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Secretory Phospholipase A_2 Induces Phospholipase Cγ-1 Activation and Ca^{2+} Mobilization in the Human Astrocytoma Cell Line 1321N1 by a Mechanism Independent of Its Catalytic Activity

Marita Hernández,[1] Maria José Barrero,[1] Javier Alvarez, Mayte Montero, Mariano Sánchez Crespo,[2] and María Luisa Nieto

Instituto de Biología y Genética Molecular, Facultad de Medicina, Consejo Superior de Investigaciones Científicas and Universidad de Valladolid, 47005-Valladolid, Spain

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The effect of secretory phospholipase A_2 ($sPLA_2$) on intracellular Ca^{2+} signaling in human astrocytoma cells was studied. $sPLA_2$ increased cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_c$) in both Ca^{2+}-containing and Ca^{2+}-free medium, thus suggesting Ca^{2+} release from intracellular stores. The activation by $sPLA_2$ of arachidonate release via cytosolic PLA_2 ($cPLA_2$) was also independent of extracellular Ca^{2+}. As $sPLA_2$ requires Ca^{2+} for activity, these results indicate that both Ca^{2+} mobilization and $cPLA_2$ activation induced by $sPLA_2$ are unrelated to phospholipase activity but dependent on signaling mechanisms. The $sPLA_2$-induced $[Ca^{2+}]_c$ peak was sensitive to *Bordetella pertussis* toxin and inhibited by caffeine, suggesting its mediation by inositol 1,4,5-trisphosphate (IP_3). $sPLA_2$ induced tyrosine phosphorylation and membrane targeting of phospholipase Cγ-1 (PLCγ-1). Moreover, the Ca^{2+} peak was sensitive to protein tyrosine kinase inhibitors. $sPLA_2$ activates two signaling pathways: one leading to the activation of the MAP kinase/$cPLA_2$ cascade and another leading to PLCγ activation and Ca^{2+} release. © 1999 Academic Press

Type IIA secretory phospholipase A_2 ($sPLA_2$) is induced in a variety of immunoinflammatory processes as an acute phase protein (1–5). The role of this protein in these processes is not clear, and there is not conclusive evidence for its direct participation as a phospholipase in the release of bioactive lipids such as arachidonic acid (AA), platelet-activating factor and lysophosphatidic acid (LPA). It has recently been shown that $sPLA_2$ activates intracellular signaling pathways involving mitogen-activated protein (MAP) kinase and cytosolic phospholipase A_2 ($cPLA_2$) (6, 7), thus leading to the release of arachidonate and mitogenesis. Some of these effects have been attributed to the generation of lysophospholipids by $sPLA_2$ (8), whereas on the other hand, the reported existence of plasma membrane receptors for $sPLA_2$ (9–11), might explain the intracellular effects of these proteins via intracellular signaling pathways. In this connection, the activation by $sPLA_2$ of MAP kinases and $cPLA_2$ in the human astrocytoma cell line 1321N1, has been related to the engagement of a plasma membrane binding structure (7). Taken together these data indicate that $sPLA_2$ has a double role in the inflammatory processes: (i) a defensive/digestive function linked to the phospholipase activity, requiring millimolar Ca^{2+}, and mainly exerted on prokaryotic cells, e.g., bacteria (12), and (ii) a cell signaling function exerted through the binding to specific plasma membrane receptors on eukaryotic cells.

To obtain further insight into the aforementioned signaling effects, we enlarged the study of $sPLA_2$ signaling effects by addressing its effects on Ca^{2+} mobilization, which constitutes a prototypical mechanism of cell activation triggered by both agonists that engage G-protein coupled receptors and receptors possessing intrinsic protein tyrosine-kinase activity. The role of intracellular Ca^{2+} signaling in $sPLA_2$-induced cell activation has deserved little attention. In fact, Polgar *et al.* (13) have described the induction of a Ca^{2+} transient by $sPLA_2$ in human platelets, but this has been

[1] The first two authors contributed equally to this work.

