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Mini Review

Phospholipase A_2, reactive oxygen species, and lipid peroxidation in CNS pathologies

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The importance of lipids in cell signaling and tissue physiology is demonstrated by the many CNS pathologies involving deregulated lipid metabolism. One such critical metabolic event is the activation of phospholipase A_2 (PLA_2), which results in the hydrolysis of membrane phospholipids and the release of free fatty acids, including arachidonic acid, a precursor for essential cell-signaling eicosanoids. Reactive oxygen species (ROS, a product of arachidonic acid metabolism) react with cellular lipids to generate lipid peroxides, which are degraded to reactive aldehydes (oxidized phospholipid, 4-hydroxynonenal, and acrolein) that bind covalently to proteins, thereby altering their function and inducing cellular damage. Dissecting the contribution of PLA_2 to lipid peroxidation in CNS injury and disorders is a challenging proposition due to the multiple forms of PLA_2, the diverse sources of ROS, and the lack of specific PLA_2 inhibitors. In this review, we summarize the role of PLA_2 in CNS pathologies, including stroke, spinal cord injury, Alzheimer's, Parkinson's, Multiple sclerosis-Experimental autoimmune encephalomyelitis and Wallerian degeneration. [BMB reports 2008; 41(8): 560-567]

INTRODUCTION

Phospholipids are important components of all mammalian cells, and perform a number of biological functions, including the following: 1) form lipid bilayers that provide the structural integrity necessary for protein function, and 2) serve as precursors for a variety of lipid second messengers. Deregulated lipid metabolism is of particular salience in the context of CNS disorders and injuries, as the brain has the highest lipid concentration after adipose tissue. Recent reviews on lipid signaling in CNS pathologies (1-3) and other disorders (4) are now available. This review focuses specifically on the role of phospholipase A_2 (PLA_2) in CNS injury and disorders. The role of ROS in conjunction with lipoxygenases will be discussed by Dr. Jae-Hong Kim in the accompanying mini-review.

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PLA_2 enzymes occur in multiple forms

PLA_2 enzymes cleave fatty acids at the *sn*-2 position of glycerophospholipids, thereby yielding free fatty acids and lysophospholipids. This reaction is of particular importance when the fatty acid released from the *sn*-2 position is arachidonic acid (ArAc), as it can be metabolized by cyclooxygenases and lipoxygenases into a variety of bioactive eicosanoids, including prostaglandins, thromboxanes, and leukotrienes. The other PLA_2 products, namely the lysophospholipids, are also biologically active and are crucial to cell signaling (5, 6).

Nearly 22 different PLA_2 enzymes have been identified in a variety of mammalian tissues (5), and have been broadly classified into three families on the basis of their calcium requirement for catalytic activity, as calcium-dependent cytosolic ($cPLA_2$) and secretory ($sPLA_2$) types and calcium-independent forms ($iPLA_2$). The PLA_2 enzymes are currently systematically classified into groups on the basis of their nucleotide and amino acid sequences. The $cPLA_2$s (α, β, γ; group IVA, IVB, and IVC) have molecular masses of 85, 114 and 61 kDa, respectively. $cPLA_2$a requires μM Ca^{2+} concentrations for binding to lipid substrates, and preferentially hydrolyzes ArAc in the *sn*-2 position of phospholipids. $cPLA_2$a is regulated via phosphorylation on Ser-505, Ser-727, and Ser-515 mediated by MAPK, MEK1, and calcium- and calmodulin-dependent kinase II, respectively, although the mechanism underlying the regulation of $cPLA_2$a by phosphorylation remains poorly understood (7). The $sPLA_2$s (groups I, II, III, V, X and XII) have low molecular weight (14-19 kDa), usually require mM calcium for catalytic activity, and lack specificity for the fatty acid in the *sn*-2 position. In addition to their catalytic activity as phospholipases, some forms of $sPLA_2$ can stimulate a variety of biological responses via binding to high-affinity transmembrane PLA_2 receptors. The $iPLA_2$s (group VI) have molecular masses of 84-88 kDa (8), are not selective for ArAc, and perform a crucial function in the regulation of the cell cycle through membrane phospholipid remodeling (9) . Platelet activating factor acetylhydrolases comprise PLA_2 groups VII and VIII, have molecular masses of 26-45 kDa, and can also cleave short-chain oxidized fatty acids (up to nine carbons) in the *sn*-2 position of phosphatidylcholine (PC) or phosphatidy-

lethanolamine. Lipoprotein-PLA_2 (Lp-PLA_2) is a 45 kDa group VIIA PLA_2 found in the circulating blood, and is associated with apo-B100 of LDL. Elevated Lp-PLA_2 level are associated with coronary heart disease, stroke, and dementia (10). The group VII and VIII PLA_2s may perform a protective function by removing the fatty acids damaged by oxidative stress. All major groups of PLA_2 are present in the central nervous system (2, 11).

cPLA$_2$ in cerebral ischemia

In vitro

A number of studies have demonstrated upregulation of PLA_2 in a variety of cerebral ischemia models. Organotypic hippocampal slice cultures were subjected to oxygen glucose deprivation (OGD) and re-oxygenation, an *in vitro* model for cerebral ischemia. PLA_2 activity was increased by 2-fold as compared to the controls in the hippocampal pyramidal cell layer immediately after 35 min of OGD, and the levels remained elevated at 24 h re-oxygenation. The use of PLA_2 inhibitors ($AACOCF_3$ to inhibit cPLA$_2$ and iPLA$_2$, BEL for iPLA$_2$, and LY311727 for sPLA$_2$) indicated that PLA_2 activation after OGD was attributable to cPLA$_2$. Inhibition of cPLA$_2$ also attenuated OGD-induced neuronal death, thereby indicating that cPLA$_2$ is involved in ischemic injury (5). The results of recent studies have demonstrated that the release of lysosomal cathepsins induced CA_1 hippocampal neuronal death via NMDA-mediated calcium influx, activation of cPLA$_2$, ArAc release, and subsequent ROS production in rat hippocampal slices subjected to OGD (12).

