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Phospholipase A_2 in Astrocytes

Responses to Oxidative Stress, Inflammation, and G Protein-Coupled Receptor Agonists

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Abstract

Astrocytes comprise the major cell type in the central nervous system (CNS) and they are essential for support of neuronal functions by providing nutrients and regulating cell-to-cell communication. Astrocytes also are immune-like cells that become reactive in response to neuronal injury. Phospholipases A_2 *(PLA_2)* are a family of ubiquitous enzymes that degrade membrane phospholipids and produce lipid mediators for regulating cellular functions. Three major classes of PLA_2 are expressed in astrocytes: group IV calcium-dependent cytosolic PLA_2 *($cPLA_2$)*, group VI calcium-independent PLA_2 *($iPLA_2$)*, and group II secretory PLA_2 *($sPLA_2$)*. Upregulation of PLA_2 in reactive astrocytes has been shown to occur in a number of neurodegenerative diseases, including stroke and Alzheimer's disease. This review focuses on describing the effects of oxidative stress, inflammation, and activation of G protein-coupled receptors on PLA_2 activation, arachidonic acid (AA) release, and production of prostanoids in astrocytes.

Index Entries: Phospholipases A_2; oxidative stress; cytokines; arachidonic acid; prostaglandin E_2; astrocyte; reactive gliosis.

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Introduction

Astrocytes are the most abundant cell type in the central nervous system (CNS) outnumbering neurons by a large margin. Recent studies indicate that astrocytes are not only responsible for nutrient supply in the brain but also mediate many forms of intercellular communication with neurons *(1).* Astrocytes are the major source in the CNS for synthesis of growth factors, polyunsaturated fatty acids, and lipoproteins that are necessary for postinjury neuronal repair and axonal regrowth. During neuronal insult, astrocytes are the first cells to sense injury and initiate a defense response.

Neuronal injury is often marked by neuronal excitation followed by release of excitatory neurotransmitters together with adenosine 5′-triphosphate (ATP) *(2).* ATP released into the extracellular space can activate ionotropic (P2X) and metabotropic (P2Y) receptors in neurons and glial cells and, in turn, trigger signaling pathways that result in neuronal apoptosis and astrogliosis, respectively *(2,3).* Although the underlying mechanisms whereby astrocytes become reactive have not been fully elucidated, activated astrocytes are characterized by an increased expression of glial fibrillary acidic protein (GFAP) and production of reactive oxygen species (ROS). Phospholipases A_2 *(PLA_2)* and cyclooxygenases (COX), two important enzymes for production of arachidonic acid (AA) and prostaglandin E_2 (PGE_2), respectively, are upregulated during reactive gliosis. This review is aimed at recent studies on the PLA_2/AA/PGE_2 cascade in astrocytes in response to oxidative stress and pro-inflammatory cytokines, including P2 nucleotide receptor agonists.

Phospholipases A_2 in Neurodegenerative Diseases

Phospholipases A_2, a superfamily of enzymes ubiquitously expressed in mammalian cells, are key enzymes for mediating the release of AA from membrane phospholipids. Recent studies have focused on the group IV Ca^{2+}-dependent cytosolic PLA_2 *($cPLA_2$),* the group II secretory PLA_2 *($sPLA_2$),* and the group VI Ca^{2+}-independent PLA_2 *($iPLA_2$) (4–8).* Genes encoding all of these PLA_2*s* are expressed in rat brain *(9).* Under normal conditions, cell membrane phospholipids are maintained in a critical equilibrium regulated by PLA_2 and acyltransferases that constitute a deacylation–reacylation cycle *(10).* Disturbances in this equilibrium can cause the increase in production of free radicals, excitotoxicity, mitochondrial dysfunction, and cell death by apoptosis or necrosis *(11).*

Several recent reviews have placed emphasis on the role of PLA_2-dependent increases in AA release from phospholipids and eicosanoid production in the pathogenesis of neurodegenerative diseases *(11–15).* Upregulation of PLA_2 has been reported in Wallerian degeneration *(16),* multiple sclerosis *(17),* focal and global cerebral ischemia *(18–20),* and Alzheimer's disease *(21).* Systemic treatment with kainic acid, an excitatory neurotransmitter receptor agonist, and bacterial lipopolysaccharide (LPS) can also result in upregulation of PLA_2 in the brain *(22–24).* The ability of specific PLA_2 inhibitors to diminish neuronal damage further supports an important role for PLA_2 in the neurodegenerative process *(19,23).*

