5-Hydroxyicosatetraenoate Stimulates Neutrophils by a Stereospecific, G Protein-linked Mechanism*

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We examined how 5-hydroxyicosatetraenoate (5-HETE) activates human neutrophils (PMN). 5-HETE stimulates PMN to mobilize Ca\textsuperscript{2+} but has little effect on degranulation or superoxide anion production. It nonetheless stereospecifically induced these responses in cells primed with tumor necrosis factor-\textgreek{a} and likewise induced PMN plasma membranes to bind \textsuperscript{35}S-labeled guanosine 5'-O-(thiotriphosphate) (GTP\textsuperscript{\gamma}S) and phosphohydrolyze [\gamma\textsuperscript{32}P]GTP. Pertussis toxin blocked GTP\textsuperscript{\gamma}S binding responses. Scatchard analyses of GTP\textsuperscript{\gamma}S binding indicated that 5-HETE raised the \textk\ of high affinity GTP\textsuperscript{\gamma}S binding sites without altering these sites' numbers or the parameters of low affinity GTP\textsuperscript{\gamma}S binding. Since N-formyl-Met-Leu-Phe, platelet-activating factor, and leukotriene (LT) \textsubscript{B\textsubscript{4}} have these same bioactions, receptors for the latter agents might mediate responses to 5-HETE. However, 5-HETE binding sites are down-regulatable, receptor agonists desensitized degranulation responses to itself but not to the receptor agonists, the receptor agonists desensitized to themselves but not 5-HETE, and a LT\textsubscript{B\textsubscript{4}} antagonist inhibited LT\textsubscript{B\textsubscript{4}} but not 5-HETE in all assays. Finally, PMN and their membranes took up \textsuperscript{3}H 5-HETE at 4 or 37 °C but, at both temperatures, also acylated the radiolabel into glycerolipids. Aclylation nullified assessment of 5-HETE binding and questions reports that measure the cell binding, but not metabolism, of various HETEs. Our studies thus indicate 5-HETE acts by a down-regulatable, G protein-linked mechanism and represent the best available evidence that 5-HETE does not operate through, for example, LT\textsubscript{B\textsubscript{4}}, receptors.

Cells regulate their function and communicate with each other using arachidonic acid metabolites. Most of these messenger eicosanoids, e.g. the leukotrienes (LTs), lipoxins, thromboxanes, and prostaglandins, operate by well defined mechanisms; they bind to their respective, G protein-linked receptors that, in turn, initiate reactions leading to cell function (1, 2). The actions of hydroxyicosatetraenoates (HETEs), in contrast, have evaded such simple classification. HETEs stimulate (3-16) and bind to (17-30) diverse cell types. They too, then, may act via receptors. These receptors need not be HETE receptors: HETEs bind with LT\textsubscript{B\textsubscript{4}} receptors on dermal cells (20, 21, 25, 27) or thromboxane receptors on platelets (28) and blood vessels (29, 30). Moreover, HETEs bind to cytosolic carrier proteins (31) and cells have an enormous capacity to store HETEs in their glycerolipids (3-9, 17, 32-34). The latter effects can mimic receptor binding, particularly in studies (17-21, 23, 24, 29) done at 37 °C. Finally, HETEs often influence cells only at >10 \textmu\textsuperscript{M}. Micromolar HETEs alter plasma membrane fluidity (5-7, 9, 18), activate protein kinase C (13, 35), inhibit diacylglycerol kinase (36), regulate arachidonate-metabolizing enzymes (10, 11, 37-41), and initiate toxic peroxidizing reactions (42). Such effects, rather than receptor activation, may mediate responses to HETEs. In view of these considerations, the case for unique HETE receptors remains open.

