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Role of Secretory Phospholipase A_2 in CNS Inflammation: Implications in Traumatic Spinal Cord Injury

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Abstract

Secretory phospholipases A_2 ($sPLA_2$s) are a subfamily of lipolytic enzymes which hydrolyze the acyl bond at the *sn-2* position of glycerophospholipids to produce free fatty acids and lysophospholipids. These products are precursors of bioactive eicosanoids and platelet-activating factor (PAF). The hydrolysis of membrane phospholipids by PLA_2 is a rate-limiting step for generation of eicosanoids and PAF. To date, more than 10 isozymes of $sPLA_2$ have been found in the mammalian central nervous system (CNS). Under physiological conditions, $sPLA_2$s are involved in diverse cellular responses, including host defense, phospholipid digestion and metabolism. However, under pathological situations, increased $sPLA_2$ activity and excessive production of free fatty acids and their metabolites may lead to inflammation, loss of membrane integrity, oxidative stress, and subsequent tissue injury. Emerging evidence suggests that $sPLA_2$ plays a role in the secondary injury process after traumatic or ischemic injuries in the brain and spinal cord. Importantly, $sPLA_2$ may act as a convergence molecule that mediates multiple key mechanisms involved in the secondary injury since it can be induced by multiple toxic factors such as inflammatory cytokines, free radicals, and excitatory amino acids, and its activation and metabolites can exacerbate the secondary injury. Blocking $sPLA_2$ action may represent a novel and efficient strategy to block multiple injury pathways associated with the CNS secondary injury. This review outlines the current knowledge of $sPLA_2$ in the CNS with emphasis placed on the possible roles of $sPLA_2$ in mediating CNS injuries, particularly the traumatic and ischemic injuries in the brain and spinal cord.

Keywords

Phospholipases A; spinal cord injury; ischemia; excitatory amino acids; reactive oxygen species; inflammation; lipid metabolism; cytokines

INTRODUCTION

Phospholipases A_2 (EC 3.1.1.4) are enzymes that catalyze the hydrolysis of the *sn-2* position of membrane glycerophospholipids, leading to the production of free fatty acids and

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lysophospholipids. These enzymes are of particular interest since free fatty acids can be converted to bioactive ecosainoids *via* the cycloxygenase pathway leading to increased inflammation. Additionally, the other reaction product, lysophospholipids, such as lysophosphatidic acid and lysophosphatidylcholine (LPC), are also bioactive [1] and can be converted into platelet-activating factor (PAF). Since lipids are a main constituent of the CNS and phospholipids constitute 44% of myelin [2], understanding the role of phospholipases in CNS disorders becomes a major priority.

To date, more than 27 mammalian isoforms of PLA_2 have been found which can be classified into four major categories: secretory PLA_2 ($sPLA_2$), cytosolic PLA_2 ($cPLA_2$), Ca^{2+}-independent PLA_2 ($iPLA_2$), and PAF acetylhydrolases (PAF-AH) [3–6] (Fig. 1). The 11 mammalian isozymes in the $sPLA_2$ subfamily have a low molecular mass of about 14–18 kD, require the presence of submillimolar to millimolar concentrations of Ca^{2+} for effective hydrolysis of a substrate phospholipid, and lack fatty acid selectivity [3,6,7].

The functions of $sPLA_2$s are far reaching, including digestion, exocytosis [8], and anticoagulation [9]. However, $sPLA_2$s most prominent role is in pathological conditions such as neurotrauma [10], antimicrobial activity [11–13], ischemia [14], atherosclerosis [15–17], and cancer [18–21]. In this review we will focus on the role of $sPLA_2$s in CNS pathology, particularly spinal cord injury (SCI).

Role in General Inflammation

$sPLA_2$ has had an established role in inflammation and inflammatory diseases for some time [22]. The blockade of PLA_2 holds a particular interest for pharmacologists since inhibition of $sPLA_2$ would in theory prevent the formation of inflammatory eicosanoids prior to the cyclooxygenase (COX; EC 1.14.99.1) reaction. In fact, PLA_2 is the rate limiting precursor in arachidonic acid (AA) production [23]. Therefore its blockade should eliminate the need for COX-1 versus COX-2 specificity in anti-inflammatory therapeutics. This theory has spurred the development of a large number of $sPLA_2$ inhibitors that unfortunately, have not produced the desired clinical efficacy to date [24].

