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María A. Balboa; ... et. al

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Amplification Mechanisms of Inflammation: Paracrine Stimulation of Arachidonic Acid Mobilization by Secreted Phospholipase A₂ Is Regulated by Cytosolic Phospholipase A₂-Derived Hydroperoxyeicosatetraenoic Acid¹

María A. Balboa, Rebeca Pérez, and Jesús Balsinde²

In macrophages and other major immunoinflammatory cells, two phospholipase A₂ (PLA₂) enzymes act in concert to mobilize arachidonic acid (AA) for immediate PG synthesis, namely group IV cytosolic phospholipase A₂ (cPLA₂) and a secreted phospholipase A₂ (sPLA₂). In this study, the molecular mechanism underlying cross-talk between the two PLA₂s during paracrine signaling has been investigated. U937 macrophage-like cells respond to Con A by releasing AA in a cPLA₂-dependent manner, and addition of exogenous group V sPLA₂ to the activated cells increases the release. This sPLA₂ effect is abolished if the cells are pretreated with cPLA₂ inhibitors, but is restored by adding exogenous free AA. Inhibitors of cyclooxygenase and 5-lipoxygenase have no effect on the response to sPLA₂. In contrast, ebselen strongly blocks it. Reconstitution experiments conducted in pyrrophenone-treated cells to abolish cPLA₂ activity reveal that 12- and 15-hydroperoxyeicosatetraenoic acid (HPETE) are able to restore the sPLA₂ response to levels found in cells displaying normal cPLA₂ activity. Moreover, 12- and 15-HPETE are able to enhance sPLA₂ activity in vitro, using a natural membrane assay. Neither of these effects is mimicked by 12- or 15-hydroxyeicosatetraenoic acid, indicating that the hydroperoxy group of HPETE is responsible for its biological activity. Collectively, these results establish a role for 12/15-HPETE as an endogenous activator of sPLA₂-mediated phospholipolysis during paracrine stimulation of macrophages and identify the mechanism that connects sPLA₂ with cPLA₂ for a full AA mobilization response. *The Journal of Immunology*, 2003, 171: 989–994.

The phospholipase A₂ (PLA₂)³ superfamily represents a heterogeneous group of enzymes with key roles in inflammation whose common feature is to hydrolyze the fatty acid present at the sn-2 position of phospholipids (1, 2). Multiple forms of PLA₂ have been described in mammalian tissues, including several forms of secreted PLA₂ (sPLA₂) (3), group IV Ca²⁺-dependent cytosolic PLA₂ (cPLA₂) (4), and group VI Ca²⁺-independent PLA₂ (5). Of those, cPLA₂ and two of the sPLA₂ enzymes, namely groups IIA and V, have repeatedly been shown to be responsible for generating free arachidonic acid (AA) for PG synthesis (6, 7).

An interesting feature of the AA release process is that cross-talk appears to exist between the PLA₂ involved. During stimulation of the cells with agents that promote an immediate AA mobilization response, cPLA₂ activation precedes and appears to be

required for the subsequent action of sPLA₂ (either group IIA or V) (8–12). The first evidence in support of this view was provided by studies in macrophages showing that the sPLA₂-dependent release of AA was blocked by cPLA₂ inhibitors, and restored by elevating the intracellular levels of free AA by exogenous addition of the fatty acid, which mimics cPLA₂ activation (8, 9). These results were later confirmed in other cell types (10, 11), and also by transfection studies showing a synergistic sPLA₂-dependent AA release in cells overexpressing cPLA₂ (12). Because sPLA₂ activity is particularly sensitive to the physical state of the membrane, different events that alter membrane dynamics, such as ceramide generation (13), membrane oxidation (14), and loss of membrane asymmetry (15, 16), have been proposed as possible mechanisms involved in facilitating sPLA₂ hydrolysis of the agonist-stimulated cellular membranes. However, the factor that intermediates between cPLA₂ and sPLA₂ has not been identified.

To understand better the interplay between cPLA₂ and sPLA₂ in AA mobilization in macrophages, we have examined the effects of exogenous group V PLA₂ on AA mobilization from activated human U937 macrophage-like cells. These settings mimic an instance of paracrine amplification of the inflammatory response in that exogenous sPLA₂ being released to the inflammatory foci acts on neighboring cells to increase the response.

