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Authors
Balsinde, J
Dennis, EA

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Distinct Roles in Signal Transduction for Each of the Phospholipase A2 Enzymes Present in P388D1 Macrophages*

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Jesus Balsinde and Edward A. Dennis†‡§

From the Department of Chemistry and Biochemistry, University of California at San Diego, La Jolla, California 92039-0601

Receptor-stimulated arachidonic acid (AA) mobilization in P388D1 macrophages consists of a transient phase in which AA accumulates in the cell and a sustained phase in which AA accumulates in the incubation medium. We have shown previously that a secretory group II phospholipase A2 (sPLA2) is the enzyme responsible for most of the AA released to the incubation medium. By using selective inhibitors for each of the PLA2s present in P388D1 macrophages, we demonstrate herein that the cytosolic group IV PLA2 (cPLA2) mediates accumulation of cell-associated AA during the early steps of P388D1 cell activation. The contribution of both cPLA2 and sPLA2 to AA release can be distinguished on the basis of the different spatial and temporal characteristics of activation and substrate preferences of the two phospholipase A2s (PLA2s). Furthermore, the results suggest the possibility that a functionally active cPLA2 must be necessary for sPLA2 to act. cPLA2 action precedes that of sPLA2, and overcoming cPLA2 inhibition by artificially increasing intracellular free AA levels restores extracellular AA release. Although this suggests cross-talk between cPLA2 and sPLA2, selective inhibition of one other PLA2 present in these cells, namely the Ca2+-independent PLA2, does not block, but instead enhances receptor-coupled AA release. These data indicate that Ca2+-independent PLA2 does not mediate AA mobilization in P388D1 macrophages. Collectively, the results of this work suggest that each of the PLA2s present in P388D1 macrophages serves a distinct role in cell activation and signal transduction.

Phospholipase A2 (PLA2) enzymes play a fundamental role in numerous cellular processes by generating an array of metabolites with various biological functions. PLA2-mediated hydrolysis of glycerophospholipids results in the release of arachidonic acid (AA) and lysophospholipids, which may either exert direct effects or serve as substrates for the generation of other lipid messengers such as the eicosanoids or platelet-activating factor (PAF) (1).

Mammalian cells contain multiple PLA2 forms (1), and there is considerable interest in determining the role that each PLA2 plays in mediating cellular functions. At least three different cellular PLA2s have been proposed to play a role in the mobilization of AA from phospholipids. These are the cytosolic group IV PLA2 (cPLA2) (2–4), the secretory group II PLA2 (sPLA2) (5–7), and a cytosolic Ca2+-independent PLA2 (iPLA2) (8, 9). Involvement of one or another PLA2 form appears to depend on the cell type and agonist involved.

Our laboratory has been examining the molecular mechanisms involved in AA mobilization in murine P388D1 macrophage-like cells (6, 10–12). Stimulation of these cells with nanomolar quantities of the receptor agonist PAF results in a very modest mobilization of free AA. However, preincubation of the cells with bacterial lipopolysaccharide (LPS) prior to stimulation with PAF increases the release of AA by these cells by about 2–3-fold (10). Recently, we have demonstrated that AA mobilization in response to LPS/PAF involves participation of a sPLA2 localized at the outer surface of the cell and that this enzyme accounts for the majority of the AA released to the extracellular medium (6, 12). In the current study, we have obtained further evidence using chemical inhibitors for the role of sPLA2 and have aimed at defining the roles played by the other two PLA2 forms present in P388D1 macrophages, namely cPLA2 and iPLA2.

