Published in final edited form as:

Mol Neurobiol. 2014 August ; 50(1): 6–14. doi:10.1007/s12035-014-8662-4.

Role of cytosolic phospholipase A₂ in oxidative and inflammatory signaling pathways in different cell types in the central nervous system

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Abstract

Phospholipases A₂ (PLA₂s) are important enzymes for metabolism of fatty acids in membrane phospholipids. Among the three major classes of PLA₂s in the mammalian system, the group IV calcium-dependent cytosolic PLA₂ alpha (cPLA₂α) has received the most attention because it is widely expressed in nearly all mammalian cells and its active participation in cell metabolism. Besides Ca²⁺ binding to its C-2 domain, this enzyme can undergo a number of cell-specific post-translational modifications, including phosphorylation by protein kinases, S-nitrosylation through interaction with nitric oxide (NO), as well as interaction with other proteins and lipid molecules. Hydrolysis of phospholipids by cPLA₂ yields two important lipid mediators, arachidonic acid (AA) and lysophospholipids. While AA is known to serve as a substrate for cyclooxygenases and lipoxygenases, which are enzymes for synthesis of eicosanoids and leukotrienes, lysophospholipids are known to possess detergent-like properties capable of altering micro-domains of cell membranes. An important feature of cPLA₂ is its link to cell surface receptors that stimulate signaling pathways associated with activation of protein kinases and production of reactive oxygen species (ROS). In the central nervous system (CNS), cPLA₂ activation has been implicated in neuronal excitation, synaptic secretion, apoptosis, cell-cell interaction, cognitive and behavioral function, oxidative-nitrosative stress and inflammatory responses that underline the pathogenesis of a number of neurodegenerative diseases. However, the types of extracellular agonists that target intracellular signaling pathways leading to cPLA₂ activation among different cell types and under different physiological and pathological conditions have not been investigated in detail. In this review, special emphasis is given to metabolic events linking cPLA₂ to activation in neurons, astrocytes, microglial cells, and cerebrovascular cells. Understanding the molecular

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Conflict of Interest

The authors declare no conflict of interest.

mechanism(s) for regulation of this enzyme is deemed important in the development of new therapeutic targets for treatment and prevention of neurodegenerative diseases.

Keywords

Arachidonic acid; cytosolic phospholipase A₂; neurons; astrocytes; microglial cells; cerebrovascular cells; ERK1/2; lipopolysaccharide; interferon gamma; ROS

Introduction

Fatty acids are essential components of phospholipids and are integral parts of cell membranes. Four decades ago, Dr. Nicolas Bazan made the novel observation that fatty acids in the brain undergo rapid release upon excitation due to electroconvulsive shock and cerebral ischemia [1, 2]. This observation, later regarded as the “Bazan effect”, has attracted extensive interest for neuroscientists to search for the underlying biochemical mechanism(s) for fatty acid release from membrane phospholipids. Subsequent studies led to evidence that fatty acids in membrane phospholipids undergo dynamic turnover through an “acylation-deacylation” mechanism mediated by phospholipases A₂ (PLA₂) and ATP-dependent acyl-coenzyme A: lysophospholipid acyltransferases. It is further recognized that this “acylation-deacylation” process provides an important mechanism for the release of selective fatty acids in different types of membrane phospholipids upon special demand.

Phospholipids in the central nervous system (CNS) are enriched with polyunsaturated fatty acids (PUFAs), and PLA₂s are the key enzymes for hydrolysis of the PUFAs in the *sn*-2 position of the glycerol moiety. More than 20 isoforms of PLA₂s are present in mammalian cells, and they are roughly divided into three major categories, namely, secretory (sPLA₂), calcium independent (iPLA₂), and calcium-dependent (cPLA₂). In recent years, there are increasing interests to uncover the role of different PLA₂s in regulating cell functions under physiological and pathological conditions [3–6]. Arachidonic acid (AA) and docosahexaenoic acid (DHA) are two important PUFAs offering unique physiological functions to the brain. While the release of AA has been linked to action of cPLA₂, the PLA₂ for release of DHA is less clear, although action of iPLA₂ has been suggested [7, 8]. AA is substrate for cyclooxygenases and lipoxygenases, and serves as the precursor for synthesis of eicosanoids and prostanoids that mediate a wide variety of inflammatory responses [9]. On the other hand, DHA is effective in mediating anti-inflammatory and neuroprotective responses [8], and is the precursor for biosynthesis of neuroprotectin D (NPD1) and resolvins [9–14]. An important goal here is to provide a comprehensive review of the extracellular agonists and receptor signaling pathways regulating cPLA₂ activity and/or expression in different cell types in the CNS.