Activated U937 cells exhibit an immediate AA release response when exposed to a variety of receptor-mediated and soluble agonists. This AA release is sensitive to inhibitors of cPLA₂, but not to inhibitors of other PLA₂s, implying that only cPLA₂ is responsible for the release (17–19). Results described in this work identify 12/15-hydroperoxyeicosatetraenoic acid (12/15-HPETE) as the cPLA₂-downstream product that enables exogenous group V sPLA₂ to properly act on the membranes of activated U937 macrophages.

Institute of Molecular Biology and Genetics, University of Valladolid School of Medicine, Valladolid, Spain

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² Address correspondence to Dr. Jesús Balsinde, Instituto de Biología y Genética Molecular (IBGM-CSIC), Facultad de Medicina, Universidad de Valladolid, Avenida Ramón y Cajal 7, E-47005 Valladolid, Spain. E-mail address: jbalinde@ibgm.uva.es

³ Abbreviations used in this paper: PLA₂, phospholipase A₂; AA, arachidonic acid; cPLA₂, cytosolic PLA₂; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; iPLA₂, calcium-independent PLA₂; sPLA₂, secreted PLA₂.

Materials and Methods

Materials

The [5, 6, 8, 9, 11, 12, 14, 15-³H]AA (100 Ci/mmol) was from Amersham (Arlington Heights, IL). The 12(S)-HPETE, 15(S)-HPETE, 12-hydroxyeicosatetraenoic acid (12-HETE), and 15-HETE were purchased from Cayman (Ann Arbor, MI). Lipoxygenase inhibitors were from BioMol (Plymouth Meeting, PA). DNA polymerase was from BioTools (Madrid, Spain). Primers for PCR were from MWG-Biotech AG (Ebersberg, Germany). Recombinant rat group V sPLA₂ was generously provided by A. Aarsman (Utrecht University, Utrecht, The Netherlands) (20). The specific cPLA₂ inhibitor pyrrophenone was generously provided by K. Seno (Shionogi, Osaka, Japan) (21). All other reagents were from Sigma-Aldrich (St. Louis, MO).

Cell culture

U937 cells were maintained in RPMI 1640 medium supplemented with 10% (v/v) FCS, 2 mM glutamine, penicillin (100 U/ml), and streptomycin (100 µg/ml). The cells were incubated at 37°C in a humidified atmosphere of CO₂/O₂ (1:19) at a cell density of 0.5–1 × 10⁶ cells/ml in 12-well plastic culture dishes (Costar, Cambridge, MA). Cell differentiation was induced by treating the cells with 35 ng/ml PMA for 24 h (22, 23).

AA release experiments

The cells were labeled with 0.5 µCi/ml [³H]AA for 18 h. After this period, the cells were washed and placed in serum-free medium for 1 h before the addition of 100 µg/ml Con A in the presence of 0.5 mg/ml BSA. When free AA, HPETEs, or HETEs were added to the cells, they were dissolved in ethanol. Appropriate controls were conducted to exclude an effect of the solvent. The supernatants were removed, cleared of cells by centrifugation, and assayed for radioactivity by liquid scintillation counting.

Enzyme assays

For the measurement of cellular iPLA₂, aliquots of U937 cell homogenates were incubated for 2 h at 37°C in 100 mM HEPES (pH 7.5) containing 5 mM EDTA and 100 µM labeled phospholipid substrate (1-palmitoyl-2-[³H]palmitoyl-glycerol-3-phosphocholine, sp. act. 60 Ci/mmol; American Radiolabeled Chemicals, St. Louis, MO) in a final volume of 150 µl. The phospholipid substrate was used in the form of sonicated vesicles in buffer. For group V sPLA₂ enzyme activity assay, the mammalian membrane substrate assay described by Diez et al. (24) was used.

