Inhibitors of Brain Phospholipase A₂ Activity: Their Neuropharmacological Effects and Therapeutic Importance for the Treatment of Neurologic Disorders

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References
Abstract—The phospholipase A<sub>2</sub> family includes secretory phospholipase A<sub>2α</sub>, cytosolic phospholipase A<sub>2β</sub>, plasmalogen-selective phospholipase A<sub>2γ</sub>, and calcium-independent phospholipase A<sub>2δ</sub>. It is generally thought that the release of arachidonic acid by cytosolic phospholipase A<sub>2β</sub> is the rate-limiting step in the generation of eicosanoids and platelet activating factor. These lipid mediators play critical roles in the initiation and modulation of inflammation and oxidative stress. Neurological disorders, such as ischemia, spinal cord injury, Alzheimer's disease, multiple sclerosis, prion diseases, and epilepsy are characterized by inflammatory reactions, oxidative stress, altered phospholipid metabolism, accumulation of lipid peroxides, and increased phospholipase A<sub>2</sub> activity. Increased activities of phospholipases A<sub>2</sub> and generation of lipid mediators may be involved in oxidative stress and neuroinflammation associated with the above neurological disorders. Several phospholipase A<sub>2</sub> inhibitors have been recently discovered and used for the treatment of ischemia and other neurological diseases in cell culture and animal models. At this time very little is known about in vivo neurochemical effects, mechanism of action, or toxicity of phospholipase A<sub>2</sub> inhibitors in human or animal models of neurological disorders. In kainic acid-mediated neurotoxicity, the activities of phospholipase A<sub>2</sub> isoforms and their immunoreactivities are markedly increased and phospholipase A<sub>2δ</sub> inhibitors, quinacrine and chloroquine, arachidonyl trifluoromethyl ketone, bromo- nol lactone, cytidine 5-diphosphoamines, and vitamin E, not only inhibit phospholipase A<sub>2</sub> activity and immunoreactivity but also prevent neurodegeneration, suggesting that phospholipase A<sub>2δ</sub> is involved in the neurodegenerative process. This also suggests that phospholipase A<sub>2δ</sub> inhibitors can be used as neuroprotectants and anti-inflammatory agents against neurodegenerative processes in neurodegenerative diseases.

I. Introduction

Phospholipases A<sub>2</sub> (PLA<sub>2</sub>;<sup>1</sup> EC 3.1.1.4) form an expanding superfamily of esterases that specifically cleave the acyl ester bond at the sn-2 position of membrane phospholipids to produce a free fatty acid and lysophospholipid (Farooqui et al., 2000b). Because a large proportion of cellular arachidonic acid is found esterified at the sn-2 position of membrane phospholipids, arachidonic acid and lysophospholipid are the major products of the PLA<sub>2</sub>-catalyzed reaction. Under normal conditions, some arachidonic acid is converted to inflammatory mediators, prostaglandins, leukotrienes, and thromboxanes, whereas a majority of arachidonic acid is reincorporated into the brain phospholipids (Rapport, 1999; Leslie, 2004). Arachidonic acid not only acts via conversion to inflammatory metabolites, but can also directly modulate neuronal function by various mechanisms, such as altering membrane fluidity and polarization state, activating protein kinase C, and regulating gene transcription (Katsuki and Okuda, 1995; Farooqui et al., 1997b). Another product of PLA<sub>2</sub> catalyzed reactions, 1-alkyl-2-lysophospholipid, is the immediate precursor of platelet-activating factor (PAF), another potent inflammatory mediator (Farooqui and Horrocks, 2004b).

Lysophospholipids may also change membrane fluidity and permeability. These metabolites are also involved in phospholipid remodeling and membrane perturbation. Accumulation of lysophospholipids is controlled by either reacylation to native phospholipids (Farooqui et al., 2000b) or by metabolism to water-soluble glycerophosphodiester such as glycerophosphocholine by lysophospholipases (Farooqui et al., 1985). Thus, tight regulation of PLA<sub>2</sub> activity is necessary for maintaining basal levels of arachidonic acid, lysophospholipid, and PAF for performing normal brain function.

Increased PLA<sub>2</sub> activity and excessive production of proinflammatory mediators, eicosanoids, and platelet-activating factor, may potentially lead to disease states and neuronal injury. Collective evidence from many recent studies suggests that increased PLA<sub>2</sub> activity and PLA<sub>2</sub>-generated mediators play a central role not only in acute inflammatory responses in brain but also in oxidative stress associated with neurological disorders such as ischemia, Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS) (Kalyvas and David, 2004; Phillis and O'Regan, 2004; Sun et al., 2004). PLA<sub>2</sub> contributes to the pathogenesis of the above disorders by attacking neural membrane phospholipids and releasing proinflammatory lipid mediators such as prostaglandins, leukotrienes, and thromboxanes, and PAF, and also by generating 4-hydroxynonenal (4-HNE). Thus, inhibition of PLA<sub>2</sub> activity provides an attractive approach for designing novel drugs for the treatment of inflammation and oxidative stress associated with acute neural trauma such as ischemia, spinal cord injury, and head injury and some neurodegenerative disorders such as AD, PD, and MS. A definitive proof of whether enhanced PLA<sub>2</sub> activity represents a definitive proof of whether enhanced PLA<sub>2</sub> activity represents a
part of the cellular defense mechanism or whether increased PLA2 activities contribute to the pathology of the neurological disorder awaits the development of a potent, specific, and clinically useful PLA2 inhibitor for the treatment of inflammation and oxidative stress associated with neurological disorders. The purpose of this review is to describe the current knowledge of PLA2 associated with neurological disorders. The treatment of inflammation and oxidative stress associated with neurological disorders but also on the development of potent, specific, and nontoxic inhibitors of PLA2 activity that can cross the blood-brain barrier without harm and can be used for the treatment of neurological disorders.

II. Multiplicity of Phospholipases A2 in Brain

Recent advances in molecular and cellular biology of PLA2 have led to the identification of more than 20 isoforms with PLA2 activity. PLA2 enzymes are subdivided into several groups depending upon their structure, enzymic properties, subcellular localization, and cellular function (Table 1) (Farooqui et al., 1997c; Chakraborti, 2003; Phillis and O'Regan, 2004; Sun et al., 2004). These groups include secretory phospholipase A2 (sPLA2), cytosolic phospholipase A2 (cPLA2), plasmalogen-selective phospholipase A2 (PlsEtn-PLA2), and calcium-independent phospholipase A2 (iPLA2). Each class of PLA2 is further subdivided into isozymes of which there are 14 for sPLA2, at least 4 for cPLA2, and 2 for iPLA2. Genes coding for sPLA2, cPLA2, and iPLA2 have been shown to occur in different regions of brain, in neurons, microglia, and astrocytes (Molloy et al., 1998; Zanassi et al., 1998; Balboa et al., 2002). PLA2 isoforms have been partially purified and characterized from brain tissue (Hirashima et al., 1992; Matsuzawa et al., 1996). PLA2 binds to two types of cell surface receptors, namely the N type, identified in neurons, and the M type, identified in skeletal muscles, of sPLA2 receptors (Hanasaki and Arita, 2002). Brain sPLA2 contains a secretion peptide and requires millimolar concentrations of Ca2+ for enzymic activity. It shows no selectivity for particular fatty acyl chains in the phospholipids. This enzyme is present in all regions of mammalian brain. The highest activities of sPLA2 are found in medulla oblongata, pons, and hippocampus, moderate activities in the hypothalamus, thalamus, and cerebral cortex, and low activities in the cerebellum and olfactory bulb (Thwin et al., 2003). At the cellular level, the sPLA2 transcript is found in astrocytes (Mosior et al., 1998b; Zanassi et al., 1998). sPLA2 is present in differentiated PC12 cells and in rat brain synaptic vesicles, indicating that neurons also express sPLA2 activity (Matsuzawa et al., 1996).

Rat brain synaptosomes or differentiated PC12 cells release sPLA2 upon stimulation via acetylcholine and glutamate receptors or via voltage-dependent calcium channels through depolarization. Thus, sPLA2 may play an important role in neuronal metabolism (Kim et al., 1995; Matsuzawa et al., 1996). Based on pharmacological studies, the sPLA2 released from neuronal cells may modulate the degranulation process leading to the release of neurotransmitters. Inhibitors of sPLA2 activity block this release. For the expression of neurotoxicity, the released sPLA2 binds to the presynaptic membrane, enters the lumen of the synaptic vesicle during retrieval of the vesicle from the plasma membrane, and hydrolyzes phospholipids of the inner leaflet of synaptic vesicles, changing the phospholipid composition and thus impairing its endocytosis. The stimulation of sPLA2 in synaptic vesicles correlates with the induction of vesicle-vesicle aggregation. This process plays a central role in presynaptic neurotransmission (Moskowitz et al., 1983; Matsuzawa et al., 1996; Wei et al., 2003). In brain,

| TABLE 1 | Properties of various isoforms of brain PLA2 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Property        | sPLA2           | cPLA2-α         | cPLA2-β         | cPLA2-γ         | PlsEtn-PLA2     |
| Localization    | Extracellular    | Cytosol         | Cytosol         | Cytosol         | Cytosol         |
| Molecular mass (kDa) | 14–18          | 85              | 114             | 61              | 39             |
| Effect of calcium | Stimulated (mM) | Translocation   | Translocation   | Translocation   | No effect       |
| Preferred substrate | PtdCho         | PtdCho          | PtdCho          | PtdCho          | PtdCho         |
| Fatty acid specificity | None           | AA              | AA              | AA, DHA         | Lino           |
| Human chromosome | 1               | 15              | 19              | 7               |
| CalB domain     | Absent          | Present         | Present         | Absent          | Absent         |
| Effect of AAOCF3 | No effect       | Inhibited       | Inhibited       | Inhibited       | Inhibited      |
| Effect of BEL   | No effect       | Inhibited       | Inhibited       | Inhibited       | Inhibited      |

AA, arachidonic acid; Lino, linoleic acid.
astrocytes express sPLA$_2$, which can be induced in response to proinflammatory cytokines such as tumor necrosis factor-$\alpha$ and interleukin-1$\beta$ (Lin et al., 2004). Mitochondrial fractions from rat brain, PC12, and U251 astrocytoma cell cultures contain significant sPLA$_2$ and iPLA$_2$ activities (Macchioni et al., 2004). The mechanism for a secretory protein (like sPLA$_2$) targeting an intracellular organelle (like mitochondria) remains unknown. However, it is proposed that at the molecular level, heparan sulfate, a glycosaminoglycan, may play an important role in internalization and attachment of PLA$_2$ isoforms to intracellular organelles (Farooqui et al., 1994b; Boilard et al., 2003). A reduction in the mitochondrial membrane potential causes the release of sPLA$_2$ and this sPLA$_2$ along with other PLA$_2$ isoforms may be involved in neural cell injury (Farooqui et al., 1997d; Macchioni et al., 2004).

Glutamate and its analogs stimulate sPLA$_2$ activity in a dose- and time-dependent manner (Kim et al., 1995; Xu et al., 2003). The neurotoxicity of glutamate is synergistically increased with the addition of sPLA$_2$ to cortical cultures. This observation suggests that glutamatergic synaptic activity may be modulated by sPLA$_2$ and its receptors on the neuronal surface (DeCoster et al., 2002; Kolko et al., 2002). In PC12 cells, sPLA$_2$ induces neurite outgrowth. Mutants with reduced sPLA$_2$ activity exhibit a comparable reduction in neurite-inducing activity (Nakashima et al., 2003), indicating that sPLA$_2$ performs a neurotrophin-like role in the central nervous system.