Human polymorphonuclear neutrophils (PMN) respond to HETEs (15, 43-47). They also store HETEs in glycerolipids (41, 48-51), \textomega\-oxidize and dehydrogenate 5-HETE and 12-HETE (51-56), and convert 15-HETE to lipoxins (41, 54). These transformations (41, 48-51) or, alternatively, interactions with LT\textsubscript{B\textsubscript{4}} receptors (57-59) or effector enzymes (37-39, 41) may underlie HETE-induced responses, particularly those requiring micromolar levels of the eicosanoid. Some HETE actions, in contrast, seem unexplained by these mechanisms. 5-HETE induces PMN to raise cytosolic Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]) but has little intrinsic ability to stimulate functional (e.g. degranulation) responses. The eicosanoid nevertheless markedly increases the ability of platelet-activating factor (PAF) and direct protein kinase C activators to stimulate function (59-61). Hence, 5-HETE is a partial PMN agonist with actions that are most clearly expressed in conjunction with certain other, more completely acting, stimuli. In eliciting [Ca\textsuperscript{2+}], rises, 5-HETE demonstrates nanomolar potency, structural dependence, stereospecificity, ability to desensitize to itself, and sensitivity to pertussis toxin (59). The HETE thus raises PMN [Ca\textsuperscript{2+}], by a sensitive, structurally selective, down-regulatable, G protein-dependent, and possibly receptor-mediated recognition system. On the other hand, PMN metabolism of 5-HETE has structural and stereospecificity requirements quite similar to those needed for 5-HETE biactivity (51, 54, 55). Furthermore, 5-HETE and LT\textsubscript{B\textsubscript{4}} weakly cross-desensitize PMN [Ca\textsuperscript{2+}], responses to each other. 5-HETE, while not reducing prostaglandin \textsubscript{E\textsubscript{3}} prostaglandin \textsubscript{D\textsubscript{3}} or PAF binding, weakly suppresses PMN binding of LT\textsubscript{B\textsubscript{4}}. The [Ca\textsuperscript{2+}]-raising actions of 5-HETE and LT\textsubscript{B\textsubscript{4}} are equally
sensitive to pertussis toxin (59, 64). Here as elsewhere, evidence that a HETE operates via unique receptors, rather than by, e.g., acting as a weak LTβ receptor agonist or stimulator of effector enzymes, is inconclusive. This report expands the scope of 5-HETE's actions on PMN and provides evidence that the HETE does not operate through receptors involved in transmitting PMN responses to LTβ, PAF, or N-formyl-Met-Leu-Phe.

MATERIALS AND METHODS

Reagents and Buffers—5(S)-HETE and 5(R)-HETE were synthesized from arachidonate (63). 5(S)-HETE, 5(S),20-di-hydroxy-6,8,11,14-E, Z.Z.Z-icosatetraenoe (5,20-dih-IE) and LTβ, were biosynthesized (59, 62). [35S]GTPγS (1210 Ci/mol), [35S]GTP (5000 Ci/mol), and [H]GTP+ (191 Ci/mmol) were from American Corp.; FMLP was from Calbiochem (LaJolla, CA); G/F/C filters were from Whatman, Maidstone, England; cytochalasin B, NAD+, creatine phosphate, creatine kinase, Trizma (Tris) base, bovine serum albumin (BSA), and Norii charcoal were from Sigma; pertussis toxin was from List Biological Laboratories (Campbell, CA); PAF and nuclease phosphoglycans were from Bachem (Philadelphia, PA); tumor necrosis factor-α (TNF-α) was from Genzyme (Boston, MA). LY 255283 was a gift from Lilly Research Laboratories (Indianapolis, IN). PMN were suspended in modified Hanks' balanced salt solution (1.4 mM Ca++) (59) or potassium-enriched relaxation buffer (59, 62). 5(S)-HETE binding experiments were done with five volumes of 4 °C Hanks' buffer, air-dried, incubated in 0.5 mM of methanol for 10 min, mixed with scintillation fluid, and counted for radioactivity (65). Results are reported as the fraction of added label trapped by filters loaded with reactions minus that trapped by filters loaded with reactions lacking membranes. Membranes from TNF-α and BSA-treated preparations were incubated with 10 pmol (100 nm) GTP minus that of reactions incubated with 5000 pmol (50 μM) GTP. The latter, high Kₐ values, were <8% of low Kₐ and did not change during membrane stimulation.

