   | 0.1 ± 0.4a   | GTP, 1       | 3.3 ± 0.4a   | 18.4 ± 4.8a  |
| LTB_4, 0.1| 5.1 ± 0.7     | 5.1 ± 0.7    | 0.5 ± 0.5a   | GTP, 0.1     | 3.7 ± 0.8a   | 25.5 ± 4.3a  |
| LTB_4, 0.01| 4.6 ± 0.4     | 4.6 ± 0.4    | 8.9 ± 2.3    | GTP, 0.01    | 5.0 ± 0.1    | 29.3 ± 4.0   |
| FMLP, 1  | 5.6 ± 0.4     | 5.6 ± 0.4    | 32.2 ± 0.7   | ATP_8, 1     | 6.8 ± 1.1    | 30.3 ± 1.0a  |
| PAF, 100 | 5.9 ± 0.2     | 5.9 ± 0.2    | 33.8 ± 0.3   | ATP_8, 0.1   | 6.9 ± 1.3    | 31.1 ± 2.6a  |
| IL-8, 25 | 6.7 ± 1.5     | 6.7 ± 1.5    | 26.1 ± 2.0   | ATP, 10      | 6.2 ± 0.8    | 24.7 ± 3.5   |
| C5a, 25  | 6.4 ± 1.0     | 6.4 ± 1.0    | 26.0 ± 4.9   | ATP, 1       | 5.2 ± 0.1    | 30.1 ± 2.0   |

5-[^3]H[oxo and 5-oxo are 5-[^3]H[oxoETE and 5-oxoETE, respectively. Membranes were pretreated with an agent for 0 (columns on left) or 30 (columns on right) min.

[^3]H[oxoETE binding and selectivity. Values significantly below those for BSA-treated membranes (p < 0.05, Student’s paired t test).

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teracting (Table I). This agrees with studies (7–11) showing that these two agents share 5-hydroxy, 6–7 double bond motifs yet still act on different recognition systems. FMLP, PAF, IL-8, and C5a likewise did not interfere with 5-\(^{3}H\)oxoETE binding (Table I). The latter chemotactic factors act through respective receptors that do not recognize 5-oxoETE (9). The 5-oxoETE binding site defined here, then, is unique and does not involve receptors for other chemotactic factors or metabolic enzymes, such as LTB\(_4\) \(\omega\)-hydroxylase. Third, GTP and GTP\(\gamma\)S, but not their adenosine analogs, reduced the membrane binding of 5-\(^{3}H\)oxoETE. The effect paralleled that found for the binding of \[^{3}H\]LTB\(_4\) (Table I), an agent that operates on G protein-linked receptors (4). Such receptors induce G proteins to exchange GTP for GDP and thence to dissociate into \(\alpha\) and \(\beta\gamma\) subunits. The receptors also must associate with G proteins in order to maintain a high affinity for their ligand. GTP analogs, by disrupting G proteins, convert serpentine receptors to a low affinity or nonbinding configuration (38). The nucleotide effects of Table I thus implicate receptor-G protein linkages in 5-\(^{3}H\)oxoETE binding. Because the stimulating actions of 5-HETE and 5-oxoETE require pertussis toxin-sensitive G proteins (8, 18), the structurally specific effects of GTP analogs reinforce a notion that 5-oxoETE binding sites are in fact receptors.

In conclusion, PMNs and their isolated plasma membranes utilize a triacsin C-sensitive acyl-CoA synthetase pathway to esterify 5-HETE and 5-oxoETE to glycerolipids. The pathway operates at 37 or 4 \(^\circ\)C. A second, and also temperature-insensitive, pathway reduces 5-oxoETE to 5-HETE and, in whole cells, dominates esterification: PMNs reduce 5-oxoETE before depositing it in glycerolipid. In a third pathway, 5-HETE and 5-oxoETE are \(\omega\)-oxidized. This route requires physiological temperatures and processes large amounts of substrate when acyl-CoA synthetase is blocked or saturated. Operation of these varying pathways obscures the whole-cell interactions of 5-HETE and 5-oxoETE with receptors. Plasmalemma, however, fails to oxidize, reduce, or, in the presence of triacsin C, esterify 5-oxoETE. Rather, it binds 5-oxoETE specifically and reversibly. Stringent criteria implicate these binding sites in mediating PMN responses to the 5-oxa class of eicosanoids. Finally, the effect of GTP analogs on 5-\(^{3}H\)oxoETE binding suggests that the latter sites belong to the serpentine superfamily of G protein-linked receptors.

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