Secretory *$sPLA_2$* in Astrocytes

Among the subtypes of *$sPLA_2$ (6),* $sPLA_2$-IIA is noted for its role in cardiovascular and inflammatory diseases, including arthritis, atherosclerosis, and sepsis *(25). $sPLA_2$*-IIA is expressed in rat astrocytes and its synthesis is induced by tumor necrosis factor-α (TNF-α) *(26). $sPLA_2$*-IIA mRNA is induced in the immortalized rat astrocyte cell line (DITNC) by TNF-α and interleukin-1β (IL-1β) *(27).* TNF-α and IL-1β in combination with interferon-γ (IFN-γ) also induce expression of messenger RNA (mRNA) for cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in primary astrocytes and DITNC cells *(28,29).* L-NIL, a specific inhibitor of iNOS,

decreased NO production without affecting the induction of *sPLA$_2$* in DITNC cells *(28,29).* In agreement with the involvement of nuclear factor (NF)-κB pathway *(30),* pyrrolidine dithiocarbamate (PDTC), an inhibitor of the NF-κB pathway, decreased cytokine-induced iNOS and *sPLA$_2$* mRNA expression in DITNC cells *(28).* Induction of *sPLA$_2$* by pro-inflammatory cytokines is inhibited by a number of factors, including lysophospholipids, AA, ethanol, and BN50730, a synthetic platelet-activating factor (PAF) receptor antagonist *(31–33).* However, more studies are needed to elucidate the mechanisms for transcriptional regulation of *sPLA$_2$*-IIA by these compounds.

In astrocytes, TNF-α was shown to enhance expression of mRNA for *sPLA$_2$*-IIA and *sPLA$_2$*-V with different time-courses *(34).* In addition to NF-κB, exposure of astroctyes to TNF-α also results in stimulation of mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3′ OH-kinase (PI 3-kinase). These results suggest that multiple signaling pathways might be responsible for the induction of *sPLA$_2$*-IIA and *sPLA$_2$*-V in astrocytes.

Upregulation of *PLA$_2$* in Cerebral Ischemia

Although astrocytes express multiple forms of *PLA$_2$ (35),* the relationship of these enzymes to neurodegenerative diseases has not been fully understood. Earlier studies demonstrated that upregulation of *sPLA$_2$* mRNA occurred in rodent brain in response to global and focal cerebral ischemia *(18,19).* Transient cerebral ischemia enhanced hydroxyl radical generation, lipid peroxidation, and *PLA$_2$* activity, and citicoline (cytidine-5′-diphosphocholine), an intermediate in phosphatidylcholine synthesis, was effective in attenuating ischemic injury *(36).* Based on its Ca^{2+} dependency, *sPLA$_2$* is thought to be the major *PLA$_2$* activated by the ischemic insult. Using the focal ischemia model in rat induced by occlusion of the middle cerebral artery (MCA), Lin et al. *(20)* observed a biphasic increase in the expression of *sPLA$_2$*-IIA mRNA in the ipsilateral cortex: initially, there was an increase in the ischemic cortex at 30 min after a 60-min MCA occlusion, and this was followed by a secondary increase in the penumbral area at 1 d after ischemia–reperfusion (Fig. 1). Confocal microscopy further showed *sPLA$_2$*-IIA immunoreactivity in GFAP-positive astrocytes but not in isolectin B4-positive microglial cells *(20).*

An *in situ* hybridization study carried out with rat brain sections indicated intense *cPLA$_2$* mRNA expression in the hippocampal neurons *(37,38).* At 6 h after induction of transient forebrain ischemia, a significant increase in *cPLA$_2$* mRNA expression was observed in the dentate granule cell layer *(37).* Although focal cerebral ischemia caused extensive astrogliosis in the penumbral area (as indicated by an increased expression of GFAP), no increase in *cPLA$_2$* mRNA expression was observed in the ischemic cortex *(20).* These results suggest that *cPLA$_2$* mRNA in neurons and astrocytes respond differently to ischemia, probably depending on the time-course and the type of ischemic insult.

Physiological Role of *sPLA$_2$* in the CNS

The molecular structure of *sPLA$_2$* (including groups II and V) suggests that these proteins are secreted from cells after synthesis *(39).* There is evidence that the secreted proteins associate with proteoglycan and are subsequently internalized and degraded *(40).* The group V *sPLA$_2$* is internalized into lipid-rich vesicles such as caveolin and lipid rafts *(41).*

Extracellular *sPLA$_2$,* including human *sPLA$_2$*-IIA and those from Taipan snake venom OS2, has been shown to enhance glutamate excitotoxicity and Ca^{2+} influx, resulting in neuronal apoptosis *(42–44).* There is evidence that the cytotoxic and inflammatory effects of extracellular *sPLA$_2$* are the result of their ability to activate specific muscle (M) or neuronal (N) type *sPLA$_2$* receptors *(45,46).* Studies by Hernandez et al. *(47,48)* indicated the presence of *sPLA$_2$* receptors in human 1321N1 astrocytoma cells.

