thematic review

Phospholipase A_2 in the central nervous system: implications for neurodegenerative diseases

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Abstract **Phospholipase A_2 (PLA_2) belongs to a family of enzymes that catalyze the cleavage of fatty acids from the *sn*-2 position of phospholipids. There are more than 19 different isoforms of PLA_2 in the mammalian system, but recent studies have focused on three major groups, namely, the group IV cytosolic PLA_2, the group II secretory PLA_2 ($sPLA_2$), and the group VI Ca^{2+}-independent PLA_2. These PLA_2s are involved in a complex network of signaling pathways that link receptor agonists, oxidative agents, and proinflammatory cytokines to the release of arachidonic acid (AA) and the synthesis of eicosanoids. PLA_2s acting on membrane phospholipids have been implicated in intracellular membrane trafficking, differentiation, proliferation, and apoptotic processes. All major groups of PLA_2 are present in the central nervous system (CNS). Therefore, this review is focused on PLA_2 and AA release in neural cells, especially in astrocytes and neurons. In addition, because many neurodegenerative diseases are associated with increased oxidative and inflammatory responses, an attempt was made to include studies on PLA_2 in cerebral ischemia, Alzheimer's disease, and neuronal injury due to excitotoxic agents. Information from these studies has provided clear evidence for the important role of PLA_2 in regulating physiological and pathological functions in the CNS.**—Sun, G. Y., J. Xu, M. D. Jensen, and A. Simonyi. **Phospholipase A_2 in the central nervous system: implications for neurodegenerative diseases. *J. Lipid Res.* 2004.** 45: **205–213.**

Supplementary key words astrocytes • neurons • neurodegeneration • Alzheimer's disease • cerebral ischemia

Bazan (1) recognized the important role of arachidonic acid (AA) in the central nervous system (CNS) in the '70s when he observed the rapid and transient release of this fatty acid in the brain due to seizure and cerebral ischemia. The "Bazan effect" has since stimulated over 30 years of investigations attempting to unravel mechanisms regulating AA release from membrane phospholipids in the CNS.

Phospholipids in CNS membranes are enriched in polyunsaturated fatty acids (PUFAs) (2). Metabolism of PUFA is stringently controlled by phospholipase A_2 (PLA_2) and acyltransferases—known as the "deacylation-reacylation cycle" (3–5). Under normal conditions, free fatty acids (FFAs) released by PLA_2 are rapidly taken up by membrane phospholipids through an energy-dependent process involving CoA and ATP (6). To date, limited information is available on the structure and functions of acyltransferases. However, recent advances in molecular biological techniques have aided in the identification of many genes encoding different groups of PLA_2 and have provided new information on the properties and functions of these molecules.

PLA_2 (EC3.1.1.4.) belongs to a family of enzymes that catalyze the cleavage of fatty acids from the *sn*-2 position of phospholipids. These enzymes are not only important for maintenance of cell membrane phospholipids; they also play a key role in regulating the release of AA, a precursor for synthesis of eicosanoids. In the mammalian system, more than 19 different isoforms of PLA_2 have been identified, and different PLA_2s have been shown to participate in physiological events related to cell injury, inflammation, and apoptosis (7, 8). Recent studies have focused on three major groups of PLA_2: the group IV calcium-dependent cytosolic PLA_2 ($cPLA_2$), the group II secretory PLA_2 ($sPLA_2$), and the group VI Ca^{2+}-independent PLA_2 ($iPLA_2$) (9). Dur-

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Abbreviations: AA, arachidonic acid; AD, Alzheimer's disease; COX, cyclooxygenase; $cPLA_2$, cytosolic PLA_2; DHA, docosahexaenoic acid; ERK, extracellular signal-regulated protein kinase; FFA, free fatty acid; IFNγ, interferon gamma; IL-1β, interleukin-1β; $iPLA_2$, Ca^{2+}-independent PLA_2; KA, kainic acid; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCA, middle cerebral artery; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NMDA, *N*-methyl-D-aspartic acid; PE, phosphatidylethanolamine; PE_{pl}, ethanolamine plasmalogen; PGE2, prostaglandin E2; PLA_2, phospholipase A_2; ROS, reactive oxygen species; $sPLA_2$, secretory PLA_2; TNFα, tumor necrosis factor alpha.