Forebrain ischemia

cPLA$_2$ gene expression was assessed in the rat brain via *in situ* hybridization. In the normal rat brain, faint signals were detected in hippocampal CA_1-CA_3 neurons and dentate granule cells. After 10 min of transient forebrain ischemia in male Wistar rats, the cPLA$_2$ gene was expressed intensely in dentate granule cells, which remain viable after transient ischemia, at 12 and 24 h of reperfusion. Expression in CA_1-CA_3 was increased slightly at 6 and 12 h, but returned to control levels by 24 h (5).

Increased cPLA$_2$ immunoreactivity as compared to shams was detected in astrocytes within the CA_1 region at 24 h after 30 min of 4-vessel occlusion, a time prior to histologically apparent neuronal damage. Intense labeling for cPLA$_2$ was noted throughout the hippocampal CA_1 region at 72 h after ischemia, at which time necrosis of the CA_1 neurons was observed. cPLA$_2$ was expressed selectively in activated microglia and astrocytes in regions of neurodegeneration. cPLA$_2$ activity and protein expression were increased in the hippocampal extracts at 72 h reperfusion (5).

Focal cerebral ischemia

24 h after permanent occlusion of the MCA, monocytes and macrophages were profoundly cPLA$_2$ immunoreactive and were localized principally to the core of the infarct. cPLA$_2$ immunoreactive astrocytes were detected in the penumbral region (5).

The PLA_2 inhibitor, quinacrine, ameliorated neurological deficits and reduced infarction after 2 h of transient focal cerebral ischemia (6). The results of these studies provide evidence that PLA_2 contributes to ischemic injury; however as quinacrine is a general PLA_2 inhibitor, these results do not indicate which form of PLA_2 was involved in the ischemic injury (6). Indirect evidence has been provided by studies showing that the inhibition of ArAc metabolism attenuated cerebral ischemia-induced oxidative injury, blood-brain barrier dysfunction, edema, infarction, and hippocampal neuronal death (5).

Transgenic mice deficient in cPLA$_2$ were generated via targeted disruption of its gene. After transient focal cerebral ischemia, cPLA$_2$ deficient mice evidenced smaller infarcts and fewer neurological deficits as compared to wild-type (5, 8), demonstrating a role for cPLA$_2$ in ischemic injury. We have observed no changes in total cPLA$_2$ protein expression following transient focal cerebral ischemia (13), a result which is consistent with those of other studies in which cPLA$_2$ mRNA expression evidenced no changes at 3 d after ischemia (14). In our studies, the antibody was not specific for phosphorylated cPLA$_2$ and changes in phospho-cPLA$_2$ would probably not have been detected (13). Aβ-induced phospho-cPLA$_2$ changes were apparent in AD models (15, 16). A knockout animal may not provide us with a clear understanding of the normal function of a specific gene product, as the overall phenotype can result from primary gene loss in addition to adaptive responses occurring during development and maturation (17). It should be noted that the C57BL/6J and 129/SV mouse strains utilized for transgenic studies harbor a naturally occurring mutation in the gene for sPLA$_2$ IIA (5, 8), and thus the cPLA$_2$ knockout mice were deficient in both cPLA$_2$ and sPLA$_2$ IIA. Transgenic mice expressing the human sPLA$_2$ IIA gene have been developed and are currently available from Taconic, but this mouse strain apparently has yet to be employed in stroke research to assess the function of sPLA$_2$ IIA.

Unilateral hypoxia-ischemia

After 15 min of unilateral hypoxia-ischemia in 21-d-old male Wistar rats, cPLA$_2$ immunoreactivity was selectively higher in the reactive glia in the hippocampal CA_1 region undergoing delayed neuronal death at 3 and 7 d after injury. Immunoreactivity for cPLA$_2$ was low in all regions of the uninjured control hemisphere (5).

cPLA$_2$ expression studies present the anomaly that mRNA was expressed principally in the neurons, whereas the expression of the protein was induced in the glia but was undetectable in the neurons. These differences cannot be explained by strain differences, as all of these studies used Wistar rats (5). Recent studies have demonstrated the induction of cPLA$_2$ by Aβ in cortical neuronal cultures, thereby indicating that neurons do express cPLA$_2$ protein; however, the contribution from residual non-neuronal cells in these cultures cannot be ruled out entirely (15).

sPLA$_2$ in cerebral ischemia

Forebrain ischemia

PLA_2 activities were increased significantly in the cytosolic, mi-

tochondrial, and microsomal fractions following global cerebral ischemia in gerbils, the majority of which were calcium-dependent, and measured 14 kDa, which is characteristic of $sPLA_2$. Similar results were reported in our studies of forebrain ischemia. PLA_2 activities were increased significantly in the mitochondrial and membrane fractions after 2 h of reperfusion. Maximum activity was observed at mM Ca^{2+}, thereby indicating that the membrane and mitochondrial fractions predominantly contain $sPLA_2$ (18). Quinacrine also attenuated CA_1 neuronal death in transient forebrain ischemia in the gerbil (6).

Group IIA $sPLA_2$s have been studied the most due to their involvement in inflammatory processes (19, 20). The expression of $sPLA_2$ II mRNA in rat brain after 20 min of transient forebrain occlusion evidenced biphasic increases at 1-6 h and 7 and 20 d post-ischemia (5 and references cited therein).

Focal cerebral ischemia

$sPLA_2$ IIA mRNA demonstrated biphasic increases in expression at 30 min of reperfusion after 60 min of focal cerebral ischemia and from 12 h to 3 d, which declined to sham levels by 7 d and then again increased at 14 d. The increase in $sPLA_2$ IIA mRNA occurred principally in the ischemic cortex, whereas the increases at 1 and 3 d were observed principally in the penumbra. The levels of $sPLA_2$ IIA protein were expressed principally in the penumbra at 3 d post-ischemia and the protein was localized in the GFAP-positive astrocytes but not in the microglia (14).

