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

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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 Acid

María A. Balboa, Rebeca Pérez, and Jesús Balsinde

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, eteafen strongly blocks it. Reconstitution experiments conducted in pyrophenone-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) family 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 cPLA2 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.
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-hydroxyicosatetraenoic acid (12-HETE), and 15-HETE were purchased from Cayman (Ann Arbor, MI). Lipoxigenase 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) (21). 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-[13H]palmitoylglycerol-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’-GAGTTGACATTTTAGGGTTCCG-3’), downstream primer (5’-GCCGCTGCTTATCTTAGG-3’) (25); 15-LOX-2, upstream primer (5’-TGGCTTCGCGCCTCAGCT-3’), downstream primer (5’-TGGTCTCCCTGGGATTAGTGGGA-3’) (26); and 12-LOX, upstream primer (5’-GTAAGGATGATCTACCTC-3’), downstream primer (5’-TTGGGTTTGGAGACTGGG-3’) (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 1 min. 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 [13H]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 lipoxigenase 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 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...
The sPLA$_2$-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 sPLA$_2$ activity**

The above data indicate that in common with other cell types (8–12), activation of cPLA$_2$ in U937 cells appears to facilitate the action of sPLA$_2$ on cellular membranes. To further stress this notion, $[^3]$Holeic acid release experiments were conducted. cPLA$_2$ releases little, if anything, of this fatty acid, whereas sPLA$_2$ does it readily (29, 30). Thus, determination of $[^3]$Holeic acid release allows one to separate the contribution of sPLA$_2$ to phospholipid hydrolysis from the one of cPLA$_2$ and, in turn, provides a straightforward tool to study the effect of cPLA$_2$ inhibition on sPLA$_2$ activation. Fig. 3 shows that U937 cells exposed to exogenous group V sPLA$_2$ released modest quantities of $[^3]$Holeic acid. Such a release was completely blocked by LY311727, thus confirming that it was actually due to sPLA$_2$ (not shown). When the cells were activated by Con A and then exposed to exogenous sPLA$_2$, a marked potentiation of the response occurred. Con A-activated cells released no oleic acid in the absence of exogenous sPLA$_2$, in agreement with previous data (19). Importantly, the enhanced response was blocked by treating the cells with the cPLA$_2$ inhibitor pyrrophenone (Fig. 3). The inhibitory effect of pyrrophenone could be reversed by exposing the cells to 1 $\mu$M exogenous AA for 2 min before sPLA$_2$ addition. At the concentration used, exogenous AA did not exert any effect on its own (Fig. 3). Addition of lyso-phosphatidylcholine to the cPLA$_2$-activity-deficient cells did not restore the sPLA$_2$ effect (Fig. 3). These results suggest that cPLA$_2$-derived AA, or an oxygenated metabolite, plays a role in mediating the action of sPLA$_2$ on cellular membranes.

Fig. 3A shows that exogenous sPLA$_2$ to total $[^3]$HAA mobilization in cells exposed to both Con A and exogenous sPLA$_2$ 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-HETE, the immediate product of 15-lipoxygenase action on free AA. The 15-HETE was considerably less effective than 15-HPETE in restoring the sPLA$_2$ 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 sPLA$_2$.

**FIGURE 2.** Exogenous sPLA$_2$-induced $[^3]$HAA release from U937 macrophage-like cells. The cells were untreated ( ), or treated with 100 $\mu$g/ml Con A or 100 $\mu$g/ml Con A plus 15 nM group V sPLA$_2$, as indicated ( ), for 15 min in the absence or presence of the indicated inhibitors. Pyrrophenone (Pyr) was used at 1 $\mu$M. LY311727 (LY) was used at 25 $\mu$M.

**FIGURE 3.** Arachidonic acid restores the enhancing effect of exogenous sPLA$_2$ on $[^3]$Holeic acid mobilization in activated U937 macrophage-like cells. The cells were preincubated with 1 $\mu$M pyrrophenone where indicated. Subsequently, the cells were stimulated by Con A (100 $\mu$g/ml) in the absence or presence of 15 nM exogenous group V sPLA$_2$ and in the absence or presence of 1 $\mu$M AA or 10 $\mu$M lyso-phosphatidylcholine (LysoPC), as indicated.

**FIGURE 4.** Effect of inhibitors of AA metabolism on $[^3]$HAA release in activated U937 macrophages. A, The cells were untreated ( ) or treated with 100 $\mu$g/ml Con A or 100 $\mu$g/ml Con A plus 15 nM group V sPLA$_2$, as indicated ( ), for 15 min in the absence or presence of the indicated inhibitors. All of the inhibitors were used at 25 $\mu$M. B, The cells were incubated with 25 $\mu$M ebselen where indicated. Subsequently, the cells were treated with ( ) or without ( ) 100 $\mu$g/ml Con A in the presence or absence of 15 nM sPLA$_2$, 0.5 $\mu$M 15-HPETE, 0.5 $\mu$M 15-HETE, or 0.5 $\mu$M 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 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 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 5 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.

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 the immediate response of the U937 cells if applied exogenously. This strategy is pathophysiological 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 PLAs\(_2\) (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 overexpression of 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 confirms 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 metabolism 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 elicited to these two separate responses.

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References