EXPERIMENTAL PROCEDURES

Materials—P388D1 cells were obtained from the American Type Culture Collection (Rockville, MD). LPS Re595 was the kind gift of Dr. Richard Ulevitch (Scripps Clinic and Research Foundation, La Jolla, CA). I soco’s modified Dulbecco’s medium (endotoxin <0.05 ng/ml) was from Whittaker Bioproducts (Walkersville, MD). Fetal bovine serum was from HyClone Labs. (Logan, UT). Nonessential amino acids were from Irvine Scientific (Santa Ana, CA). (5,6,8,9,11,12,14,15-3H)Arachidonic acid (specific activity, 100 Ci/mmole), (1-14C)I arachidonic acid (specific activity, 57 Ci/mmole), and [methyl-3H]choline chloride (specific activity, 79 Ci/mmole) were from New England Nuclear (Boston, MA). PAF, unlabeled fatty acids, and lysophospholipids were from Sigma. Okadaic acid was from Calbiochem (San Diego, CA) or Biomol (Plymouth Meeting, PA). The sPLA2 inhibitor 3-(3-acotamide-1-benzyl-2-thylindolyl-5-oxo)propane phosphonic acid (LY 311277) was kindly provided by Dr. Edward Mihelich (Lilly Research Laboratories, Indianapolis, IN). Methyl arachidonyl fluorophosphonate (MAFP) was from Cayman (Ann Arbor, MI). (E)-6-bromomethylene) tetrahydro-3-(1-naphthyl)-(2H)pyran-2-one (bromoaemone lactone; BEL), and 1-hexylthio-2-hexanoylamino-1,2-dideoxy-sn-glycerol-3-phosphoethanolamine (dC6SNPE) were synthesized by our laboratory by Killian Conde-Frieboes and Scott Boegeman, respectively, following previously published procedures (13, 14). Silicagel G-60 TLC plates were from Analtech (Newark, DE). Organic solvents (analytical grade) were baked (Phillipsburg, NJ) or Fisher.

Cell Culture and Staining Conditions—P388D1 cells were maintained at 37 °C in a humidified atmosphere at 90% air and 10% CO2 in
Two Different AA Pools Contribute to AA Release during Activation of P388D1 Cells—Our previous work established that AA release in LPS-primed, PAF-stimulated P388D1 cells is composed of two events: a transient phase in which AA accumulates in the cell and a sustained phase in which the fatty acid accumulates in the extracellular medium (12). We began the current study by determining whether the fatty acid liberated during these two phases arises from separate phospholipid pools. To this end, we used the methodology described by Fonteh and Chilton (17) to selectively label the AA-containing phospholipids. The cells were first labeled with \(^{3}H\)AA alone or \(^{3}H\)AA plus \(^{14}C\)AA were stimulated with 100 nM PAF for 10 min to measure extracellular AA or for 1.5 min to measure cell-associated AA. The ratio \(^{14}C\)/\(^{3}H\) of extracellular AA (E) or cell-associated AA (C) was quantitated and is shown. The \(^{14}C/^{3}H\) ratio for the major AA-containing phospholipid classes in these cells is also shown for comparison. PS, phosphatidylserine.

RESULTS

Function of Three PLA2s in Activated Macrophages

Measurement of Extracellular AA Release and Cell-associated Free AA—LPS-treated cells labeled with either \(^{3}H\)AA alone or \(^{3}H\)AA plus \(^{14}C\)AA were stimulated with 100 nM PAF for the time indicated. The supernatants were removed, cleared of detached cells by centrifugation, and assayed for radioactivity by liquid scintillation counting. For analysis of cell-associated, free AA, the cell monolayers were scraped in 1 ml of 0.5% Triton X-100. Lipids were extracted according to Bligh and Dyer (15) and separated by thin-layer chromatography using n-hexane/diethyl ether/acetic acid/water (50:40:6:0.6, v/v/v/v) as a solvent system. Authentic AA was co-chromatographed and visualized by exposing the plates to iodine vapors. Areas containing AA were scraped into scintillation vials, and the amount of radioactivity was measured by liquid scintillation counting.

Measurement of Lyso-PC Levels—For the measurement of lyso-PC, cells were labeled with 0.5 μCi/ml \(^{3}H\)choline for 3 days. The cells were activated with LPS and PAF as described above. After the indicated times, supernatants were discarded, and the cell monolayers were scraped in 1 ml of 0.5% Triton X-100. Lipids were extracted with ice-cold n-butanol and separated by thin-layer chromatography, using chloroform/methanol/acetic acid/water (50:40:6:0.6, v/v/v/v) as a solvent system (16). Spots corresponding to lyso-PC were scraped into scintillation vials, and the amount of radioactivity was measured by liquid scintillation counting.