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ing the past decade, excellent reviews describing the structure and properties of these PLA_2s in non-neural systems have appeared (7–11). In addition, two reviews focusing on PLA_2 in brain tissue have also been published (12, 13). The present review is devoted to PLA_2 in neural cells in the CNS, especially the signaling pathways regulating different PLA_2s in neurons and astrocytes (see **Fig. 1**). Because PLA_2s have been implicated in the pathology of a number of neurodegenerative diseases, an attempt was also made to include recent studies describing the different groups of PLA_2s in cerebral ischemia, Alzheimer's disease (AD), and neuronal injury due to excitotoxic agents. To facilitate updated information, a website linking data concerning PLA_2s in different neural cells and their involvement in neurodegenerative diseases has been created: http://www.pla2.com. The authors plan to update the information in this site periodically.

PLA_2

$cPLA_2$

$cPLA_2$ belongs to the group IV PLA_2s. Although three isoforms, i.e., $cPLA_2\alpha$, -β, and -γ, have been identified, the 85 kDa $cPLA_2\alpha$ has been studied most extensively. This protein is comprised of a C2 domain and multiple phosphorylation sites, including two consensus sites (S505 and S727) for phosphorylation by mitogen-activated protein kinases (MAPKs) (12) and an S515 site for Ca^{2+}/calmodulin (9). The C2 domain confers a Ca^{2+}-dependent translocation mechanism for this $cPLA_2$ (14, 15). Recent studies have provided evidence for translocation of $cPLA_2$ from the cytosol to nuclear membranes (16). Translocation of $cPLA_2$ has also been shown to participate in intracellular membrane trafficking processes, such as those governing the Golgi and endocytic pathways (17). $PLA_2\alpha$ seems to prefer hydrolysis of AA from phosphatidylcholine (9). In macrophages, as well as in other cell systems, agents including G protein-coupled receptor agonists, calcium ionophores, phorbol esters, and zymogens can activate $cPLA_2$, resulting in AA release (12). Through its linkage to receptor-mediated signaling pathways, $cPLA_2$ is an important PLA_2 for rapid AA release in cells and for modulating a number of intracellular processes.

$sPLA_2$

The $sPLA_2$ family consists of multiple groups (I, II, III, V, X, and XII) of enzymes characterized by a conserved Ca^{2+} binding loop and a conserved histidine residue in the