RT-PCR

cDNA from U937 cells was produced using the kit Cells-to-cDNA (Ambion, Austin, TX), following the manufacturer's instructions. The cDNA was then amplified by PCR using the following primers: 15-LOX (15-LOX-1), upstream primer (5'-GAGTTGACTTTGAGGTTTCGC-3'), downstream primer (5'-GCCCGTCTGTCTTATAGTGG-3') (25); 15-LOX-2, upstream primer (5'-TGCCTCTGCCATCCAGCT-3'), downstream primer (5'-TGTTCCCTGGGATTTAGATGGA-3') (26); and 12-LOX, upstream primer (5'-CGTAAGGATGATCTACCTCC-3'), downstream primer (5'-TTGGGGTTGGAGAGCTGGGG) (27). The expected sizes for PCR products using these primers were: 952, 1065, and 519 bp, respectively. PCR conditions were: 30–35 cycles, denaturation at 94°C for 1–2 min; annealing at 58°C for 75 min for 15-LOX, 60°C for 1 min for 15-LOX-2, and 63°C for 1 min for 12-LOX, and extension at 72°C for 2 min. An additional extension at 72°C for 10 min was performed at the end of the cycles. The amplified DNA was analyzed by 2% agarose gel electrophoresis and visualized by ethidium bromide.

Separation of AA metabolites

For these experiments, the cells were labeled with 5 µCi [³H]AA for 18 h and the stimulations were conducted in the absence of albumin. The supernatant was acidified to pH 3.5 with 5 M formic acid, and extracted twice with 3 ml of isopropanol-diethyl ether (1:1.5). The organic phase was dried under a stream of nitrogen, and the residue was dissolved in a few drops of chloroform-methanol (2:1, v/v) and analyzed by reverse-phase HPLC. Separation of lipoxygenase metabolites was performed on a 4.6 × 250-mm ODS reverse-phase column (Beckman, Palo Alto, CA), using an isocratic mobile phase of methanol-water-acetic acid (70:30:1) at a flow rate of 0.5 ml/min. Fractions of 0.5 ml were collected, and radioactivity content was measured by liquid scintillation counting. Retention times of the different products were identified by coelution with authentic standards (Cayman).

Data presentation

Assays were conducted in triplicate. Each set of experiments was repeated at least three times with similar results. Unless otherwise indicated, the data presented are from representative experiments, and are shown as means ± SD.

Results

Exogenous group V sPLA₂ effects on AA release from U937 cells

When PMA-differentiated U937 cells are stimulated by Con A, immediate AA release occurs by a mechanism that is entirely attributable to cPLA₂ activation, with no involvement of the one other PLA₂ that these cells express, namely iPLA₂ (18, 19). This conclusion is based on the complete inhibition of the AA release response by the specific cPLA₂ inhibitor pyrrophenone at concentrations higher than 0.5 µM (19), and the lack of any detectable effect of the iPLA₂ inhibitor bromoenol lactone even at concentrations as high as 25 µM (18). U937 cells have been reported not to exhibit measurable sPLA₂ activity (17, 28). In accordance with this, we have not detected expression of groups IB, IIA, IIC, IID, IIE, IIF, III, V, and X in U937 cells by RT-PCR (M. A. Balboa and J. Balsinde, unpublished data). Fig. 1A shows that pyrrophenone concentrations equal to those leading to complete inhibition of Con A-induced AA release had no effect on the iPLA₂ activity of U937 cell homogenates, as measured in an *in vitro* assay. Even at concentrations of 10 µM, pyrrophenone exerted little effect on cellular iPLA₂ activity (Fig. 1B). At the same concentrations, pyrrophenone also failed to minimally affect the activity of pure group V sPLA₂ (Fig. 1B).

Addition of exogenous group V sPLA₂ to the Con A-activated U937 cells resulted in a dramatic increase in the amount of [³H]AA mobilized (Fig. 2). Such an elevated response was inhibited by the specific cPLA₂ inhibitor pyrrophenone (Fig. 2), suggesting that in U937 macrophages, cPLA₂ activation modulates