B. Cytosolic Phospholipases A$_2$

Although brain tissue contains cPLA$_2$ activity, it has never been purified to homogeneity and characterized from brain. The cytosolic fraction from rat brain contains two forms of PLA$_2$ activity, PLA$_{2}$-H and PLA$_{2}$-L. PLA$_{2}$-H has an apparent molecular mass of 200 to 500 kDa. Its activity is partially inhibited by Ca$^{2+}$. In contrast, PLA$_{2}$-L has a molecular mass of 100 kDa and requires Ca$^{2+}$ (Yoshiihara and Watanabe, 1990). Based on several enzymic properties such as Ca$^{2+}$ sensitivity, molecular mass, and Ca$^{2+}$-mediated translocation, PLA$_{2}$-L seems to be identical to cPLA$_2$ (Yoshiihara et al., 1992). cPLA$_2$ prefers arachidonic acid over other fatty acids and does not use Ca$^{2+}$ for catalysis, although submicromolar Ca$^{2+}$ concentrations are needed for membrane binding (Clark et al., 1987; Farooqui et al., 2000b). Owing to the presence of a Ca$^{2+}$-dependent phospho-
lipid-binding domain at the N-terminal region, cPLA2 is translocated in a Ca\textsuperscript{2+}-dependent manner from cytosol to the nuclear or cellular membranes (Clark et al., 1987; Hirabayashi et al., 2004), in which other downstream enzymes, including the cyclooxygenases and lipoxygenases responsible for the metabolism of arachidonic acid to eicosanoids, are located. This gives cPLA2 access to its membrane-associated phospholipid substrate. However, we do not know the manner in which cPLA2 is activated by extracellular stimuli and whether this activation occurs at specific sites. The C-terminal region of cPLA2 contains the phosphorylation site and catalytic site. These sites may be involved in regulation of the enzymic activity. The activation of cPLA2 can be through serine residues, notably Ser-505 and Ser-727, by mitogen-activated protein kinase and protein kinase C (PKC) (Hirabayashi and Shimizu, 2000; Hirabayashi et al., 2004). In neural membranes, cPLA2 activity and arachidonic acid release are linked to dopamine, glutamate, serotonin, P2-purinergic, muscarinic, cytokine, and growth factor receptors through different coupling mechanisms (Table 2). Some receptors involve G-proteins and others do not. The ligand-mediated stimulation of the above receptors modulates the release of arachidonic acid and levels of other second messengers in brain tissue (Farooqui et al., 2000b).

cPLA2 activity can also be modulated through a cooperative binding mechanism with glycerophospholipids containing arachidonic acid (Burke et al., 1995) or through binding of anionic phospholipids, such as phosphatidylinositol 4,5-bisphosphate, phosphatidylinositol 3,4,5-trisphosphate, and ceramide 1-phosphate (Hirabayashi et al., 2004; Pettus et al., 2004), to a pleckstrin homology domain (Mosior et al., 1998a). Three paralogs of cPLA2 occur in brain and other non-neural tissues (Diaz-Arrastia and Scott, 1999; Farooqui et al., 2000b; Shirai and Ito, 2004). In addition, cPLA2 is localized in somata and dendrites of Purkinje cells, whereas cPLA2-α is present in the granule cells of rat brain (Shirai and Ito, 2004). In addition, cPLA2-α is predominantly found in astrocytes of gray matter (Farooqui et al., 2000b; Pardue et al., 2003), as well as in hippocampal neurons (Sandhya et al., 1998; Kishimoto et al., 1999; Strokin et al., 2003), in which under physiological conditions cPLA2-α may be involved in second-messenger generation and long-term potentiation (LTP), a mechanism involved in memory storage. More recently, the mRNAs for cPLA2-β and cPLA2-δ have been identified by reverse transcription-polymerase chain re-

Recombinantly expressed cPLA2-γ liberates arachidonic acid from phosphatidylcholine. Unlike cPLA2-α, cPLA2-γ also acts on other fatty acid residues at the sn-2 and sn-1 positions of glycerophospholipids. cPLA2-α hydrolyzes fatty acids at the sn-2 position, cPLA2-β prefers to cleave fatty acids at the sn-1 position, and cPLA2-γ efficiently hydrolyzes fatty acid at the sn-1 as well as sn-2 positions of the glycerol moiety (Song et al., 1999). The overexpression of cPLA2-γ increases the proportions of polyunsaturated fatty acids in phosphatidylethanolamine, indicating that this paralog can modulate the phospholipid composition (Asai et al., 2003). cPLA2-γ is constitutively expressed in the endoplasmic reticulum where it is involved in remodeling and maintaining membrane phospholipid composition under oxidative stress.

cPLA2-β displays much lower activity with [2-arachidonyl]PtdCho than do the other two paralogs. The genes for human cPLA2-α, -β, and -γ map to chromosomes 1, 15, and 19, respectively. Mitogen-activated protein kinase phosphorylation sites are only present in cPLA2-α and are not conserved in cPLA2-β and cPLA2-γ. cPLA2-α activity is uniformly distributed in various regions of rat brain (Farooqui et al., 2000b). Recent studies have indicated the presence of a new paralog of cPLA2. This paralog is mainly found in skin and has been named cPLA2-δ (molecular mass 109 kDa) (Chiba et al., 2004). In contrast with other cPLA2 paralogs, cPLA2-δ has a preference for linoleic acid release instead of arachidonic acid release.

Considerable information is available on cPLA2-α. From immunolabeling and in situ hybridization studies, cPLA2-α is localized in somata and dendrites of Purkinje cells, whereas cPLA2-β is present in the granule cells of rat brain (Shirai and Ito, 2004). In addition, cPLA2-α is predominantly found in astrocytes of gray matter (Farooqui et al., 2000b; Pardue et al., 2003), as well as in hippocampal neurons (Sandhya et al., 1998; Kishimoto et al., 1999; Strokin et al., 2003), in which under physiological conditions cPLA2-α may be involved in second-messenger generation and long-term potentiation (LTP), a mechanism involved in memory storage. More recently, the mRNAs for cPLA2-β and cPLA2-δ have been identified by reverse transcription-polymerase chain re-

**TABLE 2**

<table>
<thead>
<tr>
<th>Receptor Type</th>
<th>PLA2 Isoform</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate receptor</td>
<td>cPLA2, mPLA, iPLA, S2</td>
<td>Lazarewicz et al. (1990); Kolko et al. (1996); Farooqui et al. (2003a)</td>
</tr>
<tr>
<td>Dopamine receptor</td>
<td>cPLA2</td>
<td>Ross (2003)</td>
</tr>
<tr>
<td>Serotonin receptor</td>
<td>cPLA2</td>
<td>Qu et al. (2003a,b); Kurrasch-Orbaugh et al. (2003)</td>
</tr>
<tr>
<td>P2-purinergic receptor</td>
<td>cPLA2</td>
<td>Xing et al. (1994)</td>
</tr>
<tr>
<td>TNF-α receptor</td>
<td>cPLA2, S1</td>
<td>Atsumi et al. (1998); Jupp et al. (2003)</td>
</tr>
<tr>
<td>IL receptor</td>
<td>cPLA2, S2</td>
<td>Xu et al. (2003)</td>
</tr>
<tr>
<td>Interferon receptor</td>
<td>cPLA2, S1</td>
<td>Xu et al. (2003)</td>
</tr>
<tr>
<td>Growth factor receptor</td>
<td>cPLA2</td>
<td>Jupp et al. (2003); Akiyama et al. (2004)</td>
</tr>
<tr>
<td>Endothelin receptor</td>
<td>cPLA2, S2</td>
<td>Trevisi et al. (2002)</td>
</tr>
<tr>
<td>Muscarinic receptor</td>
<td>cPLA2</td>
<td>Bayón et al. (1997)</td>
</tr>
</tbody>
</table>
action analysis in human brain tissue (Pickard et al., 1999; Song et al., 1999; Hirabayashi et al., 2004), but the role of these paralogs of cPLA₂ in brain tissue remains speculative. Because cPLA₂-δ mainly occurs in skin, it is proposed that this paralog plays a critical role in inflammation in psoriatic lesions (Chiba et al., 2004).

Even though cPLA₂ isozymes are often considered to be the enzymes responsible for stimulus-mediated arachidonic acid release and eicosanoid formation, several studies also implicate sPLA₂ activation. cPLA₂ and sPLA₂ may modulate arachidonic acid metabolism in the astrocytoma cell line 1321N1 via the mitogen-activated protein kinase pathway (Hernandez et al., 2000). At the cellular level, interactions and interplay among calcium mobilization, cPLA₂ phosphorylation, and extracellular receptors of sPLA₂ may be responsible for increased eicosanoid production (Fig. 1). cPLA₂ may be a good candidate for triggering down-regulation of nitric oxide synthase activity and may thus be an important component of the cross-talk between calcium and nitric oxide-regulated signal transduction pathways in neuronal cells (Palomba et al., 2004). Under pathological conditions, the interactions among calcium, cPLA₂, and nitric oxide synthase may play an important role in the pathophysiology of neurological disorders associated with oxidative stress and inflammation (see below).

C. Plasmalogen-Selective Phospholipase A₂

This enzyme hydrolyzes arachidonic acid and docosahexaenoic acid from the sn-2 position of plasmalogens, a special type of glycerophospholipid with a vinyl ether linkage at the sn-1 position of the glycerol backbone (Farooqui and Horrocks, 2001). This enzyme has been purified and characterized from bovine brain cytosol (Hirashima et al., 1992), rabbit kidney (Portilla and Dai, 1996), and rabbit heart (Hazen and Gross, 1993). Bovine brain PlsEtn-PLA₂ has an apparent molecular mass of 39 kDa. Nonionic detergents, Triton X-100 and Tween 20, stimulate the enzyme activity. It is not inhibited by bromoenol lactone, an inhibitor that markedly inhibits iPLA₂. Low micromolar concentrations of ATP have no effect on PlsEtn-PLA₂ activity, but 2 mM ATP markedly inhibits its activity. Bovine brain PlsEtn-PLA₂ is inhibited by 5,5'-dithiobis(2-nitrobenzoic acid, iodoacetate, and N-ethylmaleimide in a dose-dependent manner (Farooqui et al., 1995). Various polyvalent anions, citrate > sulfate > phosphate, and metal ions, Ag⁺, Hg²⁺, and Fe³⁺, also inhibit this enzyme in a dose-dependent manner. Glycosaminoglycans markedly inhibit bovine brain PlsEtn-PLA₂ with an inhibition pattern of heparan sulfate > hyaluronic acid > chondroitin sulfate > heparin. This PLA₂ is also inhibited by N-acetylenuraminic acid, gangliosides, and sialoglycoproteins (Yang et al., 1994b). Other glycosphingolipids, such as cerebrosides and sulfatides, have no effect. However, ceramide markedly stimulates PlsEtn-PLA₂ activity in a time-and dose-dependent manner (Latorre et al., 2003). Treatment of rat brain slices with Staphylococcus aureus sphingomyelinase or C2-ceramide produces a marked decrease in PlsEtn levels, suggesting stimulation of PlsEtn-PLA₂ activity. Bromoenol lactone, a potent inhibitor of iPLA₂, does not affect this stimulation, but quinacrine and gangliosides, nonspecific inhibitors of PlsEtn-PLA₂, completely block it (Latorre et al., 2003; Yang et al., 1994a,b). These studies have led to the suggestion that the degradation of plasmalogen by PlsEtn-PLA₂ is a receptor-mediated process (Farooqui and Horrocks, 2001; Farooqui et al., 2003a; Latorre et al., 2003) and may involve an interaction between plasmalogen metabolism and sphingolipid metabolism.

PlsEtn-PLA₂ has been localized immunohistochemically in neurons and astrocytes (Farooqui and Horrocks, 2001). The colocalization of PlsEtn-PLA₂ with glial fibrillary acidic protein suggests that this PLA₂ is predominantly associated with astrocytes. This is in contrast with cPLA₂-α, which is present in neurons as well as astrocytes (Sandhya et al., 1998; Kishimoto et al., 1999). cPLA₂ (molecular mass 85 kDa) and PlsEtn-PLA₂ (molecular mass 39–42 kDa) release arachidonic acid and docosahexaenoic acid in rat brain astrocytes and cyclic AMP and Ca²⁺ regulate these enzymes differentially (Strokin et al., 2003). Because plasmalogens are major phospholipids of neural membranes, PlsEtn-PLA₂ may be mainly involved in generating docosahexaenoic acid (DHA), a 22-carbon essential fatty acid with 6 double bonds. This fatty acid is highly enriched in synaptosomal membranes, synaptic vesicles, and growth cones and accounts for >17% by weight of the total fatty acids in the brain of adult rats (Hamano et al., 1996).

D. Calcium-Independent Phospholipases A₂

The brain cytosolic fraction contains an 80-kDa Ca²⁺-independent PLA₂ activity. This enzyme has been purified from rat brain to homogeneity using multiple column chromatographic procedures with a very low yield. The purified enzyme has a specific activity of 4.3 μmol/min/mg. The peptide sequence of this enzyme has considerable homology to sequences of the iPLA₂ from P388D1 macrophages, Chinese hamster ovary cells, and human B lymphocytes (Yang et al., 1999). This iPLA₂ hydrolyzes the sn-2 fatty acid from PtdCho with preferences linoleoyl > palmitoyl > oleoyl > arachidonyl group. iPLA₂ has an unique amino acid sequence containing a lipase consensus sequence and eight ankyrin repeats. This enzyme is strongly inhibited by bromoenol lactone, and ATP augments its activity. iPLA₂ is present in all brain regions with the highest activity in striatum, hypothalamus, and hippocampus. The gene encoding iPLA₂ has been identified (Molloy et al., 1998). Alternative splicing can generate multiple iPLA₂ isoforms with distinct tissue distribution and localization (Larsson et al., 1999).
Truncated splice variant proteins that prevent the formation of active iPLA_2 tetramers may negatively regulate iPLA_2 (Larsson et al., 1998; Seashols et al., 2004). Native iPLA_2 is a homotetramer that is potentially formed through interactions between N-terminal ankyrin repeats (Ackermann and Dennis, 1995). Five splice variants of iPLA2 occur in various tissues (Larsson et al., 1998; Shirai and Ito, 2004). One splice variant (iPLA2-1) lacks exon 9 (165 base pairs), whereas the other four variants (iPLA2-2, iPLA2-3, iPLA2-ankyrin-1, and iPLA2-ankyrin-2) contain exon 9. The presence of this exon makes these splice variants membrane-bound because exon 9 encodes hydrophobic amino acids (Larsson et al., 1998; Shirai and Ito, 2004). Rat cerebellum contains iPLA2-1 and iPLA2-2 or iPLA2-3, but not iPLA2-ankyrin-1 or iPLA2-ankyrin-2. From immunolabeling studies in rat brain, granule cells, stellate cells, and the nucleus of Purkinje cells contain iPLA2 (Shirai and Ito, 2004). Strong signals of iPLA2 immunoreactivity are observed in olfactory bulb, hippocampus CA1-3, dentate gyrus, and brain stem. In non-neural cells, the cleavage of iPLA2 by caspase-3 is associated with the execution of apoptosis (Atsumi et al., 1998, 2000). The proposed role of iPLA2 in phospholipid remodeling and apoptosis is based on the use of bromoeno lactone, thought to be a specific inhibitor, but this compound actually inhibits other enzymes such as diacylglycerol lipase and phosphatidate phosphohydrolase (see below). This makes it difficult to define the role of iPLA2 in phospholipid metabolism (Farooqui et al., 2000a; Pérez et al., 2004). iPLA2 may play an important role not only in long-term potentiation and long-term depression and activity-dependent changes in synaptic strength believed to underlie certain forms of learning and memory in the hippocampus (Fitzpatrick and Baudry, 1994; Wolf et al., 1995; Fujita et al., 2001) but also in neural cell proliferation, apoptosis, and differentiation (Farooqui et al., 2004a).