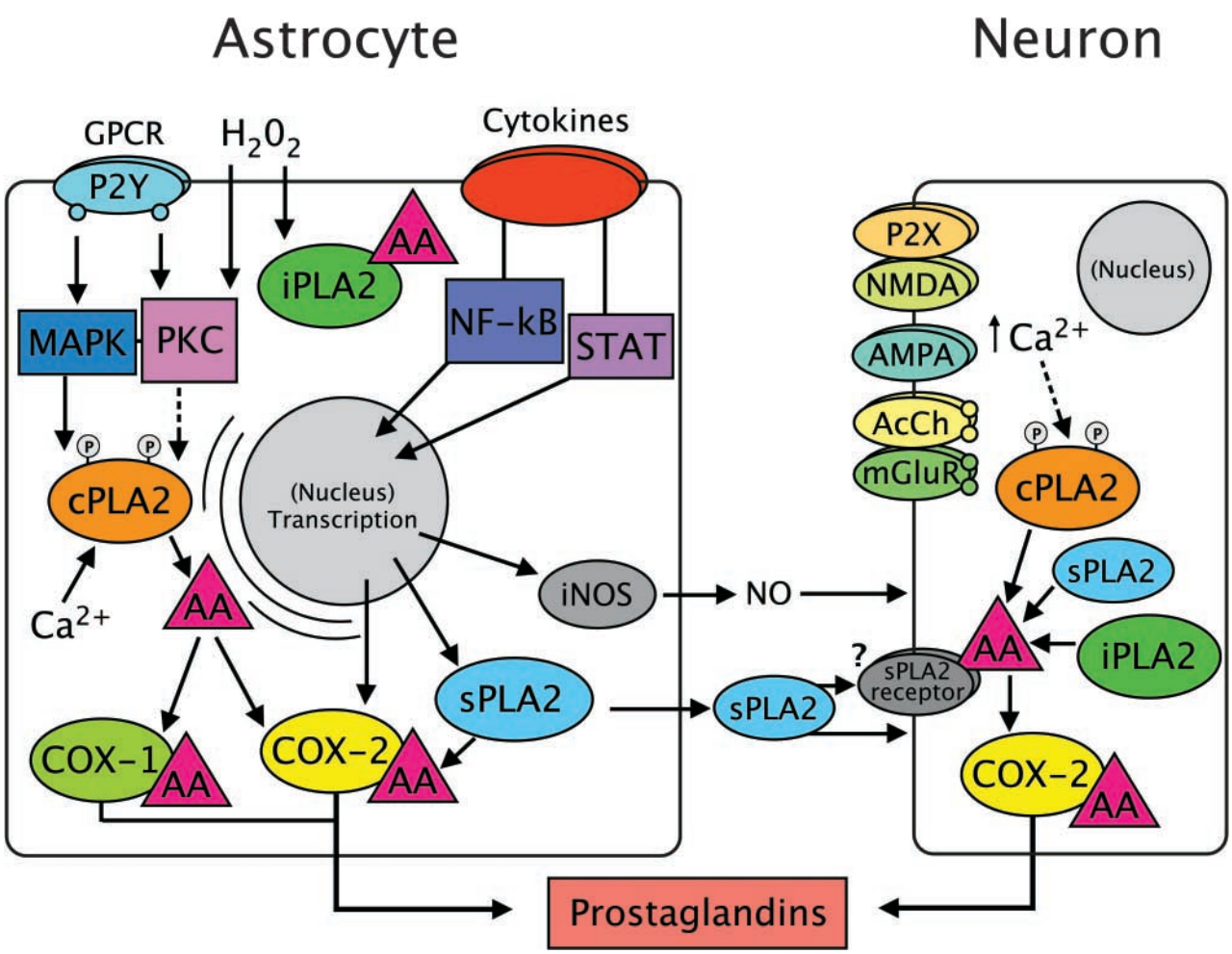

Fig. 1. A scheme representing different PLA_2 pathways in astrocytes and neurons.

catalytic domain (9). The group II $sPLA_2$s, including IIA, IIC, IID, IIE, and IIF isoforms, are low-molecular-weight proteins (~14 kDa) with secretory sequences. Genes for many of the group II $sPLA_2$ isoforms are clustered in chromosome 1 (18). These enzymes do not have strict fatty acid specificity and tend to act on anionic phospholipids in the presence of high concentrations of Ca^{2+} (9). Of the group II $sPLA_2$s, the IIA enzyme has been studied extensively because of its involvement in inflammatory processes in the peripheral systems (8, 11). In the CNS, group IIA $sPLA_2$ mRNA is expressed in cultured astrocytes and can be induced in response to proinflammatory cytokines [tumor necrosis factor alpha (TNFα), interleukin-1β (IL-1β), and interferon gamma (IFNγ)] (19–22). Upregulation of group IIA $sPLA_2$ mRNA expression was observed in brain as a result of global cerebral ischemia (23).

$iPLA_2$

The $iPLA_2$ family is comprised of group VIA and VIB. Group VIA enzyme has at least five splice variants, all with ankyrin repeats, whereas group VIB $iPLA_2$ lacks ankyrin repeats but consists of a signal motif for peroxisome localization (9). Fractionation of bovine brain cytosol by column chromatography resulted in two fractions, a 110 kDa $iPLA_2$ fraction, which prefers hydrolysis of diacyl-glycero-3-phosphoethanolamine, and a 39 kDa $iPLA_2$ fraction, which selectively acts on 1-alkenyl-2-acyl-glycero-3-phosphoethanolamine (ethanolamine plasmalogen, PE_{pl}) (13). Although $iPLA_2$s are generally regarded as housekeeping enzymes for the maintenance of membrane phospholipids, recent studies have revealed novel functional roles for this group of enzymes, i.e., regulation of vascular smooth muscle contraction (24) and apoptotic processes (25). A study by Yang et al. (26) indicated that >70% of PLA_2 activity in normal rat brain could be attributed to $iPLA_2$.

PLA_2 IN ASTROCYTES

Astrocytes are the major cell type in the CNS and play multiple functional roles in providing nutrient support to neurons, modulating Ca^{2+} homeostasis, and regulating neurotransmission, as well as mediating host defense functions. Astrocytes have been shown to contain all major groups of PLA_2 (27). Therefore, these cells have been used to study the roles of different groups of PLA_2 in normal physiological and pathological functions.