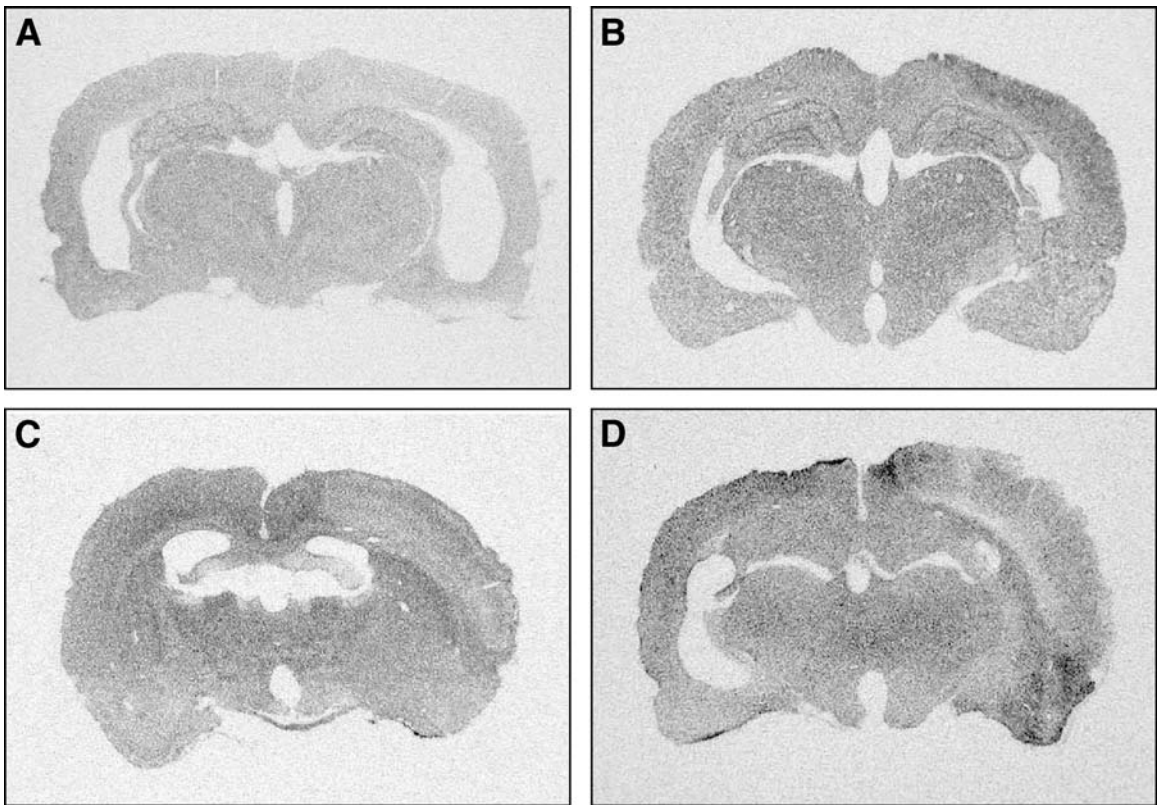

Fig. 1. *In situ* hybridization of *sPLA$_2$*-IIA mRNA after induction of focal ischemia in rat brain. Focal cerebral ischemia was induced in rats by occlusion of the MCA. Coronal brain sections were prepared and hybridized with [^{33}P]-labeled *sPLA$_2$*-IIA mRNA riboprobe, as described elsewhere *(20).* Sections shown indicate *sPLA$_2$*-IIA mRNA distribution in **(A)** sham-operated control or **(B)** 30 min, **(C)** 1 d, or **(D)** 3 d after a 60-min MCA occlusion. (Reproduced with permission from ref. *20.*)

Exogenous *sPLA$_2$* caused AA release in these cells similar to the response elicited by lysophosphatidic acid (LPA), a G protein-coupled receptor agonist. Stimulation of 1321N1 cells with *sPLA$_2$* also caused an electromobility shift in *cPLA$_2$,* indicative of its activation, as well as activation of extracellular signal-regulated kinase (ERK), c-Jun, and p38, suggesting that crosstalk exists between *cPLA$_2$* and *sPLA$_2$* in mediating an inflammatory response.

cPLA$_2$ in Astrocytes

Group IV *cPLA$_2$* is activated by phosphorylation and Ca^{2+}-dependent translocation from the cytosol to the cell membrane. The presence in *cPLA$_2$* of a consensus phosphorylation site for MAPK at Ser^{505} suggests a direct link between *cPLA$_2$* and signaling pathways that activate MAPK *(4,5,49,50).* In addition, *cPLA$_2$* can be phosphorylated by protein kinase C (PKC) and calmodulin kinase II (CAMKII) *(51),* although these phosphorylation sites have not been clearly delineated.

In most studies with cultured cells, AA release from cell membrane phospholipids is monitored by incubating cells with [^{14}C-AA] bound to bovine serum albumin (BSA). The labeled AA is rapidly incorporated into phospholipids within several hours, where it is found distributed among phospholipids, mainly phospha-

tidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylethanolamine plasmalogen (PEpl) *(52).* In immortalized astrocytes (DITNC), Ca^{2+} ionophore A23187 can cause AA release and translocation of *cPLA$_2$* from the cytoplasm to the membrane *(52).* However, analysis of the radioactive material in the culture medium by high-performance thin-layer chromatography (HPTLC) revealed that in addition to the release of AA, A23187 also induced the release of labeled phospholipids. These results suggest that the Ca^{2+} ionophore can form holes in cell membranes large enough for the release of small vesicles *(52).*