The calcium-dependent group IV cytosolic PLA₂ (cPLA₂α) is a 97 kDa protein constitutively expressed in nearly all brain cells. Investigation on cPLA₂ has gained special attention lately because not only is its requirement for calcium binding in the C2 domain, this molecule can also undergo a number of post-translational modifications through phosphorylation and S-nitrosylation. In particular, several active serine/threonine residues are susceptible to phosphorylation by protein kinases that are involved in important

signaling pathways, e.g., ERK1/2 and p38 MAPK at Ser505, Mnk1 at Ser727, and CaMKII at Ser515 [15]. In human epithelial cells, S-nitrosylation by nitric oxide (NO) of an active cysteine residue can enhance cPLA₂ activity by several folds [16]. cPLA₂ also contains cationic domains for binding zwitterionic lipids such as phosphatidylinositol 4,5-bisphosphate (PIP₂) [5], ceramide-1-phosphate (C1P), and lactosylceramide [5, 17, 18]. These properties suggest active modulation of cPLA₂ through interactions with lipids and intracellular signaling molecules.

In the CNS, cPLA₂ activation has been implicated in the pathogenesis of a number of neurodegenerative diseases, including experimental autoimmune encephalomyelitis [19, 20], Alzheimer's disease [21–26], Neimann-Pick disease [27], tumorigenesis [28], stroke [29–31], alcoholism [32, 33] as well as a number of affective disorders [34]. However, few studies have elucidated the extracellular agonists and signaling pathways leading to cPLA₂ activation in these disease conditions.

Role of cPLA₂ in neuronal excitation and synaptic plasticity

Neuronal stimulation by ionotropic glutamate receptor agonists, such as N-methyl-D-aspartic acid (NMDA), is known to elicit a massive and rapid influx of Ca²⁺ and leading to activation of Ca²⁺-dependent enzymes, mitochondrial dysfunction and neuronal apoptosis. Activation of the NMDA receptor is also marked by a rapid production of reactive oxygen species (ROS) which is attributed to activation of NADPH oxidase [35, 36]. Studies in our laboratory further linked NMDA-induced ROS production with activation of the MEK1/2-ERK1/2 pathway and phosphorylation of cPLA₂ [35]. These findings demonstrated activation of cPLA₂ together with neuronal excitation and oxidative stress. In primary cultures of cortical and hippocampal neurons, treatment with a PLA₂ inhibitor methylarachidonyl-fluorophosphonate (MAFP), resulted in altered neuronal morphology, and reduced neurite outgrowth and viability [37]. Stereotaxic injection of the PLA₂ inhibitor to the hippocampal CA1 area also caused a reduction of neuronal membrane fluidity which is thought to contribute to impairment in memory function [38].

In the peripheral nervous system, cPLA₂ is implicated in injury of primary sensory neurons and pain behavior (tactile allodynia) [39]. The increase in cPLA₂ activity is attributed to stimulation of the ionotropic P2X receptors and subsequent activation of MAPK and CaMKII [40]. In fact, phosphorylation of cPLA₂ by CaMKII was also observed in vascular smooth muscle cells following stimulation by norepinephrine. Under this condition, phosphorylation of cPLA₂ by CaMKII (at S515) occurred prior to the phosphorylation by ERK1/2 (at S505), and complete phosphorylation by both kinases were required for AA release [41]. There is further evidence that upon stimulation by norepinephrine, CaMKII promoted translocation of cPLA₂ from cytosol to the nuclear envelope [42]. In fact, cPLA₂ and CaMKII appeared to be present in close proximity in the dorsal root ganglion cells, and stimulation of the ganglion cells with ATP promoted the translocation of p-cPLA₂ to the plasma membrane [40]. Since CaMKII is regarded an important protein kinase in modulation of synaptic plasticity and long-term potentiation [43], these studies presented a possible link between CaMKII and cPLA₂ in modulating neuronal activities under disease conditions.