Response to receptor agonists

Although many G protein-coupled receptors are expressed in astrocytes, there is considerable interest in the P2Y nucleotide receptors in these cells. One reason for this interest is that in the brain, ATP is stored at high concentrations in synaptic vesicles and is coreleased with neurotransmitters during neuronal excitation (28). Therefore, P2Y receptors in astrocytes may constitute an important mechanism for mediating communication between neurons and glial cells. Activation of P2Y receptors by extracellular nucleotides such as ATP/UTP has been shown to cause an increase in intracellular Ca^{2+} concentrations ($[Ca^{2+}]i$) as well as activation of a number of signaling pathways (29). In astrocytes, activation of P2Y receptors is implicated in reactive gliosis, a pathological condition associated with a number of neurodegenerative diseases (30–32). Several studies have demonstrated the ability of ATP/UTP to stimulate AA release in astrocytes (33–36). A study by Xu et al. (36) further demonstrated the role of the extracellular signal-regulated protein kinase (ERK) and protein kinase C (PKC) pathways for enhancing $cPLA_2$ phosphorylation and stimulating AA release in murine astrocytes. ATP, acting on the $P2Y_2$ receptors in astrocytes, could also mediate the release of docosahexaenoic acid (DHA) (37). However, ATP-mediated release of DHA was shown to involve $iPLA_2$ instead of $cPLA_2$ (37). These results indicate the ability of ATP to stimulate multiple pathways that lead to activation of different PLA_2 isoforms.

The activation of endothelin receptor, another G protein-coupled receptor, was shown to cause AA release in astrocytes (38) and in smooth muscle cells (39, 40). The study with smooth muscle cells further demonstrated the involvement of PKCα and p38 MAP kinase in endothelin-1-mediated AA release (39). Many G protein-coupled receptors are linked to phospholipase C and the release of inositol trisphosphates and diacylglycerols, which are second messengers for Ca^{2+} mobilization and activation of PKC, respectively. Increases in both $[Ca^{2+}]i$ and PKC are important factors in the translocation and phosphorylation of $cPLA_2$. However, studies in NIH3T3 cells stably expressed with the serotonin $5HT_{2A}$ receptor indicated that instead of the phospholipase C pathway, 5HT-stimulated PLA_2 and AA release involved in both the Gi/o-associated G-mediated ERK1/2 and the $G_{12/13}$-coupled, Rho-mediated p38 MAP kinase pathways (41, 42). These results illustrate the complexity of different intracellular signaling pathways in the regulation of $cPLA_2$.

Response to oxidative agents

Reactive oxygen species (ROS) are produced in biological systems through both enzymatic and nonenzymatic mechanisms. Excessive generation of ROS in the CNS has been implicated in neuronal damage resulting from cerebral ischemia and in AD. Oxidant compounds such as H_2O_2 have been shown to cause perturbation of cell membrane integrity and alteration of mitochondrial function, resulting in an increase in $[Ca^{2+}]i$ (43, 44). In fact, H_2O_2 is a naturally occurring oxidant produced by a number of intracellular reactions, and excessive production of this compound is associated with signaling pathways (45) responsible for activating PKC (46), tyrosine kinase (47), and MAPK (45, 48). In astrocytes, H_2O_2 can alter phospholipid-hydrolyzing enzymes, including phospholipase D (49) and PLA_2 (50). A study in murine astrocytes further indicated the involvement of $cPLA_2$ and $iPLA_2$ in AA release induced by H_2O_2 (51). On the other hand, a study in mesangial cells that were transfected with $cPLA_2$ and/or $sPLA_2$ demonstrated the involvement of $cPLA_2$ and $sPLA_2$ in H_2O_2-induced AA release (52).

Response to inflammatory agents

Astrocytes can readily respond to proinflammatory agents and lipopolysaccharides (LPS), causing the induction of a number of genes through activation of the nuclear factor κB pathway. In primary rat astrocytes, cytokines (TNFα, IL-1β, and IFNγ) stimulated the increase in prostaglandin E2 (PGE2) production, which was preceded by an increase in cyclooxygenase-2 (COX-2) and $sPLA_2$ mRNA but not COX-1 and $cPLA_2$ mRNA (22). In another study in rat astrocytes, LPS also increased expression of $sPLA_2$ mRNA but not $cPLA_2$ mRNA (27). Although cytokines did not increase $cPLA_2$ mRNA levels, an increase in $cPLA_2$ phosphorylation was observed (22). The study by Xu et al. (22) further indicated the role of $sPLA_2$ in cytokine-induced production of PGE2. Because C57Bl/6 mice lack the group IIA $sPLA_2$ gene due to a frame shift mutation (53), astrocytes isolated from these mice were less responsive to cytokines in the production of PGE2 than were astrocytes from rat brain (22). On the other hand, a study with lung fibroblasts isolated from $cPLA_2$-deficient mice also showed less PGE2 production as compared with fibroblasts from the wild-type mice, suggesting an important role for $cPLA_2$ in the PGE2 pathway (54). Taken together, these results suggest the involvement of both $sPLA_2$ and $cPLA_2$ in cytokine-mediated PGE2 production.