Data Presentation—Except for the data shown in Fig. 1, agonist-stimulated AA release is expressed by subtracting the basal rate observed in the absence of agonist and inhibitor. These background values were in the range of 2000–3000 cpm for extracellular \(^{3}H\)AA and 500–1000 cpm for cell-associated free \(^{3}H\)AA. Assays were carried out in duplicate or triplicate. Each set of experiments was repeated at least three times with similar results. Unless otherwise indicated, the data presented are from representative experiments.

In contrast, the \(^{14}C/^{3}H\) ratio for cell-associated free AA in unstimulated cells was 0.7 ± 0.1, that is, slightly higher than that observed in PAF-activated cells (0.4 ± 0.1). Within error, there was no difference between the \(^{14}C/^{3}H\) ratio for cell-associated AA in unstimulated cells (1.0 ± 0.2) versus PAF-stimulated cells (0.8 ± 0.1), suggesting that intracellular resting levels of AA may also derive from all major phospholipid classes.

PLA2 Inhibition Studies—A useful approach to study the involvement of distinct PLA2s in AA mobilization is the use of selective inhibitors for each of these enzymes. Because each PLA2 group exhibits different catalytic properties and substrate preferences (1), inhibitors based on these characteristics should allow one to distinguish among the different cellular PLA2s. The PLA2 inhibitors used in this work are based on the aforementioned properties.

Using antisense RNA technology, we have previously demonstrated that group II sPLA2 is responsible for at least 60–70% of the AA released to the incubation medium but is not involved in raising cellular AA levels shortly after cell activation with PAF (12). In the current study, pharmacological inhibition of sPLA2 was accomplished by incubating the cells either with the water-soluble phospholipid analog, diC6SNPE (14), or the indole derivative LY311727, which is an indomethacin analogue (18). diC6SNPE inhibits human synovial group II PLA2 with an IC\(_{50}\) of 27 μM when assayed in a spectrophotometric assay with 2 mM substrate. At concentrations up to 100 μM, diC6SNPE has no effect on pure human group IV cPla2.

\(^{2}\) S. C. Boegeman, and E. A. Dennis, unpublished data.
nor does it affect PLA$_2$ activity from P388D$_1$ cell homogenates as measured toward 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine vesicles in the presence of Ca$^{2+}$ and β-mercaptoethanol. In addition, diC$_6$SNPE does not inhibit pure Ca$^{2+}$-independent PLA$_2$ from P388D$_1$ cells (13). The properties of the indole derivative LY311727 as a potent and selective inhibitor of sPLA$_2$ have recently been reported (18).

sPLA$_2$ inhibition by either diC$_6$SNPE or LY311727 markedly decreased the extracellular release of $[^3H]$AA from prelabeled P388D$_1$ cells (Figs. 2A and 3A). No effect of these inhibitors was detected on the accumulation of cell-associated free $[^3H]$AA (Figs. 2B and 3B). These data are fully consistent with our previous data using antisense RNA technology to block sPLA$_2$ activity (12).

Involvement of group IV cPLA$_2$ was initially investigated by using MAFP (19). This compound is an irreversible inhibitor of the cPLA$_2$ and has no effect on the sPLA$_2$ (19). We have confirmed in our laboratory these findings and in addition have found that MAFP does not appreciably affect arachidonoyl-CoA synthetase, lysophosphatidylcholine- or arachidonoyl-CoA acyltransferase, or CoA-independent transacylase activities in homogenates from MAFP-treated cells. Fig. 4 shows that MAFP strongly inhibited AA mobilization in PAF-activated cells. Whereas MAFP inhibited the extracellular release of $[^3H]$AA from prelabeled cells by about 75% (Fig. 4A), the PAF-induced accumulation of cellular $[^3H]$AA was almost completely blocked by the inhibitor (Fig. 4B).