### III. Involvement of Phospholipase A_2 Activity in Brain Injury

A growing body of evidence suggests the involvement of isoforms of PLA2 in neurotransmitter release, LTP, long-term depression, membrane repair, neurodegeneration, neural cell proliferation, differentiation, and apoptosis (Fig. 2) (Farooqui et al., 1997c). It is not known which PLA2 isoform performs which central nervous system function. The multiplicity of PLA2 and the interplay among lipid mediators generated by PLA2 in brain tissue provide diversity in function and specificity of various isoforms of this family of enzymes in the regulation of enzymic activity in response to a wide range of extracellular signals. However, this diversity complicates the analysis of their function (Fig. 1). The complexity of this problem becomes obvious when one considers the coupling of various isoforms of PLA2 with different receptors in a single neural cell and tries to associate PLA2 activity with specific neuronal functions and disease processes.

Isoforms of PLA2 may not function interchangeably but act in parallel to the transducer signal (Farooqui et al., 1997d). Various isoforms of PLA2 probably act on different cellular pools of phospholipids located in different types of neural cells and these isoforms may be regulated by different coupling mechanisms involving common second messengers (Sun et al., 2004). The synthesis of eicosanoids depends not only on PLA2 activity for the generation of arachidonic acid, but also on cyclooxygenase or lipoxygenase activities (Sun et al., 2004).
Tight regulation of PLA₂ activity is necessary for normal brain function (Farooqui et al., 2000b; Phillis and O'Regan, 2004).

Activities of PLA₂ isoforms are also regulated through modulation of gene expression. Interleukin (IL)-1α, (Xu et al., 2003), tumor necrosis factor (TNF)-α (Pirianov et al., 1999; Tong et al., 1999; Jupp et al., 2003), interferon-γ (Xu et al., 2003), and several growth factors induce these enzymes (Jupp et al., 2003; Akiyama et al., 2004; Wang et al., 2004b). Regulatory interactions and coupling between PLA₂ activity and the expression of genes encoding cyclooxygenase and lipoxygenases have been suggested (Doug et al., 2003). In brain tissue, the coupling between cPLA₂ depends not only on the type of neural cell but also on the stimulatory status of neural cell involved.

Stimulation of PLA₂ isoforms may contribute to brain damage in several ways:

1. The loss of essential phospholipids with the accumulation of free fatty acids and lysophospholipids may have a detergent-like effect on neuronal membranes.

2. Free fatty acids can uncouple oxidative phosphorylation, which results in mitochondrial dysfunction (Schapira, 1996). Arachidonic acid produces mitochondrial swelling in glial cells and induces changes in membrane permeability by regulating ion channels (Farooqui et al., 1997b,c). It also inhibits glutamate uptake. In the nucleus, arachidonic acid may also interact with elements of gene structure, such as promoters, enhancers, suppressors, and others, in a specific manner that is not shared by eicosanoids or other fatty acids. These interactions modulate gene expression (Farooqui et al., 1997c).

3. Polyunsaturated fatty acid-induced oxidative stress is accompanied by an increase in AP-1 and NF-κB activity and gene expression (Mazière et al., 1999). Another consequence of increased polyunsaturated fatty acid-mediated oxidative stress is the activation and inactivation of redox-sensitive proteins (Wang, 2003).

4. PAF, formed from the acetylation of lysophospholipids, not only can activate leukocytes and microglia but also can induce inflammation at endothelial and neuronal cell surfaces.

5. The accumulation of free fatty acids can trigger an uncontrolled “arachidonic acid cascade”. This sets the stage for increased production of prostaglandins, leukotrienes, and thromboxanes. Cyclooxygenases and lipoxygenases catalyze these reactions. Prostaglandins, leukotrienes, and thromboxanes are collectively called eicosanoids. They play important roles in the generation and maintenance of inflammation in neural cells. The arachidonic acid cascade also produces 4-HNE and reactive oxygen species (ROS) such as superoxide anion, hydroxyl, alkoxy, and peroxy radicals and hydrogen peroxide.

4-HNE has three functional groups that confer to its molecule a very high reactivity toward thiol and amino groups. 4-HNE has been reported to cause a number of deleterious effects in cells including inhibition of DNA synthesis, disturbance in calcium homeostasis, and inhibition of mitochondrial respiration. All of these processes may result in neuronal injury in ischemia and glutamate-mediated neurotoxicity (Farooqui et al., 1997c). In brain tissue, 4-HNE also produces alterations in the function of key membrane proteins including glucose transporter, glutamate transporter, and sodium potassium ATPase (Friguet et al., 1994; Jamme et al., 1995). Thus, high levels of this metabolite are toxic for brain tissue (Farooqui et al., 1997c).

ROS inactivate membrane proteins and DNA (Berlett and Stadtman, 1997). The reaction between ROS and proteins or unsaturated lipids in the plasma membrane leads to a chemical cross-linking of membrane proteins and lipids and a reduction in membrane unsaturation. This depletion of unsaturation in membrane lipids is associated with decreased membrane fluidity and decreases in the activity of membrane-bound enzymes, ion channels, and receptors (Ray et al., 1994).

6. An uncontrolled sustained increase in calcium influx through increased phospholipid degradation can lead to increased membrane permeability and stimulation of many enzymes associated with lipolysis, proteolysis, and disaggregation of microtubules with a disruption of cytoskeleton and membrane structure (Farooqui and Horrocks, 1991, 1994). The most compelling evidence for the involvement of cPLA₂ in neurodegeneration comes from reports indicating that mice with a targeted deletion of the gene that encodes cPLA₂ show reduced infarct size after cerebral ischemia (Bonventre et al., 1997) and resistance to MPTP-induced neurotoxicity (Klivenyi et al., 1998). Studies on brain lipid metabolism in cPLA₂ knockout mice indicate there is no net change in unesterified arachidonic acid. However, there was a 50% reduction in esterified arachidonic acid in phosphatidylcholine, indicating involvement of cPLA₂ in wild-type mice (Rosenberger et al., 2003). The knockout mice also have reduced rates of arachidonic acid incorporation into ethanolamine and choline glycerophospholipids but elevated rates into phosphatidylcholine. cPLA₂-deficient mice also show a 62% reduction in the rate of formation of prostaglandin (PG) E₂, suggesting a coupling between cPLA₂ and cyclooxygenase activities (Murakami et al., 1997; Bosetti and Weerasinge, 2003). The deletion of cPLA₂ also causes dysregulation of...
insulin-like growth factor-1 signaling and stimulates striated muscle growth (Obata et al., 2003).

IV. Physiological and Pharmacological Effects of Phospholipase A2 Inhibitors

The stimulation of PLA2 isoforms, release of arachidonic acid, and generation of platelet-activating factor are important events in the inflammation and oxidative stress associated with acute neural trauma and chronic neurological disorders (Farooqui and Horrocks, 1994; Phillis and O'Regan, 2004). Treatment of these disorders requires potent and selective inhibitors of PLA2 activity that can be used as drugs. The problem with available inhibitors of PLA2 isoforms has been their specificity. Many PLA2 inhibitors originally thought to be selective for a specific PLA2 isoform are now known to not only inhibit other PLA2 isoforms but also block activities of different enzymes (Farooqui et al., 1999; Cummings et al., 2000; Fuentes et al., 2003). For example, in non-neural cells, arachidonyl trifluoromethyl ketone inhibits not only cPLA2 activity but also cyclooxygenase and acyltransferase activities (Cummings et al., 2000; Fuentes et al., 2003). Methyl arachidonyl fluorophosphate, another inhibitor of brain cPLA2, also inhibits bovine brain iPLA2.

The discovery of potent and specific inhibitors of PLA2 isoforms is an important approach, not only for establishing functional roles of a given PLA2 isoform in a specific type of neural cell in brain tissue but also for treating oxidative stress and inflammation caused by neurodegenerative process. Studies on this important topic are beginning to emerge (Farooqui et al., 1999; Cummings et al., 2000; Miele, 2003a; Scott et al., 2003; Clark and Tam, 2004). These studies are complicated not only by the lack of information on the availability of specific inhibitors but also by the occurrence of isoforms of PLA2 activity and in vivo effects of PLA2 inhibitors on enzymic activity. Furthermore, the effect of inhibitors on the physical state of substrate aggregates in neural membranes remains unknown. In searching for good cPLA2 inhibitors, kinetic analysis is not enough to evaluate whether an inhibitor can block PLA2 activity by affecting the interfacial quality of phospholipid in lipid bilayer or by directly inhibiting the interaction between the phospholipids and the active site of the enzyme. An ideal inhibitor should have regional specificity and should be able to reach the site where cells are under oxidative stress and inflammatory and neurodegenerative processes are taking place.

Neurons are more susceptible to free radical-mediated neuroinflammation and oxidative stress than glial cells (Adibhatla et al., 2003; Ajmone-Cat et al., 2003). In fact, activated glial cells, including astroglia and microglia, sustain inflammatory processes initiated by arachidonic acid-generated metabolites. This suggests that signals modulating the induction, expression, and stimulation of PLA2 isoforms may play an important role in neurodegenerative diseases associated with neuroinflammation and oxidative stress (Farooqui and Horrocks, 1994; Farooqui et al., 2003b, 2004b). For the successful treatment of inflammatory and oxidative stress in neurological disorders, timely delivery of a well-tolerated, chronically active, and specific inhibitor of PLA2 that can bypass or cross the blood-brain barrier without harm is required. Some nonspecific PLA2 inhibitors (see below) have been used for the treatment of ischemia, spinal cord injury, and AD (Sano et al., 1997), but no compound with real clinical potential has emerged.

A. Arachidonyl Trifluoromethyl Ketone

Arachidonyl trifluoromethyl ketone (Fig. 3) is a potent inhibitor of cPLA2. NMR studies show that the carbon chain of AACOCF3 binds in a hydrophobic pocket and the carbonyl group of AACOCF3 forms a covalent bond with the serine 228 in the active site, generating a charged hemiketal oxoanion that interacts with a positively charged group of the enzyme (Street et al., 1993; Trimble et al., 1993). AACOCF3 is a 500-fold more potent inhibitor of cPLA2 than sPLA2 (Trimble et al., 1993), indicating that it is a more selective inhibitor of cPLA2 than sPLA2. This inhibitor also blocks cyclooxygenase activity (Riendeau et al., 1994).

Because of its physicochemical properties, AACOCF3 can readily penetrate into cell membranes. At 5 to 20 μM it essentially blocks all liberation of arachidonic acid in thrombin-stimulated platelets, in Ca2+ ionophore-stimulated human monocytes, and in interleukin 1-stimulated mesangial cells (Gronich et al., 1994). AACOCF3 inhibits bovine brain cPLA2 and iPLA2 in a dose-dependent manner with IC50 values of 1.5 and 6.0 μM, respectively.

The treatment of NG 108-15 cells with AACOCF3 decreases initial neurite formation in a concentration-dependent manner (Smalheiser et al., 1996). The pharmacological blockade of cPLA2 by a low concentration, 10 μM, of AACOCF3 significantly inhibits neuronal death in the CA1 region of rat hippocampus. In primary neuronal cultures, this PLA2 inhibitor prevents caspase-3 activation and neurodegeneration induced by β-amyloid peptide (Aβ) and human prion protein peptide (Bate et al., 2004), suggesting the role of PLA2 isoforms in neurodegenerative processes (Farooqui and Horrocks, 1994; Farooqui et al., 1997c). In primary neuronal cultures, AACOCF3 also abolishes methylmercury (MeHg2+) -mediated stimulation of cPLA2 and arachidonic acid release (Shanker et al., 2004), suggesting that cPLA2 plays an important role in MeHg2+-induced neurotoxicity. Similarly, in astrocytes MeHg2+-induced ROS generation is strongly inhibited by AACOCF3 (Shanker and Aschner, 2003). In neural cell cultures AACOCF3 also shortens the association of PKC-γ with plasma membrane indicating that this isoform of PKC
may be involved in neuronal plasticity (Yagi et al., 2004). AACOCF3 also blocks L-buthionine sulfoximine toxicity in glutathione-depleted mesencephalic cultures (Kramer et al., 2004).

AACOCF3 induces the dispersal of Golgi stack and trans-Golgi network resident proteins. This suggests that cPLA2 isozymes play a crucial role in membrane trafficking and in maintenance of Golgi architecture. In non-neural cultured cells, AACOCF3 inhibits the expression of IL-2 at both the mRNA and protein levels, indicating that cPLA2 may have marked effects on T-cell function (Amandi-Burgermeister et al., 1997; Ouyang and Kaminski, 1999). AACOCF3 inhibited DNA fragmentation during apoptosis in U937 cells, but failed to affect the morphological changes that occur during apoptosis, suggesting that the use of AACOCF3 may distinguish between cytoplasmic and nuclear events that occur during apoptotic cell death (Vanags et al., 1997). AACOCF3 is described as a specific inhibitor of cPLA2, but recent studies in non-neural cells indicate it may also inhibit cyclooxygenases and 5-lipoxygenase (Cummins et al., 2000; Fuentes et al., 2003). These observations strongly suggest that AACOCF3 is not a specific inhibitor of cPLA2.