Cytokines such as IL-1 were shown to enhance ATP-mediated stimulation of PLA_2 and AA release in astrocytes (35). A study by Xu et al. (22) showed that exposure of rat astrocytes to proinflammatory cytokines (TNFα, IL-1β, and IFNγ) for 16 h followed with $cPLA_2$ agonists (ATP and PMA) for 30 min resulted in 70-fold higher production of PGE2 as compared with astrocytes stimulated with ATP and PMA without prior cytokine exposure. Results from the above studies suggest that in pathological conditions associated with an increase in inflammation, a sustained increase in proinflammatory cytokines in the brain may enhance the response of G protein-coupled receptors to produce higher levels of eicosanoids. In a recent study in our laboratory, exposure of murine astrocytes to 1L-1β for 12 h resulted in an increase in COX-2 and $cPLA_2$ immunoreactivity (G. Y. Sun et al., unpublished observations). Confocal microscopic examination indicated that COX-2 and $cPLA_2$ are localized in the perinuclear area (**Fig. 2**). In a recent report by Pardue, Rapoport, and Bosetti (55), colocalization of $cPLA_2$ and COX-2 were also observed in the cerebellum of Rhesus monkey brain (55).

In addition to IL-1β, other cytokines, such as TNFα, can also activate COX-2 and stimulate signaling pathways leading to $cPLA_2$ phosphorylation and AA release (56). As shown in the study with human astrocytoma cells (1321N1), TNFα-stimulated phosphorylation of $cPLA_2$ involved the c-Jun and p38 MAP kinase pathways but not the ERK pathway (56). In addition to group IIA $sPLA_2$, group V $sPLA_2$ was also present in astrocytes, and TNFα stimulated both types of $sPLA_2$, albeit through different time courses and different pathways (57). These studies demonstrate that different cytokines can activate different isoforms of $sPLA_2$ in astrocytes.

PLA_2 IN MICROGLIAL CELLS

In addition to astrocytes, little is known about PLA_2 in other types of glial cells, such as the microglial cells and the oligodendroglial cells. This is due in part to difficulties in isolating sufficient quantities of these cells for biochemical analysis. Microglial cells are immune-active cells and exhibit many properties similar to those of macrophages and astrocytes (58). Therefore, there is substantial interest in the role of PLA_2 in the inflammatory responses in these cells. In N9 microglial cells, PLA_2 inhibitors could inhibit LPS-induced TNFα release, suggesting an involvement of PLA_2 in the cytokine pathway (59). Although the murine-derived BV-2 microglial cells lack the group IIA $sPLA_2$, they contain high levels of $cPLA_2$ (G. Y. Sun, unpublished observations). In BV-2 cells, AA release stimulated by IFNγ and PMA was PKC and ERK dependent, sug-