[2] To whom correspondence should be addressed. Fax: 34-983-423588. E-mail: mscres@ibgm.uva.es.

Abbreviations used: PLA_2, phospholipase A_2; $sPLA_2$, secretory PLA_2; $cPLA_2$, cytosolic PLA_2; $pPLA_2$, pancreatic PLA_2; $[Ca^{2+}]c$, cytosolic $[Ca^{2+}]$; IP_3, inositol 1,4,5-trisphosphate; PIP_2, phosphatidylinositol bisphosphate; PLCγ, phospholipase Cγ; AA, arachidonic acid; EGTA, ethylenglycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; BSA, bovine serum albumin; mannose-BSA, *p*-aminophenyl-α-D-mannopyranoside–BSA; LPA, lysophosphatidic acid; MAP kinase, mitogen-activated protein kinase; PTX, *Bordetella pertussis* toxin.

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related to the generation of the lipid agonist thromboxane A_2 as a consequence of the catalytic effect of the enzyme on the plasma membrane phospholipids. We have investigated here the effects of $sPLA_2$ on intracellular Ca^{2+} signaling in the human astrocytoma cell line 1321N1. This cell line has been shown to release Ca^{2+} from intracellular stores via IP_3 formation when stimulated with agonists such as carbachol (14) or thrombin (15), which bind to plasma membrane receptors coupled to PTX-insensitive G-proteins (16–18). Our results suggest that $sPLA_2$, as well as pancreatic PLA_2 ($pPLA_2$), activate Ca^{2+}-release from intracellular stores by acting on a plasma membrane binding site coupled to phospholipase Cγ-1 (PLCγ-1), thus leading to the activation of this enzyme and to the formation of IP_3. In contrast to $sPLA_2$-induced $cPLA_2$ and MAP kinase activation (7), Ca^{2+}-release induced by $sPLA_2$ was sensitive to PTX.

MATERIALS AND METHODS

Reagents. $sPLA_2$ was purified from plasma of patients with septicemia as previously described (12).This yielded a single protein on SDS/PAGE with the N-terminal amino acid sequence of type-IIA human PLA_2. [^{3}H]Arachidonic acid (100 Ci/mmol) was from Amersham International, Bucks, UK. Porcine pancreatic PLA_2 was from Sigma Chemical Co., Saint Louis, MO. Purity of the enzyme was confirmed by SDS/PAGE and Coomassie Blue staining, which disclosed a single protein band in the area of the 14 kDa molecular mass marker. Rabbit polyclonal anti-$cPLA_2$ antibody was from Santa Cruz Biotechnology Inc., Santa Cruz CA (sc-454). Monoclonal anti-phospholipase Cγ-1 (PowerClonal) and monoclonal anti-phosphotyrosine antibody clone 4G10 were from Upstate Biotechnology, Lake Placid, NY.

Cell culture and metabolic labeling of 1321N1 cells. Cells were cultured in DMEM containing 5% fetal calf serum at 37°C in an atmosphere containing 5% CO_2. Labeling with [^{3}H]AA was performed in cells that had been deprived of fetal calf serum for 16 h to render them quiescent. Labeling with [^{3}H]AA was carried out for 2 h in the presence of 0.3 μCi [^{3}H]AA/ml. After labeling, cells were washed at 37°C for four to five times with medium, and finally allowed to equilibrate at 37°C before addition of agonists or vehicle solution. The release of labeled [^{3}H]AA was assessed in the culture medium.

Immunoblot of $cPLA_2$. Cell lysates from preconfluent 1321N1 cells were loaded into a 10% SDS/PAGE, and transferred to polyvinyldifluoride membrane (Immobilon P, Millipore Corp., Bedford, MA) using a liquid transfer module. The membranes were blocked with dry milk for 2 h, washed with Tris-buffered saline and used for immunoblot using a rabbit polyclonal anti-$cPLA_2$ antibody. This was followed by incubation with sheep anti-rabbit IgG-horseradish peroxidase conjugated antibody, and detection with the Amersham ECL system.