P388D$_1$ macrophages possess a third PLA$_2$ enzyme, namely a cytosolic iPLA$_2$ that shows no preference for AA-containing phospholipids; in fact, it prefers palmitoyl over arachidonoyl residues (20). Recent evidence from our laboratory indicates that MAFP also inhibits pure iPLA$_2$ from P388D$_1$ cells. Therefore, at least part of the MAFP-sensitive AA mobilization could be mediated by the iPLA$_2$ in addition to the cPLA$_2$. The iPLA$_2$

3 J. Balsinde, I. D., Bianco, and E. A. Dennis, unpublished data.

4 Lio, Y.-C., Reynolds, L. J., Balsinde, J., and Dennis, E. A. (1996) Biochim. Biophys. Acta, in press.

5 L. J. Reynolds, and E. A. Dennis, unpublished data.
activities, measured in homogenates from BEL-treated cells: cPLA₂, sPLA₂, arachidonyl-CoA synthetase, lysophosphatidylcholine:arachidonoyl-CoA acyltransferase, and CoA-independent transacylase (22).

The effect of BEL on PAF-induced AA mobilization from LPS-primed P388D₁ cells is shown in Fig. 5. At concentrations up to 50 nM, which totally block cellular iPLA₂ (22), BEL was ineffective in inhibiting either the extracellular release of [³H]AA (Fig. 5A) or the accumulation of cellular free fatty acid (Fig. 5B). Instead, BEL enhanced both basal and PAF-stimulated [³H]AA mobilization, although the ratio of stimulated versus unstimulated release remained the same at all BEL concentrations. The enhancing effect of BEL on P388D₁ macrophage AA release is probably related to its inhibitory action on cellular fatty acid incorporation into phospholipid (22). The lack of any inhibitory effect of BEL on [³H]AA release demonstrates that the iPLA₂ does not significantly contribute to this release. Therefore, the MAFF-sensitive [³H]AA release should be ascribed to the cPLA₂.

Priming of AA Release by Okadaic Acid—It is well established that cellular cPLA₂ activity is regulated by phosphorylation (2–4). Okadaic acid, a protein phosphatase inhibitor, also induces activation of cPLA₂ by preventing dephosphorylation of the enzyme (4). Therefore, this reagent was used to further evaluate the involvement of cPLA₂ in the PAF activation. As shown in Fig. 6A, pretreatment with okadaic acid resulted in the cells becoming sensitized for an enhanced [³H]AA release in response to PAF. Importantly, when okadaic acid was added along with LPS during priming, a strong potentiation of the AA release response was observed. Okadaic acid not only increased both extracellular (Fig. 6B) and cell-associated [³H]AA (Fig. 6C), but also augmented cellular lyso-PC levels in cells pre-labeled with [³H]choline (Fig. 6D). These data stress the ability of okadaic acid to amplify LPS/PAF activation of AA mobilization, thus supporting the involvement of the phosphorylation-regulated cPLA₂ in this process. Interestingly, when the experiments depicted in Fig. 6 were carried out in the presence of the sPLA₂ inhibitor diC₆SNPE (50 μM), extracellular [³H]AA release was still inhibited by 45 ± 11% (mean ± S.E., n = 3), indicating that augmentation of cPLA₂ activity by okadaic acid does not result in a full response unless a functionally active sPLA₂ is present. diC₆SNPE did not affect cell-associated free AA levels in okadaic acid-treated cells.

cPLA₂ Activation Precedes That of sPLA₂—From our previous results using antisense RNA technology (6, 12) as well as our current data using the sPLA₂ inhibitors diC₆SNPE and LY311727, it is apparent that this enzyme accounts for a major portion of the [³H]AA released into the incubation medium after 10 min of activation with PAF. However, our data using MAFF clearly show that selective inhibition of cPLA₂ results in at least 75% inhibition of extracellular [³H]AA release (Fig. 4A). Inasmuch as MAFF does not directly inhibit sPLA₂ (see above), an explanation for these data could be that activation of cPLA₂ is necessary for sPLA₂ to act. Should this be the case, cPLA₂ activation must precede that of sPLA₂. Evidence in support of this view was obtained by investigating the time course of total [³H]AA mobilization (i.e. cell-associated plus extracellularly released [³H]AA) in cells treated with either MAFF or diC₆SNPE to selectively block cPLA₂ or sPLA₂, respectively. As shown in Fig. 7, inhibition of [³H]AA release by diC₆SNPE was only apparent after 2–3 min of cell activation, a time frame at which cell-associated AA nearly drops to levels occurring in unstimulated cells (12). On the other hand, inhibition of AA release by MAFF was already observed at the earliest point measured, i.e. 1 min (Fig. 7), demonstrating that the action of cPLA₂ on cellular phospholipids precedes that of sPLA₂.