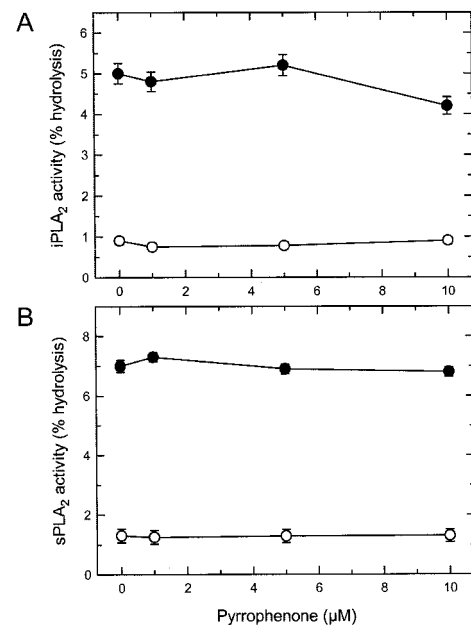


FIGURE 1. Effect of pyrrophenone on PLA₂ activities. *A*, Homogenates from U937 cells (●) were assayed for calcium-independent PLA₂ activity in the presence of the indicated concentrations of pyrrophenone. ○, Denote control incubations in the absence of homogenate. *B*, Recombinant group V sPLA₂ was assayed for activity using a natural membrane as substrate. [³H]AA-labeled membranes were incubated without (○) or with (●) 15 nM group V sPLA₂ for 1 h in the presence of the indicated concentrations of pyrrophenone.

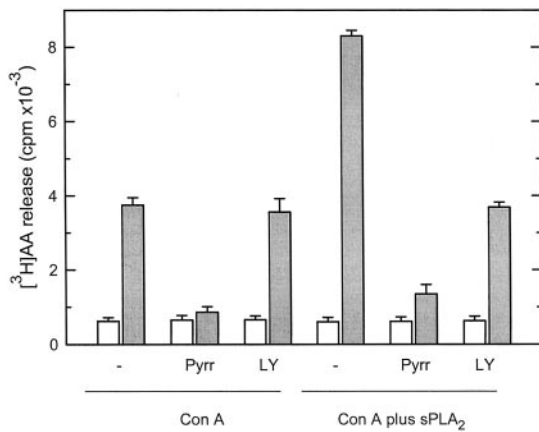


FIGURE 2. Exogenous sPLA₂-induced [³H]AA release from U937 macrophage-like cells. The cells were untreated (□) or treated with 100 μg/ml Con A or 100 μg/ml Con A plus 15 nM group V sPLA₂, as indicated (▣), for 15 min in the absence or presence of the indicated inhibitors. Pyrrophenone (Pyrr) was used at 1 μM. LY311727 (LY) was used at 25 μM.

sPLA₂. The sPLA₂-dependent AA release was blocked by the specific inhibitor LY311727, indicating that the hydrolytic activity of the enzyme is required for the response to take place (Fig. 2).

Role of 12/15-HPETE in facilitating sPLA₂ activity

The above data indicate that in common with other cell types (8–12), activation of cPLA₂ in U937 cells appears to facilitate the action of sPLA₂ on cellular membranes. To further stress this notion, [³H]oleic acid release experiments were conducted. cPLA₂ releases little, if anything, of this fatty acid, whereas sPLA₂ does it readily (29, 30). Thus, determination of [³H]oleic acid release allows one to separate the contribution of sPLA₂ to phospholipid hydrolysis from the one of cPLA₂ and, in turn, provides a straightforward tool to study the effect of cPLA₂ inhibition on sPLA₂ activation. Fig. 3 shows that U937 cells exposed to exogenous group V sPLA₂ released modest quantities of [³H]oleic acid. Such a release was completely blocked by LY311727, thus confirming

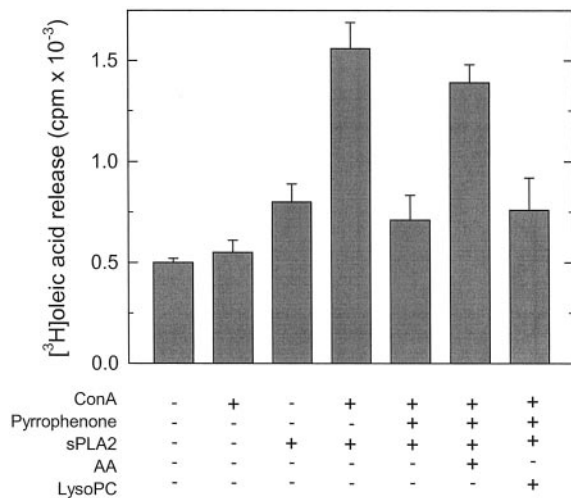


FIGURE 3. Arachidonic acid restores the enhancing effect of exogenous sPLA₂ on [³H]oleic acid mobilization in activated U937 macrophage-like cells. The cells were preincubated with 1 μM pyrrophenone where indicated. Subsequently, the cells were stimulated by Con A (100 μg/ml) in the absence or presence of 15 nM exogenous group V sPLA₂ and in the absence or presence of 1 μM AA or 10 μM lyso-phosphatidylcholine (LysoPC), as indicated.