Organization of the pre- and post-synaptic structures is extremely complex. The presence of cPLA₂ in the synaptic area suggests possible role for this enzyme in regulating synaptic activity. There is evidence for cPLA₂ to modulate vesicle formation, for promoting neurite outgrowth and maintenance of growth cone activity. In the dorsal root ganglion, Semaphorin 3A (a class of secreted and membrane protein) can trigger a signaling pathway involving cPLA₂ activation, AA release and synthesis of 12(S)-hydroxyeicosatetraenoic acid (12(S) HETE), and in turn, mediates neuronal growth cone activity [44]. In agreement with the role of cPLA₂ in neuronal membrane and synaptic activity, hippocampal neurons isolated from cPLA₂ knockout (KO) mice showed measurable differences in the nuclei and soma volume, and structure of the synaptic cleft as compared with neurons from wild-type animals [45]. In the hippocampus and cerebellum, the PKC-ERK1/2-cPLA₂ pathway has also been implicated in long-term synaptic plasticity and regulation of AMPA receptor trafficking [46].

Albeit at lower levels as compared to stimulation by NMDA, oligomeric amyloid-beta peptide (A β) can also stimulate ROS production in neurons and subsequently, phosphorylate ERK1/2 and cPLA₂, [35]. However, prolonged exposure of neurons to A β led to a decrease in NMDA response and an increase in mitochondrial dysfunction [47]. Studies by Kriem's group demonstrated the ability for A β to activate cPLA₂ and subsequently mitochondrial dysfunction and neuronal apoptosis [21, 48]. Besides stimulation of phospho-cPLA₂, A β exposure also led to activation of neutral sphingomyelinase and ceramide, and together, neuronal apoptosis [49]. Mitigation of ROS production in neurons, such as that using gp91ds-tat, a specific inhibitor for the gp91phox subunit of NADPH oxidase, could protect neurons from the deleterious effects of A β [47]. Studies with cPLA₂ deficient mice, as well as with different experimental models and pharmacological agents, have also demonstrated the critical role for cPLA₂ in neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and stroke [50–55]. These studies further underscore the role of cPLA₂ in neuronal metabolic signaling pathways linking to Ca²⁺ homeostasis, protein kinases activation, and oxidative-nitrosative stress.

cPLA₂ in oxidative and inflammatory signaling pathways in astrocytes

Our earlier studies with astrocytes demonstrated the ability for ATP/UTP to stimulate G-protein-coupled P2Y₂ receptor and signaling pathways leading to activation of PKC-dependent and independent phosphorylation of ERK1/2 and cPLA₂ [56]. Besides ATP, phorbol ester (PMA) can also increase in phospho-cPLA₂, AA release and production of prostaglandin E2 (PGE2) in astrocytes [57]. This last study further demonstrated a priming effect for pro-inflammatory cytokines (such as TNF α , IL-1 β and IFN γ) to enhance the production of PGE2 following short term exposure to ATP and PMA [57]. Other agents such as lipopolysaccharides (LPS) can stimulate cPLA₂ and PGE2 production through an ERK1/2-dependent pathway in astrocytes [58]. In fact, a number of other stimuli, including ammonia [59], alcohol [60], ceramide [61], bradykinin [62, 63], and diethylmaleate/iodoacetate [64] have been shown stimulate cPLA₂ and engage in astrocytic inflammatory and oxidative pathways.

Aggregated A β can also confer cytotoxic effects to astrocytes. Using specific inhibitors for cPLA $_2$ and iPLA $_2$, the study by Zhu et al. (2006) demonstrated ability for A β to incur a time-difference activation of cPLA $_2$ and iPLA $_2$ in alteration of mitochondrial membrane depolarization [65]. Oligomeric A β (1–42) increased phosphorylation of cPLA $_2$ in astrocytes through activating the NADPH oxidase and MAPK pathways, and subsequently, this led to perturbation of mitochondrial function, impairment in ATP production, and increase in oxidative stress. Menadione, a redox-active compound capable of inducing intracellular ROS production in astrocytes, was shown to stimulate cPLA $_2$ through p38 MAPK and ERK1/2, and subsequently, leading to an increase in actin polymerization and cytoskeletal protrusions [66]. Astrocyte plasma membranes became more molecularly ordered due to oxidative stress induced by menadione [66]. Down-regulation of cPLA $_2$, either with a pharmacological inhibitor or by RNA interference, could abrogate the menadione-induced morphological changes in astrocytes. These studies suggest an important role for ROS in activation of astrocytes, and signaling pathways involving cPLA $_2$ to elicit biochemical, morphological, and biophysical changes reminiscent of reactive astrocytes in pathological conditions.