We next explored whether the addition of metabolites result-
Regulation of AA Mobilization by Two Different PLA2 Enzymes—By using antisense RNA technology to block the expression of cPLA2, we previously demonstrated that an extracellular pool of this enzyme is responsible for at least 60–70% of the AA released from P388D1 macrophages after activation with LPS/PAF (6). By using an anti-sPLA2 monoclonal antibody, Pfelschifter et al. (7) have estimated a similar contribution of sPLA2 to extracellular AA release during receptor stimulation of rat mesangial cells. In the current study, we have utilized a third and different strategy to block sPLA2 activity, i.e. the use of diC6SNPE and LY311727, two selective and structurally unrelated sPLA2 inhibitors, and have confirmed that this enzyme mediates a major portion of the AA released to the incubation medium. Although these three different strategies emphasize the very important role of sPLA2 in AA release, they also stress that another effector enzyme is involved as well. Moreover, both antisense inhibition of sPLA2 and pharmacological inhibition of the enzyme by diC6SNPE and LY311727 have highlighted the fact that sPLA2 is not the enzyme that mediates the small burst of cell-associated free AA that occurs shortly after PAF stimulation. Use of multiple selective inhibitors in this study has provided evidence that the second effector enzyme in AA release in PAF-stimulated LPS-primed P388D1 cells is the cPLA2. Thus, the current work, along with our previous data (6, 12), establishes that cPLA2, acting intracellularly, and sPLA2, acting on the outer surface of the cell, both mediate AA release in response to PAF receptor stimulation. It is important to note that inhibition of sPLA2 by either antisense techniques (6, 12) or chemical inhibitors (this study) does not result in complete inhibition of AA release to the extracellular medium. This suggests that under PAF activation conditions, a portion of the AA released intracellularly by the cPLA2 may exit the cell and mix with the fatty acid liberated by the sPLA2.

The notion that both cPLA2 and sPLA2s mediate receptor activation of AA release may represent a signaling mechanism common to agonists that elicit short-term (i.e. PAF) or long-term responses (i.e. cytokines and growth factors). Work by Slankwich et al. (23, 24) in cytokine-stimulated rat mesangial cells and by Murakami et al. (5) in cytokine-stimulated human endothelial cells has also suggested that both PLA2s may participate in regulating AA mobilization in these cells, their relative contribution being dependent on the agonist used. However, there are other cell systems such as platelets, in which a role for sPLA2 in AA release could not be demonstrated (25). Moreover, AA release in thrombin-stimulated platelets could be completely blocked by inhibiting the cPLA2, suggesting that in this system, cPLA2 is perhaps the only effector involved in AA release (25).

Domin and Rozengurt (26) have recently demonstrated that AA mobilization in Swiss 3T3 cells treated with platelet-derived growth factor follows a bimodal kinetics. In this system, cPLA2 activation appears to be responsible for the small burst of AA mobilization that occurs during the first 20 min following agonist stimulation. However, the major component of agonist-induced AA release was found to be due to another unidentified effector. Because these data are very reminiscent of the situation in PAF-stimulated LPS-primed P388D1 cells, it is tempting to speculate that the second effector involved in the system studied by Domin and Rozengurt (26) is the sPLA2. Although it is certain that the time course of the AA release responses in
platelet-derived growth factor-stimulated Swiss 3T3 cells and in PAF-stimulated P388D1 cells are clearly distinct, this is most likely due to cell type differences and especially to the very distinct nature of the agonists employed. As a matter of fact, when 3T3 cells are challenged with a short burst agonist such as bombesin, a rapid and transient increase in cell-associated free AA is observed shortly after receptor occupancy (27).