that it was actually due to sPLA₂ (not shown). When the cells were activated by Con A and then exposed to exogenous sPLA₂, a marked potentiation of the response occurred. Con A-activated cells released no oleic acid in the absence of exogenous sPLA₂, in agreement with previous data (19). Importantly, the enhanced response was blocked by treating the cells with the cPLA₂ inhibitor pyrrophenone (Fig. 3). The inhibitory effect of pyrrophenone could be reversed by exposing the cells to 1 μM exogenous AA for 2 min before sPLA₂ addition. At the concentration used, exogenous AA did not exert any effect on its own (Fig. 3). Addition of lyso-phosphatidylcholine to the cPLA₂-activity-deficient cells did not restore the sPLA₂ effect (Fig. 3). These results suggest that cPLA₂-derived AA, or an oxygenated metabolite, plays a role in mediating the action of sPLA₂ on cellular membranes.

Fig. 4A shows that the contribution of exogenous sPLA₂ to total [³H]AA mobilization in cells exposed to both Con A and exogenous sPLA₂ was markedly reduced by the unspecific 15-lipoxygenase inhibitor ebselen (31), but not by inhibitors of cyclooxygenase (aspirin), 5-lipoxygenase-activating protein (MK-886) (32), or platelet-type 12-lipoxygenase (baicalein) (33). Moreover, inhibition by ebselen could be overcome by addition of 15-HPETE, the immediate product of 15-lipoxygenase action on free AA. The 15-HETE was considerably less effective than 15-HPETE in restoring the sPLA₂ effect, and 12-HPETE also restored it completely (Fig. 4B). These results suggest that the hydroperoxy group of 15-HPETE (and of 12-HPETE) is responsible for the enhancing effect on sPLA₂.

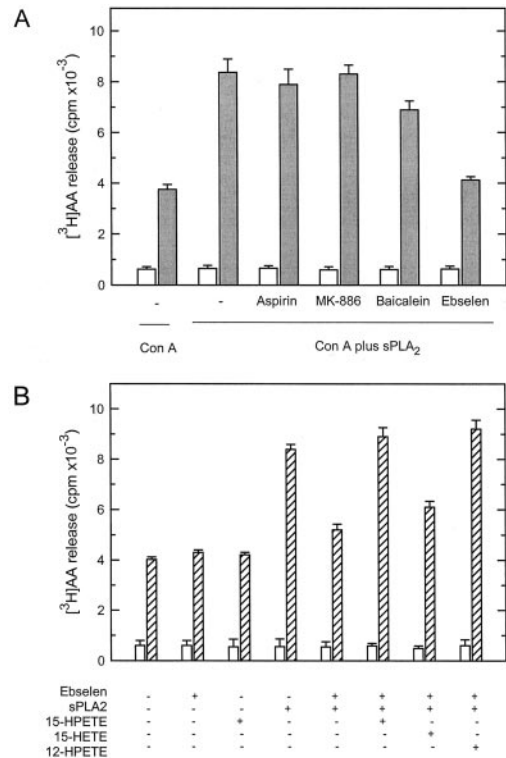


FIGURE 4. Effect of inhibitors of AA metabolism on [³H]AA release in activated U937 macrophages. *A*, The cells were untreated (□) or treated with 100 μg/ml Con A or 100 μg/ml Con A plus 15 nM group V sPLA₂, as indicated (▣), for 15 min in the absence or presence of the indicated inhibitors. All of the inhibitors were used at 25 μM. *B*, The cells were incubated with 25 μM ebselen where indicated. Subsequently, the cells were treated with (▨) or without (□) 100 μg/ml Con A in the presence or absence of 15 nM sPLA₂, 0.5 μM 15-HPETE, 0.5 μM 15-HETE, or 0.5 μM 12-HPETE, as indicated.