**B. Methyl Arachidonyl Fluorophosphonate**

MAFP (Fig. 3) is an irreversible inhibitor of bovine brain cPLA2 (IC50 0.5 μM) and has no effect on sPLA2. It inhibits enzymic activity by reacting with a serine residue at the active site. MAFP also inhibits bovine brain iPLA2 in a dose-dependent manner with an IC50 value of 0.75 μM. At 5.0 μM, MAFP completely inhibits bovine brain iPLA2 activity. In addition, MAFP inhibits Aβ-mediated stimulation of cPLA2 activity in cortical neuronal cultures (Kriem et al., 2005).

MAFP induces irreversible inhibition of the enzymatic hydrolysis of arachidonyl ethanolamide (anandamide) by fatty acid amid hydrolase. Based on various pharmacological studies with cannabinoid CB1 receptors and MAFP, it has been suggested that MAFP is an irreversible cannabinoid CB1 receptor antagonist (Fernando and Pertwee, 1997).

Because MAFP interacts with several PLA2 isoforms and with fatty amid hydrolase, it cannot be considered as a specific inhibitor of cPLA2. MAFP also blocks L-buthionine sulfoximine toxicity in glutathione-depleted mesencephalic cultures (Kramer et al., 2004). Intrathecal injections of MAFP in rats produce antinociceptive...
effects (Ates et al., 2003), suggesting that PLA₂ isoforms may play some role during pain states (see below).

C. Bromoenol Lactone

BEL (Fig. 3) is a potent inhibitor of bovine brain iPLA₂ and PlsEtn-PLA₂ with IC₅₀ values of 60 and 40 nM, respectively. BEL has a structural resemblance to plasmalogen. It inhibits brain cPLA₂ and sPLA₂ at a very high concentration (500 μM). The injection of BEL (10 μM) into postsynaptic CA1 pyramidal neurons produces a robust increase in the amplitude of α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor-mediated excitatory postsynaptic currents, suggesting that iPLA₂ plays an important role in AMPA-mediated synaptic plasticity (St-Gelais et al., 2004). AACOCF₃ and palmityl trifluoromethyl ketone, which mainly interact with cPLA₂, have no effect on AMPA-mediated synaptic transmission. The inhibition of iPLA₂ by BEL and the enhancement of AMPA subunit immunoreactivity in brain homogenates and slices support the above electrophysiological studies. Taken together, these results support the hypothesis that BEL-mediated antagonism of AMPA receptors is involved in long-term potentiation and long-term depression during the regulation of synaptic plasticity. Based on the blockage of induction of hippocampal long-term potentiation, brain iPLA₂ may be involved in learning and memory (Wolf et al., 1995; Fujita et al., 2001).

Intracerebroventricular injections of BEL, 3 nmol, markedly affect spatial performance in mice (Fujita et al., 2000), indicating that iPLA₂ is involved in spatial memory formation. BEL also modulates intracellular membrane trafficking (Kuroiwa et al., 2001; Brown et al., 2003) by inhibiting iPLA₂ activity in membrane tubule formation during reassembly of the Golgi complex. In addition, BEL treatment also interferes with membrane fusion events during endocytosis and exocytosis.

D. Benzenesulfonamides and Alk oxybenzamidines

Benzenesulfonamide (Fig. 3) and its pipiderine derivative are the most potent inhibitors of membrane-bound heart PLA₂ activity with IC₅₀ values of 28.0 and 9.0 nM, respectively (Oinuma et al., 1991). Intravenous injections of these inhibitors protect rats against ischemic damage in acute myocardial infarction. These compounds are relatively metabolically stable in plasma with half-lives of 1 to 2 h. These compounds also inhibit brain cPLA₂ activity in a dose-dependent manner with IC₅₀ values of 23.0 and 10.0 nM, respectively. Nothing is known about their ability to cross the blood-brain barrier also remains unknown.

E. 3-(Pyrrrol)-2-propionic Acid

Lehr (1996, 1997a,b) has synthesized many compounds (Fig. 3) that inhibit cPLA₂ activity in platelets with IC₅₀ values varying from 0.5 to 10 μM. Effects of these compounds on other isoforms of PLA₂ remain unknown. These inhibitors have not been used to block the activity of brain PLA₂ isoforms and have not been injected into intact animals or animal models of neurological disorders; therefore, nothing is known about their tolerance, half-lives, and toxicity. The ability of these inhibitors to cross the blood-brain barrier also remains unknown.

F. 2-Oxoamide and 1,3-Disubstituted Propan-2-ones

Long-chain 2-oxoamides of γ-aminobutyric acid and γ-norleucine (AX006, AX007, and AX008) (Fig. 3) reversibly inhibit cPLA₂ activity in a dose- and time-dependent manner (Kokotos et al., 2004). These inhibitors block the LPS-mediated release of arachidonic acid due to the stimulation of cPLA₂ in murine P388 D1 macrophages with IC₅₀ values of 8.0, 7.6, and 4.6 μM, respectively. These IC₅₀ values are lower than the IC₅₀ value reported for MAFP (25 μM). This strongly suggests that 2-oxoamides are more potent inhibitors of cPLA₂ than MAFP. Anti-inflammatory and analgesic activities of 2-oxoamides have been tested in the rat paw carrageenan-mediated edema assay. Carrageenan-induced edema in rat paw can be prevented with 2-oxoamides (Kokotos et al., 2004). Effects of these inhibitors on brain cPLA₂ remain unknown.

1,3-Disubstituted propan-2-ones (Fig. 4) were recently synthesized (Connolly et al., 2002). These compounds inhibit cPLA₂ activity with an IC₅₀ value of 0.03 μM in an in vitro assay. They are 10-fold more effective than 2-oxoamides and AACOCF₃ in inhibiting cPLA₂ activity. 1,3-Disubstituted propan-2-ones inhibit arachidonic acid production in HL60 cells with an IC₅₀ value of 2.8 μM. These inhibitors have not been injected into intact animals or animal models of neurological disorders; therefore, nothing is known about their tolerance, half-lives, and toxicity. The ability of these inhibitors to cross the blood-brain barrier also remains unknown.

G. Choline Derivatives with a Long Aliphatic Chain

Recently, a new class of hydrophobic inhibitors that partition into the lipid bilayer and compete with monomers of the glycerophospholipid substrate was described previously (Burke et al., 1999). These compounds include 2-(2-benzyl-4-chlorophenoxy)ethylidimethyl-n-oc-tadecyl-ammonium chloride and 2-(2-benzyl-4-chlorophenoxy)ethylidimethyl-n-octyl-ammonium bromide (Fig. 4). Both compounds inhibit cPLA₂ activity in a competitive manner with IC₅₀ values of 5 and 13 μM, respectively. The length of the N-alkyl chain plays an anti-inflammatory effect in vivo on carrageenan-mediated rat paw edema.
important role in the degree of inhibition. Shortening of the N-alkyl chain considerably decreases the percentage of inhibitor partitioned into the glycerophospholipid bilayer and increases the IC\textsubscript{50} value. These effects may be due to diminished hydrophobic interaction between the shorter alkyl chain and the fatty acid tails of the glycerophospholipids making up the bilayer. In contrast, lengthening of the N-alkyl chain increases the percentage of inhibitor partitioned into the lipid bilayer and decreases the IC\textsubscript{50} value. The synthesis of these inhibitors represents an important step in the development of potent in vivo cPLA\textsubscript{2} inhibitors because these compounds inhibit enzymic activity at the interface and provide a weak interaction with the glycerophospholipid bilayer (Burke et al., 1999). Based on kinetic studies, the potential in vivo efficacy of these intracellular inhibitors can be more potent than other inhibitors such as AA-COCF\textsubscript{3}. These inhibitors have not been used for in vivo studies so nothing is known about their tolerance, toxicity, and half-life. It is also not known whether these inhibitors can cross blood-brain barrier.

**H. Pyrrolidine-Based Inhibitors of Phospholipase A\textsubscript{2}**

Pyrrolidine-containing compounds (Seno et al., 2000; Ghomashchi et al., 2001) markedly inhibit cPLA\textsubscript{2-\alpha} in vitro and block arachidonate release in Ca\textsuperscript{2+} ionophore-stimulated non-neural cells (Seno et al., 2000). The structure of the most potent inhibitor, pyrrolidine-1, is shown in Fig. 4. In a fluorometric assay, pyrrolidine-1 inhibits cPLA\textsubscript{2-\alpha} activity in a dose-dependent manner with an IC\textsubscript{50} value of 0.18 \(\mu\)M. In a mixed-micelle assay system, this compound inhibits cPLA\textsubscript{2-\alpha} activity with an IC\textsubscript{50} value of 1.8 \(\mu\)M. This difference in the IC\textsubscript{50} value may be due to the differences in interface concentrations of pyrrolidine-1 in the different assay systems. The treatment of Chinese hamster ovary cells with pyrrolidine-1 results in marked inhibition of A23187-induced arachidonic acid release with an IC\textsubscript{50} value of 0.2 to 0.5 \(\mu\)M. The degree of inhibition approaches 100% with 4 to 10 \(\mu\)M pyrrolidine-1 (Ghomashchi et al., 2001). Similarly, the treatment of Madin-Darby canine kidney cells with pyrrolidine-1 also results in marked inhibition of ATP-induced arachidonic acid release with an IC\textsubscript{50} value of 0.8 \(\mu\)M. It must be stated here that pyrrolidine-1 also inhibits cPLA\textsubscript{2-\gamma} and iPLA\textsubscript{2-\beta} at a very high concentration but it does not inhibit sPLA\textsubscript{2}.

Pyrrophenone, a triphenylmethylthioether derivative of pyrrolidine, is a 39-fold more potent inhibitor (\(K_i\) 4.2 nM) of cPLA\textsubscript{2} activity than pyrrolidine-1 (Ono et al., 2002). Pretreatment of rats with pyrrolidine dithiocar-
baminate, a powerful thiol antioxidant, protects against kainate (KA)-mediated neurotoxicity (Shin et al., 2004). In vitro studies indicate that pyrrophenone may be a potential therapeutic agent for inflammatory diseases (Ono et al., 2002). Pyrrolidine-containing PLA2 inhibitors have not been injected in animal models of neurological disorders so their half-life and side effects remain unknown.

I. Antimalarial Drugs

All isoforms of bovine brain PLA2 are strongly inhibited by antimalarial drugs in a dose-dependent manner with rank order potency of chloroquine > quinacrine > hydroxychloroquine > quinine (Lu et al., 2001b). Chloroquine, quinacrine, hydroxychloroquine, and quinine inhibit bovine brain cPLA2 with IC50 values of 125, 200, 185, and 250 μM. It is suggested that among PLA2 isoforms, PlsEtN-PLA2 and cPLA2 may be associated with proximal events involved in the induction and maintenance of inflammatory processes after ischemic and traumatic brain injuries (Faroqui et al., 2004b) and sPLA2 may be involved in intensification (Han et al., 2003) of inflammation during later stages of the inflammatory reaction. At low concentrations (<50 μM), these inhibitors have no effect on the growth of neuron-enriched cultures from rat brain cortex, but at high concentrations (>1000 μM), these inhibitors are toxic.

J. Lithium Ion and Carbamazepine

Lithium ion is a mood stabilizer. It has been used for the treatment of bipolar disorders for almost a half-century (Corbella and Vieta, 2003). Lithium ion has a neuroprotective effect on brain tissue. Chronic lithium ion administration in rats result in 50% reduction in mRNA and protein levels of cPLA2 with no changes observed in iPLA2 and sPLA2 protein during these studies (Chang and Jones, 1998; Rintala et al., 1999). Lithium ion does not reduce phosphorylated cPLA2 protein. Thus, the decrease in brain cPLA2 enzyme activity induced by chronic lithium ion treatment is due to down-regulation of cPLA2 transcription (Weerasinghe et al., 2004). This down-regulation of cPLA2 transcription may be responsible for a selective reduction of arachidonic acid turnover compared with docosahexaenoic acid in rat brain phospholipids (Basselin et al., 2003). The labeling of Purkinje cell dendrites with cPLA2 and cyclooxygenase (COX-2) antibodies is inhibited by lithium ion, indicating the functional coupling at brain synapses between cPLA2 and COX-2 enzymes (Weerasinghe et al., 2004).

An anticonvulsant drug, carbamazepine (CBZ), has been used for the treatment of bipolar disorders for many years. Chronic administration of CBZ not only inhibits cPLA2 activity but also alters its protein and mRNA levels. In contrast, it did not affect iPLA2 and sPLA2 activities and protein levels (Ghelardoni et al., 2004). These effects are accompanied by a decrease in COX-2 activity and prostaglandin E2 levels in brain tissue, suggesting that CBZ blocks the cPLA2-mediated release of arachidonic acid and its conversion via COX-2 to prostaglandin E2 (Ghelardoni et al., 2004). The protein levels of other arachidonic acid-metabolizing enzymes such as 5-lipoxygenase and cytochrome P450 and their reaction product leukotriene B4 are not affected by CBZ. Thus, nonspecific PLA2 inhibitors, such as lithium ion and CBZ, may have beneficial effects, not only in neurological diseases (see below), but also in bipolar disorders and manic-depressive patients.

