We investigated whether biologically relevant concentrations of the mono-hydroxyeicosatetraenoic acids (mono-HETEs) modulate platelet functions. We report that 15-HETE, an eicosanoid produced by endothelial cells, granulocytes, and lymphocytes, potentiated platelet aggregation, nucleotide release, and elevation in intracellular calcium levels induced by a threshold concentration of thrombin (0.025 U/mL). Significant potentiation effects on these responses were observed at concentrations between 1 and 100 nmol/L. 15-HETE at these concentrations enhanced thrombin-induced platelet aggregation by 32% to 57%, nucleotide release by 40% to 65%, and elevation of intracellular calcium by 31% to 52% (P < .05 to .01). Both 12-HETE and 5-HETE, the structural isomers of 15-HETE, also potentiated thrombin-induced platelet aggregation and nucleotide release. While 12-HETE showed a small but significant effect at 100 pmol/L, 5-HETE had effects similar to those of 15-HETE at micromolar concentrations. To understand the mechanism of the HETE modulation of platelet functions, we studied the effect of 10 and 100 nmol/L 15-HETE on the production of sn-1,2-diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (1,4,5-IP3). 15-HETE enhanced thrombin-induced production of DAG and 1,4,5-IP3 in a time- and concentration-dependent manner. 15-HETE also potentiated agonist-induced phosphorylation of the 47-Kd platelet protein. These studies demonstrate an important modulatory role for 15-HETE on platelet functions. Since this eicosanoid is elevated in pathologic states associated with platelet hyperfunction, including diabetes mellitus and atherosclerosis, an elucidation of its mechanism(s) of action appears relevant to our understanding of the genesis of atherothrombotic vascular disease.

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Mammalian cells and tissues generate several oxygenated derivatives from arachidonic acid, including a variety of hydroxyeicosatetraenoic acids (HETEs). These HETEs are derived primarily from the lipoxygenase pathways. Lipoxigenases metabolize arachidonic acid to hydroperoxyeicosatetraenoic acids (HPETEs), which are unstable and are either reduced by cellular peroxidases to the corresponding stable HETEs, or metabolized to other products including leukotrienes, di-HETEs, and lipoxins. The major mono-HETEs identified and quantitated in blood plasma and serum include 15-HETE, 12-HETE, and 5-HETE. 15-HETE is produced via the 15-lipoxygenase pathway by endothelial cells, lymphocytes, and granulocytes. While platelets generate only 12-HETE, neutrophils also convert arachidonic acid to 5-HETE.

15-Lipoxygenase products appear to regulate numerous cellular functions. Both 15-HETE and 15-HPETE affect the activities of enzymes involved in arachidonic acid mobilization and metabolism in many cells, including phospholipase A2 activity in platelets and neutrophils, cyclooxygenase and prostacyclin synthetase activities in endothelial cells, 12-lipoxygenase activity in platelets, 5-lipoxygenase activity in neutrophils, and T lymphocytes, and 15-lipoxygenase activity in neutrophils. Besides modulating the activities of the enzymes involved in arachidonic acid metabolism, 15-HETE and 15-HPTE also affect many biological functions of the cellular elements involved in hemostasis. 15-HETE stimulates endothelial cell proliferation and migration and smooth muscle cell migration, and most recently has been shown to cause neovascularization. 15-HETE also modulates chemotaxis, aggregation, and activation of leukocytes.

Studies from this laboratory and others have documented increased production of 15-HETE by vascular tissues and in serum obtained from pathologic conditions associated with platelet hyperfunction, including diabetes mellitus and atherosclerosis. We have demonstrated that the plasma levels of this eicosanoid were markedly elevated in the cord blood obtained from the diabetic milieu when compared with control neonates. The 15-HETE levels measured in the plasma from control neonates and the infants of diabetic mothers were in the range of 1 to 275 nmol/L. Since albumin binds 90% to 95% of the plasma HETEs, effective concentrations of free HETEs in plasma are lower than the actual total concentrations measured. Thus, the free 15-HETE concentrations achievable in vivo are approximately in the range of 100 pmol/L to 30 nmol/L. In our present study, we report that 15-HETE, at physiologically relevant concentrations, potentiates platelet aggregation and nucleotide release. These effects are mediated via enhanced production of the phosphoinositide-derived second messengers—inositol-1,4,5-trisphosphate (1,4,5-IP3) and sn-1,2-diacylglycerol (DAG), and subsequent activation of protein kinase C.