Assay of PLCγ-1 phosphorylation and membrane targeting. For detection of tyrosine phosphorylation of PLCγ-1 cell lysates were subjected to immunoprecipitation using anti-phosphotyrosine antibody. The immune complex was recovered using GammaBind G-Sepharose. After washing three times with Nonidet-P-40-buffer and twice with LiCl buffer, the beads were resuspended in Laemmli sample buffer and subjected to SDS/PAGE. The extent of tyrosine phosphorylation of PLCγ-1 was determined by immunoblot with anti-PLCγ-1 monoclonal antibody. Targeting of PLCγ-1 to the cell membrane was assessed by separate processing of membrane fraction and cytosol obtained by centrifugation at 105,000*g* in an Optima TL ultracentrifuge (Beckman, Palo Alto, CA) using a TLA 100.2 rotor.

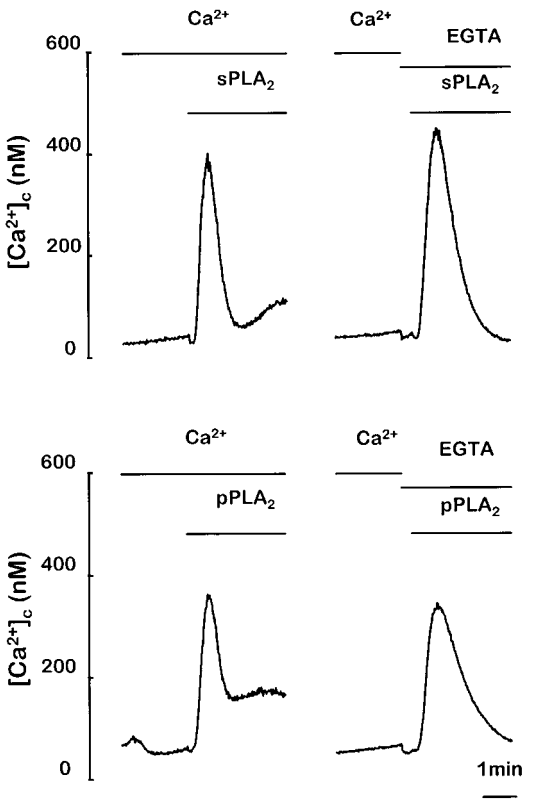

FIG. 1. Effect of the extracellular [Ca^{2+}] on the $sPLA_2$ and $pPLA_2$-induced [Ca^{2+}]$_c$ peak. 1321N1 cells were loaded with fura-2, placed in the spectrophotometer cuvette and perfused with medium containing either 1 mM Ca^{2+} or 0.5 mM EGTA, as indicated. 0.1 μg/ml $sPLA_2$ and 0.8 μg/ml $pPLA_2$ were added when indicated in the figure.

Measurement of [Ca^{2+}]$_c$. The measurements were performed as described previously (19). Briefly, cells were grown in glass coverslips for 3 days in culture medium and then deprived of fetal calf serum. The cell-coated coverslips were loaded with fura-2 by incubation for 1 h at room temperature with 4 μM fura-2/AM (Molecular Probes, Eugene, Oregon). Glass coverslips were then placed at an angle of 45° in the thermostatized sample compartment of a Cairn Research Spectrophotometer, that allowed rapid (30–300 Hz) alternation of up to six different excitation wavelengths. [Ca^{2+}]c values were calculated at 1-s periods from the ratio of the fluorescences excited at 340 and 380 nm, and emitted above 520 nm (20). The effect of different stimuli was tested by perfusing new media containing the effectors.