K. Vitamin E and Gangliosides

Vitamin E (α-tocopherol) is another nonspecific inhibitor of cPLA2 activity (Douglas et al., 1986). It modulates the production of arachidonic acid and eicosanoids (Tran et al., 1996). It inhibits bovine brain cPLA2 and iPLA2 activities in a dose- and time-dependent manner with IC50 values of 500 and 750 μM, respectively. Vitamin E crosses the blood-brain barrier and with time accumulates in brain (Pentland et al., 1992). It reduces lipid peroxidation and stabilizes neuronal membranes. Several reports indicate that ischemia is accompanied by an increase in PLA2 activity and a reduction in the levels of vitamin E and glutathione (Faroqui et al., 1994a). A deficiency of vitamin E and selenium in rats leads to a biphasic increase in iPLA2 activity in non-neural cells (Burgess and Kuo, 1996), once again supporting the view that PLA2 activity is regulated by vitamin E. Vitamin E may modulate activities of PLA2 isozymes by two mechanisms: first, by direct incorporation into substrate vesicles; and second, by stimulation of the activity of PLA2 isoforms by up-regulating the rate of their synthesis at the transcriptional or translation level (Tran et al., 1996).

GM1 and GM3 gangliosides also inhibit cPLA2 and PlsEtN-PLA2 activities in a dose-dependent manner (Yang et al., 1994a,b). With PlsEtN-PLA2, the IC50 values for GM1 and GM3 gangliosides were 150.0 and 75.0 μg/ml, respectively. The IC50 values for brain cPLA2 for GM1 and GM3 were 250.0 and 100.0 μg/ml, respectively. The mechanism of inhibition by gangliosides remains unknown. However, the orientation of N-acetyleneuraminic acid residues in glycoconjugates is important for inhibitory activity (Yang et al., 1994b). Gangliosides not only stabilize neural membranes but also regulate calcium influx and enzyme activities associated with signal transduction.

L. Cytidine 5-Diphosphoamines

CDP-arnes are key intermediates in the biosynthesis of phosphatidylcholine and phosphatidylethanolamine. CDP-choline (citicoline) decreases cPLA2 stimulation and hydroxyl radical generation after transient cerebral ischemia (Adibhatla and Hatcher, 2003). This process results in lowering of the concentration of free fatty acids in a dose- and time-dependent manner. CDP-
choline protects neural membranes, not only by accelerating the re-synthesis of phospholipids but also by quenching free radicals generated by PLA₂ isozymes (Rao et al., 2001; Adibhatla et al., 2002). A mixture of CDP-ethanolamine and CDP-choline may be more effective than CDP-choline alone (Murphy and Horrocks, 1993).

**M. Long-Chain Polyunsaturated Fatty Acids**

Long-chain polyunsaturated fatty acids are normal constituents of neural membrane phospholipids and products of the PLA₂-catalyzed reaction. They include arachidonic acid (belonging to the n-6 class), eicosapentaenoic acid (EPA), and DHA (belonging to the n-3 class). Arachidonic acid is released by the action of cPLA₂, and EPA and DHA are released by the action of PlsEtn-PLA₂ on neural membrane phospholipids. In vitro, the addition of these fatty acids to the reaction mixture inhibits the PLA₂-catalyzed reaction in a dose- and time-dependent manner. This inhibition can be reversed by the addition of bovine serum albumin. The in vivo effects of these fatty acids on brain metabolism are quite complex. Arachidonic acid is metabolized to prostaglandins, leukotrienes, and thromboxanes by cyclooxygenases and lipoxygenases. These metabolites can cause vasoconstriction and hence compromise blood flow and oxygen delivery to brain tissue. EPA competes with arachidonic acid at the cyclooxygenase level to produce the 3-series prostaglandins (PGE₃, PGI₃, and TXA₃) or the 5-series leukotrienes (LTB₅, LTC₅, and LTD₅). These metabolites are less active than the corresponding arachidonic acid-derived compounds (Anderle et al., 2004). For example, TXA₃ is less active than TXA₂ in aggregating platelets and constricting blood vessels (James et al., 2000; Calder and Grimble, 2002). DHA is not a substrate for cyclooxygenase. It inhibits cyclooxygenase activity and is metabolized to docosanoids (Fig. 5). Docosanoids include 10,17S-docosatrienes and 17S-resolvins (Hong et al., 2003; Marcheselli et al., 2003; Serhan et al., 2004). They not only antagonize the effects of arachidonic acid-generated metabolites but also act potently on leukocyte trafficking as well as down-regulating the expression of cytokines in glial cells (Hong et al., 2003; Marcheselli et al., 2003; Mukherjee et al., 2004; Serhan et al., 2004). The specific receptors for these bioactive lipid metabolites occur in neural and non-neural tissues. These receptors include resolvin D receptors (ResoDR1), resolvin E receptors (resoER1), and neuroprotectin D receptors (NPDR). Characterization of these receptors in brain tissue is in progress. The generation of docosanoids may

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**FIG. 5.** Chemical structures of docosahexaenoic acid and its metabolites. a, docosahexaenoic acid; b, eicosapentaenoic acid; c, 10,17S-docosatriene; d, 16,17S-docosatriene; e, 7,16,17S-resolvin; and f, 4,5,17S-resolvin. These metabolites retard the actions of arachidonic acid and its eicosanoid metabolites.
be an internal protective mechanism for preventing brain damage (Horrocks and Farooqui, 2004; Mukherjee et al., 2004; Serhan et al., 2004).

**N. Phospholipase A2 Antisense Oligonucleotides and Interfering RNA**

Antisense oligonucleotides have been synthesized to inhibit isoforms of PLA2 (Locati et al., 1996; Yoo et al., 2001; Laktionova et al., 2004). These antisense oligonucleotides efficiently inhibit the activity of various isoforms of PLA2 and also block their expression. They may be used for the treatment of inflammation and oxidative stress in neurological disorders. It must be emphasized here that the in vivo effects of antisense oligonucleotides may not be predictable from in vitro studies, partly because of the potential for the activation of the immune system by nucleotides (Endres and von Schacky, 1996). Furthermore, for neurological disorders there may be additional problems, including the efficient delivery of antisense oligonucleotides to a specific brain region in vivo and the high cost in manufacturing large quantities of antisense oligonucleotides.

An important development for inhibiting the enzymic activity of PLA2 isoforms has been the discovery of the RNA interference (RNAi) technique. This technology takes advantage of the evolutionary adaptation of neural cells to silence a gene whose corresponding double-stranded RNA molecule is present in the cell (Hannon, 2002). Introduction of a synthesized double-stranded RNA specific for a PLA2 isoform gene may cause rapid and prolonged reduction of mRNA and protein expression of that PLA2 isoform in brain tissue. RNAi has been developed for iPLA2 in non-neural cells (Shinzawa and Tsujimoto, 2003). It inhibits protein expression and iPLA2 activity in a dose- and time-dependent manner. In vivo injections of RNAi have not been made in intact animals, so the therapeutic importance of RNAi remains unknown.

**V. Phospholipase A2 Activity in Kainic Acid-Induced Neural Cell Injury**

KA is a nondegradable analog of glutamate. It is 30- to 100-fold more potent than glutamate as a neuronal excitant. Systemic administration of KA in adult rats induces persistent seizures and seizure-mediated brain damage (Lerma, 1997; Ben-Ari and Cossart, 2000). KA produces selective degeneration of neurons, especially in striatal and hippocampal areas of brain after intraventricular and intracerebral injections (Ben-Ari and Cossart, 2000). Axons and nerve terminals are more resistant to the destructive effects of KA than the cell soma. The mechanisms underlying KA-induced neuronal damage are quite complex. These mechanisms not only include inhibition of mitochondrial function and increases in intracellular calcium ion, due to depolarization, but also free radical generation (Farooqui et al., 2001). Our nuclear microscopic studies have clearly indicated a steady increase in calcium ion at 1, 2, and 3 weeks after KA injection (Ong et al., 1999). This increase in calcium ion may be responsible for the stimulation of many calcium-dependent enzymes including isoforms of PLA2 (Sandhya et al., 1998), calpain II (Ong et al., 1997), and endonucleases during KA-induced neurotoxicity.

Systemic administration of KA into adult rats markedly increases cPLA2 immunoreactivity in neurons at 1 and 3 days after injection. The cPLA2 immunoreactivity is also increased in astrocytes at 1, 2, 4, and 11 weeks after KA injection (Sandhya et al., 1998). Collective evidence suggests that an increase of cPLA2, PlsEtn-PLA2, and sPLA2 activities in KA-induced toxicity may be involved in neurodegeneration, whereas the elevation of cPLA2 activity in astrocytes may be associated with gliosis (Sandhya et al., 1998). We have also observed a decrease in 4-HNE and glutathione levels in KA-mediated toxicity.

Our immunocytochemical studies are supported by neurochemical studies on neuronal cell cultures (Kim et al., 1995; Thwin et al., 2003; Farooqui et al., 2003a,b). Treatment of neuron-enriched cultures with KA results in the stimulation of cPLA2, PlsEtn-PLA2, and sPLA2 activities in a dose- and time-dependent manner (Fig. 6) (Farooqui et al., 2003a,b; Thwin et al., 2003). This stimulation of cPLA2 activity in neuron-enriched cultures can be blocked by a nonspecific cPLA2 inhibitor, quinacrine (Farooqui et al., 2003b), a PlsEtn-PLA2 inhibitor, BEL (Farooqui et al., 2003a), a sPLA2 inhibitor, 12-epi-sclaradial (Thwin et al., 2003), or by a KA/AMPA receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (Farooqui et al., 2003a). During KA neurotoxicity, the increased cPLA2 immunoreactivity is accompanied by an increase in the 4-HNE immunoreactivity and a decrease in glutathione immunoreactivity (Ong et al., 2000) in hippocampus, indicating alterations in cellular redox. The reduction in glutathione levels may be due either to the formation of a 4-HNE-glutathione adduct or to decrease in cysteine uptake caused by KA-mediated neurotoxicity. Both processes increase the vulnerability to decrease in cysteine uptake caused by KA-mediated neurotoxicity. Both processes increase the vulnerability...
of neurons to oxidative stress. All of these events, along with the decrease in ATP levels, are closely associated with cell death (Faroqqui et al., 2004b).

The molecular mechanism(s) by which KA stimulates cPLA$_2$ activity are not fully understood. However, excessive stimulation of the KA receptors may produce a marked increase in the level of intracellular Ca$^{2+}$ due to membrane depolarization. Overstimulation of KA receptors may also induce the release of proinflammatory cytokines. Ca$^{2+}$ stimulates the enzymic activity through translocation to neural membranes, whereas cytokines may activate cPLA$_2$ activity through phosphorylation.

Like KA, intracerebroventricular injections of carrageenan, a high molecular weight sulfated polygalactose, and lipopolysaccharide, a major glycolipid component of Gram-negative bacterial outer membranes, also produce an increase in cPLA$_2$ activity and neuronal death in the hippocampus (Ong et al., 2003a; Rosenberger et al., 2004). Unlike KA, intracerebroventricular injections of carrageenan do not produce convulsions.

During KA-induced neurotoxicity, neuronal loss, as shown by a decrease in GluR1 staining, is visible in Nissl sections at 3 days after injection. In contrast, carrageenan injections do not produce a decrease in GluR1 staining until the 3rd day after injection, and cell death can be seen in Nissl sections only from 1 week after injection. Finally, during carrageenan neurotoxicity glial cell induction of cPLA$_2$ occurs earlier than KA-mediated neurotoxicity (Ong et al., 2003a). Like KA-mediated neurotoxicity, carrageenan injections induce the expression of cyclooxygenase immunoreactivity (Sandhya et al., 1998; Ibuki et al., 2003). This response appears at 3 h and becomes most prominent at 6 h after carrageenan injection. Intrathecal administration of a cyclooxygenase-2 inhibitor at 2 h after carrageenan injection produces a prominent therapeutic effect on hyperalgesia. These studies once again support the coupling between PLA$_2$ and cyclooxygenase enzymes. Similarly, intracerebroventricular injections of lipopolysaccharide produce elevations in cPLA$_2$ activity but not in sPLA$_2$ and iPLA$_2$ activities (Rosenberger et al., 2004).

The molecular mechanism of neuronal loss in carrageenan- and lipopolysaccharide-mediated toxicity is not fully understood. These agents may produce neuroinflammation by activating microglial cells and releasing proinflammatory and cytotoxic factors such as interleukin-1, tumor necrosis factor-$\alpha$, and nitric oxide (Serhan et al., 2004; Wang et al., 2004a). All these factors are known to up-regulate cPLA$_2$ activity in a neurotoxin-mediated model of neurodegeneration and represent early steps in generation and maintenance of inflammatory processes and oxidative stress (Faroqqui et al., 2002).

Our recent studies have indicated a significant increase in the number of 4-HNE-immunoreactive pyramidal neurons and in cPLA$_2$ activity in the hippocampus at 1 day, 1 week, and 2 weeks after a single intracerebroventricular injection of 1 $\mu$l of 10 mM ferrous ammonium citrate, an agent that produces severe oxidative stress (Ong et al., 2005). Intense 4-HNE labeling is observed at 1 day postinjection. 4-HNE immunoreactivity is markedly decreased after 2 weeks postinjection. A significant increase in cPLA$_2$ immunoreactivity is also observed in pyramidal neurons at 1 week postinjection. Despite the increased 4-HNE and cPLA$_2$ labeling, no loss of neurons is observed. Electron microscopic studies showed that 4-HNE- or cPLA$_2$-positive neurons have features of injured neurons, but they were still viable. The reduction of 4-HNE immunoreactivity in neurons at 2 weeks after oxidative injury, and the lack of cell loss at any of the time intervals, indicates that the hippocampal pyramidal neurons have a remarkable ability to recover from a single episode of severe oxidative injury (W.-Y. Ong et al., 2005, manuscript submitted for publication).