5-HETE Uptake and Metabolism—10⁷ PMN or 5 μg of membrane protein were incubated in 1 ml of Hanks' buffer at 37 or 4 °C for 20 min, exposed to 100 pmol [3H]-5-HETE ≥ 5 μM 5-HETE, and incubated for 5–80 min at 4 °C. For uptake, reactions were passed through GF/C filters. For GTP hydrolysis, membranes from 5-HETE uptake and metabolism studies, reactions were placed on ice, centrifuged (11,000 × g, 5 s, 4 °C), and quickly washed four times with 1 ml of 4 °C Hanks' buffer. Cells and pooled substrates were extracted separately by an acidic Bligh and Dyer method and analyzed on TLC (pre-activated (180 °C for 3 h) silica gel plates developed to 15 cm with ethyl ether/hexane/glacial acetic acid (60:40, v/v/v)). Zones (0.5 cm) were scraped from the plates, incubated with 0.5 μM of methanol for 10 min, mixed with scintillation fluid, and counted for radioactivity. In selected experiments, identity of isolated material was verified by its elution with 5-HETE or 5,20-dih-IE on reverse-phase HPLC or, alternatively, sensitivity to phospholipase A₂, lipase, and saponification (61). The latter three reactions converted 90% of label migrating with glycerolipids to label migrating with 5-HETE on TLC. Membrane metabolic studies stopped reactions with acidic Bligh and Dyer extraction solvents and analyzed radiolabel as in cellular studies.

Pertussis Toxin Treatment—Pertussis toxin (8 μg) was activated at 30 °C by incubation with 20 μl of buffer (pH 8) containing 40 mM dithiothreitol, 100 mM triethanolamine, and 160 μg of BSA. After 30 min, reactions were diluted with 160 μl of the same buffer containing 4 μg of membrane protein and 15 mM NAD+. After 30 min, 20 μl membrane samples were added to 50 μl of binding buffer containing (11,000 × g, 5 s, 4 °C) to obtain supernatants, which were assayed for supern oxide anion production. Results are compared to membranes that were identically treated except for being incubated with 8 μg of BSA in place of pertussis toxin.

RESULTS

Ca²⁺ Transients—LTB₄, 5(S)-HETE, and FMLP stimulated PMN to raise [Ca²⁺]. Each stimulus induced responses that peaked at ~15 s and then completely reversed over the ensuing 1–2 min. LY 255283, an LTβ, competitive antagonist (68), reduced the Ca²⁺-mobilizing actions of all three stimuli but had much greater effects on LTβ, Fig. 1 exemplifies these results by giving the [Ca²⁺], rise found 15 s after stimulation of PMN treated with 0, 1, or 3 μM LY 255283. The antagonist reduced the amplitude of Ca²⁺ transient responses but did not alter the kinetics of these responses and, by itself, did not influence [Ca²⁺]. Thus, LY 255283 lacked intrinsic Ca²⁺ mobilizing effects and showed some nonspecific ability to desensitize [Ca²⁺], rises. Nevertheless, it discriminated the [Ca²⁺]-raising actions of LTβ, B from those of 5(S)-HETE and FMLP.

Degranulation and O₂⁻ Production—5(S)-HETE failed to degranulate PMN (first dose-response curve in each panel of Fig. 2), whereas TNF-α caused small amounts of lysozyme to
be released (first data point for each curve of Fig. 2). In combination, in contrast, the two agents proved capable of releasing remarkably large amounts of lysozyme. Responses increased with TNF-α (3–3200 units/ml and 5(S)-HETE (5–5000 nM) concentrations. For all stimuli combinations, however, PMN incubated with TNF-α for 7.5–15 min were more responsive to 5(S)-HETE than cells treated with TNF-α for 22.5 or 30 min (Fig. 2), whereas PMN exposed to TNF-α for 3.75 or 60 min had minimal responses to 5(S)-HETE (not shown). Companion experiments helped interpret these results. First, TNF-α and 5(S)-HETE also cooperated to release β-glucuronidase (Fig. 3, right panel) and trigger O₂⁻ production (Table I). The TNF-α/5(S)-HETE interaction therefore is not limited to secondary granule enzyme (lysozyme) release but also includes release of a primary granule enzyme (β-glucuronidase) as well as stimulation of the oxidative burst. Second, 5(R)-HETE was much weaker than 5(S)-HETE in stimulating TNF-α-primed PMN to release lysozyme and β-glucuronidase (Table I). The antagonist by itself failed to elicit degranulation and, in contrast to results in [Ca²⁺]i assays, did not suppress 5(S)-HETE-induced degranulation. This finding further differentiates the PMN-stimulating actions of 5(S)-HETE from LTB₄. Finally, PMN did not release the cytotoxic enzyme, lactic acid dehydrogenase, in any of the assays. Degranulation, therefore, was not due to PMN cytolysis. We conclude that 5(S)-HETE acts by a stereospecific, selectively desensitizable mechanism to stim-

**TABLE I**

| Stimulus          | BSA | TNF-α  | Priming agent | 5(S)-HETE | 5(R)-HETE |
|-------------------|-----|--------|---------------|-----------|-----------|
| 5(S)-HETE         | 0.001 ± 0.001* | 0.340 ± 0.018* | 0 ± 0 | 0.070 ± 0.003* |

*Mean ± S.E. (n = 3–5) maximal rates of O₂⁻ release in nmol/min/10⁷ PMN. All values were corrected for the O₂⁻ release that was not inhibited by superoxide dismutase.