Nucleotide Receptor Agonists Stimulate *cPLA$_2$* and AA Release in Astrocytes

Many G protein-coupled receptors (GPCRs) transmit signals through activation of polyphosphoinositide hydrolysis by phospholipase C, resulting in generation of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerols (DAG), which are second messengers for intracellular Ca^{2+} mobilization and activation of PKC, respectively. Several G protein-coupled P2Y receptor subtypes are expressed in astrocytes, and ATP and uridine 5′-triphosphate (UTP) are equipotent agonists for eliciting intracellular calcium mobilization in these cells *(53,54).* Subsequent studies suggest that induction of prostaglandin release by ATP/UTP probably involves activation of a $P2Y_2$ receptor subtype and *cPLA$_2$ (55–59).* Ishimoto et al. *(60)* have shown that ATP-induced *PLA$_2$* activation was mediated by a pertussis toxin-sensitive G protein. In rat astrocytes, AA release caused by ATP, UTP, and 2-methylthio-ATP was linked to activation of the $P2Y_1$ and $P2Y_2$ receptor subtypes *(59).* Furthermore, ATP/UTP-induced $P2Y_2$ receptor activation is linked to signaling pathways that lead to mitogenic responses in astrocytes *(3,61–63).* The presence of an integrin-binding motif in the first extracellular loop of the $P2Y_2$ receptor is required to maintain the receptor in a high-affinity state through direct interaction with $\alpha_v\beta_3/\beta_5$ integrins *(64).* In this regard, the $P2Y_2$ receptor differs from other P2Y receptors in its ability to activate a number of signaling pathways that regulate inflammation and reorganization of cytoskeletal proteins (Weisman et al., this issue; *[64]*). These events might play a role in the induction of reactive gliosis after excitotoxic insults *(65).*

In primary mouse astrocytes, phorbol myristoyl acetate (PMA) and ATP stimulated signaling pathways leading to phosphorylation of ERK and *cPLA$_2$ (66).* However, although stimulation of the epidermal growth factor (EGF) receptor in astrocytes also resulted in phosphorylation of ERK and *cPLA$_2$,* EGF did not elicit AA release in these cells. These results suggest that phosphorylation of *cPLA$_2$* by ERK1/2 is not sufficient to cause AA release. Other results demonstrated that ATP and PMA induced AA release through activation of signaling pathways with different sensitivities to PKC and ERK1/2 inhibitors *(66).* GF109203x, a PKC inhibitor, or U0126, an inhibitor of MEK1/2 (the kinases that phosphorylate ERK1/2), partially inhibited ATP-mediated AA release, whereas pretreatment of astrocytes with both PKC and MEK1/2 inhibitors completely inhibited ATP-mediated *cPLA$_2$* phosphorylation and AA release (Fig. 2). These results suggest a role for PKC and ERK1/2 in ATP-mediated activation of *cPLA$_2$* in murine astrocytes *(66).* In kidney cells, ATP-mediated AA release also was inhibited by U0126 but was independent of *cPLA$_2$* phosphorylation and its translocation from cytosol to membrane *(67).*

Analysis of mouse and rat astrocytes indicated similar and different PKC isoforms between the two species. As shown in Fig. 3, mouse and rat astrocytes express PKCα, PKCι, and PKCλ, whereas mouse astrocytes also express PKCε and rat astrocytes also express PKCδ and low levels of PKCε. Short-term treatment of mouse astrocytes with PMA resulted in translocation of PKCα from the cytoplasm to membrane and AA release. Prolonged treatment with PMA for 24 h caused