We also noted a 4-fold increase in $sPLA_2$ IIA mRNA within the ipsilateral cortex as compared to the contralateral cortex after 1 h of middle cerebral artery occlusion (MCAO) and 24 h of reperfusion in spontaneously hypertensive rats (13). $sPLA_2$ protein expression was increased significantly in the ipsilateral cortex over 7 d of reperfusion after 1 h of MCAO (21).

In cases of permanent MCAO, PLA_2 activity reached a maximum at 8 h and was increased by 2-fold as compared to the activity seen in the contralateral cortex. The $sPLA_2$ inhibitor, indoxam, significantly reduced infarct volume. *In vitro*, $sPLA_2$ IIA induced complete neuronal death in cultured rat cortical neurons, but exerted no detectable effect on the survival of astrocytes (5 and references cited therein).

PLA_2 in spinal cord injury (SCI)

The first event after SCI is the depolarization and opening of voltage-dependent ion channels, and the consequent massive release of neurotransmitters, including glutamate. The accumulation of intracellular calcium initiates mitochondrial dysfunction, and the activation of nitric oxide synthase (NOS) and PLA_2. The resultant generation of free radicals (comprised of different species including reactive nitrogen species (RNS), ROS and other radicals) and subsequent lipid peroxidation is thought to constitute a major pathway of secondary injury in SCI (22). PLA_2 activity and $cPLA_2$ expression were increased significantly after SCI; $cPLA_2$ expression was confined to neurons and oligodendrocytes. Mepacrine, a PLA_2 inhibitor, attenuated spinal neuronal death *in vitro* and PLA_2-induced demyelination *in vivo* (22).

PLA_2 in neurodegenerative diseases

The mRNA expression of pro-inflammatory $sPLA_2$ IIA was upregulated in Alzheimer's Disease (AD) brains as compared to non-dementia elderly brains. $sPLA_2$ IIA immunoreactive astrocytes in the AD hippocampus were associated with Aβ plaques (23). Aβ and NMDA induced $cPLA_2$ phosphorylation, and ArAc release via the activation of NADPH oxidase, ROS generation, and ERK1/2 activation in cortical neuronal cultures (15, 24). Aβ induced mitochondrial dysfunction through $iPLA_2$ and $cPLA_2$ in cultured cortical astrocytes (16).

Free radical generation and lipid peroxidation perform a significant function in Parkinson's Disease (PD). It is believed that one of the factors responsible for this is phospholipase activation in the substantia nigra, a notion that is supported by the fact that $cPLA_2$ deficient mice are resistant to 1-methyl-4-phenyl-1,2,3,6-tetrohydropyridine (MPTP) induced neurotoxicity (6), an animal model for PD.

Experimental autoimmune encephalomyelitis (EAE)

can be induced in animals via immunization against myelin antigens (25) and is an animal model for multiple sclerosis (MS), owing to similarities in its histopathology and clinical course. Recent studies have demonstrated that $cPLA_2$ performs a pivotal function in EAE (25, 26). $cPLA_2$, which can be induced by the cytokine tumor necrosis factor-α (TNF-α) (27), was expressed abundantly in EAE lesions. The blockage of $cPLA_2$ resulted in a marked reduction in both the onset and progression of the disease (25), thereby indicating that $cPLA_2$ performs a significant function in both the induction and effector phases of EAE. A second study demonstrated that $cPLA_2$ null mice were resistant to EAE (26). The treatment of EAE rats with the nonapeptide $sPLA_2$ inhibitor, CHEC-9, significantly attenuated $sPLA_2$ activity, EAE symptoms, and ED-1 positive microglia/macrophages (28). Recently, MS patients were found to have elevated levels of $sPLA_2$ activity in the urine (28). These studies demonstrate that both $cPLA_2$ and $sPLA_2$ inhibition may prove to be effective treatment options for MS.

Reactive oxygen species (ROS) contribute to stroke injury

ROS, including hydrogen peroxide (H_2O_2) and the superoxide radical ($O_2^{\bullet -}$) are generated by a number of cellular oxidative metabolic processes, such as xanthine oxidase, NADPH oxidase, the metabolism of ArAc by cyclooxygenases (COX) and lipoxygenases (LOX) (29, 30), and the mitochondrial respiratory chain. Cardiolipin is an exclusive inner mitochondrial phospholipid which is essential for mitochondrial electron transport (31). The hydrolysis of cardiolipin by mitochondrial $sPLA_2$ could disrupt the mitochondrial respiratory chain, thereby resulting in increased ROS generation. ROS can also be formed non-enzymatically, for example via the autoxidation of catecholamines (18).

Sources of ROS and the involvement of PLA_2 are summarized in Fig. 1. Reports in the recent literature suggest that COX-2 does not directly generate ROS during ArAc oxidative metabolism, but does form free radicals (i.e., tyrosyl radicals on the protein and carbon-centered radicals on ArAc) (32). ROS production was elevated in a stroke model, but was not attenuated in COX-2 deficient mice, nor by the COX-2 inhibitor NS398 (33). Mice deficient in the nox2 subunit of $O_2^{\bullet -}$-producing NADPH oxidase (33) evidenced reduced ROS generation, thereby implicating NADPH oxidase as a significant source of ROS in the stroke model. Although there have been many reports in the relevant literature concerning ROS production by COX, this may be attributable to secondary ROS generation induced by a variety of eicosanoids. However, in the studies cited above (33), COX-2 inhibition or knockout would be expected to attenuate any secondary ROS production attributable to COX-2 metabolites. This apparent incongruity may be due to the specific time points selected. The studies of Kunz, *et al*. demonstrated increased ROS generation over 2 h to 3 days. ROS production following the inhibition of COX-2 by NS398 or in COX-2 null mice was determined at 2 h and 3 days, which does not preclude the possibility of COX-2 dependent ROS generation during the intermediate 2-48 h reperfusion times. NS398 did reduce ROS generation in the hippocampal slices subjected to OGD as measured by DHE fluorescence, thus leaving open the notion that COX-2 may contribute, at least indirectly, to ROS generation (12).