MATERIALS AND METHODS

Blood Collection and Preparation of Washed Platelets

Venous blood was collected after informed consent from human volunteers (age 24 to 45 years) who had not ingested any medication in the previous 2 weeks. The blood was anticoagulated using 9

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Measurement of DAG in Platelets

Washed platelets were prepared and suspended in HEPES-buffered salt solution, pH 7.4. Platelet aggregation was determined in a Lumi-Aggregometer (Chrono-log, Haverton, PA), and adenosine triphosphate (ATP) release was monitored with luciferin-luciferase as previously described.21,22

Measurement of Platelet Intracellular Calcium

Platelets were loaded with Fura-2, washed, and suspended in HEPES-buffered salt solution.26,27 Changes in intracellular calcium levels were monitored spectrofluorometrically in a Fluorolog Spectrophotofluorometer (Spx Industries, Edison, NJ).24,25 Fluorescence measurements were made with excitation wavelengths at 340 and 380 nm and the emission wavelength set at 505 nm. Intracellular calcium concentration, [Ca2+]i, was calculated using the CM software provided with the instrument.

Measurement of DAG in Platelets

For the assay of DAG, washed platelets were suspended in HEPES-Tyrode buffer, pH 7.4,26 and stimulated with thrombin in the presence or absence of the indicated concentration of 15-HETE. Lipids were extracted,28 and aliquots of the lipid extracts were separated on a 5% to 18% gradient polyacrylamide sodium dodecyl sulfate (SDS) slab gels. Dried gels were autoradiographed, phosphorylated bands were identified, and the band corresponding to the 47-Kd protein was quantitated by liquid scintillation counting.

Measurement of 1,4,5-IP3 in Platelets by Complementary Methodologies

Analysis of 1,4,5-IP3 by radioreceptor assay. Washed human platelets suspended in HEPES-Tyrode buffer26 were stimulated with thrombin in the presence or absence of the indicated concentration of 15-HETE. Aliquots were removed before and at varying time intervals after the addition of the agonist into tubes containing an equal volume of 20% ice-cold trichloroacetic acid (TCA). 1,4,5-IP3 in the TCA extract was assayed using a commercially available radioreceptor assay kit (New England Nuclear, Boston, MA).

Studies with 32P-phosphate- or 14C-inositol-labeled platelets. Human platelets were labeled with 32P-phosphate29 or 14C-inositol,30 and suspended in HEPES-Tyrode buffer. Inositol phosphates were isolated using Supelco LC-NH2 solid-phase extraction tubes (Supelco, Bellefonte, PA), and analyzed by reverse phase high-performance liquid chromatography (HPLC) in a Spectra-Physics Liquid Chromatograph (Spectra-Physics, San Jose, CA) using a Whatman SAX analytical column and a gradient of 2 mol/L ammonium phosphate buffer, pH 3.3, as the eluting buffer at a flow rate of 2 mL/min.31 Fractions of 500 μL were counted in an LKB Mini-Beta Liquid Scintillation Counter (Pharmacia LKB Nuclear, Gaithersburg, MD). [γ-32P]-inositol phosphate standards were run before and after sample analysis. The radioactive peaks were identified by comparing their retention times with those of reference compounds.

Phosphorylation of 47-Kd Protein in Platelets

Phosphorylation of the 47-Kd protein was studied as previously described with 32P-phosphate–loaded washed platelets. Proteins were separated on a 5% to 18% gradient polyacrylamide sodium dodecyl sulfate (SDS) slab gels. Dried gels were autoradiographed, phosphorylated bands were identified, and the band corresponding to the 47-Kd protein was quantitated by liquid scintillation counting.