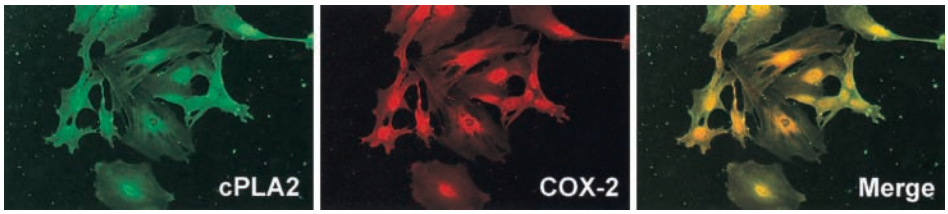

Fig. 2. Confocal microscopy showing localization of cytosolic PLA_2 ($cPLA_2$) and cyclooxygenase-2 (COX-2) in the perinuclear area of primary murine astrocytes following treatment with interleukin-1β (IL-1β) (10 ng/ml) for 12 h. Astrocytes were plated on coverslips coated with poly-D-lysine and grown to 50% confluence. Cells were treated with IL-1β and were washed with Balch Buffer II (BBII) containing 25 mM HEPES, 75 mM potassium acetate, and 5 mM EGTA. Immediately after treatment, cells were fixed with 2% paraformaldehyde in phosphate-buffered saline (pH 7.2) at 25°C for 30 min. Cells were permeabilized and blocked by incubation for 30 min at 25°C with 0.1% (w/v) saponin and 10% (v/v) normal donkey serum diluted in BBII. Cells were incubated with primary antibodies (1:50 rabbit polyclonal anti-$cPLA_2$ IgG or 1:100 goat polyclonal anti-rat COX-2 IgG) diluted in BBII. Incubation with secondary antibodies (1:200 Cy3-conjugated donkey anti-rabbit IgG or 1:200 Cy5-conjugated donkey anti-goat IgG) was carried out at 25°C for 4 h. After three washes with BBII, the coverslips were rinsed with distilled water and mounted on glass slides with Mowilol. The stained cells were viewed with a confocal microscope (Bio-Rad Lasershop 2000).

gesting the involvement of $cPLA_2$ in mediating the AA release in these cells (G. Y. Sun, unpublished observations). In human microglial cells, LPS was capable of inducing COX-2 mRNA expression and PGE2 production (60). These results suggest that PLA_2 may also play a role in mediating the inflammatory cascade in microglial cells.

PLA_2 IN NEURONS

AA is regarded as a neuromodulator in the CNS, and PLA_2 is thought to have a role in neuronal plasticity (61). Neuronal excitation resulting from depolarization with high concentrations of potassium (62) and stimulation with excitatory neurotransmitter agonists such as α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) (63), *N*-methyl-D-aspartic acid (NMDA) (64), and glutamate (65), as well as muscarinic cholinergic agonists (66), can stimulate AA release in neurons. Furthermore, oxidant compounds such as H_2O_2 could further enhance AA release stimulated by a number of neurotransmitter agonists (67). Although there is evidence for the involvement of Ca^{2+} and $cPLA_2$ in the AA release from neurons, the signaling pathways leading to $cPLA_2$ activation remain unclear.

Studies in vivo have demonstrated $cPLA_2$ mRNA expression in hippocampal neurons (68). Infusion of NMDA into the hippocampus resulted in the activation of $cPLA_2$ and COX-2 expression and the production of PGE2 and PGF2α (69). Upregulation of $cPLA_2$ expression was also shown in injured cervical neurons and dorsal root ganglia (70, 71). However, a study by Morioka et al. (72) indicated that cultured rat neurons expressed both $cPLA_2$ and $sPLA_2$ and that both PLA_2s were involved in the cytokine-induced release of substance P from these neurons. Using specific antibodies, the study by Matsuzawa et al. (73) detected group IIA $sPLA_2$ in brain synaptosomes. Furthermore, release of group IIA $sPLA_2$ from synaptosomes was observed upon depolarization with high concentrations of potassium (73). Thus, these studies suggest the presence of $sPLA_2$ in neurons. Studies by Kolko et al. (74–77) have provided evidence for the presence of a neuronal receptor for group IIA $sPLA_2$. Low concentrations of $sPLA_2$-OS2 (from Taipan snake venom) enhanced glutamate excitotoxicity, leading to neuron death (78). Injection of $sPLA_2$ into the brain caused epileptic seizure (79) as well as neuronal apoptosis (80). Some of these effects were attributed to the ability of $sPLA_2$ to increase Ca^{2+} influx through stimulation of an L-type voltage-sensitive Ca^{2+} channel (81).

PLA_2 IN NEUROBLASTOMA CELLS

Most tumor cells contain elevated levels of PLA_2, and increased production of eicosanoids has been implicated in cell growth. Using specific PLA_2 inhibitors, van Rossum et al. (82) demonstrated the involvement of $cPLA_2$ activity in cell cycle progression, especially from G1 to S phase in neuroblastoma (N2A) cells. In human neuroblastoma LA-N-1 cells, an isoform of $iPLA_2$ was shown to specifically utilize phosphatidylethanolamine (PE) and PE-plasmalogen as substrates (83). Differentiation of these cells with retinoic acid was marked by an increase in $iPLA_2$ activity in the nuclei, suggesting a role for this PLA_2 in regulating nuclear membrane functions (83). In another human neuroblastoma cell line, SK-N-SH, $iPLA_2$ was involved in IL-1β stimulation of COX-2 expression and PGE2 secretion (84) and in neuronal injury induced by hypoxia-hypoglycemia (85). Therefore, studies with neuroblastoma cells have revealed novel functions of $iPLA_2$.