cPLA $_2$ in oxidative and inflammatory signaling pathways in microglial cells

Microglial cells are myeloid lineage cells and the principle resident immune cells in the CNS. Microglial cells exhibit multiple functions and are regarded as a major source for inflammatory responses associated with different types of brain injury. Microglial cells respond vividly to LPS which stimulates cPLA $_2$ and PGE $_2$ production through a signal transduction pathway involving sphingomyelinase and p38 MAPK [67]. ATP also can induce cPLA $_2$ and PGE $_2$ release from microglial cells and this action is attributed to the activation of P2X $_7$ receptors [68]. However, PGE $_2$ production due to stimulation of P2X $_7$ receptors appeared to involve COX1 and not COX2. In another study, infection of macrophage with *Candida albicans* resulted in rapid activation of cPLA $_2$, AA release, and production of eicosanoids, and the production of PGE $_2$ was also mediated through COX1 [69]. Since COX1 is constitutive as compared to COX2 which is induced through the NF- κ B pathway, these studies demonstrate that rapid response of cPLA $_2$ to agonists can produce inflammatory events without involvement of the transcriptional processes. Chronic infusion of LPS to brains has been shown to cause inflammatory responses with increases in TNF α , iNOS and microglial activation [70]. Under this condition, the increase in cPLA $_2$ and production of 5-LOX was attributed to the action of COX-2.

Studies from our laboratory have demonstrated the involvement of ERK1/2 in LPS-IFN γ -induced production of NO and ROS in microglial cells [71, 72]. In agreement with results from a study by Ribeiro et al. (2013), our study also indicated an increase in phospho-cPLA following LPS-IFN γ treatment (unpublished data) [73]. A study with rat primary microglial cells further showed an increase in the expression of total cPLA $_2$ at 6–8 hours after treatment with LPS [74]. In the BV-2 microglial cells, LPS-induced cPLA $_2$ activation was mediated by ERK1/2 and JNK but not p38 MAPK [73]. Furthermore, cPLA $_2$ siRNA or its inhibitor, AACOCF $_3$, attenuated LPS-induced NO and ROS production as well as iNOS and p67phox expression in microglial cells [73, 74]. Taken together, these studies demonstrated the critical role of cPLA $_2$ in mediating inflammatory responses in microglial cells.

Superoxide anions generated by NADPH oxidase can react with NO to form peroxynitrite (ONOO⁻), a highly toxic radical with potent ability to damage cell membranes. Oxidation of PUFAs in membrane phospholipids can produce 4-hydroxy-2-nonenal (4-HNE), another reactive lipid peroxidation product which can form protein adducts [75] and thus is used as a good marker for assessing oxidative stress in brain tissue and brain injury [36]. In a study with the Ra2 murine microglial cells, 4-HNE was shown to upregulate cPLA₂ expression as well as increased phosphorylation through a pathway involving ERK1/2 and p38 MAPK [75].

Similar to neurons and astrocytes, aggregated A β can also confer toxic effects on microglial cells, as demonstrated by increased production of ROS and upregulation of phospho-cPLA₂ expression and cPLA₂ activity [76]. Antisense cPLA₂ and pyrrophenone, a cPLA₂ specific inhibitor, were effective in abolishing ROS, iNOS and PGE₂ production induced by A β .

IFN γ , or type II interferon, is a cytokine critical for innate and adaptive immunity against viral and bacterial infections, and in autoinflammatory and autoimmune diseases. Although IFN γ is produced predominantly by natural killer T cells and lymphocytes, microglial cells are capable of responding to this cytokine, which is known to stimulate the canonical JAK-STAT pathway for producing transcription factors such as interferon-gamma-activated sites (GAS) and IFN regulatory factors (IRF). In microglial cells, activation of GAS is necessary for induction of the iNOS gene by IFN γ and LPS [77]. In our study with immortalized microglial cells (BV-2 and HAPI), IFN γ not only can activate the canonical JAK-STAT pathway but also induce a non-canonical pathway involving Raf-Ras and MEK1/2, which in turn, lead to activation of ERK1/2 [72] as well as cPLA₂ (unpublished data). Indeed, IFN γ -induced stimulation of p-ERK1/2 has become a key signaling pathway for activation of a number of cytoplasmic proteins, including NADPH oxidase subunits for ROS production, filopodia formation, and IKK α for the NF- κ B pathway in these microglial cells (Fig 1).