Values were significantly (p < 0.05, Student’s paired t test) above unstimulated PMN. By itself, TNF-α induced minimal (< 0.001 nmol/min/10⁷ PMN) O₂⁻ release.

**TABLE II**

| Desensitization of degranulation responses in TNF-α-primed PMN |
|---------------------------------------------------------------|
| Stimulus           | BSA | 5(S)-HETE | LTB₄ | PAF | fMLP |
| 5(S)-HETE          | 5.9 ± 0.7  | 3.7 ± 2.1  | 12.0 ± 0.9 | 5.7 ± 1.8 | 5.0 ± 2.2 |
| LTBA₄              | 2.4 ± 0.2  | 1.8 ± 0.4  | 1.0 ± 0.2  | 2.4 ± 0.2  | 2.2 ± 0.4  |
| PAF                | 0.8 ± 0.2  | 0.8 ± 0.2  | 0.8 ± 0.2  | 0.8 ± 0.2  | 0.8 ± 0.2  |
| fMLP               | 11.5 ± 0.5 | 11.5 ± 0.5 | 11.5 ± 0.5 | 11.5 ± 0.5 | 11.5 ± 0.5 |

*Percentage of total cell lysozyme released ± S.E. for 7 experiments. All data were corrected for the release induced by TNF-α alone. Experiments using various other concentrations of the agonists as desensitizers and stimuli yielded similar results. β-Glucuronidase release was similarly effected by the desensitizing protocol.

Values were significantly lower (p < 0.01, Student’s paired t test) than those for PMN not exposed to a desensitizing stimulus.
Effect of LY 255283 on PMN degranulation responses

PMN were incubated at 37 °C with BSA or 1000 units/ml TNF-α for 10 min, exposed to 0 or 1 μM LY 255283 for 2.5 min, treated with cytochalasin B for 2.5 min, and challenged for 5 min with 500 nM 5(S)-HETE or 3 nM LTB₄.

| Stimulus       | Lysozyme | β-Glucuronidase |
|----------------|----------|-----------------|
| BSA            | BSA      | BSA             |
| 5(S)-HETE      | 0.8 ± 0.9 α 14.2 ± 1.8 0.8 ± 0.4 13.4 ± 2.6 |
| 5(S)-HETE + LY | 2.4 ± 1.4 18.6 ± 2.6 1.1 ± 0.7 16.1 ± 1.5 |
| 5(R)-HETE      | 8.8 ± 0.9 29.6 ± 4.2 8.6 ± 1.9 25.3 ± 4.4 |
| LTB₄           | 4.4 ± 1.0 α 19.0 ± 4.1 4.2 ± 1.3 15.4 ± 5.8 α |

* Percentage of total cell enzyme released ± S.E. for 6 experiments. All data are corrected for release induced by BSA or TNF-α alone.
  * Values were significantly (p < 0.005, Student’s paired t test) lower than those induced by the same stimulus in the absence of LY 255283.

![Figure 4. Stereospecificity of 5-HETEs in activating PMN G proteins.](image)

For GTPyS binding (upper panels), plasmaemalaa (100 ng of protein) was incubated with 5000 amol of [³²⁵]GTPyS, 320 nM GDP, and 0 (shaded bars) or 5, 50, 500, or 5000 (successive open bars) nM 5(S)- or 5(R)-HETE for 30-120 min. Data are the mean amol of [³²⁵]GTPyS bound per 200 ng of membrane protein after correction for the amount of label released in experiments incubating membranes with 5000, rather than 10, pmol of GTP. Data are thus low Kₐ hydrolysis values. Asterisks indicated those values significantly greater (p < 0.05, Student’s paired t test) than unstimulated membranes.