RESULTS

$sPLA_2$ and $pPLA_2$ elicit Ca^{2+} mobilization in 1321N1 astrocytoma cells. Figure 1 shows that addition of either $sPLA_2$ or $pPLA_2$ to 1321N1 astrocytoma cells produced a rapid and transient peak of [Ca^{2+}]$_c$. The increase in [Ca^{2+}]$_c$ took place immediately after the addition of the phospholipase, lasted for about 1 min, and then [Ca^{2+}]$_c$ rapidly returned to resting values, even in the continuous presence of the PLA_2. This [Ca^{2+}]$_c$ peak induced by both $sPLA_2$ and $pPLA_2$ was not modified when extracellular Ca^{2+} was substituted by

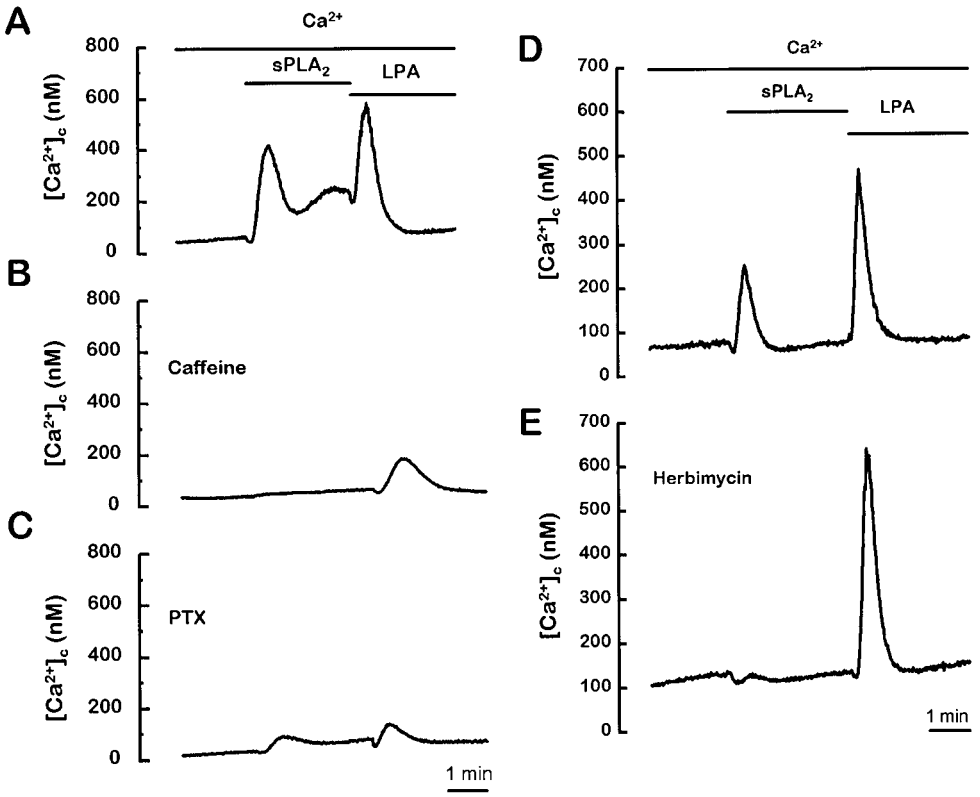

FIG. 2. Effect of caffeine, PTX and herbimycin A on the $sPLA_2$ and LPA-induced $[Ca^{2+}]_c$ signal. 1321N1 cells were placed in medium containing 1 mM Ca^{2+} and perfused with either 0.1 μg/ml $sPLA_2$ or 2 μM LPA, as indicated. In the experiment shown in B, 10 mM caffeine was also present along the experiment. In the experiment shown in C, cells were incubated overnight with PTX (500 ng/ml). The effects of herbimycin on the $sPLA_2$- and LPA-induced $[Ca^{2+}]_c$ peaks are shown in D and E. Herbimycin A was used at the dose of 5 μM for 4 h at 37°C prior to stimulation with $sPLA_2$.

