Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 7270–7274, August 1993
Pharmacology

Transmembrane signaling in human polymorphonuclear neutrophils: 15(*S*)-Hydroxy-(5*Z*,8*Z*,11*Z*,13*E*)-eicosatetraenoic acid modulates receptor agonist-triggered cell activation

ROBERT J. SMITH*†, JAMES M. JUSTEN*, ELDON G. NIDY‡, LAUREL M. SAM*, AND JOHN E. BLEASDALE§

Departments of *Hypersensitivity Diseases Research, ‡Medicinal Chemistry Research, and §Metabolic Diseases Research, The Upjohn Co., 301 Henrietta Street, Kalamazoo, MI 49001

Communicated by Minor J. Coon, March 22, 1993 (received for review November 2, 1992)

ABSTRACT **15(*S*)-Hydroxy-(5*Z*,8*Z*,11*Z*,13*E*)-eicosatetraenoic acid (15-HETE) exerted a time- and concentration-dependent inhibition of superoxide anion (O_2^-) production and exocytosis of both azurophil and specific granule constituents from human polymorphonuclear neutrophils (PMN) stimulated with the receptor-specific agonists, *N*-formylmethionylleucylphenylalanine (FMLP), platelet-activating factor, and leukotriene B_4, but not that elicited by phorbol 12-myristate 13-acetate. 15-HETE did not alter the binding of FMLP to its specific receptors on PMN but, rather, appeared to interfere with a subsequent process in signal transduction. Receptor-coupled production of inositol 1,4,5-trisphosphate ($InsP_3$) and increases in cytosolic free calcium elicited with FMLP, platelet-activating factor, and leukotriene B_4 were suppressed by 15-HETE. 15-HETE did not, however, inhibit the mobilization of ^{45}Ca from intracellular stores elicited by the addition of $InsP_3$ to permeabilized PMN. 15-HETE suppressed O_2^- production and increases in intracellular [Ca^{2+}] induced when cell-surface receptors were bypassed and the PMN were activated directly by the guanine nucleotide-binding protein (G protein) activators aluminum fluoride (AlF_4^-) and mastoparan. 15-HETE, however, did not perturb all G protein functions because cAMP production in FMLP-activated PMN was essentially unaffected by 15-HETE. These data support the proposition that 15-HETE modulates receptor-triggered activation of PMN either by uncoupling G protein stimulation of phospholipase C or by directly inhibiting phospholipase C, thus inhibiting the $InsP_3$-dependent rise in intracellular [Ca^{2+}] that is prerequisite for PMN responsiveness to receptor agonists.**

Leukotrienes generated by the 5-lipoxygenation of arachidonic acid in various cell types are prominent mediators of inflammatory and pulmonary disorders (1–3). In contrast, the principal eicosanoid generated by lipoxygenase activity in eosinophils (4, 5), lung (6), and fibroblasts (7) is the 15-lipoxygenase product, 15(*S*)-hydroxy-(5*Z*,8*Z*,11*Z*,13*E*)-eicosatetraenoic acid (15-HETE), which is also produced by polymorphonuclear neutrophils (PMN) (8, 9), endothelial cells (10), epithelial cells (11), macrophages (12), and placenta (13). 15-HETE has been identified in the airways of asthmatics (14) and may also play a role in the development of arteriosclerotic plaques (15) and in the vascular complications associated with pregnancy-induced hypertension (13). 15-HETE has been reported to inhibit murine splenocyte proliferation (16), interleukin 2-induced cell blastogenesis (17), and mixed lymphocyte reactions (18), but 15-HETE stimulates cytotoxic suppressor T-cell generation (19).

Although PMN play a major role in host defense, the proteinases, reactive oxygen species, and eicosanoids released from activated PMN may themselves cause tissue injury associated with inflammatory disease (20). Because 15-HETE generated by PMN and other cells may modulate PMN interactions, the purpose of this investigation was to characterize the effects of 15-HETE on PMN stimulated with pathophysiological agonists and to define the mechanism(s) by which 15-HETE modulates receptor-coupled activation of PMN.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Reagents. *N*-Formylmethionylleucylphenylalanine (FMLP) (Sigma), leukotriene B_4 (LTB_4) (Upjohn), and platelet-activating factor (PAF) were prepared in dimethyl sulfoxide, methanol, and 0.15 M NaCl containing bovine serum albumin at 2.5 mg/ml, respectively. Cytochalasin B (CB; Aldrich), phorbol 12-myristate 13-acetate (PMA) (LC Services, Woburn, MA), 15-HETE (Upjohn), and fura-2 A/M (Molecular Probes) were dissolved in ethanol, dimethylsulfoxide, methanol, and dimethyl sulfoxide, respectively. The small amounts of dimethyl sulfoxide, ethanol, and methanol (final concentration of 0.1%) used as vehicle did not alter cell viability, degranulation, O_2^- production, fura-2 A/M fluorescence, or $InsP_3$ production.

Preparation of PMN and Measurement of Degranulation and O_2^- Production. Human PMN were purified by standard techniques (21) (purity ≥ 98%, viability > 98%) and suspended in modified PBS, pH 7.4/138 mM NaCl/8.1 mM Na_2HPO_4/1.5 mM KH_2PO_4/2.7 mM KCl/1.0 mM $CaCl_2$/1.0 mM $MgCl_2$/0.1% glucose. After exposure to agonist, as described in *Results*, PMN were centrifuged at 750 × *g* (4°C) for 3 min, and the clear supernatants were assayed for lactate dehydrogenase, myeloperoxidase (MPO), and vitamin B_{12}-binding protein (B_{12}-BP) activities (21). The net agonist-induced discharge of granule constituents (MPO, B_{12}-BP) from PMN is expressed as percentage of total activity released by 0.2% Triton X-100. O_2^- production was measured as superoxide dismutase-inhibitable reduction of ferricytochrome *c* (22).