The 15-HPETE also enabled sPLA₂ action on cellular membranes in cells deficient in cPLA₂ activity, and 12-HPETE reproduced the effect (Fig. 5). Collectively, the results of Figs. 4 and 5 suggest that 12/15-HPETE is/are the factor(s) that, lying downstream of cPLA₂, enables sPLA₂ to properly act on cellular membranes. The 15-HPETE and 12-HPETE are both produced by reticulocyte-type 15-lipoxygenase in human myeloid cells, with 15-HPETE being the major product (34). However, because the relative proportion of products synthesized varies among species, this enzyme is also frequently called 12/15-lipoxygenase, and hereinafter it will be referred as such. This enzyme is also called 15-lipoxygenase-1 to distinguish it from the more recently described 15-lipoxygenase-2 (26). RT-PCR analysis of RNA from PMA-differentiated U937 cells revealed that these cells did express 12/15-lipoxygenase (Fig. 6A). PCR product specificity was confirmed by DNA sequence analysis. No expression of 12-lipoxygenase (platelet type) or 15-lipoxygenase-2 could be demonstrated (data not shown).

Production of 15-HPETE by activated U937 cells

Reverse-phase HPLC determinations were conducted to verify whether activated U937 macrophage-like cells produced 12/15-lipoxygenase metabolites in a cPLA₂-dependent manner. Stimulation of the [³H]AA-labeled U937 cells with Con A resulted in a significant production of 15-[³H]HPETE (Fig. 6B). Low levels of 12-[³H]HPETE were also detected (Fig. 6B). Unstimulated [³H]AA-labeled U937 cells did not produce significant amounts of these products. Importantly, when the experiments were conducted in the presence of pyrrophenone to block cPLA₂ activity, a strong inhibition of 12/15-[³H]HPETE production was detected (93 ± 2% inhibition, mean ± SD, n = 3). Thus, activated cells produce 12/15-lipoxygenase products downstream of cPLA₂ activation.

The 12/15-HPETE enhance sPLA₂ activity

The 12/15-lipoxygenase is known to catalyze endogenous membrane oxidation, which may have profound effects on cellular physiology (34). Given that sPLA₂ are particularly sensitive to physical changes of the membranes (35), it is likely that the hydroperoxy metabolites produced by 12/15-lipoxygenase may act to influence sPLA₂ activity by altering membrane structure. To in-

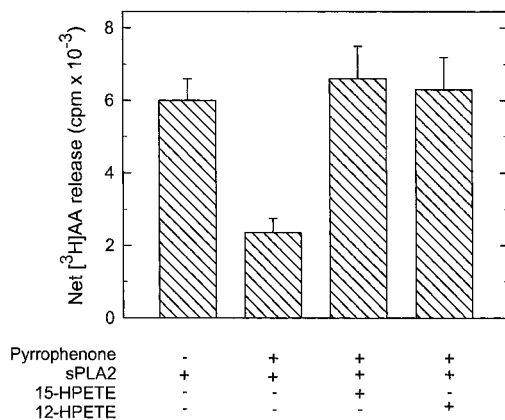


FIGURE 5. The 12- and 15-HPETE restore the enhancing effect of exogenous sPLA₂ on [³H]AA mobilization in activated U937 macrophages. [³H]AA-labeled Con A-activated cells were preincubated with 1 μM pyrrophenone for 15 min where indicated. Subsequently, 0.5 μM 15-HPETE or 12-HPETE was added 3 min before adding 15 nM group V sPLA₂, as indicated. To highlight the contribution of sPLA₂ to the [³H]AA release, the responses in the absence of sPLA₂ were subtracted from those in the presence of sPLA₂ at each condition.