Data Analysis

Since simultaneous comparison of control with treatments was not possible in all studies (especially ATP release and calcium measurements), and since decay of platelet metabolic activities occurs with time, a control was assayed after every two treatments. Each treatment was then compared with the immediate control. The results were expressed as percent control response. Each of the experiments described were repeated from four to 10 times as indicated, using different platelets obtained from different donors on different days. The statistical significance of difference in a treatment series was determined by analysis of variance (ANOVA). Individual treatments in a treatment series were then compared with the control using Dunnett’s test.34

Materials

15-HETE, 12-HETE, 5-HETE, HHT, prostacyclin, and TxB2 standards were obtained from Biomol Research Laboratories (Plymouth Meeting, PA). The HETE preparations were purified by reverse phase HPLC before use. Arachidonic acid, ATP, luciferin-luciferase, and DAG were purchased from Sigma Chemical (St Louis, MO). Fura-2AM was obtained from Calbiochem-Behring (La Jolla, CA). DAG kinase was purchased from Lipidex (Westfield, NJ). Bovine thrombin was purchased from Park Davis (Morris Plains, NJ). [1-14C]-arachidonic acid (50 to 60 mCi/mmol), [γ-32P]-ATP (5,000 Ci/mmol), [32P]-phosphate (8,000 to 9,000 Ci/ mmol), [14C]-inositol (342 mCi/mmol), and [3H]-labeled inositol phosphate standards were obtained from Amersham (Arlington Heights, IL) or New England Nuclear.
RESULTS

Effect of HETEs on Platelet Aggregation

Incubation of washed human platelets with thrombin (0.01 to 0.1 U/mL) caused platelet aggregation in a concentration-dependent manner, with maximal responses (20% to 100% aggregation) achieved by 2 to 10 minutes depending on the concentration of thrombin used. In 10 different experiments, thrombin (0.025 U/mL) induced 20% to 35% aggregation by 3 minutes, reaching the maximal response (40% to 50% aggregation) by 6 to 8 minutes. For our subsequent studies with HETEs, a final threshold concentration of 0.025 U/mL thrombin was used unless otherwise indicated.

As shown in a representative experiment (Fig 1), 15-HETE affected both phases of platelet aggregation induced by this threshold concentration of thrombin, ie, the primary response—rate of aggregation, and the final response—maximal aggregation. Both responses were potentiated by 40% to 80% at concentrations between 1 and 100 nmol/L 15-HETE. However, this hydroxyeicosanoid had no effect on the aggregation changes induced by the concentration of thrombin that caused maximal platelet aggregation (100%), ie, 0.1 U/mL thrombin used in our studies. In six different experiments, we evaluated the effect of 15-HETE (0.1 to 100 nmol/L) on the extent of platelet aggregation induced at 3 minutes in response to stimulation with threshold doses of thrombin. When compared with the control, 15-HETE enhanced platelet aggregation by 25% ± 15% (mean ± SE), 32% ± 9% (P < .05), 57% ± 10% (P < .01), and 55% ± 21% (P < .01) at 0.1, 1, 10, and 100 nmol/L, respectively.

12-HETE, the lipoxygenase metabolite of arachidonic acid in platelets, and the structural isomer of 15-HETE, showed a smaller stimulatory effect at 10 and 100 pmol/L (18% ± 5% and 26% ± 9%, n = 6) with statistical significance (P = .05) achieved at the 100 pmol/L concentration only. While 5-HETE had no effect at concentrations between 1 nmol/L and 10 µmol/L, platelet aggregation was enhanced at a 5-HETE concentration of 30 µmol/L (66% ± 18%, n = 6, P < .01). None of the mono-HETEs alone induced spontaneous platelet aggregation in stirred suspensions at concentrations between 1 pmol/L and 100 µmol/L.

Effect of HETEs on Nucleotide Release by Platelets

In the representative experiment depicted in Fig 2, control platelets released 2.35, 3.86, and 4.51 nmol ATP per 10⁹ platelets in response to stimulation by thrombin at 3, 4, and 5 minutes, respectively, with 15-HETE enhancing the thrombin-induced nucleotide release in a concentration- and time-dependent manner. Thrombin-induced nucleotide release at 3 minutes was increased by 20% to 125% in the presence of added 15-HETE (1 and 100 nmol/L). In six different experiments, we measured the nucleotide release by platelets at 3 minutes in response to stimulation with thrombin in the presence or absence of 15-HETE. At concentrations of 0.1, 1, 10, and 100 nmol/L, 15-HETE enhanced thrombin-induced ATP release by 12% ± 13%, 40% ± 10% (P = .05), 65% ± 16% (P < .01), and 64% ± 19% (P < .01), respectively. This response of 15-HETE on nucleotide release paralleled the response seen on platelet aggregation with a similar maximal stimulatory concentration.