Measurement of Specific Binding of FMLP to PMN. PMN (2.5×10^7 cells per ml of modified PBS) were treated for 3 min at 37°C with either 15-HETE (20 μM) or vehicle (methanol), and aliquots (0.7 ml) were transferred to 1.5-ml polypropylene tubes that contained [^{3}H]FMLP (0.5 nM, 76.6 Ci/mmol; 1 Ci = 37 GBq) and unlabeled FMLP at various concentrations (0.1–300 nM) in 2 μl of dimethyl sulfoxide. After either 15 min at 37°C or 60 min at 0°C, aliquots (200 μl in triplicate) were

Abbreviations: 15-HETE, 15(*S*)-hydroxy-(5*Z*,8*Z*,11*Z*,13*E*)-eicosatetraenoic acid; PMN, polymorphonuclear neutrophils; FMLP, *N*-formylmethionylleucylphenylalanine; PAF, platelet-activating factor; PMA, phorbol 12-myristate 13-acetate; $InsP_3$, inositol 1,4,5-trisphosphate; LTB_4, leukotriene B_4; GTP[γS], guanosine 5′-[γ-thio]triphosphate; PKC, protein kinase C; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; MPO, myeloperoxidase; B_{12}-BP, vitamin B_{12}-binding protein; CB, cytochalasin B; PLC, phospholipase C.

†To whom reprint requests should be addressed.

centrifuged (9000 × *g* for 90 sec at 4°C) through 200 μl of silicone oil (GE SF-1250, R. H. Carlson Co., Greenwich, CT), and bound [^{3}H]FMLP in the cell pellets was measured by scintillation spectrometry. Binding data were analyzed by using a nonlinear least-squares program (damping Gauss–Newton algorithm) (23).

Measurement of Intracellular Calcium Mobilization. The mobilization of intracellular calcium in PMN, as reflected in a rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), was monitored in fura-2-loaded PMN, as described (24).

Measurement of InsP_3 and cAMP Production in PMN. (*i*) For whole cells, PMN (2×10^6–10^7 in 0.2 ml) were pretreated for 3 min at 37°C with either 15-HETE (at various concentrations) or vehicle (methanol, 0.1% final concentration). Cytochalasin B (5 μg/ml) and FMLP (100 nM) were then added, followed at various intervals by 0.07 ml of ice-cold trichloroacetic acid (20%). InsP_3 in trichloroacetic acid extracts was quantitated by using a competitive radiobinding assay (21), and cAMP was measured by using an RIA (25). (*ii*) For streptolysin-O-permeabilized cells, PMN (10^7) in 0.35 ml of permeabilization buffer (137 mM NaCl/2.7 mM KCl/20 mM Pipes buffer, pH 6.8/5.6 mM glucose/2 mM $MgCl_2$/bovine serum albumin at 1 mg/ml) were preincubated for 2 min at 37°C with either 15-HETE or vehicle (methanol, 0.2%). Streptolysin-O (12.5 units) in 0.1 ml of permeabilization buffer supplemented with 10 mM EGTA was added, and incubation at 37°C was continued for a further 10 min. Guanosine 5′-[γ-thio]triphosphate (GTP[γS]; 100 μM) was then added in 0.05 ml of permeabilization buffer/4.67 mM $CaCl_2$/100 mM LiCl/10 mM MgATP/10 mM sodium 2,3-diphosphoglycerate. After 1 min, incubations were quenched with 0.072 ml of ice-cold 40% trichloroacetic acid, and InsP_3 in trichloroacetic acid extracts was quantitated (21).

Measurement of GTPase Activity in PMN Membranes. PMN (5×10^8 in 50 ml of modified PBS) were treated for 5 min at room temperature with either 15-HETE (20 μM) or vehicle (methanol, 0.5%) and lysed; membranes were then isolated, as described (21). FMLP-stimulated GTPase activity in membranes was assayed as described (21), except that before FMLP (500 nM) addition, some membranes were preincubated for 5 min at room temperature with 15-HETE (at various concentrations), or vehicle [GTPase assay buffer supplemented with fatty acid-free bovine serum albumin (2.5 mg/ml)].

Protein Kinase C (PKC) Activity and Redistribution in PMN. PKC activity in the PMN subcellular fractions was quantitated by measuring phosphorylation of histone IIIS using [^{32}P]ATP as described (24). Subcellular redistribution of PKC in stimulated PMN was determined (21).