PLA_2 IN NEURODEGENERATIVE DISEASES

Information regarding the roles of different types of PLA_2 in neurodegenerative diseases is sketchy, primarily because of the complex cellular network and the presence of different cell types in the brain. In this review, an attempt was made to cover studies on PLA_2 in cerebral ischemia, AD, and neurodegeneration due to excitotoxic compounds. For a better coverage of PLA_2 in other neurological and psychiatric disorders, including alcoholism, epilepsy, schizophrenia, and affective disorders, readers are encouraged to visit our web site (http://www.pla2.com).

Cerebral ischemia

Cessation of blood flow in cerebral ischemia (stroke) is known to trigger a number of physiological and biochemical changes, including rapid energy depletion, release of excitatory amino acid transmitters, neuronal membrane depolarization, and influx of Ca^{2+}. Many of these changes are associated with an increase in oxidative stress, resulting in the production of ROS, which in turn, are important factors underlying delayed neuron cell death (86). In the rat focal cerebral ischemia model induced by the occlusion of the middle cerebral artery (MCA), a biphasic increase in FFAs was observed, one during the ischemic period and another at ~16 h after reperfusion (87). In the early phase of ischemia, FFA accumulation was attributed to activation of Ca^{2+}-dependent $cPLA_2$ as well as to an inhibition of the energy-dependent reacylation process (88). The second phase of FFA increase was attributed to upregulation of the group IIA $sPLA_2$ in reactive astrocytes in the penumbral area (G. Y. Sun, unpublished observations). The increase in $sPLA_2$, together with that of other lipid mediators in reactive astrocytes, is in agreement with the increased inflammatory response observed during this period of cerebral ischemia (89). In another form of MCA occlusion induced by a photochemical mechanism, an increase in group IIA $sPLA_2$ activity was associated with ischemia-induced neuronal apoptosis (80). Indoxam, a specific $sPLA_2$ inhibitor, was shown to offer protection against the ischemia-induced damage (80).

In the forebrain model of global cerebral ischemia, delayed neuron death was found in the hippocampal CA1 area 2 to 7 days after ischemia-reperfusion. A study by Lauritzen, Heurteaux, and Lazdunski (23) indicated a biphasic upregulation of group IIA $sPLA_2$ mRNA in rat brain after transient global ischemia. In another study,

analysis of fatty acids using different phospholipase inhibitors provided evidence that the FFA release resulting from global ischemia-reperfusion was mainly due to the activation of the Ca^{2+}-dependent $cPLA_2$ (90). In immature rats following hypoxic ischemia, neuron death in the CA1 hippocampal area was accompanied by an increase in COX-2 and $cPLA_2$ immunoreactivity (91). The increase in $cPLA_2$ immunoreactivity occurring 72 to 168 h after the ischemic insult was attributed to an increase in glial activity (91). An immunohistochemical study also indicated an increase in $cPLA_2$ in reactive glia after global cerebral ischemia (92). Thus, neuronal damage due to ischemic injury may involve COX-2, $cPLA_2$, and $sPLA_2$, depending on the cell type, time course, and type of ischemic insult.

AD

Increased deposition of amyloid plaques infiltrated by reactive astrocytes and microglial cells is a major pathological landmark of AD. Aggregated forms of amyloid β (Aβ) peptides, particularly Aβ1-42, have been shown to elicit cytotoxic effects resulting in neuron cell death (93, 94). Aβ also stimulated astrocytes to release cytokines and nitric oxide (95), supporting the increase in oxidative and inflammatory responses in AD (93, 96, 97). Indeed, in an animal model of amyloidosis, increased lipid peroxidation was observed prior to deposition of amyloid plaques (98).

There is evidence for changes in membrane phospholipids in the AD brain. A study by Farooqui, Rapoport, and Horrocks (99) revealed a decrease in PE_{pl} in the AD brain. In the study by Han, Holtzman, and McKee (100), a decrease in plasmenyl phospholipids was detected in the white matter of AD brain during the early stage of the disease, when only mild cognitive impairments were apparent. Plasmalogens are synthesized in peroxisomes. However, it is not clear whether the decrease in these phospholipids in the AD brain is associated with a peroxisomal disorder. Because PE_{pl}s in brain are highly enriched in DHA (2), a deficiency in these phospholipids may also lead to a decrease in DHA, which in turn, may have important implications in brain function, including learning ability (101). Recent studies in vitro demonstrated the ability of PE_{pl} to protect cholesterol-rich membranes from oxidative stress (102). Furthermore, deficiency in PE_{pl} could lead to altered cholesterol transport (103).