EGTA. As shown in Fig. 1, the $[Ca^{2+}]_c$ peaks obtained under these conditions were identical in both height and duration to those obtained in normal Ca^{2+}-containing medium. This result shows unequivocally that the $[Ca^{2+}]_c$ peak is due to Ca^{2+}-release from intracellular stores and indicates that this effect is unrelated to the phospholipase activity of both $sPLA_2$ and $pPLA_2$, which requires millimolar $[Ca^{2+}]$ (21). As to the signaling pathway leading to Ca^{2+}-release from intracellular stores by $sPLA_2$, the most probable one is the inositol phosphate cascade, in view of its widespread involvement in Ca^{2+} mobilization and its reported coupling to a series of agonist receptors in 1321N1 cells. Figure 2 shows the effect of pharmacological treatments with both caffeine and PTX on the $[Ca^{2+}]_c$ peak elicited by $sPLA_2$ and LPA. The effects of LPA were also tested in every case, because this phospholipid activates MAP kinase and $cPLA_2$ (7), it is known to act in many cell types on specific receptors coupled to phospholipase C activation and IP_3 production (revised in Ref. 22), and it has been proposed as an effector of $sPLA_2$ effect on human platelets (8). Figure 2A shows the $[Ca^{2+}]_c$ peaks induced by consecutive additions of $sPLA_2$ and LPA to 1321N1 cells. Figure 2B shows that addition of caffeine did not produce any effect by itself, thus suggesting that these cells lack caffeine-sensitive ryanodine receptors, but inhibited the $[Ca^{2+}]_c$ peaks induced by both agents. Caffeine is known to inhibit IP_3-induced Ca^{2+}-release (23), and therefore the results shown in Fig. 2B are consistent with the hypothesis that Ca^{2+}-release induced by both $sPLA_2$ and LPA is mediated by IP_3. Figure 2C shows that the $[Ca^{2+}]_c$ peaks induced by both $sPLA_2$ and LPA were sensitive to PTX, suggesting the involvement of a PTX-sensitive G-protein in the pathway leading from the engagement of the plasma binding structure to IP_3 production. The same results were obtained using $pPLA_2$ (data not shown). The sensitivity to PTX suggests that this signaling pathway activated by $sPLA_2$ is different from that recently described leading to activation of MAP kinase and $cPLA_2$ (6, 7), which is insensitive to PTX. To investigate the possible relationship between both pathways, we studied first the Ca^{2+}-dependence of the activation by $sPLA_2$ of $cPLA_2$ by measuring the release of $[^3H]$AA induced by $sPLA_2$ both in the presence and in the absence of extracellular Ca^{2+}. The results showed that there was no significant difference in the amount of the $[^3H]$AA released under both conditions (248% of

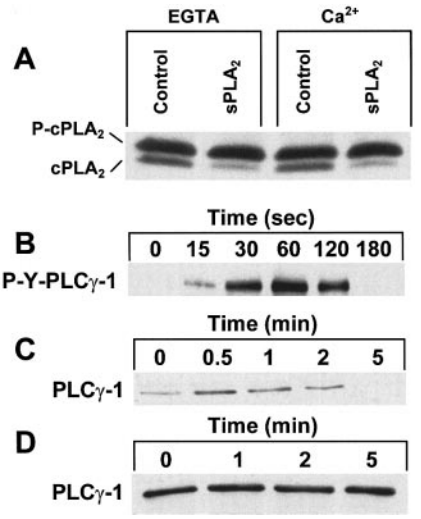

FIG. 3. Effect of $sPLA_2$ on $cPLA_2$ phosphorylation and tyrosine phosphorylation of phospholipase Cγ-1. 1321N1 cells were incubated with 0.1 μg/ml $sPLA_2$ for 15 min in the standard medium containing 1.8 mM $CaCl_2$ or in a medium supplemented with 5 mM EGTA (EGTA). The cell lysate was used to assess the band shift of $cPLA_2$ (A), or used for immunoprecipitation with anti-phosphotyrosine monoclonal antibody, SDS/PAGE separation of the immunoprecipitate, and blotting with anti-phospholipase Cγ-1 antibody (B). In an independent experiment, the cell lysate was used for ultracentrifugation at 105,000*g* and separate processing of the membrane (C) and cytosol (D) fractions, SDS/PAGE and immunoblotting with anti-phospholipase Cγ-1 antibody. P, phosphorylated; P-Y, phosphotyrosine.