Measurement of ^{45}Ca Efflux from Permeabilized PMN. PMN [25×10^6 cells per ml of KCl·Hepes buffer (100 mM KCl/20 mM NaCl/30 mM Hepes), pH 7.0, containing 1 mM EGTA, 1 mM dithiothreitol, and protease inhibitors at 1 μl/ml, aprotinin at 10 mg/ml, leupeptin at 10 mg/ml, soybean trypsin inhibitor at 25 mg/ml] were prewarmed for 5 min and then incubated (no shaking) with streptolysin-O (GIBCO, 10 units/ml) at 37°C for 10 min. Permeabilized PMN were washed twice in KCl·Hepes buffer/1 mM EGTA, resuspended at 100×10^6 cells per ml in KCl·Hepes buffer/0.1 mM EGTA, added to an equal volume of KCl·Hepes buffer/4 mM MgATP/2 mM $MgCl_2$/400 nM antimycin A/oligomycin at 4 μg/ml/30 μM ^{45}Ca, and placed in a shaking incubator for 20 min at 37°C. Cells loaded with ^{45}Ca (150 μl) were incubated with 75 μl of vehicle (KCl·Hepes buffer/1 mM EGTA/6 mM vanadate) or InsP_3 (with or without 15-HETE) for 1 min. A portion (150 μl) of the reaction mixture was then layered on 150 μl of silicone oil (GE SF-1250), which was layered on a 30-μl cushion of 12% sucrose and centrifuged (9000 × *g* for 90 sec at room temperature). ^{45}Ca retained in the cell pellet was expressed as a percentage of that present initially.

Data Analysis. Data were analyzed with one-way ANOVA and Student's *t* test.

RESULTS

Receptor Agonist-Stimulated Granule Exocytosis from 15-HETE-Treated PMN. 15-HETE exerted a concentration-dependent (0.3–20 μM) inhibition of FMLP-, LTB_4-, and PAF-induced discharge of azurophil (MPO) and specific granule (B_{12}-BP) constituents from CB-treated PMN (Fig. 1). IC_{50} values were 3 μM (FMLP), 7 μM (LTB_4), and 10 μM (PAF) for MPO discharge; and 7 μM (FMLP), 20 μM (LTB_4), and >20 μM (PAF) for B_{12}-BP discharge. Maximal suppression of PMN degranulation with 15-HETE (20 μM) was 97% (FMLP), 62% (LTB_4), and 58% (PAF) for MPO release ($P < 0.01$); and 68% (FMLP), 52% (LTB_4), and 38% (PAF) for B_{12}-BP release ($P < 0.01$). However, PMA-stimulated degranulation was unaffected by 15-HETE (Fig. 1).

Influence of 15-HETE on the PMN Oxidative Burst. FMLP, LTB_4, and PAF each triggered O_2^- production in PMN that was suppressed by 15-HETE (0.1–20 μM) (Fig. 2). IC_{50} values were 0.5 μM (PAF), 3 μM (FMLP), and 10 μM (LTB_4). As observed with granule exocytosis, 15-HETE exerted no effect on the PMN oxidative burst elicited with PMA. 15-HETE did not suppress O_2^- production by a cell-free xanthine/xanthine oxidase O_2^--generating system (data not shown), indicating that 15-HETE was not functioning as a free radical scavenger but most likely was inhibiting a more proximal event in PMN activation.

The release of cytoplasmic lactate dehydrogenase from 15-HETE-treated PMN was insignificant (<5% of total cell activity). The inhibitory action of 15-HETE, therefore, is probably not a nonspecific consequence of cell toxicity.

Effect of 15-HETE on Specific Binding of [^{3}H]FMLP to PMN. PMN were pretreated with either 15-HETE (20 μM) or vehicle (methanol) for 3 min at 37°C before measuring equilibrium binding of FMLP at 0°C and at 37°C. At 0°C, binding of FMLP to PMN was not significantly affected by 15-HETE. B_{max} values were 40,400 ± 5200 sites per cell and 43,400 ± 3300 sites per cell in the absence and presence of 15-HETE, respectively. K_d values were 27 ± 9 nM and 25 ± 5 nM without and with 15-HETE, respectively. At 37°C, measurement of FMLP binding was complicated by sequestration/internalization of FMLP receptors, but there was no apparent

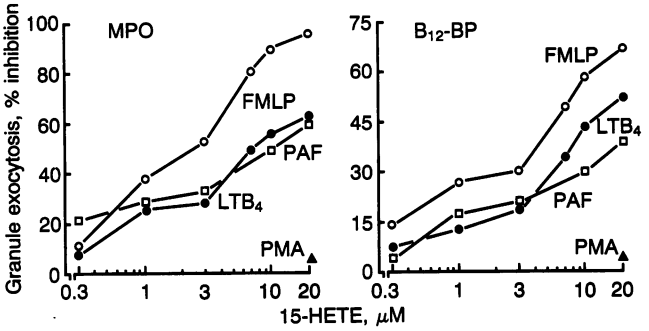

FIG. 1. Suppression by 15-HETE of PMN degranulation elicited with various agonists. PMN (5×10^6) were preincubated with or without 15-HETE for 3 min at 37°C. CB (5 μg/ml) and FMLP, PAF, or LTB_4 at 10 nM were then added for 2 min. PMN were also incubated with CB and 10 nM (B_{12}-BP release) or 100 nM (MPO release) PMA for 2 min. FMLP, LTB_4, PAF, and PMA triggered the release of 7.7 ± 0.4, 10.4 ± 0.2, 11.9 ± 1.6, and 5.8 ± 0.7% of total-cell MPO activity, respectively; and 9.9 ± 1.4, 13.1 ± 2.0, 16.5 ± 1.7, and 18.5 ± 1.7% of total-cell B_{12}-BP activity, respectively. Total cell activities were 1.1 ± .03 ΔA_{460} (MPO) and 2049.6 ± 348 ng of B_{12}-BP per 5×10^6 cells (B_{12}-BP). Data are the means of three separate experiments done in duplicate (SEMs ≤ 10% of the mean).