Endogenous defenses that detoxify ROS include enzymatic systems such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px), and non-enzymatic antioxidants including ascorbic acid, vitamin E, β-carotene, and glutathione (GSH) (5). Neurons may be particularly vulnerable to free radical damage, as they harbor low levels of GSH (34). While ROS have been proposed to perform crucial functions in the coordination and regulation of a number of cellular signaling pathways (redox signaling), oxidative stress results when ROS formation exceeds the capacity of anti-oxidant defense systems (35). The results of studies utilizing transgenic mouse models have provided evidence suggesting that ROS contribute to ischemic brain injury. Mice overexpressing either SOD1 or GSH-Px-1 evidenced significantly smaller infarcts than were seen in the wild-type counterparts after focal cerebral ischemia. Conversely, ischemic injury was increased in SOD1-deficient mice (35).

ROS and RNS initiate lipid peroxidation

The highly reactive hydroxyl radical (•OH) is formed from H_2O_2 in the presence of divalent metal ions, particularly Fe^{2+} and Cu^{2+}, *via* the Fenton reaction. Once formed, •OH reacts almost instantaneously with a host of cellular components, including polyunsaturated fatty acids of membrane lipids. Nitric oxide (•NO) is formed via the enzymatic oxidation of L-arginine to citrulline by nitric oxide synthases (NOS) and functions as a crucial regulator of vascular response and neuronal signaling (36). $O_2^{\bullet -}$ does not induce lipid peroxidation directly, but can react with •NO to form peroxynitrite ($ONOO^-$), a strong oxidant that can initiate lipid peroxidation.

Lipid peroxidation products can alter cellular function

The peroxidation of lipids can disrupt the organization of the membrane, inducing changes in fluidity and permeability, inhibition of metabolic processes, and alterations in ion transport (37). Damage to mitochondria induced by lipid peroxidation can result in further ROS generation. In the presence of oxygen radicals, the double bonds of unsaturated fatty acids of phospholipids can become oxidized. The scission of the oxidized polyunsaturated fatty acids results in the formation of phospholipid aldehydes including oxidized phosphatidylcholine (OxPC), and aldehyde cleavage fragments including malondialdehyde (MDA), 4-hydroxynonenal (HNE), and acrolein (38, 39). These aldehydes, in turn, bind covalently to proteins and alter their functions. Among all α,β-unsaturated aldehydes, acrolein is by far the strongest electrophile and evidence the highest level of reactivity with proteins. Overproduction of lipid peroxides and al-

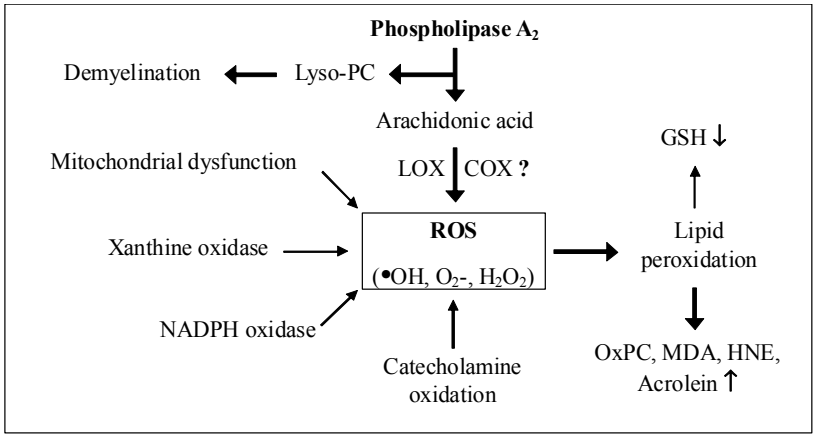

Fig. 1. Contribution of PLA_2 to ROS formation and lipid peroxides. The role of COX-2 in ROS generation is currently under examination (33).

dehyde products can induce the depletion of GSH via detoxification by GSH-Px and glutathione *S*-transferase (18).

The presence of OxPC on the apoptotic cell surface has been detected using EO6 monoclonal antibodies that bind exclusively to OxPC and OxPC-protein adducts (40). The oxidation of PC to OxPC on apoptotic cells may enhance pro-inflammatory signals (41, 42). The existence of OxPC has been demonstrated in the brains of multiple sclerosis patients using EO6 monoclonal antibodies (40). Although multiple sclerosis (a brain disorder) and stroke (brain injury) have specific differences, inflammatory response and ROS production are common factors between the two.

Lipid peroxidation in cerebral ischemia

Global cerebral ischemia

Transient forebrain ischemia in the gerbil (which lacks a Circle of Willis and thus collateral blood flow) is characterized by delayed hippocampal CA_1 neuronal death. Neurodegeneration is evident by 3 d reperfusion and culminates in neuronal death by 6 d (43). Five min of forebrain ischemia and 6 h of reperfusion in the gerbil resulted in significantly increased levels of MDA, HNE, and lipid hydroperoxides in the cortex, striatum, and hippocampus and thus preceded the onset of neuronal death (44). These increases persisted over 4 d of reperfusion in the ischemia-vulnerable hippocampus.

In another study (39 and references cited therein) involving 3 min of global cerebral ischemia, a transient increase in nuclear HNE immunoreactivity was noted in the hippocampal CA_1 neurons with little or no reactivity in the cytosol. By way of contrast, cytoplasmic immunoreactivity increased markedly from 8 h to 7 d of reperfusion. At 7 d, HNE immunoreactivity was noted within the reactive astrocytes in the CA_1 region. Similar results were noted in our studies in 10 min forebrain ischemia and 24 h of reperfusion in the gerbil. Immunohistochemical studies have demonstrated localization of HNE in the ischemia-susceptible hippocampal CA_1 region (5). HNE immunoreactivity was strongest in the nuclei, with significant labeling also detected in the cytoplasm. Little or no HNE immunoreactivity was noted in the shams.