Different Phospholipid Sources for the Two PLA2s—Given the fact that cPLA2 and sPLA2 mobilize AA from activated P388D1 cells with different spatial and temporal characteristics, it would seem possible that the two enzymes utilize different AA pools. We explored this issue by selectively labeling the different AA-containing phospholipids with [3H]AA and [14C]AA. In P388D1 cells, two of the major AA-containing phospholipids, namely PC and PI, are labeled with exogenous radioactive AA very rapidly (within minutes), whereas PE is labeled more slowly (12). This phenomenon, along with the nonuniform distribution of arachidonoyl moieties in different phospholipid classes, allowed us to label the phospholipids with AA to different [14C]/[3H] ratios. Calculation of the [14C]/[3H] ratio for the AA released by PAF at two different locations, i.e. cell-associated and outside the cell, gives two very different values. This result strongly suggests that cell-associated free AA and extracellular free AA arise from different pools.

By comparing the [14C]/[3H] ratios in free AA with those in the phospholipids, we could delineate the origin of cell-associated AA and extracellular AA. Although the interpretation of these data may be complicated by the phenomenon of mixing AA pools as well as the molecular heterogeneity of each phospholipid class, some definite conclusions can be reached. The fact that the [14C]/[3H] ratio for extracellular AA is considerably lower than that of cell-associated AA suggests that most of the extracellular free AA arises from PE, but this is not the case for cell-associated AA. As a matter of fact, PE is the only phospholipid whose [14C]/[3H] ratio is comparable with that of extracellular AA. Following a similar rationale, it can be concluded that all major phospholipids contribute to the early burst in cell-associated AA, although their relative contribution cannot be estimated from our data. Because cPLA2 is responsible for raising the levels of cell-associated free AA, involvement of all major phospholipid classes in this process is consistent with the notion that this enzyme does not distinguish among phospholipid head groups (28, 29).

It is generally assumed that the phospholipids are asymmetrically distributed in cellular membranes, PC being localized primarily at the outer leaflet and PE at the inner leaflet of the plasma membrane (30). Thus the notion that the extracellular AA release arises primarily from PE would seem, at a first glance, unexpected. However, sPLA2, the enzyme primarily responsible for mobilizing AA to the extracellular medium, has been reported to prefer PE over any other phospholipid when these are presented in a natural membrane system (29, 31). It is possible that the sPLA2 preference for PE in these studies could be caused by a higher proportion of PE relative to other phospholipids in these membranes, because studies with vesicles containing various kinds of phospholipids have failed to detect any head group specificity (32). However, studies in platelets have demonstrated that during activation, a rapid translocation of AA-containing PE from the inner to the outer leaflet of the plasma membrane may occur. Such a translocation would permit the AA-containing PE to be readily accessible to the extracellular sPLA2. Interestingly, recent work by Fourcade et al. (34) has suggested that loss of membrane asymmetry resulting from movement of phospholipids from the inner to the outer leaflet of the membrane may play a key role in regulating the activity of extracellular sPLA2.

Cross-talk between the PLA2s—The results of this study raise the possibility that cross-talk may exist between the mechanisms of activation of cPLA2 and sPLA2. We have found that cPLA2 becomes activated before sPLA2 begins to act and
that inhibition of cPLA2 by MAFP leads to a very marked inhibition of total AA release induced by PAF, higher than is expected if one considers that sPLA2 is responsible for at least two-thirds of the AA released to the extracellular medium (6, 12). Thus the question arises as to whether cPLA2-mediated events are required for the action of sPLA2. We explored this issue by directly adding the PLA2 by-products, namely free fatty acids and lysophospholipids, shortly before agonist addition to cells in which cPLA2 had been inactivated by MAFP. Exogenous AA, but not other fatty acids or lysophospholipids, was able to restore the extracellular [3H]AA release in response to PAF. Treating the cells with exogenous AA has the effect of increasing cell-associated free fatty acid levels well above those found in untreated cells, thereby mimicking cPLA2 activation. No effect of was seen when exogenous AA was added in the absence of PAF or when a sPLA2 inhibitor was used, suggesting that the effect may be specific.