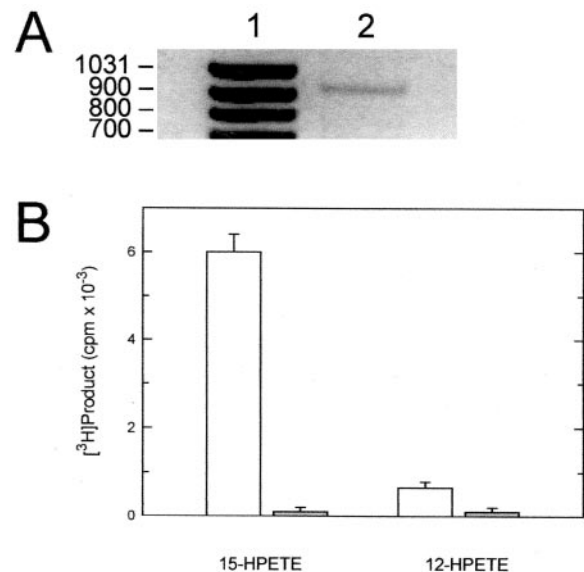


FIGURE 6. Involvement of 12/15-lipoxygenase in AA mobilization in U937 cells. A, RT-PCR analysis of 12/15-lipoxygenase in U937 cells. cDNA from U937 cells was obtained and amplified by PCR using the primers set described in *Materials and Methods*. Lane 1, Corresponds to a 1000-bp ladder; lane 2, corresponds to the analysis of the product (952 bp) obtained by PCR from U937 cDNA. B, The 12- and 15-HPETE production by activated U937 macrophages. The [³H]AA-labeled cells were treated with 100 μg/ml Con A for 15 min in the presence (gray bars) or absence (open bars) of 1 μM pyrrophenone, as indicated. The 12/15-HPETE production was determined by reverse-phase HPLC, as described in *Materials and Methods*.

investigate this possibility, sPLA₂ activity measurements were conducted using the natural membrane assay described by Diez et al. (24). In this system, purified [³H]AA-labeled membranes are used as substrate. Addition of 15-HPETE to the assay mix resulted in a marked increase in sPLA₂ activity (Fig. 7). Such an increase was not observed if 15-HETE was added instead. The 12-HPETE exerted the same stimulatory effect as 15-HPETE. As a positive control, H₂O₂-oxidized membranes were used (19), and marked increases in sPLA₂ activity were observed as well (data not shown). Thus, membrane peroxidation sensitizes membranes to sPLA₂ attack.

Discussion

Major immunoinflammatory cells such as macrophages and mast cells mobilize AA for PG production in two temporally distinct

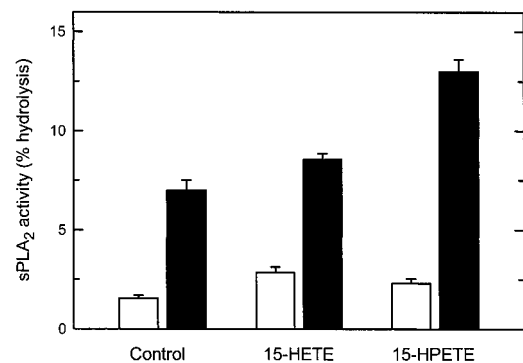


FIGURE 7. The 12/15-HPETE enhance sPLA₂ activity using a natural membrane as substrate. [³H]AA-labeled membranes were incubated with (■) or without (□) 15 nM group V sPLA₂ for 1 h in the presence of 0.5 μM 12/15-HETE, 0.5 μM 12/15-HPETE, or neither, as indicated.

phases, namely the immediate and delayed pathways (6, 10, 35–37). The immediate pathway, which is typically triggered by Ca^{2+} -mobilizing agonists, goes on for short periods of time (up to 1 h) and occurs at the expense of pre-existing effectors. The delayed pathway, spanning several hours, is strikingly dependent on protein synthesis. Both pathways appear to involve two PLA_2 effectors, namely cPLA_2 and sPLA_2 , although the mechanisms dramatically differ. In general terms, cPLA_2 appears to act as the initiator and key regulator of the response, while sPLA_2 amplifies the cPLA_2 -generated signal. The sPLA_2 may act both as an autocrine and paracrine effector (i.e., on the same cells that secreted it, or on neighboring cells), ensuring in this manner an efficient amplification of the response (6, 10, 30, 35–37).

In this work, we have studied the interactions between cPLA_2 and exogenous sPLA_2 during the immediate AA release response triggered by Con A on U937 macrophage-like cells. AA release in this cellular system depends on cPLA_2 activation, as judged by complete inhibition of the response by the highly selective cPLA_2 inhibitor pyrrophenone (19) (Fig. 1). Although no sPLA_2 activity has been demonstrated to occur in U937 cells (28), this study shows that sPLA_2 can still participate in the immediate response of the U937 cells if applied exogenously. This strategy is pathophysiologically sound in that it mimics a paracrine mechanism for amplification of the inflammatory response. In turn, the use of exogenous enzyme provides a straightforward means to study the influence of cPLA_2 activation on the action of sPLA_2 .