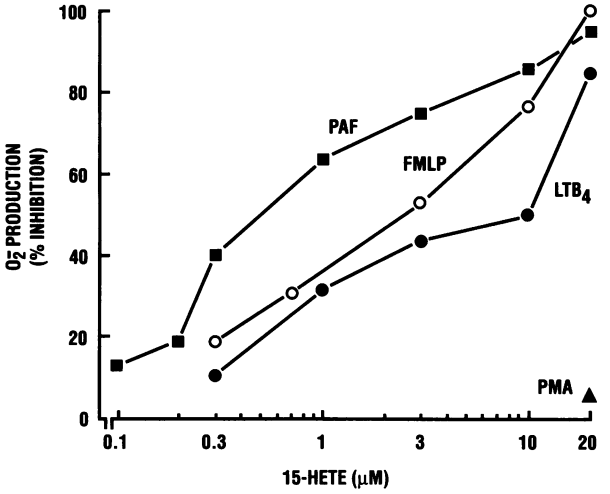

FIG. 2. Inhibition by 15-HETE of superoxide anion production in activated PMN. Cells (5×10^6) were preincubated with or without 15-HETE for 3 min followed by a 5-min exposure to CB and FMLP (10 nM), PAF (100 nM), LTB_4 (500 nM), or PMA (3 nM) at 37°C. O_2^- generated spontaneously and in response to agonists was 0.1 ± 0.03 (spontaneous), 9.4 ± 1.7 (FMLP), 5.9 ± 0.7 (LTB_4), 16.9 ± 3.1 (PAF), and 11.6 ± 1.7 (PMA) nmol of ferricytochrome *c* reduced per 3 min per 5×10^6 cells. Data are the means of four experiments done in duplicate (SEMs ≤ 10% of the mean).

reduction in the affinity of binding sites for FMLP on PMN exposed to 15-HETE (20 μM).

15-HETE Modulates InsP_3 Production in Activated PMN. FMLP (100 nM) stimulated a transient accumulation of InsP_3 in PMN (Fig. 3) that was maximal after 5 sec (305% above that in unstimulated cells). 15-HETE suppressed this InsP_3 production (IC_{50} = 10 μM). Maximal suppression (65%, $P < 0.001$) was seen with 30 μM 15-HETE (Fig. 3). 15-HETE did not affect basal InsP_3 in unstimulated PMN.

Agonist-Induced Changes in $[Ca^{2+}]_i$ in PMN Exposed to 15-HETE. FMLP, LTB_4, and PAF increased $[Ca^{2+}]_i$ in fura-2-loaded PMN (Fig. 4). 15-HETE caused a concentration-dependent inhibition of intracellular calcium mobilization induced by FMLP, PAF, and LTB_4 with IC_{50} values for 15-HETE of ≈20, 5, and 3 μM, respectively. 15-HETE (20 μM) alone, however, did not alter $[Ca^{2+}]_i$ in PMN (Fig. 4).

InsP_3-Induced ^{45}Ca Efflux from Permeabilized PMN Treated with 15-HETE. Streptolysin O-permeabilized PMN, which were preloaded with ^{45}Ca, retained 92.8 ± 0.7% of the total-cell-associated ^{45}Ca during a subsequent 30-min incubation period but only 50.7 ± 4.6% ($\bar{x}$ ± SEM, n = 3) when InsP_3 (30 μM) was present. 15-HETE (1–20 μM) affected neither InsP_3-triggered ^{45}Ca efflux from PMN nor passive ^{45}Ca efflux from unstimulated cells. Furthermore, 15-HETE was also inactive if preincubated with permeabilized PMN before InsP_3 addition. The calcium ionophore ionomycin, was used as a positive control and triggered the efflux of 62.4 ± 7.9% of total-cell-associated ^{45}Ca in PMN, which was unaltered in the presence of 15-HETE (20 μM).

GTP[γS]-Stimulated InsP_3 Production in Permeabilized PMN Treated with 15-HETE. Exposure of streptolysin O-permeabilized PMN to GTP[γS] (100 μM) resulted in an accumulation of InsP_3 to 160% above that in unstimulated cells {GTP[γS] (100 μM), 52.3 ± 3.5 vs. control, 21.6 ± 5.2 pmol per 10^7 cells, $\bar{x}$ ± SEM, n = 3}. 15-HETE (1–30 μM) did not affect GTP[γS]-induced InsP_3 production in PMN (50.4 ± 0.5 pmol per 10^7 cells with 30 μM 15-HETE). Similarly, basal InsP_3 in unstimulated permeabilized PMN was unaffected by 15-HETE [control, 19.6 ± 1.1 vs. 15-HETE (30 μM), 21.6 ± 5.2 pmol per 10^7 cells].

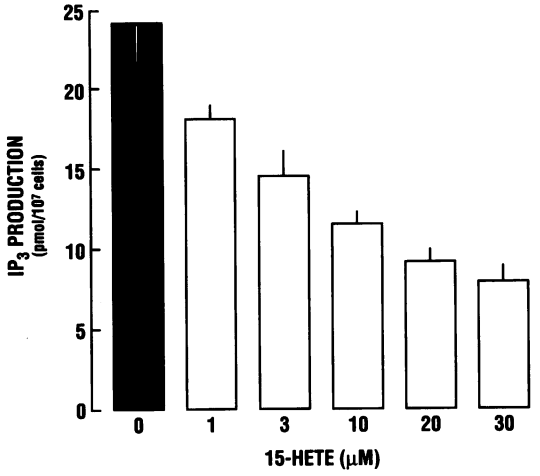

FIG. 3. Suppression by 15-HETE of agonist-triggered InsP_3 (IP_3) production in PMN. Cells (10^7) were preincubated with or without 15-HETE for 1 min followed by a 5-sec incubation with CB and FMLP (100 nM) at 37°C. Data are the means ± SEMs of four separate experiments done in triplicate.