12-HETE potentiated thrombin-induced nucleotide release only at 100 pmol/L (27% ± 8% over thrombin control, n = 6, P < .05), while 5-HETE enhanced thrombin-induced nucleotide release only at concentrations in the micromolar range (63% ± 30% at 10 µmol/L, n = 6, P = .05; 116% ± 18% at 30 µmol/L, P < .01). In the absence of thrombin, the three mono-HETEs alone had no effect on nucleotide release at concentrations between 1 pmol/L and 100 µmol/L.
Fig 2. Time-dependent effects of 15-HETE on thrombin-induced nucleotide release by platelets. Washed human platelets were stimulated with 0.025 U/mL thrombin in the presence or absence of the indicated concentrations of 15-HETE. ATP release was monitored using luciferin-luciferase. Results presented are from a representative experiment repeated six times with similar results.

**Effect of 15-HETE on the Changes in Platelet Intracellular Calcium**

The effect of 15-HETE (10 and 100 nmol/L) on time-dependent changes in the thrombin-induced release of intracellular calcium is shown in Fig 3. In this representative experiment, the intracellular calcium level in the control platelets was elevated from a basal value of 115 nmol/L to 286 nmol/L in response to activation by thrombin alone. 15-HETE potentiated the thrombin-induced peak response by 40% and 85% at 10 and 100 nmol/L, respectively. In six different experiments, 15-HETE, at concentrations of 0.1, 1, 10, and 100 nmol/L, produced a stimulatory effect of 18% ± 16%, 31% ± 9% (P < .05), 52% ± 14% (P < .01), and 31% ± 9% (P < .05), over the peak intracellular calcium release induced by thrombin in control platelets.

**Effect of 15-HETE on DAG Production in Platelets**

For these studies, concentrations of 10 and 100 nmol/L of 15-HETE were used, since we had previously observed maximal potentiation effects on platelet functions at these levels. 15-HETE potentiated thrombin-induced DAG production in a concentration- and time-dependent manner (Fig 4). DAG mass in unstimulated platelets was 144 ± 23 pmol/10^9 platelets (mean ± SE, n = 6). In response to thrombin treatment, the DAG level in control platelets was...
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15-HETE enhanced thrombin-induced 1,4,5-IP₃ release in a time- and concentration-dependent manner, with statistically significant changes noted at all time points evaluated. For example, at 30 seconds, thrombin-induced release of 1,4,5-IP₃ was increased by 80% (P < .05), and 220% (P < .01) in the presence of 10 and 100 nmol/L 15-HETE, respectively. Similar effects were also observed in the two complementary assays using either ³²P-phosphate- or ¹⁴C-inositol-labeled platelets (Table 1). In these latter studies, the effects of 15-HETE on the agonist-induced release of radiolabeled 1,4,5-IP₃ were evaluated following a 10-second incubation with thrombin.

**Effect of 15-HETE on the Phosphorylation of the 47-Kd Protein**

The effect of 15-HETE on the phosphorylation of the 47-Kd protein is shown in Fig 6. The baseline phosphorylation of the 47-Kd protein in unstimulated platelets was 40,235 ± 2,358 cpm/10⁹ platelets (mean ± SE, n = 4). 15-HETE (10 and 100 nmol/L) had no effect on this basal protein phosphorylation. Incorporation of ³²P-phosphate...
The presence of the indicated concentrations of 15-HETE. Inositol potentiated thrombin-induced phosphorylation of the 47-Kd protein was increased by 6,671\times 10^2 \text{ cpm/10}^9 \text{ platelets.}

Agonist-induced response was determined as described in the legend to Fig 4. Baseline phosphorylation of the 47-Kd protein in unstimulated platelets was 40,235 \pm 827 \text{ cpm/10}^9 \text{ platelets. Individual treatments were significantly different from the respective controls at } ^*P < .05 \text{ and } ^{**}P < .01.

with an increase in $^{32}$P-phosphate incorporation observed at all time points evaluated (Fig 6). For example, at 30 seconds, 10 and 100 nmol/L 15-HETE enhanced protein phosphorylation by 123\% ($P = .05$) and 205\% ($P < .01$), respectively.