![Figure 5. Specificity of LY 255283 in blocking PMN membrane GTPyS binding responses.](image)

For GTPyS binding (upper panels), plasmaemalaa (100 ng of protein) were incubated with LY 255283 for 5 min and then exposed to 50 pm [³²⁵]GTPyS ± 100 μM GTPyS, 320 nM GDP, and 5(S)-HETE (left panels) or LTB₄ (right panels) for 30 (upper panels) or 60 (lower panels) min. Data are the mean amol of [³²⁵]GTPyS bound per 100 ng of membrane protein above that found in unstimulated but otherwise identically treated membranes for 7 experiments ± S.E. LY 255283 by itself did not alter binding but, as indicated by asterisks, significantly reduced (p < 0.05, Student’s paired t test) the membrane binding response to LTB₄. Note the differences in y axes.
TABLE IV
Effects of various stimuli on the parameters of GTPyS binding to PMN plasma membranes

PMN plasma membranes were incubated (2 h, 30 °C) with 5 pmol [3H]GTPyS plus GTPyS, 320 nM GDP, 4 mM free Mg2+, and 125 µg/ml BSA ± the indicated stimulus. Reactions were filtered, counted for radiolabel binding, and evaluated for binding sites using LIGAND. In all cases, LIGAND indicated the presence of two binding sites (p < 0.02, F-distribution).

| Treatment          | High affinity | Low affinity |
|--------------------|--------------|-------------|
|                    | Kd (nM) | Bmax (fmol/50 µg of membrane protein) | Kd (nM) | Bmax (fmol/50 µg of membrane protein) |
| BSA                | 1.3 ± 0.14    | 8.0 ± 0.86  | 0.05 ± 0.01  | 14 ± 1.2  |
| 5 µM 5(S)-HETE     | 1.8 ± 0.17    | 5.3 ± 1.30  | 0.06 ± 0.03  | 15 ± 1.7  |
| 100 nM PAF         | 2.1 ± 0.28    | 6.7 ± 0.77  | 0.05 ± 0.01  | 15 ± 1.1  |
| 100 nM FMLP        | 2.7 ± 0.31    | 6.1 ± 0.54  | 0.06 ± 0.01  | 16 ± 1.0  |
| 100 nM LTBP        | 3.7 ± 0.48    | 5.4 ± 1.13  | 0.07 ± 0.03  | 17 ± 1.2  |

*Kd (10^6 liter/mol) and Bmax (fmol/50 µg of membrane protein) ± S.E. for 8–11 experiments.

†Values were significantly greater (p < 0.05, Student’s unpaired t test) than BSA-treated membranes.

Fig. 6. GTPγS binding responses. PMN plasma membranes (50 or 100 ng) were incubated with 50 pmol [3H]GTPγS ± 100 µM GTP, 320 nM GDP, and the indicated stimuli for 30 min. Results are the rise in the fractional specific binding of radiolabel above unstimulated membranes per 100 ng of membrane protein for 7–17 experiments and generally had S.E. of <15% of reported mean values.

TABLE V
Effects of pertussis toxin on the GTPγS binding responses of PMN membranes

PMN plasma membrane isolates (4 µg of protein) were incubated with 0 or 8 µg/ml pertussis toxin in a final reaction volume of 320 µl for 30 min and then diluted into a final reaction (100 µl) containing 50 pmol [3H]GTPγS ± 100 µM GTP, 320 nM GDP, and 12.5 µg of BSA ± a test stimulus. After 15– or 30-min incubations, reactions were assayed for label binding.

| Stimulus          | Incubation time (pertussis toxin concentration) | 15 min | 30 min |
|-------------------|-----------------------------------------------|--------|--------|
| 5(S)-HETE (500 nM) | 0 25 µg/ml                                    | 0      | 25 µg/ml|
| PAF (3 nM)        | 0 25 µg/ml                                    | 0      | 25 µg/ml|
| LTBP (3 nM)       | 0 25 µg/ml                                    | 0      | 25 µg/ml|
| FMLP (10 nM)      | 3.7 ± 0.5                                    | 1.4 ± 0.4  | 4.3 ± 0.7  | 1.6 ± 0.4  |

*Percentage of ambient radioactivity specifically bound ± S.E. per 250 ng of membrane protein for 4 experiments.