There is accumulating evidence for the involvement of specific PLA_2s in AD brain pathology. In two separate studies, a decrease in PLA_2 activity was found in the parietal and temporal cortex (104), as well as the prefrontal cortex, of the AD brain (105). On the other hand, immunohistochemical studies showed an increase in $cPLA_2$ immunoreactivity associated with the glial fibrillary acidic protein-positive astrocytes in the AD brain (106). In a recent gene array study, profiling of 12,633 genes in the hippocampal CA1 area of AD patients indicated an increase in $cPLA_2$ and COX-2 expression, as well as upregulation of a number of apoptotic and proinflammatory genes (107). These findings are in agreement with the increased oxidative and inflammatory responses and presence of reactive astrocytes associated with AD pathology (93–98).

Studies in human neuroblastoma LA-N-2 cells demonstrated the ability of Aβ to enhance the activity of a number of phospholipases (108). Nicotine, a cholinergic agonist, inhibited an Aβ-induced increase in PLA_2 activation (109). Aβ was shown to cause oxidative damage to neurons, resulting in increased Ca^{2+} influx and activation of apoptotic pathways (96, 97). The ability of PLA_2 inhibitors to attenuate Aβ-induced ROS production indicates the involvement of PLA_2 in Aβ cytotoxicity (110).

Neurodegeneration mediated by excitotoxic agents

Excitotoxic compounds such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-OH-dopamine have been shown to cause neurodegeneration resulting in Parkinson-like symptoms. Quinacrine, a nonselective PLA_2 inhibitor, significantly reduced MPTP-induced dopamine loss (111). Mice deficient in $cPLA_2$ were shown to exhibit more resistance to MPTP neurotoxicity than wild-type mice, further supporting a role of $cPLA_2$ in mediating MPTP neurotoxicity (112).

Kainic acid (KA) is a subclass of glutamate receptor agonists, and systemic and/or local administration of this compound can result in seizures and neurodegeneration. Upregulation of $cPLA_2$ expression was found in hippocampal neurons after injury induced by injection of KA into the brain (113). Quinacrine, the PLA_2 inhibitor, was shown to attenuate the KA-induced increase in $cPLA_2$ mRNA expression as well as in neuronal damage (114). Electron microscopic examination indicated two phases of upregulation of $cPLA_2$ in the hippocampus following KA injection. The first phase was attributed to an increase in $cPLA_2$ in the neurons, and the second phase (after 1 week) was attributed to an adaptive response associated with gliosis (114). Epileptic seizure due to KA-mediated kindling also resulted in an upregulation of COX-2 and $cPLA_2$ in hippocampal neurons (115). These studies demonstrate a role for $cPLA_2$ in hippocampal neuronal injury resulting from excitotoxic compounds.

SUMMARY

A review of recent studies clearly demonstrates the important role of PLA_2 in mediating normal and pathological functions in the CNS. However, studies of PLA_2 in the CNS are complicated by the presence of different types of cells and the complex signaling pathways generated by different agonists (Fig. 1). Studies with astrocytes in culture reveal a link between $cPLA_2$ and the G protein-coupled receptors and $sPLA_2$ and the transcriptional pathways induced by proinflammatory cytokines. These studies have provided new information on mechanisms for regulating different groups of PLA_2 in neural cells in the CNS.

These studies also reveal several areas requiring further studies: *1*) Because $iPLA_2$ comprises a large portion of PLA_2 activity in the CNS, future studies should be directed toward a better understanding of the structure and function of different isoforms of $iPLA_2$ in the brain. *2*) Because AA release is associated with neuronal excitation,

further studies are needed to clearly identify the types of PLA_2 and the signaling pathways regulating AA release in neurons. *3*) Studies with astrocytes may provide more information regarding the physiological roles of $cPLA_2$ in intracellular trafficking as well as in other intracellular functions. *4*) Because microglial cells have been implicated in the pathology of many neurodegenerative diseases, more studies are needed to demonstrate the role of PLA_2 in the inflammatory response of these cells. With the advancement of molecular biological techniques, and as specific antibodies targeted to different PLA_2s become more readily available, it can be projected that more studies will focus on defining the roles of different PLA_2s in neurodegenerative diseases. It is anticipated that the new information will be important for the development of novel therapeutic strategies to combat damage resulting from upregulation of PLA_2 in the affected neurodegenerative disorders.

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