Influence of 15-HETE on Mastoparan-Stimulated Intracellular Calcium Mobilization in PMN. In the presence of extracellular calcium (1.2 mM) mastoparan induced a rapid rise in $[Ca^{2+}]_i$ in PMN that was suppressed 34% by 15-HETE at 30 μM ($P < 0.05$) but was unaffected by 10 μM 15-HETE (Fig. 5). In the absence of extracellular calcium, mastoparan triggered a smaller, rapid rise in $[Ca^{2+}]_i$ that was suppressed by 15-HETE at 10 μM and 30 μM by 48% ($P < 0.01$) and 67% ($P < 0.01$), respectively (Fig. 5).

Influence of 15-HETE on the AlF_4^--Triggered Oxidative Burst in PMN. AlF_4^- stimulated a concentration-dependent production of O_2^-, wherein 0.8 ± 0.2, 3.5 ± 0.6, 10.2 ± 2.3, and 14.0 ± 2.6 nmol of O_2^- per 5×10^6 cells ($\bar{x}$ ± SEM, n = 5) were generated with 10, 13, 15, and 20 mM AlF_4^-, respectively. 15-HETE inhibited (IC_{50} = 15 μM) O_2^- production in AlF_4^- (20 mM)-activated PMN; 76% inhibition occurred at 30 μM 15-HETE ($P < 0.01$).

Effect of 15-HETE on Basal and FMLP-Stimulated GTPase Activity of PMN Membranes. FMLP (100 nM) significantly ($P < 0.05$) stimulated (108% above basal) GTPase activity of PMN membranes. 15-HETE (30 μM) caused a small, but significant ($P < 0.05$), inhibition of basal (16%) and FMLP-induced GTPase activity (14%) in a membrane fraction from 15-HETE-pretreated PMN (Fig. 6*A*). However, when mem-

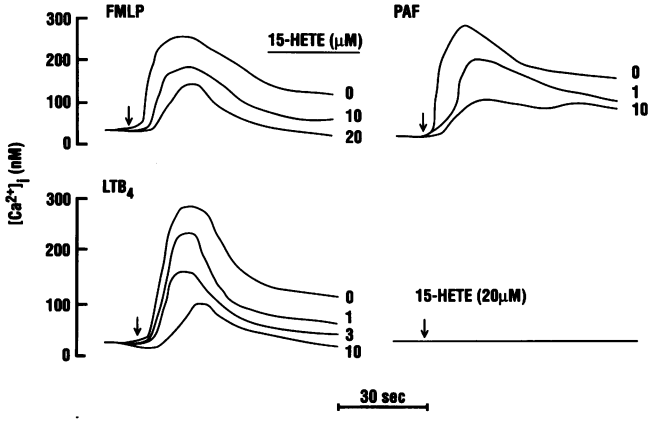

FIG. 4. Receptor agonist-induced changes in $[Ca^{2+}]_i$ in PMN exposed to 15-HETE. PMN (7.5×10^6 in 3 ml) were preincubated with or without 15-HETE for 3 min before addition (at arrows) of various agonists (10 nM final). Resting $[Ca^{2+}]_i$ was 31 ± 3 nM. Data are typical from five experiments.

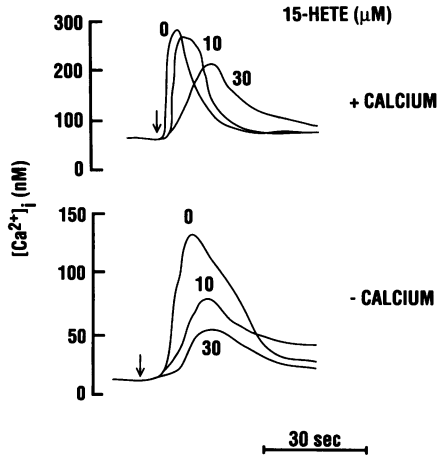

FIG. 5. Influence of 15-HETE on the mastoparan-triggered rise in $[Ca^{2+}]_i$ in PMN. Cells (5×10^6 in 2 ml) were preincubated with or without 15-HETE for 3 min with extracellular calcium (1.2 mM) or without (calcium-free Hepes buffer plus 2 mM EGTA) before addition of 30 μM mastoparan at the arrows. Resting $[Ca^{2+}]_i$ was 65 ± 3 nM and 20 ± 1 nM with and without extracellular calcium, respectively. Data are typical from four experiments.

branes prepared from unstimulated PMN were exposed to 15-HETE (1–30 μM), basal GTPase activity was slightly enhanced, and FMLP-stimulated GTPase activity was unaffected by 15-HETE (Fig. 6*B*).