VI. Protection of Kainic Acid-Induced Neural Cell Injury by Phospholipase A$_2$ Inhibitors

In our studies on KA-induced toxicity in rat brain hippocampal slices and primary neuronal cells in culture, activities of PLA$_2$ isoforms and their immunoreactivities are markedly increased (Lu et al., 2001a,b; Farooqui et al., 2003a,b). Quinacrine and other inhibitors of cPLA$_2$ activity, such as AACOCF$_3$ and BEL, could block this increase in PLA$_2$ activities and immunoreactivity (Fig. 7). Detailed investigations on the effect of quinacrine indicate that this drug not only blocks the cPLA$_2$ enzymic activity but also inhibits the expression of its mRNA (Ong et al., 2003b). Thus, 3 days after KA injection into the right lateral ventricle of rats, cPLA$_2$ mRNA levels were up-regulated 6- to 13-fold in the injected right hippocampus and 5- to 9-fold in the noninjected contralateral hippocampus. cPLA$_2$ mRNA levels remained at the low basal level in phosphate-buffered saline-injected control rat hippocampus. One week later, cPLA$_2$ mRNA was up-regulated 3- to 6-fold in the injected right hippocampus and 3- to 8-fold in the noninjected contralateral side. Administration of quinacrine prevented the KA-mediated increases in cPLA$_2$ mRNA levels. In rat brain hippocampal slices, other antimalarial drugs, including chloroquine, hydroxychloroquine, and quinine, also produce a significant reduction of the increased cPLA$_2$ immunoreactivity. This suggests that the increased cPLA$_2$ immunoreactivity after KA-induced neuronal damage is regulated at the mRNA level and antimalarial drugs inhibit this increase (Ong et al., 2003b).

Similarly surfactin, a surfactant lipopeptide with antibiotic, antifungal, and anticoagulant properties, inhibits cPLA$_2$ activity in a dose- and time-dependent manner. Our studies on KA-induced neurotoxicity in rat hippocampal slice cultures indicate that surfactin significantly reduces neurodegeneration, up-regulation of cPLA$_2$ activity, and 4-HNE formation. This cPLA$_2$ inhib-
itor can be used to prevent KA-induced neurotoxicity (Farooqui et al., 2004b). Vitamin E and ganglioside, the nonspecific inhibitors of cPLA2, protect neurons from KA-mediated neurotoxicity (Ortiz et al., 2001), supporting our proposal that PLA2 inhibitors act as neuroprotective agents in KA-mediated neurotoxicity.

The molecular mechanism of action of PLA2 inhibitors in KA-induced neurotoxicity may include the following possibilities:

1. PLA2 inhibitors may inhibit cPLA2 activity by binding to phospholipid substrates (Blackwell and Flower, 1983).
2. PLA2 inhibitors may block cPLA2 activity by interacting with the active site (Lu et al., 2001b).
3. PLA2 inhibitors, such as quinacrine, may inhibit cPLA2 by blocking the expression of the mRNA for cPLA2 in kainic acid-induced neurotoxicity, as indicated by our northern blot studies (Ong et al., 2003b).
4. PLA2 inhibitors might modulate KA receptors by controlling the levels of eicosanoids. The latter are known to be involved in the regulation of hippocampal glutamate receptors (Chabot et al., 1998). PLA2 inhibitors can modulate the expression of cytokines, NF-kB, adhesion molecules, and brain proteases (Burgermeister et al., 1999). All these molecules are associated with the induction and maintenance of inflammatory processes after neuronal injury.
5. PLA2 inhibitors may exert their neuroprotective effects by binding to an AP-1 site and suppressing or lowering transcription of genes for cPLA2 isozymes. In addition to their effect on cPLA2 activity and gene expression, PLA2 inhibitors, such as quinacrine, have also been shown to have anesthetic and anti-inflammatory effects. These effects may be important in preventing neuronal degeneration after KA-induced neurotoxicity. Collective evidence strongly suggests that cPLA2 inhibitors can be used as potential therapeutic agents for treatment of inflammation and oxidative stress in the KA-mediated model of neural cell injury.

VII. Phospholipase A2 Activity in Neurological Disorders

It is now well established that activities of PLA2 isoforms are up-regulated in acute neural trauma, ischemia, spinal cord injury, and head injury, and many neurodegenerative diseases (Fig. 8) with substantial inflammatory and oxidative components such as AD, PD, and MS (Kalyvas and David, 2004; Phillis and O’Regan, 2004; Sun et al., 2004). Many studies indicate that neuroinflammation in these neurological disorders is accompanied by the activation of astrocytes and microglia and the release of inflammatory cytokines. These cytokines propagate inflammation in turn by a number of mechanisms including the up-regulation of PLA2 isoforms, generation of eicosanoids and platelet-activating factor, stimulation of nitric-oxide synthase, and calpain activation (Farooqui and Horrocks, 1991, 1994; Farooqui et al., 2000b). In this context, among the various approaches under investigation as therapies for neurotrauma and neurodegenerative diseases, there is growing support for strategies that prevent inflammatory reactions during neurodegeneration.

A. Ischemia

Ischemic injury produces the stimulation of cPLA2 and PlsEtn-PLA2 in brain tissue (Edgar et al., 1982; Rordorf et al., 1991; Clemens et al., 1996). The stimulation of these enzymes results in the massive release of free fatty acids in brain, the Bazan effect (Farooqui et
The formation of free fatty acids, depletion of ATP, and alterations in ion homeostasis induce membrane dysfunction that may lead to cellular injury (Farooqui and Horrocks, 1991, 1994). The stimulation of PlsEtn-PLA$_2$ may occupy a proximal position in the injury pathway, initiating neural cell injury, whereas cPLA$_2$ may participate by hydrolyzing PtdCho and amplifying the injury process (Sapirstein and Bonventre, 2000; Farooqui et al., 2003a,b). The mechanisms of stimulation of isoforms of PLA$_2$ in ischemic injury are not known. Covalent modification, such as phosphorylation, may be involved in the stimulation process (Edgar et al., 1982). An increased expression of cPLA$_2$ mRNA and sPLA$_2$ mRNA also occurs after transient forebrain ischemia (Lin et al., 2004; Sun et al., 2004). The role of cPLA$_2$ in neuronal damage is strongly supported by studies on cPLA$_2$ knockout mice (Bonventre et al., 1997; Sapirstein and Bonventre, 2000; Tabuchi et al., 2003). After transient middle cerebral artery occlusion, cPLA$_2$ knockout mice develop smaller infarcts, less brain edema, and less neurological deficits than control mice, indicating a reduced susceptibility of cPLA$_2$ knockout mice to ischemic neurodegeneration. Primary neural cell cultures prepared from cPLA$_2$-deficient mice generate significantly smaller amounts of prostaglandins and leukotrienes (Uozumi and Shimizu, 2002). This suggests that the cPLA$_2$ deletion contributes to a decrease in arachidonic acid supply to cyclooxygenase-2 and a resultant decrease in prostaglandins synthesis (Hong et al., 2001).

### B. Alzheimer’s Disease

Activities of cPLA$_2$ and PlsEtn-PLA$_2$ are markedly higher in nucleus basalis and hippocampal regions of AD brain compared with age-matched control brains (Fig. 9) (Stephenson et al., 1996, 1999; Farooqui et al., 1997a, 2003a,b). A similar elevation in COX-2 activity is also found in hippocampal neurons of AD patients compared with age-matched control subjects (Sugaya et al., 2000). In an earlier study, a significantly lower Ca$^{2+}$-dependent PLA$_2$ activity was found in the parietal and frontal cortices in AD patients compared with age-matched, nondementia patients and control subjects (Gattaz et al., 1995). The method for determining enzymic activity is probably responsible for this discrepancy. Recent studies by this group (Gattaz et al., 2004) also indicate that iPLA$_2$ activity of platelets from AD patients is markedly lower than that from control subjects. This suggests that more studies are required on the determination of activities of PLA$_2$ isoforms not only in different regions of AD brain but also on various stages of AD (initial stage,
moderately advanced stage, and advanced AD) in human populations. It should be kept in mind that the progress of neurodegeneration varies considerably during the development of AD. This process is strongly influenced by genetic factors. The level of the inflammatory response within the brain is a central factor influencing the neurodegenerative process through the release of inflammatory mediators that are supplied by PLA2 and COX-2 catalyzed reactions.

The elevation of phospholipid degradation metabolites, phosphomonooesters and phosphodiesters, in AD brain supports our finding of increased cPLA2 and PlsEtn-PLA2 activities. The increase in phosphomonooester and phosphodiester correlates with pathological markers of AD, such as neurofibrillary tangles and senile plaques (Pettegrew, 1989). Changes in brain membrane phospholipid and high-energy phosphate metabolism occur before any clinical manifestation of AD (Pettegrew et al., 1995), suggesting that abnormal signal transduction due to disturbed phospholipid metabolism may be an important feature of AD. The aldehydic product of arachidonic acid metabolism, 4-HNE, colocalizes with intraneuronal neurofibrillary tangles and may contribute to the cytoskeletal derangement found in AD. Alterations in phospholipid metabolism may be closely associated with the loss of synapses and neurons and the formation of senile plaques and neurofibrillary tangles in AD (Pet-

The exact cause of increased cPLA2 and PlsEtn-PLA2 activities in AD brain is not fully understood. However, there are several possibilities. Aβ, which accumulates in AD, activates cPLA2 activity (Lehtonen et al., 1996; Kanfer et al., 1998). Treatment of cortical cultures with Aβ stimulates cPLA2 activity. This stimulation is blocked not only by the cPLA2 inhibitor MAPF but also by cPLA2 antisense oligonucleotides (Kriem et al., 2005), strongly suggesting the involvement of cPLA2 in the pathogenesis of AD (Kriem et al., 2005). The second possibility is that the activation of astrocytes and microglia in AD may result in expression of cytokines, TNF-α, IL-1β, and IL-6, that are known to stimulate cPLA2 activity (Xu et al., 2003; Rosales-Corrall et al., 2004). Finally, the Aβ-mediated influx of calcium ions promotes the activation and translocation of cPLA2 from cytosol to neural membranes. This results in breakdown of the membranes and abnormal signal transduction in AD (Faroqui and Horrocks, 1994). β-Amyloid-mediated changes in calcium ion signaling underlie not only its action on long-term potentiation but also on many calcium-dependent enzymes (Faroqui and Horrocks, 1994), which may render neurons vulnerable to neurodegenerative process. At this stage it is not known whether elevation of cPLA2 and PlsEtn-PLA2 activities is the cause or the consequence of neurodegenerative process and whether changes in activities of PLA2 isoforms are primary or secondary. Thus, more studies on the involvement of PLA2 isoforms in pathogenesis of AD are required.

C. Experimental Model of Parkinson’s Disease

PD is characterized by a selective degeneration of the dopaminergic neurons of the substantia nigra. Free radicals and lipid peroxides also play an important role in the pathogenesis of PD. They are generated by the action of PLA2 isoforms and produce oxidative stress in dopaminergic neurons in the substantia nigra. Mice deficient in cPLA2 activity are resistant to MPTP neurotoxicity. This resistance strongly suggests that cPLA2 is closely associated with the pathophysiology of PD (Klivenyi et al., 1998). In brain, MPTP is converted to its toxic metabolite, 1-methyl-4-phenylpyridinium ion (MPP+), in the presence of monoamine oxidase B. MPP+ is actively taken up into nigrostriatal neurons where it inhibits mitochondrial oxidative phosphorylation, leading to neuronal cell death (Singer et al., 1987).

The involvement of cPLA2 in PD pathogenesis is supported by MPP+-mediated toxicity in GH3 cell cultures (Yoshinaga et al., 2000). MPP+-mediated neurodegeneration is accompanied by the stimulation of PLA2 and arachidonic acid release in GH3 cells. This release of arachidonic acid can be blocked by AOCOCF3, a specific inhibitor of cPLA2. Once again, this finding suggests the involvement of cPLA2 in MPP+-mediated neurodegeneration. It is interesting to note that in the MPTP-induced model of parkinsonism, quinacrine protects dopaminergic neurons from neurodegeneration (Tariq et al., 2001). In this system quinacrine may act not only as a PLA2 inhibitor but also as a membrane stabilizer and antioxidant (Tariq et al., 2001; Turnbull et al., 2003).

D. Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis

MS and EAE, an animal model for MS, are inflammatory demyelinating diseases of the brain and spinal cord that result in motor and sensory deficits. A marked increase in PLA2 activity occurs in brain tissue from MS patients (Huterer et al., 1995). cPLA2 is highly expressed in EAE lesions (Trigueros et al., 2003; Kalyvas and David, 2004; Phillis and O’Regan, 2004) and inhibition of this enzyme results in a remarkable reduction in the onset and progression of EAE. The reduction in EAE severity correlates with cPLA2 activity and its downstream mediators such as COX-2, the LTB4 receptor 2, and various chemokines and cytokines (Kalyvas and David, 2004). The induction and maintenance of neuroinflammation in MS patients may involve cPLA2. sPLA2 activity is markedly elevated in EAE and LPS-
mediated neurotoxicity (Pinto et al., 2003). An extracellular sPLA₂ inhibitor, N-derivatized phosphatidylethanolamine linked to a polymeric carrier, blocks central nervous system inflammation under both in vivo and in vitro conditions. These interesting observations suggest that more studies are required on the involvement of PLA₂ isoforms in neurodegenerative processes in MS.