control in the presence of Ca^{2+} compared with 236% in the presence of 5 mM EGTA). Moreover, $sPLA_2$ induced a similar release of [^{3}H]AA (213% of control) in cells depleted of Ca^{2+} by prolonged incubation in Ca^{2+}-free medium and in the presence of 200 nM thapsigargin, a potent inhibitor of the endoplasmic reticulum Ca^{2+} pump. Thus suggesting that activation of $cPLA_2$ by extracellular $sPLA_2$ does not require an increase in $[Ca^{2+}]_c$. In keeping with these findings, the decrease in electrophoretic mobility of $cPLA_2$ (band-shift), which is produced as a consequence of its phosphorylation on Ser-505 (24), was observed both in the presence and absence of Ca^{2+} in the extracellular medium (Fig. 3A).

Phospholipase Cγ-1 is phosphorylated in tyrosine in response to $sPLA_2$. The question then arises as to the characterization of both the phospholipase C isozyme involved in the hydrolysis of phosphatidylinositol bisphosphate (PIP_2) and the mechanism of activation triggered by $sPLA_2$. Since PLCγ is activated by protein tyrosine phosphorylation and then targeted to the plasma membrane, we studied the effect of $sPLA_2$ on the phosphorylation of PLCγ. Figure 3B shows that PLCγ-1 is rapidly phosphorylated in tyrosine after $sPLA_2$ binding, with a time course consistent with the rapid activation of Ca^{2+}-release observed, since the phosphorylated PLCγ-1 was already detected 15 s after $sPLA_2$ addition and completely disappeared three min afterwards. In addition, incubation of 1321N1 cells with $sPLA_2$ produced a detectable targeting of PLCγ-1 to the plasma membrane (Fig. 3C) even though the enzyme has a preferential location to the cytosol (Fig. 3D), thus suggesting anchoring of the enzyme to putative signaling complexes adjacent to the membrane. A functional connection between the phosphorylation of PLCγ-1 and Ca^{2+} mobilization induced by $sPLA_2$ was disclosed in experiments carried out in the presence of the tyrosine kinase inhibitor herbimycin A (Fig. 2E), which abrogated the Ca^{2+} mobilization elicited by $sPLA_2$, whereas it did not modify the effect of LPA, pointing again to the existence of distinct mechanisms in the response to both agonists.

DISCUSSION

We report here that $sPLA_2$ triggers both activation of the inositol phosphate cascade and release of Ca^{2+} from intracellular stores in 1321N1 astrocytoma cells. This effect cannot be attributed to its phospholipase A_2 activity and the ensuing release of bioactive lipids from membrane phospholipids, since $sPLA_2$ catalytic activity requires the presence of $[Ca^{2+}]$ in the millimolar range and $sPLA_2$ produces the same effects on intracellular Ca^{2+} even in the complete absence of extracellular Ca^{2+}. The effects of $sPLA_2$ on $[Ca^{2+}]_c$ in 1321N1 cells differ from those recently shown in platelets (13). In that case, $sPLA_2$ required the presence of millimolar $[Ca^{2+}]$, and was proposed to act through the generation of a lipid platelet agonist(s) from plasma membrane phospholipids. In 1321N1 cells our findings rather suggest an effect of $sPLA_2$ on intracellular $[Ca^{2+}]$ mediated by direct triggering of a plasma membrane signaling structure, e.g., a receptor, as previously suggested for the activation of the MAP kinase/$cPLA_2$ cascade (7). It could be argued, however, that the effect of $sPLA_2$ on intracellular Ca^{2+} observed in this study could be due to the generation of intracellular lipidic messengers after the $sPLA_2$-induced activation of $cPLA_2$ and the subsequent release of either AA (13) or lysophosphatidylcholine (6). However, significant activation of $cPLA_2$ by $sPLA_2$ requires at least 5 min (7), while the $sPLA_2$-induced $[Ca^{2+}]_c$ peak occurs with no measurable delay. Moreover, analysis of the AA metabolites produced by 1321N1 cells only shows significant production of eicosanoids after prolonged incubation with agonists able to induce cyclooxygenase-2 expression (Hernández, Bayón, Sánchez Crespo, and Nieto, unpublished work). Therefore, the most likely interpretation of the $sPLA_2$ effect on Ca^{2+} signaling should be the direct activation by this protein of a plasma membrane receptor coupled somehow to Ca^{2+}-release from intracellular stores.