Receptor Agonist-Elicited Redistribution of PKC in 15-HETE-Treated PMN. FMLP significantly increased (149% above control, $P < 0.01$) the PKC activity associated with the extractable particulate (membrane) fraction of PMN (Fig. 7). 15-HETE caused a concentration-dependent inhibition of this FMLP-triggered redistribution of PKC to the PMN membrane fraction with maximal suppression (89%, $P < 0.01$) at 20 μM. Although 15-HETE suppressed receptor agonist-induced PKC redistribution to a PMN membrane

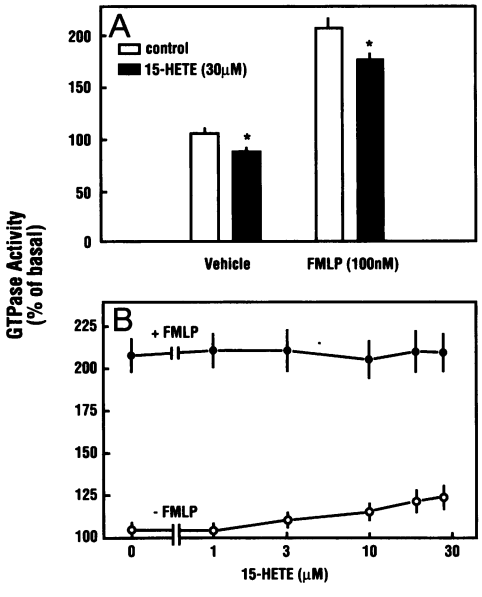

FIG. 6. Effect of 15-HETE on basal and FMLP-stimulated GTPase activity of PMN membranes. (*A*) PMN (5×10^8) were preincubated for 5 min with or without 15-HETE (30 μM). Membranes were prepared from these PMN (21) and incubated with FMLP (500 nM) for 10 min at 37°C. (*B*) PMN membranes were prepared from untreated PMN and then incubated with or without 15-HETE for 5 min, followed by a 10-min incubation with or without FMLP (500 nM). GTPase activities shown are means ± SEMs of three separate experiments done in triplicate.

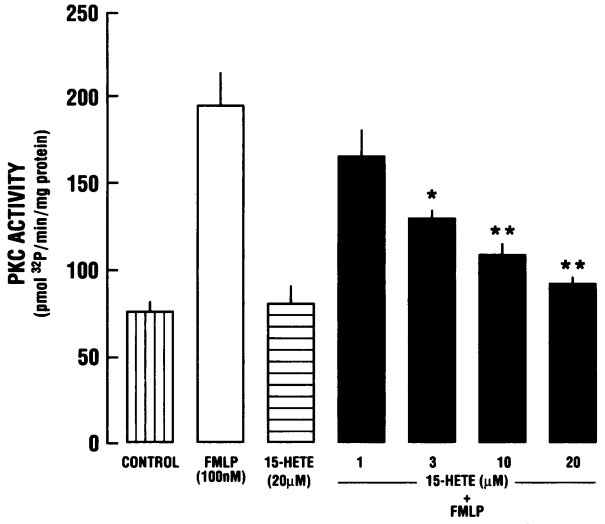

FIG. 7. Influence of 15-HETE on the FMLP-induced association of PKC with a membrane fraction of PMN. Cells (4×10^7) were preincubated with CB for 2 min followed by a 3-min incubation either with or without 15-HETE. PMN were then incubated with or without FMLP (100 nM) for 1 min at 37°C. Data are the means ± SEMs of four separate experiments done in duplicate. *, $P < 0.05$ and **, $P < 0.01$ vs. FMLP alone.

fraction, it had no direct effect on PKC activity [control, 148.8 ± 31.8 vs. 15-HETE (20 μM), 160.7 ± 18.9 pmol of $^{32}PO_4$ per min per mg of protein] under conditions where PMA stimulated PKC activity (183% above control), and staurosporine (30 nM) suppressed PKC activity by 63%.

cAMP Production in PMN Exposed to 15-HETE. FMLP (100 nM) stimulated a rapid increase in cAMP in PMN with peak concentrations seen after 20 sec (control, 0.34 ± 0.03 vs. FMLP, 1.26 ± 0.22 pmol per 10^6, $\bar{x}$ ± SEM, $n = 4$). Basal cAMP in PMN (before FMLP addition) was unaffected by 15-HETE (30 μM), and the 20-sec peak of FMLP-induced cAMP production in 15-HETE-pretreated cells (1.3 ± 0.22 pmol per 10^6 cells) was not significantly different from that in cells pretreated with vehicle. Only 300 sec after FMLP addition was there a small but significant ($P = 0.02$) increase in FMLP-induced cAMP [FMLP, 0.67 ± 0.03 vs. FMLP plus 15-HETE (30 μM), 0.85 ± 0.05 pmol per 10^6 cells, $\bar{x}$ ± SEM, $n = 4$].

DISCUSSION

We found that 15-HETE inhibited PMN degranulation and O_2^- production elicited by structurally diverse receptor-specific agonists (FMLP, PAF, LTB_4), but not PMA. It seemed unlikely that 15-HETE was interfering directly with the specific binding of each of these agonists with their respective cell-surface receptors, but an indirect effect on receptor binding (e.g., by interaction with a common coupled G protein) could not be precluded. However, 15-HETE had a negligible effect on specific binding of [^{3}H]FMLP to intact PMN; this result suggested that the inhibition by 15-HETE of PMN activation was at a postreceptor level. A well-defined component of PMN activation by receptor agonists is a G protein-dependent activation of a phospholipase C (PLC) that generates InsP_3 and 1,2-diacylglycerol. 15-HETE was found to inhibit InsP_3 production in PMN stimulated with receptor agonists, but not basal InsP_3. This inhibition by 15-HETE of receptor-coupled InsP_3 production was itself sufficient to suppress the InsP_3-triggered rise in $[Ca^{2+}]_i$ in PMN activated by FMLP, PAF, or LTB_4. 15-HETE did not affect InsP_3-stimulated efflux of ^{45}Ca from permeabilized PMN, suggest-

ing that its site of action was entirely proximal to $InsP_3$ production.