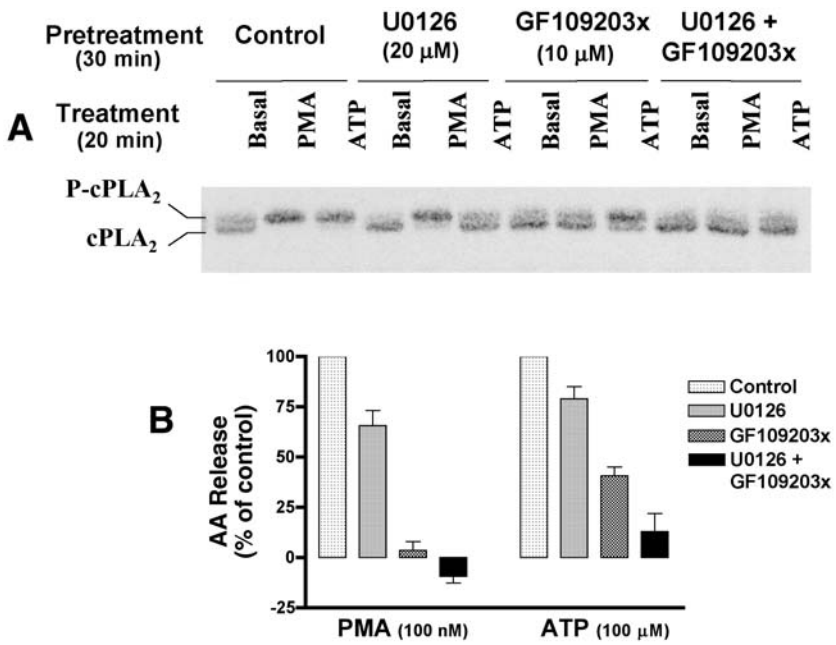

Fig. 2. Effects of PKC and ERK1/2 inhibitors on phosphorylation of *cPLA2* and AA release. **(A)** Astrocytes were pretreated with vehicle (control), GF109203x (10 μ*M*), or U0126 (20 μ*M*) for 30 min at 37°C, followed by incubation with vehicle (basal), PMA (100 n*M*), or ATP (100 μ*M*) for 20 min at 37°C. Cell lysates were prepared and subjected to Western analysis of *cPLA2*. Phosphorylation of *cPLA2* was indicated by an electrophoretic mobility shift of the enzyme. **(B)** [^{14}C]-AA-Labeled astrocytes were treated as in (A) and labeled-AA release in response to PMA or UTP was determined as described elsewhere *(66).* The effects of inhibitors were expressed as a percentage of the net stimulated AA release in the absence of inhibitors (control). (Data were abstracted from Fig. 7 in ref. *66.*)

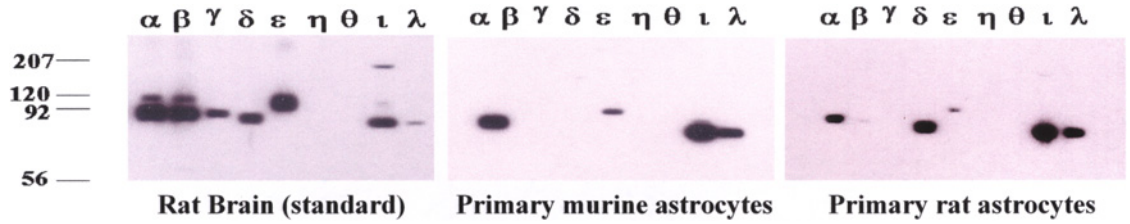

Fig. 3. PKC isoforms in primary murine and rat astrocytes. Cell lysates prepared from astrocytes were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunodetection with selective antibodies to PKC isoforms was performed with the Multiscreen Apparatus (Bio-Rad). Rat brain proteins were used as standards.

downregulation of PKCα and PKCε *(66).* In contrast with results from rat astrocytes *(59),* prolonged treatment of mouse astrocytes with PMA to downregulate conventional and novel PKC isoforms did not affect ATP-mediated AA release *(66).* These discrepancies might well be the result of the differences in PKC isoforms between the two species. Another possibility

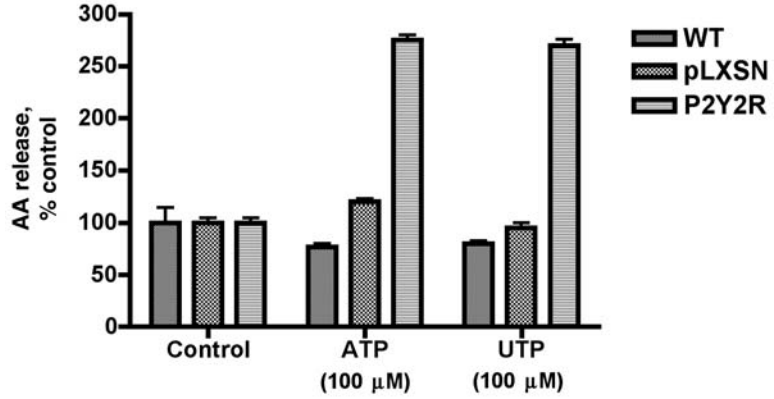

Fig. 4. ATP or UTP stimulates AA release through activation of the P2Y$_2$ receptor. Wild-type human 1321N1 astrocytoma cells were transfected with vector alone (pLXSN) or vector containing human P2Y$_2$ receptor cDNA (P2Y2R). Cells were prelabeled with [1-^{14}C]-AA (0.1 μCi; NEN, Boston, MA) for 4 h and then stimulated with ATP (100 μ*M*) or UTP (100 μ*M*) for 30 min at 37°C. Results are expressed as net AA release as a percentage of unstimulated controls and are means ±SD of triplicate samples representing one typical experiment.

might be the result of the absence of functional *sPLA$_2$*-IIA in astrocytes in C57B1/6 mice as a result of missense mutation in the *sPLA$_2$*-IIA gene *(68).*

Because several P2Y receptor subtypes are expressed in astrocytes, multiple receptors could mediate ATP-stimulated AA release. We have demonstrated a link between the P2Y$_2$ receptor and *cPLA$_2$* activation in human 1321N1 astrocytoma cells expressing a recombinant P2Y$_2$ receptor *(63,69).* Overexpression of the human P2Y$_2$ receptor in 1321N1 cells that lack endogenous P2 receptors resulted in an increase in ATP/UTP-mediated AA release as compared to the wild-type cells and the vector-transfected controls (Fig. 4).