Spinal microglial cells are activated during spinal cord injury and have been implicated in the pathogenesis of neuropathic pain [78]. Spinal microglial cells are susceptible to stimulation by LPS which induces the increase in COX-1 and COX-2 and production of PGE₂ and NO through the p38 MAPK pathway [79]. Interestingly, a recent study indicated a role for lysophosphatidic acid (LPA) for microglial stimulation upon spinal cord injury and neuropathic pain [80]. In addition, this study further demonstrated that NMDA and neurokinin 1 receptors, cPLA₂, iPLA₂ and microglial activation, as well as LPA1 and LPA3 receptors, were all involved in nerve injury-induced LPA production and neuropathic pain [81]. Obviously, more studies are needed to investigate the underlying mechanisms linking spinal microglial cells and neurons in neuropathic pain.

cPLA₂ and signaling pathways in cerebrovascular cells

The brain's microvascular structure is a multicellular complex comprised of endothelial cells, astrocytes and pericytes, and together, these cells are responsible for many biological processes such as regulation of the blood-brain barrier (BBB), angiogenesis, transmigration of monocytes, and atherogenesis. Previous studies have shown that activation of the PKC-ERK1/2-cPLA₂ pathway is important in endothelial functions [82]. Similar to astrocytes,

cPLA₂ phosphorylation/activation in cerebral endothelial cells is regulated by oxidative signaling through NADPH oxidase and ERK1/2 [83]. Vascular endothelial growth factor (VEGF) is important in endothelial angiogenesis and is responsible for cell growth, migration, and tubulogenesis in endothelial cells. VEGF receptors are coupled to the downstream signaling pathway involving activation of Src-PLD1-PKC γ , and in turn, stimulation of cPLA₂ [84]. The important role of cPLA₂ in mediating VEGF-induced DNA synthesis, migration, and tube formation in human retinal microvascular endothelial cells was further supported by depleting cPLA₂ with siRNA [84].

Gram negative pathogens, such as *E. coli*, can invade brain microvascular endothelial cells and cross the BBB, and this type of infection can be a major cause of neonatal meningitis [82]. PLA₂ and their metabolites have been implicated in mediating communication between endothelial cells and pericytes through signaling pathways involving PKC α and MAPK/ERK [85, 86]. In a study with human brain microvascular endothelial cells, cPLA₂, AA release and synthesis of cysteinyl leukotrienes (LTs) was induced upon an invasion of Group B *Streptococcus*, a pathogen important in the development of neonatal meningitis [87]. *Cronobacter sakazakii*, another opportunistic pathogen that targets endothelial cells, is involved in neonatal sepsis and meningitis through activation of the PI3K/Akt and cPLA₂ signaling pathway leading to disruption of actin microfilaments [88]. The important role of cPLA₂ in *C. sakazakii*-induced Akt signaling and actin rearrangement can be demonstrated by treating cells with the cPLA₂ inhibitor AACOCF₃. Results of these studies well demonstrated the important role of cPLA₂ in mediating damaging effects induced by pro-inflammatory pathogens.

Targeting cPLA₂ and upstream signaling pathways to mitigate neurodegenerative processes

Increased oxidative stress and chronic inflammation are common factors associated with many age-related neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and stroke. In a cerebral ischemia model induced by middle cerebral artery occlusion (MCAO) in mice, a transient and rapid increase in total and phosphorylated-cPLA₂ expression was observed in the ischemic hemisphere immediately after MCAO and returned to control levels by 2 hours after reperfusion [55]. The early increase in cPLA₂ expression was coupled to the increase in COX-2, oxidative stress, and upregulation of phospho-p38 and pERK1/2 MAPK [55]. In another study using the MCAO model in rats, increases in expressions of phospho-p38 MAPK were observed from 6 hours and phospho-cPLA₂ three days after ischemia/reperfusion [89]. Under this condition, administration of the p38 MAPK inhibitor SB203580 could suppress phospho-cPLA₂ activation and attenuate BBB extravasation and subsequent induction of edema due to ischemia/reperfusion. AACOCF₃, the cPLA₂ inhibitor, is also effective in reducing ischemia-induced infarction [90]. Recent studies also suggest effective application of AACOCF₃ to ameliorate injuries in animal models of other neurodegenerative diseases, including experimental autoimmune encephalomyelitis [74].