A perturbation of the lipid bilayer or “membrane rearrangement,” initiated by an agonist/receptor interaction, appears to be required to activate sPLA2 at the outer surface of the cell (35). The data reported herein suggest that, in addition to phospholipid translocation (34), such a membrane rearrangement may involve a transient elevation of free AA mediated by receptor-activated cPLA2. Thus, our results suggest to appear as a new role for free AA in cellular signaling, i.e. to help regulate the accessibility of sPLA2 to its substrate in the membrane.

However, such an intracellular elevation of free AA is not itself sufficient to elicit the cellular response. Other additional signals that occur at the earliest stages of PAF activation, such as inositol phospholipid turnover, Ca2+ mobilization, or protein phosphorylation (11) are also required for the AA release process to fully take place. When all of these signals are induced sufficiently, sPLA2 begins to hydrolyze phospholipids at the outer surface of the cell, and this results in full AA mobilization. According to this model, augmentation of any of these early signals could result in an increased liberation of AA to the extracellular medium. This is what occurs in the experiments using okadaic acid, wherein augmentation of cPLA2 activity and hence cell-associated free AA levels dramatically enhance the extracellular AA release, provided sPLA2 is functional.

We should emphasize that the above model of cross-talk between cPLA2 and sPLA2 has to be regarded as a working model and not as an established one. Much of our evidence in favor of a causal relationship between cPLA2 and sPLA2 rests on the use of the phosphorylfluoride MAFP, a highly reactive compound. It cannot be ruled out at this time that MAFP is exerting some other undesired effects or that the sPLA2-activating effect of exogenous AA is unrelated to cPLA2.

iPLA2 Role in AA Mobilization—Another striking feature of the current work is the role of the one other PLA2 present in P388D1 cells, i.e. the iPLA2, during PAF activation. We have investigated this issue by conducting studies with BEL, a selective inhibitor of the iPLA2. Our results clearly show that BEL does not inhibit AA release, ruling out a significant role for the iPLA2 in this release. In a previous report, we demonstrated that the steady-state level of lysophospholipids in BEL-treated cells is decreased by about two-thirds and that this effect directly correlates with the inhibition of AA esterification into phospholipids as well as the inhibition of cellular iPLA2 activity (22). Moreover, BEL does not have any effect on the AA reacylating enzymes arachidonoyl-CoA synthetase and lysophospholipid:arachidonoyl-CoA acyltransferase (22). Based on these previous data, our current finding that the unstimulated levels of [3H]AA are increased in media from BEL-treated cells may be explained as the consequence of the diminished capacity of these cells to reacylate AA into membrane phospholipids. Thus, the basal level of AA, being produced by constitutively active enzymes different from the iPLA2, increases because the iPLA2 is blocked, thereby lowering the availability of acceptor.