**Effect of 15-HETE on TxA2 Formation**

To determine whether TxA2, the platelet proaggregatory eicosanoid, was involved in mediating the HETE effect, we investigated the effect of 15-HETE on TxA2 (measured as TxB2) and other eicosanoid formation in $^{14}$C-arachidonic acid–labeled platelets. In six different experiments, control platelets released 1,549 $\pm$ 238 cpm TxB2/10^9 platelets (mean $\pm$ SE) following a 3-minute incubation with thrombin (Table 2). 15-HETE at concentrations between 1 nmol/L and 100 nmol/L had no effect on thrombin-induced TxB2 formation. Further, in two different experiments, in vivo aspirinized platelets aggregated and released nucleotides in the presence of 15-HETE in a manner similar to 15-HETE–treated platelets obtained from the same donor before aspirin ingestion, although TxB2 formation was markedly inhibited (by 93\%) in the platelets obtained post-aspirin. The thrombin-induced production of HHT and 12-HETE was not affected by 15-HETE at concentrations between 1 and 100 nmol/L (Table 2).

**DISCUSSION**

There are many critical biochemical events of platelet activation that could potentially be affected by the HETEs. The rapid turnover of phosphoinositides is a key event that occurs following stimulation of platelets by a variety of agonists, including thrombin. Both platelet aggregation and release responses appear to be mediated via this signal transduction pathway.\textsuperscript{35,36} Activation of phospholipase C, as a consequence of agonist-receptor binding, results in the generation of two intracellular messengers—1,4,5-IP3 and DAG from phosphatidylinositol-4,5-bisphosphate. These messenger molecules affect divergent pathways of platelet activation: 1,4,5-IP3 releases calcium from intracellular stores,\textsuperscript{35,36} and DAG activates a calcium and phospholipid-dependent kinase, protein kinase C.\textsuperscript{37} Protein kinase C phosphorylates a 47-Kd protein in platelets, which is thought to be involved in the fusion of granular membranes with those of the surface-connected canalicular system.\textsuperscript{38} The increase in cytosolic calcium activates a calcium/
calmodulin-dependent myosin light chain (MLC) kinase that phosphorylates MLC. This initiates interaction of myosin with actin molecules and contraction of the actin-myosin cytoskeleton. These two distinct biochemical events together mediate the platelet responses, ie, platelet shape change, aggregation, and secretion. The increase in cytosolic calcium in platelets also causes the activation of phospholipase A2. Released arachidonic acid is converted via the cyclooxygenase pathway to PGH2 and TxA2,41,42 which enhance agonist responses through an interaction with specific TxA2/PGH2 receptors.40 Arachidonic acid is also metabolized via the lipoxygenase pathway to 12-HETE,41,42 although a role for 12-HETE in platelet activation has not been clearly defined.

In this study, 15-HETE, a lipoxygenase metabolite of arachidonic acid in endothelial cells and white blood cells, has been shown to potentiate thrombin-induced platelet aggregation, nucleotide release, and elevation of intracellular calcium. In addition, 12-HETE and 5-HETE, the structural isomers of 15-HETE, and the lipoxygenase metabolites of arachidonic acid in platelets and neutrophils, respectively, also enhanced agonist-induced platelet aggregation and nucleotide release when evaluated under similar experimental conditions. However, the mono-HETEs showed differential dose-responses on platelet functions. While 15-HETE potentiated thrombin-induced platelet functions at nanomolar levels, 12-HETE responses were observed at picomolar levels, and the 5-HETE effect was seen at concentrations in the micromolar range. Further, the magnitude of the potentiation effects of these mono-HETEs varied, with 15-HETE being the most potent eicosanoid. Potentiation of platelet functions by these mono-HETEs was not due to a detergent effect, since the eicosanoids enhanced platelet functions at differing concentrations. Further, the mono-HETEs alone had no effect.

To delineate the mechanism(s) by which the mono-HETEs enhanced agonist-induced platelet functions, 15-HETE was used as a prototype mono-HETE in our biochemical studies, since the latter eicosanoid appeared to be the most effective mediator of the HETE effects, with activity noted at physiologically relevant concentrations. Studies centered on whether the mono-HETE of interest mediated its effects via enhanced production of the intracellular messengers—1,4,5-IP3 and DAG, and/or the platelet proaggregatory eicosanoid TxA2. Since significant changes in biological responses (aggregation and release) and DAG and 1,4,5-IP3 production were observed at similar 15-HETE concentrations, it appears that the HETE-induced potentiation of platelet functions is mediated via the enhanced production of these second messengers. While we have used a recently described mass measurement assay to quantitate the amount of DAG produced, the changes in 1,4,5-IP3 production were studied using three complementary procedures, and an increase in 1,4,5-IP3 in response to this eicosanoid was observed in all instances. Further, 15-HETE also potentiated agonist-induced phosphorylation of the 47-Kd platelet protein—one of the terminal biochemical changes seen in response to 1,4,5-IP3 and DAG production in activated platelets. Our findings also demonstrate that the platelet proaggregatory eicosanoid TxA2 was not involved in mediating the HETE effects. This conclusion was supported by the findings that 15-HETE (1 to 100 nmol/L) had no effect on TxA2 formation, and that in vivo aspirinated platelets responded to 15-HETE in a manner similar to control platelets.