The inhibition by caffeine of $sPLA_2$-induced Ca^{2+}-release is consistent with the involvement of IP_3 in the mechanism of Ca^{2+} mobilization. Interestingly, several evidences suggest that this signaling pathway operates

simultaneously, but with no significant cross-talks, with the pathway leading to the activation of MAP kinase and $cPLA_2$ (7). First, the activation of Ca^{2+}-release takes place via a PTX-sensitive G protein, while the other pathway is insensitive to PTX. Second, both the activation of $cPLA_2$ and the release of AA takes place even in Ca^{2+}-depleted cells, where no $[Ca^{2+}]_c$ increase can occur. Third, Ca^{2+}-release is an early event that takes place just a few seconds after $sPLA_2$ binding, while significant activation of $cPLA_2$ or MAP kinase requires 5 min (7). Therefore, although we cannot exclude the possibility that an interaction between both pathways could take place at a later step during the physiological cell activation, we can conclude that: i) activation of MAP kinase and $cPLA_2$ does not require an increase in $[Ca^{2+}]_c$. A similar dissociation between AA release and Ca^{2+} mobilization has been described previously in this cell line in response to the cholinergic agonist carbachol (25); and ii) the activation of the inositol phosphate cascade by $sPLA_2$ does not require previous activation of $cPLA_2$ or the MAP kinase pathway.

Since our data indicate that Ca^{2+}-release depends on IP_3, this points to the involvement of PLC. PLC activation by receptors can be achieved either by allosteric activation of PLCβ isoforms or by tyrosine phosphorylation of PLCγ isoforms. Because the calcium signal is coupled to G_i proteins, it would be assumed that the receptor signals through PLCβ. However, we have found that the Ca^{2+} signal on astrocytoma cells also depends on tyrosine phosphorylation, since herbimycin pretreatment blocks the calcium response. Furthermore, $sPLA_2$ induces a transient tyrosine phosphorylation of PLCγ-1 and its translocation to the plasma membrane.

Engagement of PLCγ isoforms has recently been associated to G-protein coupled receptors, e.g., bradykinin B2 receptor in vascular endothelial cells (26), and it has also been shown a functional linkage between PTX sensitive-G proteins and PLCγ in the mediation of Ca^{2+} mobilization. Thus, activation of PLCγ-1 by epidermal growth factor has been reported to require both phosphorylation and a PTX-sensitive G protein (27). In this way, it has been demonstrated that tyrosine phosphorylation is essential but not sufficient to allow expression of PLCγ activity, because targeting to the plasma membrane is also required. Since PLCγ can interact via its pleckstrin homology domain with both the lipid product of phosphatidyl inositol 3-kinase and the $\beta\gamma$ subunits ($G_{\beta\gamma}$) of heterotrimeric G proteins, and these signaling molecules can mediate PLCγ targeting to cell membranes, our findings suggest that binding of $sPLA_2$ to its plasma membrane receptor induces both activation of a tyrosine kinase activity able to phosphorylate PLCγ-1 and activation of a PTX-sensitive G-protein that would facilitate its translocation to the plasma membrane. Although $sPLA_2$ receptor is neither a classical G-protein coupled receptor nor a tyrosine kinase receptor, its functional association with these signaling pathways can be explained by two mechanisms: the first one could be an interaction of a transmembrane or cytoplasmic domain with intracellular signaling proteins. In fact, a recent report revealed that heterotrimeric G-proteins can be activated in the absence of specific G-protein receptors via shear stress (28). It has also been showed that CD14, a glycosylphosphatidylinositol-anchored protein containing neither transmembrane nor cytoplasmic amino acid sequences can physically associate with G_α proteins and Src kinases, thus leading to the involvement of these transducing molecules in bacterial lipopolysaccharide/CD14 signaling (29). The second mechanism could involve transactivation of tyrosine kinase receptors (EGF or PDGF) as a branch of the $sPLA_2$ signal transduction pathway that leads to the activation of PLCγ. Since recent evidence suggests that G-protein coupled receptors, e.g., muscarinic receptors (30), LPA receptors (31), and angiotensin II receptors (32) transactivate EGF receptors to transmit a portion of their signal, the possibility of a role for EGF receptor in $sPLA_2$ signaling deserves a detailed attention. Preliminary experiments carried out in the presence of the EGF receptor kinase inhibitor AG1478 have shown an inhibition of Ca^{2+} mobilization in 1321N1 cells without affecting the response elicited by $sPLA_2$. This result would argue against the transactivation hypothesis. Nevertheless, this is not a conclusive evidence as yet, and further studies are being developed to assess the actual contribution of growth factor receptors to the $sPLA_2$ signal pathway.