Receptor-independent $InsP_3$ production in permeabilized PMN stimulated with GTP[γS] was little affected by 15-HETE. Because 15-HETE-sensitive components of PMN activation could possibly be rendered unresponsive in permeabilized cells, we used the cell-penetrating G protein activator mastoparan (30) to investigate further the possibility that 15-HETE interferes with G protein–PLC coupling. Mastoparan triggers a pertussis toxin-sensitive $InsP_3$ production (31), a rise in $[Ca^{2+}]_i$ (31), as well as degranulation and O_2^- production in PMN (32). We found that mastoparan induced a rapid rise in $[Ca^{2+}]_i$ that was inhibited by 15-HETE at concentrations at which $InsP_3$ production, intracellular calcium mobilization, and functional responsiveness were also suppressed. Interestingly, 15-HETE was more potent in suppressing the increase in $[Ca^{2+}]_i$ in mastoparan-activated PMN when calcium was absent from the extracellular medium. It has been proposed that mastoparan also elicits $InsP_3$-*independent* changes in $[Ca^{2+}]_i$, either by functioning as an ionophore itself (33) or by triggering the opening of calcium channels in the plasma membrane (31, 34). This $InsP_3$-independent action of mastoparan is relatively insensitive to 15-HETE.

15-HETE also suppressed the PMN oxidative burst elicited with another G protein activator AlF_4^- (35). In contrast to mastoparan, however, the AlF_4^--induced rise in $[Ca^{2+}]_i$ is quite slow (36), and PMN responsiveness to AlF_4^- is pertussis toxin-insensitive (37). This result suggests that 15-HETE may interfere with more than one G protein. 15-HETE exerted a small, but statistically significant, inhibitory effect on basal and FMLP-stimulated GTPase activity of PMN membranes. It should be noted, however, that not all G protein functions are perturbed by 15-HETE in stimulated PMN. For instance, 15-HETE essentially did not alter FMLP-stimulated G protein-dependent cAMP production in PMN. This finding suggests that cAMP is not involved in the mechanism of 15-HETE action.

Pathophysiological agonists such as FMLP (21, 40) and interleukin 8 (41) stimulate the redistribution of PKC to a plasma membrane compartment. 15-HETE inhibited FMLP-induced redistribution of PKC most likely because it suppressed receptor-coupled diacylglycerol production (42). In addition, in some cells 15-HETE is preferentially incorporated into phosphatidylinositol, which yields altered species of diacylglycerol when the cells are hormonally stimulated (39). This may result in inhibition of PKC redistribution, PKC activity, and ultimately PMN responsiveness. However, this effect of 15-HETE mediated via incorporation of 15-HETE into phospholipids is slower than the rapid responses to 15-HETE reported here. Lipoxin A_4 is also generated in response to FMLP by PMN that have incorporated 15-HETE (29, 38), and lipoxin A_4 suppresses $InsP_3$ production (28). Complicating the interpretation of the latter observation, however, are the findings that lipoxin A_4 itself triggers a rise in $[Ca^{2+}]_i$ in PMN (27) and lipoxins A_4 and B_4 do not suppress PMN activation by various agonists (26). Lipoxins, therefore, are unlikely to be critically involved in the mechanism by which 15-HETE inhibits PMN activation.

PLC-dependent processes are critical to receptor-triggered PMN activation. Our data show that 15-HETE inhibits $InsP_3$ production, intracellular calcium mobilization, and PKC redistribution as well as granule exocytosis and the oxidative burst in PMN stimulated by receptor agonists. These observations, together with the suggestion that 15-HETE regulates receptor-coupled signal transduction at either the level of the PLC or its regulation by G proteins, provide insight into the mechanism of action of this eicosanoid as an autocrine or paracrine modulator of PMN responsiveness. It follows, therefore, that agents that modulate 15-HETE production have the potential to interfere with PMN involvement in inflammatory diseases.