P2Y$_2$ receptor activation by ATP also might mediate the release of docosahexaenoic acid (DHA) in astrocytes *(70).* However, ATP-induced DHA release was shown to involve *iPLA$_2$* and not *cPLA$_2$,* and it was Ca^{2+} independent and inhibited by haloenol lactone suicide substrate, a selective *iPLA$_2$* inhibitor *(70).* Other receptor agonists such as bradykinin, glutamate, and thrombin also stimulated the release of AA and DHA in astrocytes *(70).* Endothelin, a GPCR agonist, also has been shown to cause AA release in astrocytes, although the pathway is not clearly understood *(71).*

Activation of *PLA$_2$* in Astrocytes in Response to Oxidative Stress

Although astrocytes generally have a more potent antioxidant defense system than neurons, this system is stressed when astrocytes become reactive as a result of an increased production of ROS. Excessive ROS production is known to cause oxidative modifications of lipids, proteins, and nucleic acids and has been implicated as a major underlying cause of neurodegenerative diseases, including stroke, traumatic brain injury, Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease. Oxidizing agents such as H_2O_2 can activate enzymes involved in phospholipid metabolism, in particular phospholipase D *(72–75).* H_2O_2 also stimulates *PLA$_2$* in astrocytes *(76)* and neurons *(77).* Exposure of astrocytes to H_2O_2 causes activation of intracellular kinases and increases the expression of c-fos and c-jun, immediate early proto-oncogenes *(76).* Some of the effects mediated by H_2O_2 were attributable to induction of *PLA$_2$* and

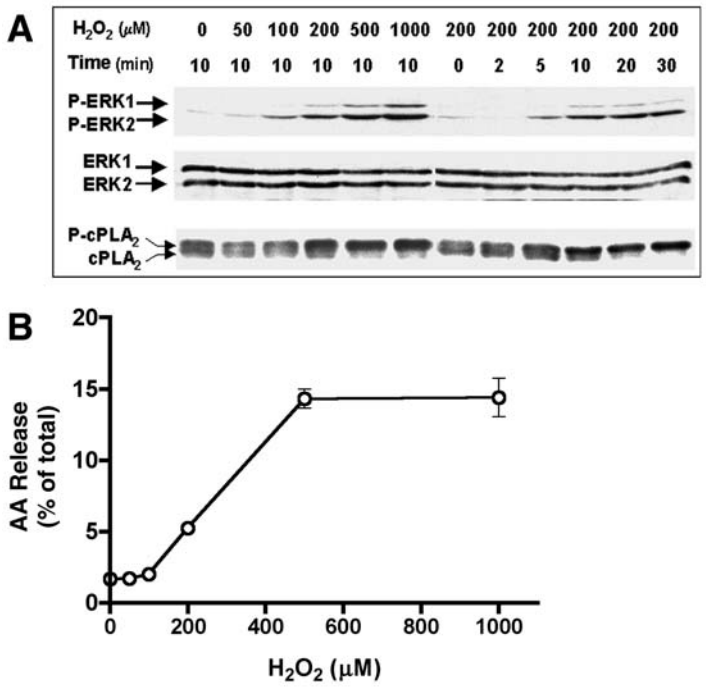

Fig. 5. **(A)** H_2O_2-stimulated phosphorylation of ERK1/2 and *cPLA₂*. Primary murine astrocytes were stimulated with the indicated doses of H_2O_2 for 0–30 min at 37°C. Then, cell lysates were subjected to Western analysis using anti-phospho-ERK, anti-total ERK and anti-*cPLA₂* antibodies. **(B)** H_2O_2-induced dose-dependent AA release from murine primary astrocytes. Primary astrocytes were prelabeled with [1-^{14}C]-AA (0.1 μCi; NEN, Boston, MA) for 4 h and then stimulated with the indicated concentration of H_2O_2 for 30 min at 37°C, as described elsewhere *(29)*. AA release is presented as a percentage of the total [^{14}C]-labeled AA in cell extracts and represents the means ± SD of results from three experiments performed in triplicate. (Reproduced with permission from ref. *29*.)

subsequent release of AA metabolites. In murine astrocytes, H_2O_2 can stimulate phosphorylation of ERK and *cPLA₂* as well as AA release (Fig. 5) *(29)*. Treatment of murine astrocytes with inhibitors of *cPLA₂* and *iPLA₂* suggests involvement of both types of *PLA₂* in mediating AA release. Treatment with U0126 and GF109203x completely inhibited phosphorylation of ERK and *cPLA₂* but only partially inhibited AA release, further suggesting that H_2O_2-mediated AA release in murine astrocytes is mediated by both *cPLA₂* and *iPLA₂* *(29)*. In mouse peritoneal macrophages, *iPLA₂* plays the major role in ROS-mediated AA release *(78)*. Similar to ATP-stimulated AA release, H_2O_2-stimulated AA release in murine astrocytes was not affected by downregulation of the conventional and novel PKC isoforms by prolonged treatment with PMA. Although it is known that tyrosine phosphorylation of PKC isoforms is induced by oxidative stress *(79)*, more studies are needed to define the roles of PKC isoforms and *PLA₂* in the mechanisms underlying the effects of oxidative stress.

Prostaglandin Production in Astrocytes

Prostaglandin synthesis is mediated by cyclooxygenases (COX-1 and COX-2) downstream of the *PLA₂* pathway and is an important step in the generation of an inflammatory response in astrocytes. COX-2 is a target for

nonsteroidal anti-inflammatory drugs in the treatment of inflammation *(80)*, and increased expression of COX-2 in astrocytes is associated with many pathological conditions *(81,82)*. COX-2 mRNA and protein expression in astrocytes are induced in response to the pro-inflammatory cytokines IL-1β and LPS *(83–86)*. ATP also can induce reactive gliosis and increase in COX-2 expression and prostaglandin production *(87)*. In addition, primary astrocytes treated with IL-β for 24 h became more sensitive to ATP-induced AA release, suggesting involvement of P2Y receptors and *cPLA$_2$* in the cytokine-mediated production of AA *(58)*.