As regards the plasma membrane receptors, the activation of Ca^{2+}-release was inhibited by heparin and not by mannose-BSA (not shown), while the activation of MAP kinase/$cPLA_2$ was inhibited by both. Although this may be taken as an argument for the presence of two different receptors, we have discussed previously (7) that the inhibition by both compounds of the activation of the MAP kinase/$cPLA_2$ cascade makes it difficult the assignment of a particular structure to the binding receptor, and suggests that there could be more than one binding structure or, alternatively, a scarce selectivity for these compounds. Therefore, no matter what may be the receptor(s) involved in the activation of each signaling pathway by $sPLA_2$, the common inhibition by heparin suggests that they are closely related.

In summary, our data suggest that $sPLA_2$ interacts with a plasma membrane binding structure(s), and triggers the activation of two different and non-interacting signaling pathways. The first one is insensitive to PTX and involves the activation of the MAP kinase cascade and the phosphorylation and activation of $cPLA_2$ (7). The second one would lead to the activation of PLCγ (followed by IP_3 production and Ca^{2+} mobilization) both by tyrosine phosphorylation and

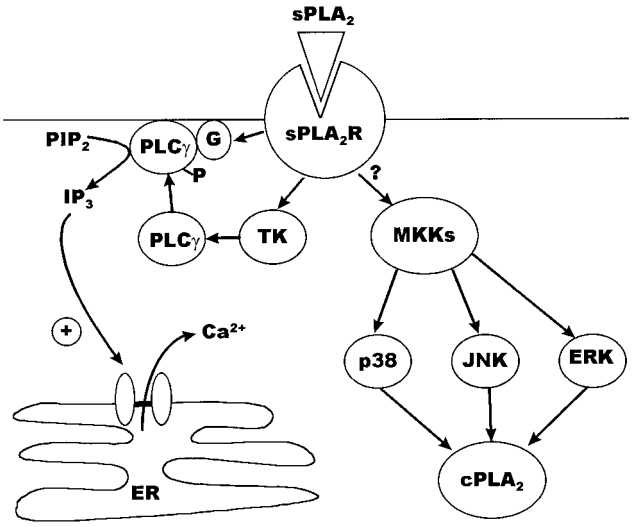

FIG. 4. Proposed scheme of signaling mechanisms triggered by $sPLA_2$. $SPLA_2R$, cell membrane binding structure for $sPLA_2$; TK, protein tyrosine kinase; PIP_2, phosphatidylinositol bisphosphate; IP_3, inositol trisphosphate; PLCγ, phospholipase Cγ; P, phosphorylated; MKK, MAP kinase kinase; p38, p38 isoform of MAP kinase; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinases including p42 and p44; ER, endoplasmic reticulum.

through a PTX-sensitive G-protein. LPA is able to activate also both pathways, but activation of the MAP kinase cascade in response to this agonist should have a different starting point, namely a PTX-sensitive G protein. Figure 4 shows a model of the signaling pathway we propose, where the identification of the protein tyrosine kinases and the docking proteins that serve as scaffolds for the receptor regulated PLCγ activity remains the subject of further studies.

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