Lipid peroxidation was evaluated via thiobarbituric acid assays in rat brains after 30 min of forebrain ischemia and up to 72 h of reperfusion. Lipid peroxide levels were unchanged during ischemia and 1 h reperfusion but increased between 8 and 72 h of recirculation in the ischemia-vulnerable hippocampus, with the greatest increase observed at 48 h (5 and references cited therein).

HNE immunoreactivity was assessed post-mortem in a group of patients who had experienced global cerebral ischemic (cardio-respiratory) attacks and had subsequently died. We noted a substantial increase in the number of HNE-positive neurons and glia in the hippocampi of the ischemic group as compared to the age- and sex-matched controls (45).

Focal cerebral ischemia

Relative generation of OxPC in the ischemic hemisphere *vs* contralateral region was assessed via Western blot assay following transient focal cerebral ischemia and 1 day reperfusion. Our studies demonstrated an increase in OxPC-modified protein (~15 kDa) in the ischemic cortex as compared to the contralateral cortex (Fig. 2). The identity of the modified 15-kDa protein currently remains unknown (40).

The proteasome is a large 700 kDa complex which performs the majority of protein degradation, and is responsible for the removal of most oxidized, aggregated, or damaged proteins. The activity of the proteasome complex was shown to decrease after transient focal cerebral ischemia in mice, without any corresponding reduction in the protein expression of the proteasome subunits. Levels of HNE-modified proteasome complex subunits increased at as early as 1 h of reperfusion, thereby suggesting that the loss of proteasome activity was attributable to the modification by HNE. GSH-Px-deficient mice evidenced further increased levels of HNE-modified proteasome subunits (46).

Using an antibody against HNE-modified proteins, no HNE immunoreactivity was detected at 1 h, but was detectable in neurons within the infarcted zone at 3 h and in the boundary between the infarcted and non-infarcted zones over 6-48 h of reperfusion following 3 h of focal cerebral ischemia in the rats (39 and references cited therein). Bcl-2 expression attenuates ischemic injury. In transient forebrain ischemia, the hippocampal CA_1 neurons that are destined to die stop generating Bcl-2 protein (47), and it is conceivable that increased levels of HNE-modified proteins are attributable, at least in part, to this lack of Bcl-2 expression.

In permanent focal cerebral ischemia in the rat, HNE immunoreactivity increased in the ipsilateral hemisphere 4 h after the induction of MCAO. HNE immunoreactivity extended beyond the region of ischemic damage, which suggests that

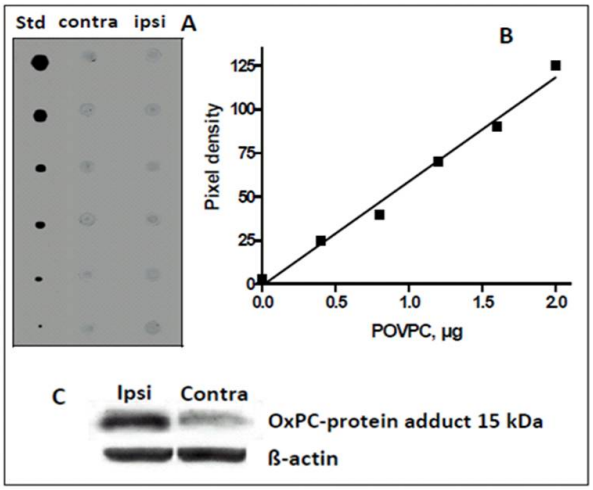

Fig. 2. (A) Dot blot of POVPC standard (1-palmitoyl-2-(5'-oxo)-valeryl-sn-glycero-3-phosphorylcholine) and lipid extracts from contra- and ipsi-cortex after MCAO/24 h reperfusion using EO6 antibodies. Free OxPC in the brain extracts was quite low. (B) Standard curve generated from pixel density of POVPC. (C) Western blot of OxPC-protein adduct in the ipsi-cortex after tMCAO.

HNE-modified proteins accumulated in the tissues prior to the development of infarction (5 and references cited therein). In another study, MDA and conjugated diene levels were elevated significantly in the ipsilateral cortex as compared to the contralateral cortex after 60 min of permanent MCAO (5).

EPC-K1 (a phosphodiester of vitamin C and E that inhibits PLA_2 activity and lipid peroxidation) reduced spatial learning deficits after 20 min of transient global ischemia (4-vessel occlusion) in male Wistar rats. In another study, EPC-K1 induced significant reductions in both cerebral lipid peroxidation and infarcts in transient focal cerebral ischemia, thereby indicating the contribution of lipid peroxidation to ischemic brain injury (5).

To date, studies concerning aldehyde products in the ischemic brain have focused on HNE and have demonstrated increased lipid peroxidation in several cerebral ischemia models. Recent studies have suggested that acrolein may function as a novel biochemical marker for the diagnosis of stroke; however, acrolein formation was attributed to the polyamine oxidase pathway (48, 49).

Lipid peroxidation in neurodegenerative diseases

A number of studies have demonstrated increased lipid peroxidation in cases of AD, thereby supporting the notion that oxidative damage performs a relevant role in AD (50). Recent studies demonstrated increased levels of HNE and acrolein in the brain tissue from patients affected by mild cognitive disorder and early AD, thereby indicating that lipid peroxidation occurs early in the pathogenesis of AD (50). ROS may also perform a function in amyloid deposition in AD, as oxidizing conditions induce protein cross-linking and the aggregation of Aβ peptides, and also contribute to the aggregation of tau protein (2).

Multiple sclerosis (MS) is an inflammatory demyelinating autoimmune disease which affects the CNS, but its underlying cause remains elusive. In MS, the immune system attacks the myelin sheath of nerve cell fibers in the brain and spinal cord. Thiobarbituric acid reactive substances and F_2-isoprostane levels have been shown to be elevated in the CSF of MS patients, and HNE has been associated with MS lesions, which indicates that lipid peroxidation also occurs in cases of MS (1).