Group V sPLA_2 is produced by human and murine macrophages and mast cells, and has been repeatedly shown to play key roles in proinflammatory AA signaling (38) and, importantly, to be capable of activating cells in the vicinity of those that secreted it (25). Unlike the group IIA enzyme, group V sPLA_2 can act on the outer membrane of otherwise unstimulated cells (24, 25). However, the effect is more prominent on agonist-activated cells (39), reflecting the need for some kind of membrane rearrangement for group V sPLA_2 to fully express its hydrolytic activity (35).

Our previous studies in murine macrophages demonstrated that the elevated activity that group V sPLA_2 displays toward agonist-activated cells can be greatly diminished if cellular cPLA_2 activity is blocked, indicating the existence of cross-talk between the two signaling PLA_2 s (8, 9). Importantly, the inhibitory effect of cPLA_2 can be overcome by exogenous AA, suggesting that a cPLA_2 -derived AA metabolite intermediates between cPLA_2 and sPLA_2 (8, 9). In this study, we provide direct evidence that such a metabolite is 12/15-HPETE, the immediate product of 12/15-lipoxygenase action on free AA. Thus, cell treatment with ebselen blocks the enhanced action of group V sPLA_2 on activated U937 cells, and reconstitution experiments show that overcoming 12/15-lipoxygenase inhibition by exogenous supply of 12/15-HPETE fully restores the action of sPLA_2 on the activated cells. Thus, these results support a model whereby agonist activation of cPLA_2 results in the immediate generation of free AA, which will be used by 12/15-lipoxygenase to produce 12/15-HPETE. Subsequently, 12/15-HPETE serves a signaling role by enabling full activation of group V sPLA_2 and thus allowing for a further amplification of the AA mobilization response.

The sPLA_2 -activating effect of 12/15-HPETE was not mimicked by 12/15-HETE, indicating that the hydroperoxy group of 12/15-HPETE is responsible for its biological activity. In turn, this clearly suggests a role for 12/15-HPETE-mediated oxidation of membrane phospholipids as the mechanism for membrane sensitization leading to enhanced group V sPLA_2 activity. Fully supporting this view, we have found significantly higher sPLA_2 activity in vitro when the membrane substrate was pretreated with 12/15-HPETE.

It has been recognized that a prominent biological action of 12/15-lipoxygenase metabolites on cells is to induce lipid peroxidation reactions, to initiate a series of structural membrane changes (34). For this kind of peroxidation reaction, 12/15-lipoxygenase appears to typically act on esterified substrate, not necessarily on the free fatty acid (34). The current results establish, however, that cPLA_2 generation of free AA is required, and hence, that the involvement of 12/15-lipoxygenase in sPLA_2 activation takes place through the production of free 12/15-HPETE. This confers on the system greater versatility in that the peroxidizing effect of 12/15-HPETE can be exerted at places far away from its site of synthesis. This is important because 12/15-lipoxygenase is an intracellular enzyme, while exogenous sPLA_2 acts primarily on the outer surface of the cells (24, 25, 38). Because 12/15-HPETE can readily be taken up and esterified by the cells (40), it could also be envisioned that this metabolite may exit the cells to amplify the inflammatory response.

Although the results of this study have established a cascade of events for full AA mobilization involving the sequential participation of cPLA_2 , 12/15-lipoxygenase, and sPLA_2 , elegant studies by Cho and coworkers (30) have demonstrated that in otherwise unstimulated cells, exogenous group V sPLA_2 action leads to activation of cPLA_2 and the immediate metabolite of free AA by lipoxygenase pathways. It is tempting to speculate that in analogy with the results of our study, part of the hydroperoxy fatty acids produced under these settings (25) may act to enhance sPLA_2 attack on cellular membranes and in this manner amplify the immediate response. In contrast, overexpression studies by Kuwata et al. (41) have suggested a role for 12/15-lipoxygenase in regulating the expression of group IIA sPLA_2 during the delayed phase of AA mobilization of 3Y1 fibroblastic cells. Although the mechanisms implicating 12/15-lipoxygenase in the immediate (this study) and delayed (41) AA mobilization pathways obviously differ, it is nonetheless striking that the same effectors appear to be used to elicit these two separate responses.

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