Many mouse strains, including C57B1/6 mice, lack a functional *sPLA$_2$*-IIA gene *(68)*. Astrocytes from *sPLA$_2$*-IIA-negative mice are less responsive to cytokine-induced prostaglandin production as compared to astrocytes obtained from rat brain *(88)*. Rat astrocytes contain all three major types of *PLA$_2$* (e.g., *sPLA$_2$*, *cPLA$_2$*, and *iPLA$_2$*) as well as COX-1 and COX-2. Pro-inflammatory cytokines (TNF-α, IL-1β, and IFN-γ) can induce mRNA expression of *sPLA$_2$*-IIA and COX-2 and increase production of AA, PGE_2, and NO *(88)*. Although these cytokines did not alter the levels of *cPLA$_2$* protein (Fig. 6), an increase in *cPLA$_2$* phosphorylation was observed after cytokine treatment. Results in Fig. 6 show that astrocytes exposed to cytokines for 18 h became more responsive to ATP- or PMA-stimulated PGE_2 production. ATP- or PMA-induced PGE_2 production in cytokine-primed astrocytes was inhibited by NS-398, a COX-2-specific inhibitor *(88)*. Taken together, these results suggest that cytokines induce *sPLA$_2$* and COX-2 and enhance *cPLA$_2$* reactivity, and, together, these enzymes play an important role in inflammatory responses in astrocytes (Fig. 7).

Prostaglandins are known cause fever and pain *(80)*. Toyomoto et al. *(89)* demonstrated that PG can stimulate the production of nerve growth factors in cultured astrocytes. Activation of the PGE_2 receptor also might be linked to increased gene expression of amyloid precursor protein in neurons, thus enhancing the deposit of amyloid-β peptides *(90)*. Because ATP is released during neuronal injury, it is possible that ATP acts as a trophic factor to enhance PGE_2 production through activation of *cPLA$_2$*.

Summary and Future Direction

Many studies have demonstrated that *cPLA$_2$*, *iPLA$_2$*, and *sPLA$_2$* are activated in astrocytes in response to GPCR agonists, oxidative stress, and inflammatory agents. Astrocytes appear to be particularly sensitive to ATP/UTP, which act on the $P2Y_2$ receptor and stimulate signaling pathways leading to AA release. $P2Y_2$ receptor activation triggers an increase in intracellular Ca^{2+} mobilization and activates PKC and ERK1/2, which lead to phosphorylation and translocation of *cPLA$_2$* from the cytosol to membrane *(66)*. Astrocytes respond to oxidative agents such as H_2O_2, and the increase in AA release is contributed by both *cPLA$_2$* and *iPLA$_2$ (29)*. Pro-inflammatory cytokines have been shown to enhance transcription in astrocytes of oxidative and inflammatory genes, including *iNOS, sPLA$_2$*-IIA, and *COX-2*. Furthermore, cytokines enhanced *cPLA$_2$* phosphorylation and rendered it more responsive to stimulation by GPCR agonists or PMA. These studies demonstrate the involvement of *cPLA$_2$*, *sPLA$_2$*, and COX-2 in the AA/PG cascade in astrocytes *(88)*. It seems reasonable to conclude that reactive astrogliosis observed in a number of neurodegenerative diseases is coupled with enhancement of the *PLA$_2$*/COX cascade. There is evidence that cytokine-activated astrocytes can promote recovery of CNS function *(91)*. Obviously, further studies are needed to determine the physiological and pathological effects of reactive astrogliosis in the CNS, information that will define therapeutic approaches for the treatment of neurodegenerative diseases.

Although there is insufficient information regarding protein:protein interactions involving *PLA$_2$* in astrocyte membranes, studies with other cell types indicate that *cPLA$_2$* and *sPLA$_2$*