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REFERENCES

1. Dennis, E. A. (1994) J. Biol. Chem. 269, 13057–13060
2. Lin, L. L., Wartmann, M., Lin, A. Y., Kopf, J., L., Seth, A., and Davis, R. J. (1993) Cell 72, 269–276.
3. Kramer, R. M., Roberts, E. F., Manetta, J. V., Hyslop, P. A., and Jakubowski, J. A. (1993) J. Biol. Chem. 268, 26796–26804.
4. Oiu, Z. H., de Carvalho, M. S., and Leslie, C. C. (1993) J. Biol. Chem. 268, 24506–24513.
5. Murakami, M., Kudo, I., and Inoue, K. (1993) J. Biol. Chem. 268, 839–844.
6. Barbour, S. E., and Dennis, E. A. (1993) J. Biol. Chem. 268, 21875–21882.
7. Pfleischler, J., Schalkwijk, C., Briner, V. A., and van den Bosch, H. (1993) J. Clin. Invest. 92, 2516–2523.
8. Lehman, J. L., Brown, K. A., Ramanadham, S., and Gross, R. W. (1993) J. Biol. Chem. 268, 20713–20716.
9. Ramanadham, S., Gross, R. W., Han, X., and Turk, J. (1993) Biochemistry 32, 337–346.
10. Glaser, K. B., Asnis, R., and Dennis, E. A. (1990) J. Biol. Chem. 265, 8658–8664.
11. Asnis, R., Randriamampita, C., Tsien, R. Y., and Dennis, E. A. (1994) Biochemistry 33, 543–551.
12. Balsinde, J., Barbour, S. E., Bianco, I. D., and Dennis, E. A. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 11060–11064.
13. Ackermann, E. J., Conde-Friebes, K., and Dennis, E. A. (1995) J. Biol. Chem. 270, 445–450.
14. Plesniak, L. A., Boegeman, S. C., Segelke, B. L., and Dennis, E. A. (1993) Biochemistry 32, 5009–5015.
15. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
16. Fernández, B., Baiboia, M. A., Solís-Herruzo, J. A., and Balsinde, J. (1994) J. Biol. Chem. 269, 26711–26716.
17. Fonteh, A. N., and Chilton, N. H. (1993) J. Immunol. 150, 560–570.
18. Schalkwijk, C. G., de Vet, E., Pfeilschifter, J., and van den Bosch, H. (1992) Nat. Struct. Biol. 2, 458–464.
19. Huang, Z., Liu, S., Street, I., Laliberte, F., Abdullah, K., Desmarais, S., Wang, Z., Kennedy, B., Payette, F., Riendeau, D., Weich, P., and Gresser, M. (1994) J. Med. Chem. 37, 307–308.
20. Ackermann, E. J., Kempter, E. S., and Dennis, E. A. (1994) J. Biol. Chem. 269, 9227–9233.
21. Hazen, S. L., Zupan, L. A., Weiss, R. H., Getman, D. P., and Gross, R. W. (1991) J. Biol. Chem. 266, 7227–7232.
22. Balsinde, J., Bianco, I. D., Ackermann, E. J., Conde-Friebes, K., and Dennis, E. A. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 8527–8531.
23. Schalkwijk, C. G., Pfleischler, J., Marki, F., and van den Bosch, H. (1992) J. Biol. Chem. 267, 8846–8851.
24. Schalkwijk, C. G., de Vet, E., Pfleischler, J., and van den Bosch, H. (1992) J. Biol. Chem. 267, 26864–26867.
25. Bartoli, F., Lin, H. K., Gomashachi, F., Gelb, M. H., Jain, M. K., and Aptiz, et al.
26. Domin, J., and Rozengurt, E. (1993) J. Biol. Chem. 268, 8927–8934
27. Currie, S., Smith, G. L., Crichton, C. A., Jackson, C. G., Hallam, C., and Wakeram, M. J. O. (1992) J. Biol. Chem. 267, 6056–6062
28. Hanel, A. M., Schuttel, S., and Gelb, M. H. (1993) Biochemistry 32, 5949–5958
29. Diez, E., Chilton, F. H., Stroup, G., Mayer, R. J., Winkler, J. D., and Fonteh, A. N. (1994) Biochem. J. 301, 721–726
30. Schrott, A. J., and Zwaal, R. F. A. (1991) Biochim. Biophys. Acta 1071, 313–329
31. Fonteh, A. N., Bass, D. A., Marshall, L. A., Seeds, M., Samet, J. M., and Chilton, F. H. (1994) J. Immunol. 152, 5438–5446
32. Bayburt, T., Yu, B. Z., Lin, H. K., Browning, J., Jain, M. K., and Gelb, M. H. (1993) Biochemistry 32, 573–582
33. Joo, F., Chevy, F., Colard, O., and Wolf, C. (1993) Biochim. Biophys. Acta 1149, 231–240
34. Fourcade, O., Simon, M. F., Viodé, C., Rugani, N., Leballe, F., Ragab, A., Fournié, B., Sarda, L., and Chap, H. (1995) Cell 80, 919–927
35. Kudo, I., Murakami, M., Hara, S., and Indou, K. (1993) Biochim. Biophys. Acta 1170, 217–231
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