While the stimulatory effects of 15-HETE on platelet functions that we have observed occur at physiologically relevant concentrations in the nanomolar range, other investigators have documented an inhibitory effect with both 15-HETE and 15-HPETE.44,45 However, these studies used micromolar concentrations of 15-HETE and 15-HPETE. It is interesting to note that 15-HETE stimulates endothelial cell proliferation and migration, and that these proliferative and migratory responses have also been observed at similar nanomolar concentrations of this eicosanoid. The proliferative response in endothelial cells appeared to be mediated via an enhanced production of DAG. Thus, 15-HETE appears to mediate its potentiation effect on endothelial cell and platelet functions via similar mechanisms, ie, enhanced production of phosphoinositide-derived second messengers.

There are several suggestions in the literature that 15-HETE could modulate various platelet-endothelial cell functions, especially in certain pathologic states. Besides the previous studies demonstrating an effect of 15-HETE on endothelial cell proliferation,6 smooth muscle cell migration,15 and neovascularization,14 Brown et al have reported that endothelial cells exposed to high glucose concentrations in vitro produced increased amounts of 15-HETE when compared with cells maintained under normoglycemic conditions. Using the atherosclerotic rabbit model, other investigators have shown that the aortae obtained from these animals produce increased amounts of 15-HETE when compared with controls.7,18 Both 15-HETE and 15-HPETE also decrease the production of prostacyclin in endothelial cells by inhibiting cyclooxygenase activity4 and prostacyclin synthase activity.8 While these latter effects are observed in vitro at micromolar concentrations, previous studies have also shown an inverse correlation between ex vivo production of prostacyclin and 15-HETE by vascular tissues in pathologic states including diabetes mellitus, suggesting that the products of 15-lipoxygenase in endothelial cells could function as endogenous regulators of prostacyclin production. By modulating prostacyclin production, 15-HETE and 15-HPETE could play a role in the maintenance of vascular tone. Moreover, several recent studies also indicate that both 15-HETE and 15-HPETE are potent vasoconstrictors of both arteries and veins in the pulmonary and cerebral microcirculation.7,46 Thus, 15-HETE may play an important role in the vascular changes observed in pathologic states such as diabetes mellitus and atherosclerosis by modulating platelet functions (both directly by its effect on platelet biologic responses or indirectly via prostacyclin), and by effects on vascular tone, endothelial cell and smooth muscle cell migration, and proliferation and new vessel formation.
12-HETE, the endogenous lipoxygenase product in platelets, also exhibited a small but significant stimulatory response on thrombin-induced platelet functions at picomolar levels. A recent study by Sekiya et al in bovine platelets demonstrated that 12-HETE (5 to 25 μmol/L) was a strong potentiator of platelet aggregation induced by a threshold concentration of thrombin (0.04 U/mL). However, these investigators did not evaluate platelet responses to submicromolar concentrations of this eicosanoid.

In summary, 15-HETE, at physiologically relevant concentrations, potentiates thrombin-induced platelet aggregation, nucleotide release, and elevation of intracellular calcium. Eicosanoid-induced modulation of platelet functions appear to be mediated via enhanced production of DAG and 1,4,5-IP₃. Since this eicosanoid is elevated in pathologic states associated with platelet hyperfunction, an elucidation of its mechanism(s) of action appears relevant to our understanding of the genesis of atherothrombotic vascular disease.

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15-Hydroxyeicosatetraenoic acid-mediated potentiation of thrombin-induced platelet functions occurs via enhanced production of phosphoinositide-derived second messengers--sn-1,2-diacylglycerol and inositol-1,4,5-trisphosphate

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