Wallerian/anterograde degeneration occurs when a nerve fiber is cut or crushed and the portion distal to the injury (i.e. the part of the axon separated from the neuron's cell nucleus) degenerates, a process in which PLA_2 plays an important role, namely in myelin breakdown and phagocytosis (51). Wallerian degeneration occurs after axonal injury in both the peripheral nervous system (PNS) and the CNS. The axonal degeneration is followed by the degradation of the myelin sheath and infiltration by macrophages. The macrophages, accompanied by Schwann cells, function to clear the debris from the degeneration. The PNS is much faster and more efficient at clearing myelin debris than is the CNS, and Schwann cells are the primary factor in this difference. PLA_2 expressed during the early stage of Wallerian degeneration hydrolyzes PC in the myelin to LPC and ArAc. LPC can induce further myelin breakdown while eicosanoids derived from ArAc stimulate inflammatory responses.

Increased lipid peroxidation has been reported in conjunction with Parkinson's Disease, Huntington's Disease, and amyotrophic lateral sclerosis, but the involvement of PLA_2 has yet to be demonstrated (1).

Conclusions and future perspectives

The findings thus far published reveal increases in $cPLA_2$ and $sPLA_2$ in cases of stroke (5) and neurodegenerative diseases (Table 1). Gene knockout studies have demonstrated the role of $cPLA_2$ (17) in ischemic injury, MS-EAE (26), and Parkinson's

Table 1. Role of PLA_2 in CNS pathologies

CNS pathology	Role of PLA_2
Alzheimer disease	Upregulation of PLA_2, increased lipid peroxidation (6, 50, 54). $sPLA_2$ IIA expression increased in AD rains (23). Aβ induced mitochondrial dysfunction through $iPLA_2$ and $cPLA_2$. Aβ induced $cPLA_2$ phosphorylation via NADPH oxidase and ROS production (15, 16).
Parkinson's diseases	$cPLA_2$knock out mice showed protection against MPTP toxicity (6).
Multiple sclerosis-experimental autoimmune encephalomyelitis (MS-EAE)	$cPLA_2$ is highly expressed in EAE (6). $cPLA_2$-deficient mice are resistant to EAE (26). $sPLA_2$ activity increased and inhibition by CHEC-9 blocks inflammation (28).
Wallerian degeneration	PLA_2 plays an important role in myelin breakdown and phagocytosis. PLA_2 expressed during the early stage of Wallerian degeneration hydrolyzes PC in myelin to LPC and ArAc (51). LPC can aggravate the inflammatory responses that can further up-regulate $cPLA_2$ in a positive feedback manner.
Transient focal cerebral ischemia (Stroke)	Activation of PLA_2 and increased $sPLA_2$ expression (5, 13, 14). $cPLA_2$ knockout mice showed protection (5 and references cited therein). CDP-choline attenuated $sPLA_2$ (13). PLA_2 inhibitor quinacrine reduced the infarction size (6).
Spinal Cord Injury	$cPLA_2$ expression, PLA_2 activity were increased (22); Mepacrine reduced PLA2-induced neuronal death. The studies did not directly assess the role of endogenous PLA2 in neuronal injury after SCI. Major part of the studies conducted injecting PLA2 or mellitin to spinal cord of normal rats and showed increases in TNF-α, IL-1β and HNE.

disorder (6). The high level of $cPLA_2$ expression in myelin disorders including MS-EAE (25) and Wallerian degeneration (51), and resistance against MPTP-induced Parkinsonism in $cPLA_2$ null mice suggests the potential for $cPLA_2$ pharmacological interventions. While $iPLA_2$s perform a crucial role in the regulation of the cell cycle by membrane phospholipid remodeling (9), their role in CNS pathology remains to be definitively established. A neuronal $sPLA_2$ receptor has been identified and is abundantly expressed in the brain (52), but its physiological functions, ligand interactions, and possible function in neurodegeneration have yet to be elucidated. PLA_2 activating protein, which greatly stimulates $sPLA_2$ activity and is expressed in the brain, is another factor that remains to be explored sufficiently (5). A number of group IIA $sPLA_2$ inhibitors have already been developed (53), but most of these have yet to be tested in the context of CNS pathologies.