E. Prion Diseases

Prion diseases include scrapie, found in goats and sheep, bovine spongiform encephalopathy (mad cow disease) in cattle, and fetal familial insomnia, Creutzfeldt-Jakob disease (CJD), kuru, and Gerstmann-Sträussler-Scheinker syndrome in humans (Prusiner, 2001; Grossman et al., 2003). Neuronal loss, spongiform degeneration, and glial cell proliferation are the pathological hallmarks of prion diseases. Human prion protein (PrPc) contains 209 amino acids, a disulfide bridge between residues 179 and 214, a glycosylphosphatidylinositol (GPI) anchor, and two sites of nonobligatory N-linked glycosylation at amino acids 181 and 197 (DeArmond and Prusiner, 2003). In prion diseases, the soluble PrPSc, which consists of an α-helix and random coil structures, is refolded into a β-sheet, the insoluble protease resistant isoform, PrPSc (DeArmond and Prusiner, 2003). The accumulation of PrPSc, which occurs in the cytoplasm and in secondary lysosomes as well as in neuronal plasmalemma and synaptic regions, may be responsible for the loss of cognitive function in prion diseases (Lazarewicz et al., 1990; Jeffrey et al., 1992).

PrPSc and PrP106-126, a neurotoxic prion peptide, are known to stimulate NMDA receptors. This stimulation is blocked by MK-801, memantine, and flupirtine (Mul-Williams, 2004). In this model of prion disease, neuronal PLA₂ is activated by GPI isolated from PrPc or PrPSc. The ability of GPI to activate PLA₂ is lost by either removal of the acyl chains or cleavage of the phosphatidylinositol-glycan linkage and inhibited by a monoclonal antibody that recognizes phosphatidylinositol (Bate and Williams, 2004). Furthermore, the treatment of neuronal cultures with inositol monophosphate or sialic acid provides resistance to the toxic effects of prion neurotoxic peptides. These observations strongly implicate PLA₂ in the pathogenesis of prion diseases. The involvement of PLA₂ in prion-mediated diseases may be a primary event or a secondary effect of an abnormal signal transduction process related to inflammation and oxidative stress in degenerating neurons. However, it is interesting to note that quinacrine, an acridine-based PLA₂ antagonist, inhibits PrPSc formation (IC₅₀ 300 nM), and can be used for the treatment of the human prion disease, CJD (Doh-ura et al., 2000; Korth et al., 2001; Love, 2001; Follette, 2003; May et al., 2003). The molecular mechanism involved in the inhibition of PrPSc formation by quinacrine remains unknown. However, it has been suggested that quinacrine blocks PrP106-126 formed channels (Farrelly et al., 2003). NMR spectroscopic studies indicate that the PLA₂ inhibitor, quina-crine, binds to human prion protein at the Tyr-225, Tyr-226, and Gln-227 residues of helix α3 (Vogtherr et al., 2003). Similarly, other antimalarial drugs, such as chloroquine and the phenothiazine derivatives acepromazine, chlorpromazine, and promazine, also bind to prion protein between residues 121 to 230, suggesting that Tyr-225, Tyr-226, and Gln-227 residues are necessary for the binding of antimalarial drugs and phenothiazine derivatives (Vogtherr et al., 2003) to PrPSc.

Acute and chronic prion disease, CJD (Doh-ura et al., 2000; Korth et al., 2001) and scapie, found in goats and sheep, with an overall loss of 18% found at 30 min after the compression trauma to the spinal cord, 10% of the PlsEtn is lost with an overall loss of 18% found at 30 min after the compression injury (Horricks et al., 1985). Similar results have been reported in another model of spinal cord injury in rabbits (Lušková et al., 1996).

The loss of PlsEtn after compression and ischemic injuries can be explained by the stimulation of PlsEtn-PLA₂ due to shear stress (Taylor, 1988). Stimulation of the PlsEtn-PLA₂ may result in changes in membrane fluidity and permeability, resulting in increased calcium influx, impaired mitochondrial function, and the subsequent generation of ROS. Low levels of ROS act as second messengers and produce neurodegeneration by apoptosis whereas high levels of ROS produce irreversible damage to cellular components and cause cell death by necrosis (Denecker et al., 2001). Necrosis normally occurs at the core of the injury site, whereas neural cells, including oligodendroglia, undergo apoptosis several hours or days after injury in the surrounding area.
G. Head Injury

The neurochemical changes in head injury are accompanied by widespread neuronal depolarization, accumulation of glutamate in the extracellular space, and increased levels of arachidonic acid and eicosanoids as well as leukotrienes (McIntosh et al., 1998). The release of arachidonic acid and its metabolites is due to activation of cPLA₂ (Shohami et al., 1989), as well as the phospholipase C/diacylglycerol-lipase pathway (Wei et al., 1982). The pathological consequences of alterations in phospholipid metabolism in neural trauma and neurodegenerative diseases may include release of glutamate, energy failure, stimulation of PLA₂ and diacylglycerol lipase activities, free radical damage, and alteration of membrane fluidity and permeability. These changes may influence the pattern of membrane-bound enzymes, receptors, and ion channels (Farooqui and Horrocks, 1994).

Glutamate-mediated abnormal phospholipid metabolism may be a common mechanism involved in ischemia and neurodegenerative diseases such as AD. The acute neural trauma in ischemia is accompanied by a rapid release of glutamate and sustained calcium influx at the core of the injury site but not in the surrounding area. This process may result in necrotic cell death at the core of the injury, whereas penumbral region neurons may die by apoptosis. In contrast in AD, there is not an excessive release of glutamate. However, the number of NMDA and other glutamate receptors is decreased in excessive release of glutamate. However, the number of NMDA and other glutamate receptors is decreased in the neocortex and hippocampal regions compared with that in age-matched control subjects (Geddes et al., 1992). This decrease results in an alteration of membrane fluidity and permeability. These changes may influence the pattern of membrane-bound enzymes, receptors, and ion channels (Farooqui and Horrocks, 1994).

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H. Epilepsy

Epileptic seizures are known to stimulate cPLA₂ activity and its expression with the accumulation of arachidonic acid (Visioli et al., 1994; Kajiwara et al., 1996). Studies on the pentylenetetrazol-induced model of epilepsy in rat brain indicate significant elevations in sPLA₂ activity in cortical, hippocampal, and cerebellar regions compared with the control group. The increase in sPLA₂ activity is more pronounced in hippocampal and cortical regions than in the cerebellar region (Yegin et al., 2002). At present, information on activities of PLA₂ isoforms is not available for different regions of epileptic human brain. More studies are needed on the involvement of PLA₂ isoforms in the pathogenesis of epilepsy.

I. Schizophrenia and Depressive Disorders

Marked elevations are found in iPLA₂ activity in brain tissue of schizophrenic patients (Hudson et al., 1996; Ross et al., 1997, 1999). This results in accelerated phospholipid metabolism in schizophrenia. Levels of phosphatidylcholine and phosphatidylethanolamine are decreased, whereas the levels of lysophosphatidylcholine are increased in brain, erythrocytes, platelets, and skin fibroblasts of patients with schizophrenia (Yao et al., 2000; Ross, 2003). The cause of the increased iPLA₂ activity is not known. However, abnormalities in dopamine and retinoid metabolism along with alterations in cytokines in schizophrenic patients may be responsible for the stimulation of PLA₂ isoforms (Laruelle et al., 1999; Farooqui et al., 2004a; Yao and Van Kammen, 2004). In contrast, cPLA₂ activity is markedly decreased in schizophrenic patients (Ross, 2003). Abnormalities in the promoter region of the gene encoding cPLA₂ may be responsible for the altered cPLA₂ activity in schizophrenic subjects (Hudson et al., 1996; Rybakowski et al., 2003). A dimorphic site within the first intron of cPLA₂ may also be responsible for abnormal PLA₂ activity (Ross, 2003). The wide range of phenotypic variability compounds the problem of analyzing and identifying vulnerable genes in schizophrenia. Some clinical or biochemical markers might be used to diminish this problem. Thus, more studies are needed to understand the involvement of PLA₂ polymorphism in the pathophysiology of schizophrenia (Junqueira et al., 2004). Cocaine users also have reduced cPLA₂ activity in their brain tissue (Ross and Turenne, 2002). As in schizophrenia, we suggest that dopaminergic hyperactivity in cocaine users may be responsible for the decreased cPLA₂ activity.

Studies on the association between cPLA₂ and mood disorders indicate a potential involvement of cPLA₂ polymorphism in mood disorders (Pae et al., 2004). It has been reported that genotyping and allele distributions in patients with major depressive disorders are significantly different from those of the control human subjects and a BanI polymorphism of the cPLA₂ gene may be related to the pathogenesis of major depressive disorder (Pae et al., 2004).

VIII. Use of Phospholipase A₂ Inhibitors for the Treatment of Neurological Disorders

In brain, quinacrine (Fig. 3) protects gerbil CA1 hippocampal pyramidal cells during 5 min of forebrain ischemia (Estevez and Phillis, 1997). After intravascular injections, quinacrine appears in monkey brain after 24 h (Dubin et al., 1982), indicating that this inhibitor is metabolically stable and can cross the blood-brain barrier. It is localized in neurons. It has also been admin-
istered (5 mg/kg) to rats that underwent 2 h of middle cerebral artery occlusion (Estevez and Phillis, 1997). The administration of quinacrine results in a marked reduction in neurological deficits after 24 h of reperfusion. Importantly, the effects of quinacrine persist even after 7 days. These findings are supported by biochemical and histopathological analysis that indicate a significant decrease in infarct size in quinacrine-treated rats compared with saline-treated controls. Based on these studies (Estevez and Phillis, 1997), it has been proposed that PLA2 inhibitors have cerebroprotective effects in focal as well as global models of cerebral ischemia. In organotypic hippocampal cultures oxygen/glucose deprivation produces a 2-fold increase in PLA2 activity with significant cell death. This increase in PLA2 can be blocked by AACOCF3 in a dose- and time-dependent manner (Arai et al., 2001). sPLA2 and iPLA2 inhibitors were ineffective in blocking cell death. In an attempt to evaluate the contribution of PLA2 isoforms to the release of free fatty acids, rat cerebral cortex was superfused with inhibitors of PLA2 activity. AACOCF3 markedly inhibited the efflux of arachidonic, docosahexaenoic, linoleic, palmitic, and oleic acids from the ischemic/reperfused rat cerebral cortex (Phillis and O’Regan, 2004). Exposure to the sPLA2 and iPLA2 inhibitors has minimal effect on the efflux of free fatty acids. These observations strongly suggest that cPLA2 plays an important role in ischemic injury and that PLA2 inhibitors can be used for the treatment of ischemic injury.

In addition, quinacrine has been proposed as a therapeutic agent for prion diseases (Korth et al., 2001). Quinacrine blocks prion protein peptide (PrP106-126)-mediated caspase-3 activation, supporting the involvement of cPLA2 in apoptotic cell death (Stewart et al., 2001). Quinacrine and a combination of quinacrine with chlorpromazine, a phenothiazine derivative, were used for the treatment of CJD using compassionate use as a justification (Love, 2001; Follette, 2003; Kobayashi et al., 2003). During quinacrine treatment, the initial responses of CJD patients have been positive, but within days of starting treatment, patients returned back to their previous states, indicating a transient recovery (Love, 2001; Follette, 2003; Kobayashi et al., 2003). The reason for the transient effect of quinacrine on CJD patients remains unknown. The transient effect may be due to the advanced stage of CJD. If quinacrine treatment could be started at the onset of CJD, patients would probably respond to this drug in a positive manner (Follette, 2003; Kobayashi et al., 2003). PLA2 activities were not determined during these studies. Thus, no comments can be made about the levels of arachidonic acid and its metabolites, inflammatory reactions, and oxidative stress that occur in prion-mediated neurodegeneration in CJD. More studies are required on the involvement of PLA2 isoforms and generation of proinflammatory mediators in the pathogenesis of prion diseases in animal and cell culture models.

Recent in vitro studies on prion-infected cell lines ScN2a, SMB, and ScGT1 also indicate that daily treatment of these cells with CDP, aristolochic acid, BEL, and AACOCF3 causes a significant decrease in protease-resistant prion protein compared with untreated control cells. The treatment with PLA2 inhibitors decreases protease-resistant prion protein but also reduces prostaglandin E2 levels. This observation strongly suggests that PLA2 activity may be closely related to the pathogenesis of prion diseases (Bate et al., 2004). Furthermore, corticosteroids that induce the formation of lipocortins (annexins), a family of PLA2 inhibitory proteins (Kaetzel and Dedman, 1995), also reduce the content of protease-resistant prion protein in prion-infected cell lines. This finding again supports an involvement of PLA2 in prion diseases (Bate et al., 2004). However, the use of glucocorticoids in prevention of prion diseases should be treated with caution because the chronic administration of glucocorticoids is known to produce neuronal atrophy (Abraham et al., 2001).

PAF, which is generated by the acetylation of lyosphosphatidylcholine, another product of PLA2-catalyzed reactions, increases the generation of protease-resistant prion protein, and PAF antagonists block it. The mechanism by which PAF antagonists inhibit the formation of protease-resistant prion protein remains unknown. However, PAF antagonists and PLA2 inhibitors may act by altering intracellular trafficking of cellular prion protein. These observations suggest the pivotal role of PLA2 and PAF in modulating formation of protease-resistant prion protein, an agent that is suggested to be the main cause of prion diseases (Bate et al., 2004).