†Values were significantly lower (p < 0.05, Student’s paired t test) than membranes incubated with no pertussis toxin.

uptake peaked at ~0.12 (Fig. 7). While such results might reflect receptor binding, PMN are known to process 5-HETE at 37 °C. For example, PMN suspensions exposed to 100 pmol [3H]5(S)-HETE ± 5 µM 5(S)-HETE at 37 °C for 5–80 min contained labels that migrate with triglyceride, phospholipid, 5(S),20-diHETE, and 5(S)-HETE on TLC. Labels migrating with 5,20-diHETE and 5(S)-HETE occur only in extracellular fluid, whereas cell-associated labels migrate with triglyceride and phospholipid (51). PMN incubated with 100 pmol [3H]5-HETE at 4 °C for 80 min contained extracellular radiolabel that migrated with 5(S)-HETE but not 5,20-diHETE (Fig. 8, upper left panel). The lower temperature thus blocks ω-oxidation not only of LTB4 (85) but also of 5(S)-HETE. Unexpectedly, however, cellular radioactivity in these experiments migrated exclusively with triglyceride and phospholipid (Fig. 8, upper right panel). Addition of 5 µM 5(S)-HETE to the PMN reduced label uptake without changing the general pattern of results (Fig. 8, lower panels). Similar findings occurred with PMN incubated for 40 min at 4 °C (data not shown). Indeed, plasma membrane exposed to 100 pmol [3H]5-HETE at 37 or 4 °C for 40 min formed triglyceride- and phospholipid-co-migrating radioactivity. In the presence of 5 µM 5(S)-HETE, the membranes converted lower although still measurable percentages of the label to the latter species (data not shown). Further studies found that: (a) radioactivity in the supernatants of whole cell (4 °C) incubations migrated (>90%) with 5(S)-HETE but not 5,20-diHETE on HPLC; (b) lipase treatment or saponification of cell or membrane
labels that migrated with triglyceride on TLC (from 37 or 4 °C incubates) yielded label (>98%) moving with 5(S)-HETE on TLC; and (c) phospholipase A2 treatment or saponification of labels that migrated with phospholipid on TLC (from 37 or 4 °C incubates) also yielded label moving (>98%) with 5(S)-HETE on TLC (not shown). Thus, PMN take up and acylate 5(S)-HETE into triglycerides and phospholipids at 4 as well as 37 °C. Acylation reactions keep pace with ligand uptake to effect virtually complete removal of intact 5(S)-HETE from PMN. Membranes similarly acylate 5(S)-HETE at both temperatures. In all cases, then, 5(S)-HETE uptake and metabolism occur more or less concomitantly.

5(S)-HETE has limited actions on PMN. It induces Ca2+ transients but has little or no ability to stimulate degradation or oxidative metabolism. We broadened the biological spectrum of 5(S)-HETE using TNF-α, a cytokine that primes PMN to LTB4, PAF, and fMLP (66). TNF-α, in effect, converted the HETE from an incomplete agonist to one fully capable of eliciting exocytotic and O2; production responses (Fig. 2, Table I). 5(S)-HETE furthermore desensitized TNF-α-primed PMN to itself (Table II), stimulated PMN plasma-lemmata in associate with hydrolyze [γ-32P]GTP and bind [35S] GTPγS (Fig. 4), and worked by a stereospecific (Figs. 3 and 4), pertussis toxin-sensitive (Table V) mechanism. LTB4, PAF, and fMLP had these same actions in stimulating primed PMN (66) (Table II) and PMN membranes (Fig. 6, Tables IV and V) allowing the possibility that 5(S)-HETE operated through one or more receptors for these agonists. However, 5(S)-HETE did not cross-desensitize with the agonists in assays of PMN degranulation (Table II), did not block PMN binding of [3H]fMLP or [3H]PAF (59), and was insensitive to a LTB4, antagonist (Figs. 1 and 5 and Table III). Evidently, then, responses to 5(S)-HETE do not require PAF, fMLP, or LTB4, receptors.