1. Samuelsson, B. & Serhan, C. N. (1987) in *Prostaglandins in Cancer Research*, eds. Garaci, E., Paoletti, R. & Santoro, M. G. (Springer, Berlin), pp. 3–11.
2. Spector, A. A., Gordon, J. A. & Moore, S. A. (1988) *Prog. Lipid Res.* **27**, 271–323.
3. Wasserman, M. A., Smith, E. F., III, Underwood, D. C. & Barnette, M. A. (1991) in *Lipoxygenases and Their Products*, eds. Crooke, S. T. & Wong, M. A. (Academic, San Diego), pp. 1–50.
4. Turk, J., Maas, R. L., Brash, A. R., Roberts, L. J. & Oates, J. A. (1982) *J. Biol. Chem.* **257**, 7068–7076.
5. Morita, E., Schröder, J.-M. & Christophers, E. (1990) *Scand. J. Immunol.* **32**, 497–502.
6. Hamberg, M., Hedquist, P. & Radegran, K. (1980) *Acta Physiol. Scand.* **110**, 219–221.
7. Kragballe, K., Pinnamaneni, G., Desjarias, L., Duell, E. A. & Voorhees, J. J. (1986) *J. Invest. Dermatol.* **87**, 454–498.
8. Borgeat, P. & Samuelsson, B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2148–2152.
9. Vanderhoek, J. Y., Wilhelmi, L. L., Exberg, S. L. & Karmin, M. T. (1990) *Eicosanoids* **3**, 181–185.
10. Hopkins, N. K., Oglesby, T. D., Bundy, G. L. & Gorman, R. R. (1984) *J. Biol. Chem.* **259**, 14048–14053.
11. Hunter, J. A., Finkbeiner, W. E., Nadel, J. A., Goetzl, E. J. & Holtzman, M. J. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4633–4637.
12. Humes, J. L., Opas, E. E., Galavage, M., Soderman, D. & Bonney, R. J. (1986) *Biochem. J.* **233**, 199–206.
13. Mitchell, M. D. & Koenig, J. M. (1991) *Prostaglandins Leukotrienes Essent. Fatty Acids* **43**, 61–62.
14. Murray, J. J., Tonnel, A. B., Brash, A. R., Roberts, L. J., II, Gosset, P., Workman, R., Capron, A. & Oates, J. A. (1986) *N. Engl. J. Med.* **315**, 800–804.
15. Hartung, H.-P. & Toyka, K. V. (1987) *Brain Res.* **435**, 367–370.
16. Gualde, N., Chable-Rabinovitch, H., Motta, C., Durand, J., Beneytout, J. L. & Rigaud, M. (1983) *Biochim. Biophys. Acta* **750**, 429–433.
17. Farrar, W. L. & Humes, J. L. (1985) *J. Immunol.* **135**, 1153–1159.
18. Mexmain, S., Cook, J. C., Aldigier, N., Gualde, N. & Rigaud, M. (1985) *J. Immunol.* **135**, 1361–1365.
19. Gualde, N., Alturu, D. & Goodwin, J. S. (1985) *J. Immunol.* **134**, 1125–1129.
20. Weissmann, G. (1982) *J. Lab. Clin. Med.* **100**, 322–333.
21. Smith, R. J., Sam, L. M., Justen, J. M., Bundy, G. L., Bala, G. A. & Bleasdale, J. E. (1990) *J. Pharmacol. Exp. Ther.* **253**, 688–697.
22. Smith, R. J. & Iden, S. S. (1981) *J. Reticuloendothel. Soc.* **29**, 215–225.
23. Yamaoka, T., Tanigawara, Y., Nakagawa, T. & Uno, T. (1981) *J. Pharmacobio- Dyn.* **4**, 879–885.
24. Smith, R. J., Justen, J. M. & Sam, L. M. (1988) *Inflammation* **12**, 597–611.
25. Bleasdale, J. E., Thakur, N. R., Gremban, R. S., Bundy, G. L., Fitzpatrick, F. A., Smith, R. J. & Bunting, S. (1990) *J. Pharmacol. Exp. Ther.* **255**, 756–768.
26. Serhan, C. N. & Reardon, E. (1989) *Free Radical Res. Commun.* **7**, 341–345.
27. Moores, K. E. & Merritt, J. E. (1991) *Eicosanoids* **4**, 89–94.
28. Grandordy, B. M., Lacroix, H., Mavoungou, E., Kribis, S., Crea, A. E. G., Spur, B. N. & Lee, T. H. (1990) *Biochem. Biophys. Res. Commun.* **167**, 1022–1029.
29. Serhan, C. N., Nicolaou, K. C., Webber, S. E., Veale, C. A., Dahlen, S.-E., Puustinen, T. J. & Samuelsson, B. (1986) *J. Biol. Chem.* **261**, 16340–16345.
30. Higashijima, T., Uzu, S., Nakajima, T. & Ross, E. M. (1988) *J. Biol. Chem.* **263**, 6491–6494.
31. Perianin, A. & Snyderman, R. (1989) *J. Immunol.* **143**, 1669–1673.
32. Norgauer, J., Eberle, M., Lemke, H.-D. & Aktories, K. (1992) *Biochem. J.* **282**, 393–397.
33. Nakajima, J., Yasuhara, T., Uzu, S., Wakamatsu, K., Miyazawa, T., Fukuda, K. & Tsukamoto, Y. (1985) *Peptides* **6**, Suppl. 3, 425–430.
34. Yatani, A., Imato, Y., Codina, S., Hamilton, L., Brown, A. M. & Birnbaumer, L. (1988) *J. Biol. Chem.* **263**, 9887–9895.
35. Howlett, A. C., Sternweis, P. C., Macik, B. A., Van Arsdale, P. M. & Gilman, A. G. (1979) *J. Biol. Chem.* **254**, 2287–2295.
36. English, D., Debono, D. J. & Gabig, T. G. (1987) *J. Clin. Invest.* **80**, 145–153.
37. Strnad, C. F., Parente, J. E. & Wong, K. (1986) *FEBS Lett.* **206**, 20–24.
38. Brezinski, M. E. & Serhan, C. N. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6248–6252.
39. Legrand, A. B., Lawson, J. A., Meyrick, B. O., Blair, I. A. & Oates, J. A. (1991) *J. Biol. Chem.* **266**, 7570–7577.
40. Pike, M. C., Jakoi, L., McPhail, L. C. & Snyderman, R. (1986) *Blood* **67**, 909–913.
41. Smith, R. J., Sam, L. M., Leach, K. L. & Justen, J. M. (1992) *J. Leukocyte Biol.* **52**, 17–26.
42. Christiansen, N. O. & Borregaard, N. (1989) *Scand. J. Immunol.* **29**, 409–416.