Vitamin E protects from neuronal damage induced by cerebral ischemia by inhibiting apoptosis in hippocampal neurons (Tagami et al., 1999; Zhang et al., 2004). This finding suggests that vitamin E reacts with the free radicals and prevents neuronal apoptosis produced by cerebral ischemia and reperfusion. Vitamin E also protects neurons from a toxic concentration of sodium nitroprusside, a nitric oxide donor, in a dose-dependent manner indicating that this vitamin protects brain tissue by inhibiting free radical generation and oxidative stress. Double-blind human trials of vitamin E have been performed. It slows the progression of Alzheimer’s disease (Sano et al., 1997) or has only symptomatic effects with no alteration of the progression of AD (Sano et al., 1997). In cat spinal cord, preloading with vitamin E and selenium promotes recovery after spinal cord injury (Anderson et al., 1985). Collective evidence suggests that vitamin E may be useful for the treatment of inflammation and oxidative stress in acute neural trauma and neurodegenerative diseases. Negative results in human trials are probably due to the lack of a range of redox inhibitors.
CDP-choline (citicoline) inhibits cPLA2 activity and lowers the concentration of free fatty acids in a dose- and time-dependent manner (Adibhatla et al., 2002). This compound is an intermediate in PtdCho biosynthesis that has been used for the treatment of ischemic and head injuries (Andersen et al., 1999; Dempsey and Rao, 2003). It not only restores the concentration of PtdCho after ischemic injury by increasing PtdCho synthesis from diacylglycerol but also blocks the activation of cPLA2 activity (Adibhatla et al., 2002). The decrease in cPLA2 activity may lead to a reduction in levels of arachidonic acid and reactive oxygen species, with stabilization of neural membranes. CDP-choline also protects cerebellar granule neurons from glutamate-mediated neurotoxicity (Mir et al., 2003), suggesting that CDP-choline may protect neurons from excitotoxicity. CDP-choline has been used in phase III clinical trials for stroke and is being evaluated for the treatment of AD and PD. It also improves the verbal memory of aged human subjects. These observations suggest that this cPLA2 inhibitor can be used for treating acute neural trauma as well as neurodegenerative diseases.

Neurotrophic effects of gangliosides have been demonstrated in AD, PD, spinal cord injury, and stroke (Geisler et al., 1991; Svennerholm, 1994). The mechanism underlying the ganglioside action is not fully understood. However, gangliosides are known to inhibit neurotransmitter release and cPLA2 and PIsEtN-PLA2 activities. Gangliosides also rescue neuronal cultures from death after neurotrophic factor deprivation (Ferrari et al., 1993). NMR studies indicate that GM1 ganglioside binds tightly with β-amyloid peptide and inhibits the α-helix to β-sheet conformational change in β-amyloid peptide (Mandal and Pettegrew, 2004). The interaction with GM1 ganglioside may release the inhibition of PLA2 isoforms by GM1 and β-amyloid, resulting in delay of neurodegeneration in AD. In ischemic brain, gangliosides protect neural cells by scavenging free radicals generated during reperfusion (Fighera et al., 2004).

In the MPP*-mediated cell culture model of PD (Yoshinaga et al., 2000), neurodegeneration is accompanied by the stimulation of PLA2 and arachidonic acid release in GH3 cells. This release of arachidonic acid can be blocked by arachidonyl trifluoromethyl ketone, a potent inhibitor of cPLA2, suggesting the involvement of cPLA2-mediated oxidative stress in MPP*-mediated neurodegeneration. Similarly in the MPTP-induced model of parkinsonism, quinacrine protects dopaminergic neurons from neurodegeneration (Tariq et al., 2001). In this system quinacrine may act not only as a PLA2 inhibitor but also as a membrane stabilizer and antioxidant (Tariq et al., 2001; Turnbull et al., 2003).

Polyunsaturated fatty acids of the n-3 series have many beneficial effects in the central nervous system (Farooqui and Horrocks, 2004a). Thus, EPA prevents LPS-mediated TNF-α expression by preventing NF-κB activation and protects rat hippocampus from LPS-mediated neurotoxicity (Lonergan et al., 2004; Zhao et al., 2004). In C6 glia cells, EPA modulates myelin proteolipid gene expression (Salvati et al., 2004). This fatty acid is used for the treatment of schizophrenia (Peet and Ryles, 2001; Horrobin, 2003).

Chronic preadministration of DHA prevents β-amyloid-induced impairment of an avoidance ability-related memory function in a rat model of AD (Hashimoto et al., 2002) and protects mice from synaptic loss and dendritic pathological changes in another model of AD (Calon et al., 2004). Thus, DHA is beneficial in preventing the learning deficiencies in these AD models. DHA also affects amyloid precursor protein processing by inhibiting α- and β-secretase activities (de Wilde et al., 2003; Walsh and Selkoe, 2004). Supplements of DHA produce a neuroprotective effect on β-amyloid deposition without significant toxic effects. DHA reverses the age-related impairment in LTP and depolarization-induced glutamate release. It also inhibits the production of TNF-α, interleukin-1, and interleukin-6. DHA protects the brain against ischemic and excitotoxic damage in rats (Gamoh et al., 1999; Terano et al., 1999). DHA may act as an antioxidant (Hossain et al., 1998). DHA induces antioxidant defenses by enhancing cerebral activities of catalase, glutathione peroxidase, and levels of glutathione (Hossain et al., 1999). Thus, EPA and DHA exert their neuroprotective effects by modulating cytokines, inflammation, and oxidative stress.

Treatment of Zellweger syndrome patients with purified DHA partially improves visual function, increases levels of plasmalogens, and reduces levels of saturated very long-chain fatty acids (Hossain et al., 1999). The level of DHA is also low in patients with multiple sclerosis. Fish oil supplements with vitamins improve the clinical outcome in MS patients (Nordvik et al., 2000). Collective evidence from many studies indicates that DHA supplementation restores signal transduction processes associated with behavioral deficits, learning activity in Alzheimer disease, schizophrenia, depression, hyperactivity, stroke, and peroxisomal disorders (Farooqui and Horrocks, 2004a).

All of the cPLA2 inhibitors used in these studies are nonspecific. Thus, the design and synthesis of specific cPLA2 inhibitors is urgently needed to make progress in this important area of research. The reaction catalyzed by cPLA2 is a rate-limiting step for the generation of eicosanoids, lysophospholipids, and platelet-activating factor. High levels of these metabolites are responsible for oxidative stress, inflammation, and neuronal death at the injury site. Potent PLA2 inhibitors can effectively block the above events associated with neurodegenerative processes and rescue neural cells from cell death. It is hoped that the synthesis of a new generation of cPLA2 inhibitors would have regional specificity. The inhibitors should be able to reach the injury site where neural cells
are under oxidative stress and where neurodegenerative processes are taking place.

IX. Prevention of Pain by Phospholipase A2 Inhibitors

Proinflammatory cytokines released at the site of neural trauma and nerve injury may be involved in sensitization of nociceptors leading to hyperalgesia (Walters, 1994). Injections of carrageenan into the paw or face have been widely used as a model to induce pain sensitization (Ng and Ong, 2001). We have recently studied the effect of intracerebroventricular injections of a sPLA2 inhibitor, 12-epi-scalaradial, a cPLA2 inhibitor, AACOCF3, and an iPLA2 inhibitor, bromoenol lactone, on the development of alldynia after facial carrageenan injections in two strains of mice (Yeo et al., 2004) (Fig. 10). C57BL/6J (B6) mice show an increase in alldynia from 8 h to 3 days after facial carrageenan injection. On the other hand, the BALB/c strain did not show an increase in alldynia at any time point. In both B6 and BALB/c mice, all PLA2 inhibitors significantly reduced responses to von Frey hair stimulation at 8 h and 1 day, but at 3 days only the sPLA2 inhibitor had an effect. Because BALB/c mice do not show increases in alldynia after carrageenan injection, the reduction in responses seen with PLA2 inhibitors actually means that these inhibitors produce a loss of normal sensitivity to von Frey hair stimulation. The effects of PLA2 inhibitors are unlikely to be due simply to inhibition of arachidonic acid generation, because intracerebroventricular injection of arachidonic acid also had an antinociceptive effect (Yeo et al., 2004). It is proposed that lysophosphatidylcholine mediates pain transmission in the central nervous system. The pronounced and long-lasting antinociceptive effect of 12-epi-scalaradial is consistent with our recent finding that sPLA2 induces exocytosis and neurotransmitter release in neurons and supports a key role of central nervous system sPLA2 in synaptic and pain transmission. These results suggest that PLA2 isoforms play an important role not only in pain transmission but also in nonpainful, touch, or pressure sensation. Our studies on the antinociceptive effect of PLA2 inhibitors are supported by recent studies on intrathecal injections of MAPF and AACOCF3 in rats. MAPF has a significant antinociceptive effect in the rat formalin test (Ates et al., 2003). Based on our studies, we suggest that PLA2 inhibitors may reduce alldynia and hyperalgesia in inflammation-mediated central pain.

X. Perspective and Direction for Future Studies

PLA2 isoforms along with cyclooxygenases have emerged as major players in modulating inflammation and oxidative stress in brain tissue. Elucidation of the mechanism of action of PLA2 inhibitors in vivo is a critical area of research because of the potential pharmacological benefits of these compounds as therapeutic agents for the treatment of inflammation and oxidative stress in neurotrauma and neurodegenerative diseases (Farooqui et al., 1999). Although several inhibitors of PLA2 activity have been reported in the literature (Farooqui et al., 1999; Cummings et al., 2000; Miele, 2003b), little information is available on the mechanism of their action. Different mechanisms of action are possible; e.g., an inhibitor can produce alterations in enzymic activity by perturbing the physicochemical properties of phospholipid bilayers. A PLA2 inhibitor can directly interact with the active site of an isoform, as AACOCF3, MAPF, and BEL, or it can act on an allosteric site on the enzyme molecule to bring about changes in enzymic activity. An inhibitor may also possess a detergent-like structure.

![Fig. 10. Total responses in mice treated with dimethyl sulfoxide (DMSO), PLA2 inhibitors or arachidonic acid, and carrageenan at 8 h after injection. The y-axes indicate total scratching/withdrawal/attack/escape responses in mice that received intracerebroventricular injection of DMSO (5 µl, vehicle control), PLA2 inhibitors (0.01 µmol in DMSO), or arachidonic acid (0.01 µmol in DMSO) and a facial injection of carrageenan, 8 h after injections. Data were analyzed by one-way analysis of variance with Bonferroni’s multiple comparison post hoc test. *, statistically significant differences (P < 0.05). n = 4 mice in each group. Both B6 mice (A) and BALB/c mice (B) showed significantly fewer total responses to von Frey hair stimulation of the face after injection with inhibitors to each of the three isoforms of PLA2 or arachidonic acid compared with DMSO. D + C, S + C, A + C, B + C, AA + C indicate DMSO, 12-epi-scalaradial, AACOCF3, BEL, or arachidonic acid, plus carrageenan, respectively. Data modified from Yeo et al. (2004).]
PLA2-catalyzed reactions are the rate-limiting steps for reduction pathways in neurological disorders. Because emerging understanding of the role of signal transduction processes in acute trauma and neurodegenerative diseases will differ in inhibitor sensitivities and reactivity in vivo.

In brain, PLA2 occurs in multiple forms. Thus, specific PLA2 inhibitors must be designed for individual PLA2 isoforms to define their roles in brain metabolism. The design of PLA2 inhibitors should be focused on our rapidly emerging understanding of the role of signal transduction pathways in neurological disorders. Because PLA2-catalyzed reactions are the rate-limiting steps for the production of prostaglandins, leukotrienes, and thromboxanes, the identification of PLA2-coupled receptors and their endogenous regulatory pathways that mediate proliferative, metabolic, and inflammatory signals can provide better targets for designing PLA2 inhibitors. Specificity, selectivity, harmlessness, and the ability to cross the blood-brain barrier are important qualities of a PLA2 inhibitor as a potential therapeutic agent for neurological disorders.

At this time, it is quite difficult to predict the potential side effects of the chronic use of cell-permeable, specific, or nonspecific inhibitors of PLA2. Hence, studies on the availability of specific, nontoxic potent inhibitors with greater blood-brain barrier permeability in animal models of neurodegenerative diseases are urgently needed. Surely, PLA2 isoforms from different sources will have different inhibitor sensitivities and reactivity in vivo. Nevertheless, pharmacological studies in animal models of acute trauma and neurodegenerative diseases will provide directions that should be taken to develop better PLA2 inhibitors for targeting isoforms of brain PLA2 activities.

In recent years advanced molecular biology procedures have been used in a number of studies to overcome some of the problems associated with the specificity of chemical inhibitors of PLA2. For example, antisense oligonucleotides that inhibit specific cPLA2 or iPLA2 have been developed. Transgenic mice that are deficient in or overexpress cPLA2 isoforms are now available (Bonventre et al., 1997). Overexpression of cPLA2 allows one to study the effect of increased cPLA2 activity, whereas a deficiency of cPLA2 can be helpful in studying the consequences of reduced cPLA2 activity on cellular metabolism in normal and diseased cells. RNAi for iPLA2 has also been developed. Transfection studies with RNAi of iPLA2 indicate that the levels of iPLA2 protein and iPLA2 activity are decreased in a dose-dependent manner in transfected non-neural cells (Shinzawa and Tsujimoto, 2003). Such studies are needed in neuronal cell cultures and animal models to understand the neurophysiological importance of the RNAi technique. We propose that a comparison of activities of PLA2 isoforms and intensity of signal transduction process between normal and genetically manipulated mice may provide further insight into the role of PLA2 isoforms, their ligands, and their lipid mediators in neurodegenerative processes.

Thus, the development of specific inhibitors for different PLA2 isoforms should be an important goal for future research on brain PLA2 activities. The chemical approach, together with molecular biological procedures such as RNAi and alterations in signal transduction processes in knockout mice, may provide the important need to develop specific PLA2 inhibitors that can be used to retard oxidative stress and inflammatory reactions during neurodegeneration in neurological disorders.

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