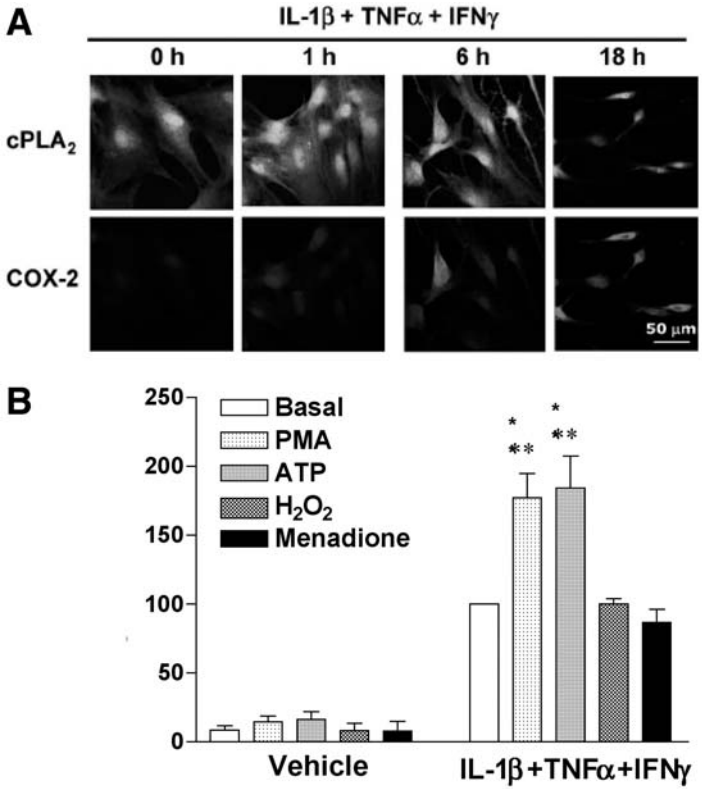

Fig. 6. **(A)** Immunocytochemical analysis of cytokine-induced *cPLA$_2$* and COX-2 expression in primary rat astrocytes. Astrocytes were incubated at 37°C with or without IL-1β (10 ng/mL), TNF-α (10 ng/mL), and IFN-γ (10 ng/mL) for the indicated time period. Cells were fixed and *cPLA$_2$* and COX-2 expression was determined by immunocytochemistry with *anti-cPLA$_2$* or anti COX-2 antibodies and secondary antibodies conjugated to Oregon Green *(cPLA$_2$)* or Texas Red (COX-2). **(B)** PGE_2 release from primary rat astrocytes in response to cytokines, PMA, ATP, H_2O_2, or menadione. Astrocytes were incubated with or without cytokine mixture [as in (A)] at 37°C for 18 h and then stimulated with vehicle (basal), PMA (100 n*M*), ATP (100 μ*M*), H_2O_2 (200 μ*M*), or menadione (100 μ*M*) at 37°C for 30 min. The medium was sampled and levels of PGE_2 were determined (see details in ref. *87*). The results are expressed as percentage increase relative to untreated (basal) controls. Data are the means ± SEM of results from three independent experiments performed in triplicate. *$p < 0.05$ compared to cytokine-treated controls. (Reproduced with permission from ref. *88*.)

interact with vimentin, a 57-kDa type III intermediate filament protein that is abundant in astrocytes *(8,67,92).* Vimentin is shown to facilitate $cPLA_2$ transport to the perinuclear area *(93)* and regulates trafficking of *cPLA$_2$* to the Golgi and lipid-rich vesicles involved in receptor internalization. There is also evidence that *PLA$_2$* are present in the caveolin and that activation of endogenous *PLA$_2$* activity within this lipid-rich structure can modulate the binding of receptor ligands *(13,41,94,95).* Furthermore, the presence of an ankyrin-binding motif in *iPLA$_2$* suggests that this *PLA$_2$* can interact with other proteins. This might have physiological relevance in astrocytes, because *iPLA$_2$* is the most abundant *PLA$_2$* isoform expressed in the brain *(96).* Future studies should focus on interactions between these and other cytoskeletal proteins to better understand how *PLA$_2$* activity is targeted to different subcellular sites to modulate astrocyte functions related to neurodegenerative diseases.

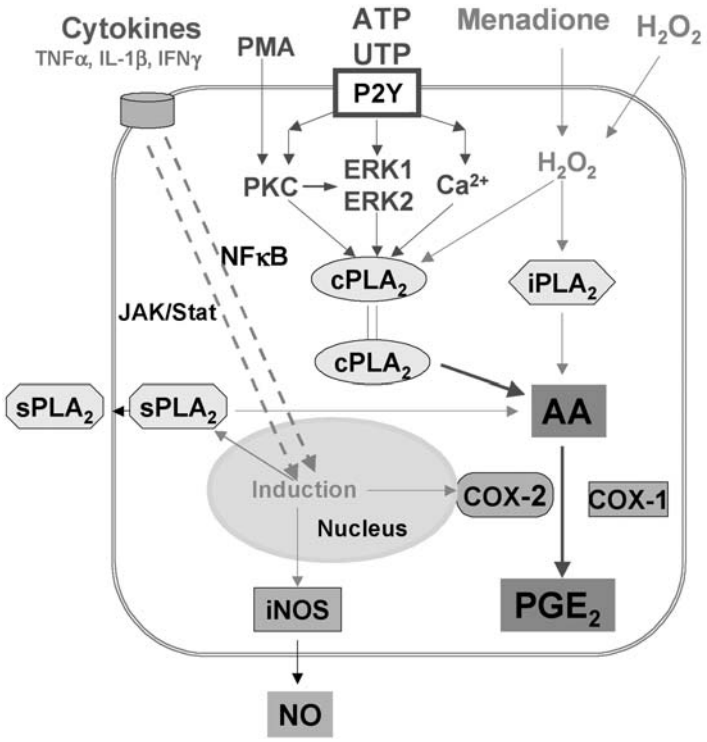

Fig. 7. Factors contributing to AA release and PGE_2 production in astrocytes. (Reproduced with permission from ref. *88.*)

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