The above results prompted a search for 5(S)-HETE binding sites. Since PMN ω-oxidize, dehydrogenate, and acylate 5(S)-HETE at 37 °C (33, 34, 35, 61), experiments were done at 4 °C. The lower temperature blocked ω-oxidation (dehydrogenation products are not detected by our methods) but acylation reactions persisted (Fig. 8). Plasma membrane also failed to ω-oxidize [3H]5(S)-HETE (our incubates omitted exogenous NADPH, a co-factor for C-20 hydroxylation) but still incorporated radiolabel into glycerolipids even at 4 °C (Figs. 8 and 9). We in fact never detected unmetabolized 5(S)-HETE in PMN. Moreover, PMN membranes (5 μg of protein) incubated with 100 pm [3H]5(S)-HETE for 40 min at 4 °C incorporated 3.4% of added label. Incorporation fell to 1.2% in reactions containing 5 μM 5(S)-HETE. Metabolic experiments done under identical conditions indicated that the membranes had deposited 3 or 1%, respectively, of label in glycerolipids after incubations with 100 pm [3H]5(S)-HETE or 100 pm [3H]5(S)-HETE plus 5 μM 5(S)-HETE. Thus, the uptake of 5(S)-HETE by PMN or their membranes reflects principally, if not exclusively, glycolipid deposition. This occurrence voids any assessment of PMN 5(S)-HETE receptors and challenges observations on HETE binding whether done at 37 °C (18–21, 23, 24, 29) or 4 °C (22, 25–28, 30). Earlier work may have included a saturable, temperature-insensitive acylation or other metabolic reaction in measurements of ligand specific binding.

Our functional studies indicate that 5(S)-HETE acts by a down-regulatable, G protein-linked mechanism that does not involve receptors for three typical agonists. Obviously, PMN receptors for other agonists, e.g. lipoxin A4 (69), prostaglandin E2, prostaglandin D2 (64), or ATP (70), could be 5(S)-HETE targets. However, prostaglandins E2 and D2 block, rather than enhance, PMN function, and excess 5(S)-HETE does not interfere with PMN binding of these prostanooids (64). Likewise, lipoxin A4 and B4, at 0.02–2 μM, do not mimic 5(S)-HETE in promoting PMN responses to PAF, and various nucleotides including ATP, while enhancing PAF-induced responses, do not cross-desensitize with 5(S)-HETE in assays of degranulation.2 Indeed, 5(S)-HETE is peculiar among Ca2+-mobilizing, pertussis toxin-sensitive stimuli in that it elicits functions like O2 production only when PMN are co-challenged with protein kinase C activators (60), PAF (61), or TNF-α (Figs. 2 and 3; Table I). We are not aware of any other PMN receptor agonist with these particular bioactions and therefore suspect that 5(S)-HETE bypasses all known receptors to stimulate PMN.

Although the data presented here are fully compatible with the notion that 5(S)-HETE acts through receptors, they do not exclude alternate PMN-stimulating mechanisms, e.g. direct G-protein activation. It is nevertheless interesting to speculate on putative 5(S)-HETE receptors. Such receptors would be directed toward cooperating with certain stimuli to elicit PMN function. We note that other PMN eicosanoid receptors localize to plasma membrane and associate with either Gα (prostaglandins E2 and D2 receptors) or Gβ (LTB4 and lipoxin A4 receptors) proteins (64, 55, 69). Our GTP binding and hydrolysis studies suggest that 5(S)-HETE receptors, if existing in PMN, are also plasmalemmal-bound and G protein-linked. As a first impression, then, the known PMN eicosanoid receptors may belong to the rhodopsin superfamily of cell surface recognition molecules and relate to each other in a manner formally analogous to, e.g., adrenergic rhodopsin superfamily receptors (71). Adrenergic receptors fall into different classes based in part on their affinities for a series of analogs. Eicosanoid receptors might also be profitable classified into, e.g., stimulatory Ei (LTB4, lipoxin A4, putative 5(S)-HETE) and inhibitory Eo (prostaglandins E2 and D2) receptors. Here, as in the adrenergic system, receptors within a single class may differ. LTB4 receptors elicit a large array of functions; lipoxin A4 receptors cause a different and putative 5(S)-HETE receptors stimulate function only in PMN co-challenged with certain other stimuli. Relevant to the last point, it seems at least possible that the latter stimuli up-regulate 5(S)-HETE receptors. TNF-α induces PMN to increase their expression of fMLP and PAF receptors (66) and conceivably might have the same effects on putative 5(S)-HETE receptors. In any event, recent studies indicate that PMN synthesize various o xo analogs of arachidonic acid metabolites (55, 56). Our studies recommend these and other novel analogs be tested for interaction with the full range of Ei receptors. Possibly, an eicosanoid analog will be found to interact with multiple Ei receptor types and thereby indicate a close relationship between these fascinating receptor systems.

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