There is increasing evidence supporting the use of botanical antioxidants to suppress oxidative and inflammatory pathways in different cell types in the CNS. A recent

comprehensive review outlined the mechanisms of actions of a number of flavonoids found in cocoa, tea, berries and citrus, to mitigate multiple age-associated events including impaired cognition and neuro-inflammation [91]. As an example, the leaf extract of *Centella asiatica*, used as an alternative medicine for memory improvement in the Indian Ayurvedic system, was shown to inhibit cPLA₂ and sPLA₂ activities in primary cortical neurons [92]. Another study with *Ginkgo biloba* extract (EGb761) showed the contribution of cPLA₂ and ERK1/2 to the signaling pathway to protect spinal cord neurons from glutamate excitotoxicity [93]. Although our studies did not include analysis of cPLA₂, we demonstrated the ability of EGCG from green tea [47] and honokiol, a polyphenol from Magnolia bark, to inhibit NMDA-induced neuronal excitation and ROS production from primary cortical neurons [72]. Study with *Buddleja officinalis* extract, a traditional Korean herbal medicine used to treat strokes, headaches, vascular diseases and neurological disorders, was shown to exert anti-inflammatory effects (LPS-induced iNOS) by suppressing the ERK1/2 and NF-κB pathways [94]. Wogonin, an active component from the root of the Chinese herbal plant, *Huang qin*, was shown to attenuate endotoxin/IFNγ-induced PGE₂ and NO production in microglial cells through the Src-ERK1/2-NF-κB pathway, albeit the exact involvement of cPLA₂ remains to be further demonstrated [95].

Conclusions and perspectives

Taken together, these studies suggest involvement of cPLA₂ in oxidative and inflammatory signaling pathways and provide evidence for its link to multiple cell specific receptors and agonists in the CNS. These characteristic properties of cPLA₂ underscore its role in regulating cell metabolism under different physiological and pathological conditions. Studies here also highlight an important link between cPLA₂ and MAPKs, especially ERK1/2 and p38 MAPK in different cell types. Recognizing this metabolic link is important because these kinases are involved in phosphorylation of many other cellular proteins, including phosphorylation of NADPH oxidase subunits required for ROS production [96]. In both neurons and microglial cells, signaling pathways leading to ERK1/2, ROS, and cPLA₂ activation have been shown to explain neuronal excitotoxicity as well as glial oxidative and inflammatory responses [35, 71]. Our studies with microglial cells further place ERK1/2 and cPLA₂ in a cross-talk mechanism between NF-κB and JAK-STAT pathways induced by LPS and IFNγ, respectively (Fig. 1). Since other types of PLA₂ are present in these cells, future studies should be directed to understanding the relationship between cPLA₂ and other PLA₂s, in particular, iPLA₂ and sPLA₂, in mediating inflammatory responses.

Acknowledgments

This work was supported in part by NIH Grants 2P01 AG08357 from NIA and P50AT006273 from the National Center for Complementary and Alternative Medicines (NCCAM), the Office of Dietary Supplements (ODS), and the National Cancer Institute (NCI). The help of Ms. Deborah Ratliff to proof-read and edit the manuscript is much appreciated.

Abbreviations

Aβ amyloid beta peptides

AA	arachidonic acid
AACOCF₃	arachidonyl trifluoromethyl ketone
BEL	bromoenol lactone
COX	cyclooxygenase
CNS	central nervous system
CAMK-II	calcium/calmodulin-dependent protein kinase-II
cPLA₂α	cytosolic phospholipase A ₂ alpha
DHA	docosahexaenoic acid
ERK1/2	extracellular signal regulated kinases 1/2
IFN_γ	interferon gamma
LOX	lipoxygenase
LPS	lipopolysaccharides
MAFP	methyalarachidonyl-fluorophosphonate
MAPK	mitogen activated protein kinases
NMDA	N-methyl-D-aspartic acid
NO	nitric oxide
PGE₂	prostaglandin E ₂
PKC	protein kinase C
PMA	phorbol myristoyl acetate
PUFA	polyunsaturated fatty acids
ROS	reactive oxygen species

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Fig. 1. cPLA2 in oxidative and inflammatory signaling pathways in microglial cells

A graphical illustration depicting the role of cPLA2 in oxidative and inflammatory signaling pathways in microglial cells. This figure demonstrates the multiple signaling pathways leading to phosphorylation of ERK1/2 and cPLA2 and the multiple links for ERK1/2 to other metabolic processes including phosphorylation of NADPH oxidase subunits leading to production of ROS and development of filopodia.