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REFERENCES

1. Adibhatla, R. M. and Hatcher, J. F. (2007) Role of lipids in brain injury and diseases. *Future Lipidol.* **2**, 403-422.
2. Adibhatla, R. M. and Hatcher, J. F. (2008, in press) Altered lipid metabolism in brain injury and disorders, in Lipids in Health and Disease, (Quinn P. J. and Wang X., eds), Subcellular Biochemistry Vol. 49. Springer-Verlag, New York.
3. Adibhatla, R. M. and Hatcher, J. F. (2008) An odyssey of plaque to stroke: a lipid perspective. www.athero.org.
4. Wymann, M. P. and Schneiter, R. (2008) Lipid signalling in disease. *Nat. Rev. Mol. Cell Biol.* **9**, 162-176.
5. Adibhatla, R. M. and Hatcher, J. F. (2006) Phospholipase A_2, reactive oxygen species, and lipid peroxidation in cerebral ischemia. *Free Radic. Biol. Med.* **40**, 376-387.
6. Farooqui, A. A., Ong, W.-Y. and Horrocks, L. A. (2006) Inhibitors of brain phospholipase A_2 activity: Their neuropharmacological effects and therapeutic importance for the treatment of neurologic disorders. *Pharmacol. Rev.* **58**, 591-620.
7. Tucker, D. E., Gijon, M. A., Spencer, D. M., Qiu, Z.-H., Gelb, M. H. and Leslie, C. C. (2008) Regulation of cytosolic phospholipase A_2a by hsp90 and a p54 kinase in okadaic acid-stimulated macrophages. *J. Leukocyte Biol.* In Press, doi:10.1189/jlb.0308197.
8. Green, J. T., Orr, S. K. and Bazinet, R. P. (2008) The emerging role of group VI calcium-independent phospholipase A_2 in releasing docosahexaenoic acid from brain phospholipids. *J. Lipid Res.* **49**, 939-944.
9. Balboa, M. A., Perez, R. and Balsinde, J. (2008) Calcium-independent phospholipase A_2 mediates proliferation of human promonocytic U937 *cells. FEBS J.* **275**, 1915-1924.
10. Adibhatla, R. M. and Hatcher, J. F. (2008) Integration of cytokine biology and lipid metabolism in stroke. *Front. Biosci.* **13**, 1250-1270.
11. Sun, G. Y., Xu, J., Jensen, M. D. and Simonyi, A. (2004) Phospholipase A_2 in the central nervous system: Implications for neurodegenerative diseases. *J. Lipid Res.* **45**, 205-213.
12. Windelborn, J. A. and Lipton, P. (2008) Lysosomal release of cathepsins causes ischemic damage in the rat hippocampal slice and depends on NMDA-mediated calcium influx, arachidonic acid metabolism, and free radical production. *J. Neurochem.* **106**, 56-69.
13. Adibhatla, R. M., Hatcher, J. F., Larsen, E. C., Chen, X., Sun, D. and Tsao, F. (2006) CDP-choline significantly restores the phosphatidylcholine levels by differentially affecting phospholipase A_2 and CTP-phosphocholine cytidylyltransferase after stroke. *J. Biol. Chem.* **281**, 6718-6725.
14. Lin, T.-N., Wang, Q., Simonyi, A., Chen, J.-J., Cheung, W.-M., Y. He, Y., Xu, J., Sun, A. Y., Hsu, C. Y. and Sun, G. Y. (2004) Induction of secretory phospholipase A_2 in reactive astrocytes in response to transient focal cerebral ischemia in the rat brain. *J. Neurochem.* **90**, 637-645.
15. Shelat, P. B., Chalimoniuk, M., Wang, J.-H., Strosznajder, J. B., Lee, J. C., Sun, A. Y., Simonyi, A. and Sun, G. Y. (2008) Amyloid β peptide and NMDA induce ROS from NADPH oxidase and AA release from cytosolic phospholipase A_2 in cortical neurons. *J. Neurochem.* **106**, 45-55.
16. Zhu, D., Lai, Y., Shelat, P. B., Hu, C., Sun, G. Y. and Lee, J. C. M. (2006) Phospholipases A_2 mediate Amyloid-β peptide-induced mitochondrial dysfunction. *J. Neurosci.* **26**, 11111-11119.
17. Rosenberger, T. A., Villacreses, N. E., Contreras, M. A., Bonventre, J. V. and Rapoport, S. I. (2003) Brain lipid metabolism in the $cPLA_2$ knockout mouse. *J. Lipid Res.* **44**, 109-117.
18. Adibhatla, R. M., Hatcher, J. F. and Dempsey, R. J. (2003) Phospholipase A_2, hydroxyl radicals and lipid peroxidation in transient cerebral ischemia. *Antioxid. Redox. Signal.* **5**, 647-654.
19. Fuentes, L., Hernandez, M., Nieto, M. L. and Crespo, M. S. (2002) Biological effects of group IIA secreted phosholipase A_2. *FEBS Lett.* **531**, 7-11.
20. Gilroy, D. W., Newson, J., Sawmynaden, P., Willoughby, D. A. and Croxtall, J. D. (2004) A novel role for phospholipase A_2 isoforms in the checkpoint control of acute inflammation. *FASEB J.* **18**, 489-498.
21. Ginsberg, M. D. (2008) Neuroprotection for ischemic stroke: Past, present and future. Neuropharmacology In Press, doi:10.1016/j.neuropharm.2007.1012.1007.
22. Liu, N.-K., Zhang, Y. P., Titsworth, W. L., Jiang, X. R., Han, S. K., Lu, P.-H., Shields, C. B. and Xu, X.-M. (2006) A novel role of phospholipase A_2 in mediating spinal cord secondary injury. *Ann. Neurol.* **59**, 606-619.
23. Moses, G. S., Jensen, M. D., Lue, L. F., Walker, D. G., Sun, A. Y., Simonyi, A. and Sun, G. Y. (2006) Secretory PLA_2-IIA: A new inflammatory factor for Alzheimer's disease. *J. Neuroinflammation* **3**, doi:10.1186/1742-2094-1183-1128.

24. Sun, G. Y., Horrocks, L. A. and Farooqui, A. A. (2007) The roles of NADPH oxidase and phospholipases A_2 in oxidative and inflammatory responses in neurodegenerative diseases. *J. Neurochem.* **103**, 1-16.
25. Kalyvas, A. and David, S. (2004) Cytosolic phospholipase A_2 plays a key role in the pathogenesis of multiple sclerosis-like disease. *Neuron* **41**, 323-335.
26. Marusic, S., Leach, M. W., Pelker, J. W., Azoitei, M. L., Uozumi, N., Cui, J., Shen, M. W. H., DeClercq, C. M., Miyashiro, J. S., Carito, B. A., Thakker, P., Simmons, D. L., Leonard, J. P., Shimizu, T. and Clark, J. D. (2005) Cytosolic phospholipase $A_2\alpha$-deficient mice are resistant to experimental autoimmune encephalomyelitis. *J. Exp. Med.* **202**, 841-851.
27. Kronke, M. and Adam-Klages, S. (2002) Role of caspases in TNF-mediated regulation of $cPLA_2$. *FEBS Lett.* **531**, 18-22.
28. Cunningham, T. J., Yao, L., Oetinger, M., Cort, L., Blankenhorn, E. P. and Greensteinm, J. I. (2006) Secreted phospholipase A_2 activity in experimental autoimmune encephalomyelitis and multiple sclerosis. *J. Neuroinflammation* **3**, doi: 10.1186/1742-2094-1183-1126.
29. Simmons, D. L., Botting, R. M. and Hla, T. (2004) Cyclooxygenase isozymes: The biology of prostaglandin synthesis and inhibition. *Pharmacol. Rev.* **56**, 387-437.
30. Phillis, J. W., Horrocks, L. A. and Farooqui, A. A. (2006) Cyclooxygenases, lipoxygenases, and epoxygenases in CNS: Their role and involvement in neurological disorders. *Brain Res. Rev.* **52**, 201-243.
31. Kirkland, R. A., Adibhatla, R. M., Hatcher, J. F. and Franklin, J. L. (2002) Loss of cardiolipin and mitochondria during programmed neuronal death: evidence of a role for lipid peroxidation and autophagy. *Neuroscience* **115**, 587-602.
32. Jiang, J., Borisenko, G. G., Osipov, A., Martin, I., Chen, R., Shvedova, A. A., Sorokin, A., Tyurina, Y. Y., Potapovich, A., Tyurin, V. A., Graham, S. H. and Kagan, V. E. (2004) Arachidonic acid-induced carbon-centered radicals and phospholipid peroxidation in cyclo-oxygenase-2-transfected PC12 cells. *J. Neurochem.* **90**, 1036-1049.
33. Kunz, A., Anrather, J., Zhou, P., Orio, M. and Iadecola, C. (2007) Cyclooxygenase-2 does not contribute to postischemic production of reactive oxygen species. *J. Cereb. Blood Flow Metab.* **27**, 545-551.
34. Dringen, R. (2000) Metabolism and functions of glutathione in brain. *Prog. Neurobiol.* **62**, 649-671.
35. Taylor, J. M. and Crack, P. J. (2004) Impact of oxidative stress on neuronal survival. Clin. *Exp. Pharmacol. Physiol.* **31**, 397-406.
36. Endres, M., Laufs, U., Liao, J. K. and Moskowitz, M. A. (2004) Targeting eNOS for stroke protection. *Trends Neurosci.* **27**, 283-289.
37. Nigam, S. and Schewe, T. (2000) Phospholipase A_2s and lipid peroxidation. *Biochim. Biophys. Acta* **1488**, 167-181.
38. Stevens, J. F. and Maier, C. S. (2008) Acrolein: sources, metabolism, and biomolecular interactions relevant to human health and disease. *Mol. Nutr. Food Res.* **52**, 7-25.
39. Uchida, K. (2003) 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. *Prog. Lipid Res.* **42**, 318-343.
40. Qin, J., Goswami, R., Balabanov, R. and Dawson, G. (2007) Oxidized phosphatidylcholine is a marker for neuroinflammation in multiple sclerosis brain. *J. Neurosci. Res.* **85**, 977-984.
41. Bratton, D. L. and Henson, P. M. (2005) Autoimmunity and apoptosis: refusing to go quietly. *Nat. Med.* **11**, 26-27.
42. Chang, M.-K., Binder, C. J., Miller, Y. I., Subbanagounder, G., Silverman, G. J., Berliner, J. A. and Witztum, J. L. (2004) Apoptotic cells with oxidation-specific epitopes are immunogenic and proinflammatory. *J. Exp. Med.* **200**, 1359-1370.
43. Adibhatla, R. M., Hatcher, J. F. and Dempsey, R. J. (2006) Lipids and lipidomics in brain injury and diseases. *AAPS J.* **8**, E314-E321.
44. Candelario-Jalil, E., Mhadu, N. H., Al-Dalain, S. M., Martinez, G. and Leon, O. S. (2001) Time course of oxidative damage in different brain regions following transient cerebral ischemia in gerbils. *Neurosci. Res.* **41**, 233-241.
45. McCracken, E., Graham, D. I., Nilsen, M., Stewart, J., Nicoll, J. A. and Horsburgh, K. (2001) 4-Hydroxynonenal immunoreactivity is increased in human hippocampus after global ischemia. *Brain Pathol.* **11**, 414-421.
46. Keller, J. N., Huang, F. F., Zhu, H., Yu, J., Ho, Y. S. and Kindy, M. S. (2000) Oxidative stress-associated impairment of proteasome activity during ischemia-reperfusion injury. *J. Cereb. Blood Flow Metab.* **20**, 1467-1473.
47. Chen, J., Graham, S. H., Nakayama, M., Zhu, R. L., Jin, K. L., Stetler, R. A. and Simon, R. P. (1997) Apoptosis repressor genes Bcl-2 and Bcl-X-long are expressed in the rat brain following global ischemia. *J. Cereb. Blood Flow Metab.* **17**, 2-10.
48. Adibhatla, R. M., Hatcher, J. F., Sailor, K. A. and Dempsey, R. J. (2002) Polyamines and CNS injury: Spermine and spermidine decrease following transient focal cerebral ischemia in spontaneously hypertensive rats. *Brain Res.* **938**, 81-86.
49. Tomitori, H., Usui, T., Saeki, N., Ueda, S., Kase, H., Nishimura, K., Kashiwagi, K. and Igarashi, K. (2005) Polyamine oxidase and acrolein as novel biochemical markers for diagnosis of cerebral stroke. *Stroke* **36**, 2609-2613.
50. Williams, T. I., Lynn, B. C., Markesbery, W. R. and Lovell, M. A. (2006) Increased levels of 4-hydroxynonenal and acrolein, neurotoxic markers of lipid peroxidation, in the brain in mild cognitive impairment and early Alzheimer's disease. *Neurobiol. Aging* **27**, 1094-1099.
51. De, S., Trigueros, M. A., Kalyvas, A. and David, S. (2003) Phospholipase A_2 plays an important role in myelin breakdown and phagocytosis during Wallerian degeneration. *Mol. Cell. Neurosci.* **24**, 753-765.
52. Nicolas, J. P., Lin, Y., Lambeau, G., Ghomashchi, F., Lazdunski, M. and Gelb, M. H. (1997) Localization of structural elements of bee venom phospholipase A_2 involved in N-type receptor binding and neurotoxicity. *J. Biol. Chem.* **272**, 7173-7181.
53. Reid, R. C. (2005) Inhibitors of secretory phospholipase A_2 group IIA. *Curr. Med. Chem.* **12**, 3011-3026.
54. Farooqui, A. A., Horrocks, L. A. and Farooqui, T. (2007) Modulation of inflammation in brain: a matter of fat. *J. Neurochem.* **101**, 577-599.