$sPLA_2$s have been linked to many inflammatory diseases. $sPLA_2$ activity is elevated in several body fluids of patients with acute pancreatitis [25]. Synovial fluid from arthritic joints of rheumatic patients contains $sPLA_2$-IIA [26,27]. Total PLA_2 activity and $sPLA_2$-IIA is enhanced in bronchoalveolar lavage fluids from patients with adult respiratory distress syndrome [28]. Increased levels of $sPLA_2$-IIA were seen in the skin of patients with psoriasis [29]. Increased group II, PLA_2 expression was found in colonic mucosa of patients with Crohn's Disease and ulcerative colitis [30] and experimental models of ischemic bowel disease in rodents [31,32]. Additionally, serum levels of $sPLA_2$, particularly group IIA, increase in patients with sepsis [33,34] and injuries [22,35], and following many types of surgeries such as cardiac surgery [36], aortobifemoral reconstruction [36], and splenectomy [12]. Levels of serum $sPLA_2$-IIA correlate with C-reactive protein in several disease states, supporting the notion that $sPLA_2$-IIA is an acute phase protein [37]. Some suggest that elevations in serum levels of $sPLA_2$-IB are a specific marker of pancreatic damage whereas elevation in $sPLA_2$-IIA are a more general marker of inflammation [22].

$sPLA_2$ has been either found in or produced by various inflammatory cells including platelets [38], mast cells [39], fibroblasts [40], macrophages [41,42] and neutrophils [43]. Macrophages isolated from peritoneal exudates of mice and rabbits secrete PLA_2 [42]. Additionally, $sPLA_2$-IIA is constitutively expressed in immune tissues, such as the spleen, thymus, tonsils, and bone marrow [26,27]. Proinflammatory cytokines such as interferon gamma (IFN-γ), interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) can induce its production in a variety of cell types such as human arterial smooth muscle cells, HepG2,

HEK293, and BRL-3A in culture [44–46]. Interestingly the AA release from cells treated with IIA, IID, IIE and V were found to be dependent on IL-1, while treatment with group X released AA irrespective of IL-1 [6]. More relevant to the CNS, TNFα, IL-1, and lipopolysaccharide (LPS) were shown to induce $sPLA_2$-IIA production in cultured astrocytes and direct injection of LPS into brain increased IIA mRNA [47]. Similarly LPS injections have been shown to increase $sPLA_2$-IIE production [48]. Consistent with its inducible nature, the promoter region of $sPLA_2$-IIA gene contains TATA and CAAT boxes as well as several elements homologous with consensus sequences for binding of transcription factors such as AP-1, C/EBPs, CREB, NF-κB, STAT, and PPARγ [37,49]. Finally, $sPLA_2$ induction can be blocked by anti-inflammatory cytokines, such as platelet-derived growth factor, transforming growth factor β, and IL-10 as well as glucocorticoids [50–52].

CLASSIFICATION, STRUCTURE, AND PROPERTIES OF $sPLA_2$

General Structure

Eleven mammalian $sPLA_2$s exist which are further divided into groups I, II, III, V, X, XII, and XIII (Fig. 1). All $sPLA_2$s are structurally related, and generally are 14–17 kD secreted enzymes, with six absolutely conserved disulfide bonds, which contribute to the high degree of stability of these enzymes. The enzymes do not have strict fatty acid specificity, unlike $cPLA_2$, but instead tend to act on anionic phospholipids in the presence of high concentrations of Ca^{2+}. The central, core protein consists of a highly conserved Ca^{2+}-binding loop (XCGXGG) and a catalytic site (DXCCXXHD) [6]. Group differentiations are then made based on the presence or absence of up to two additional unique disulfide bonds, the presence or absence of an N- or C-terminal extension, and alterations in the conserved catalytic site.

Intracellular Handling

The site of $sPLA_2$ activity has been a point of exhaustive study in recent years. While all $sPLA_2$ isoforms are capable of secretion by definition, recent work has indicated that some heterogeneity exists among their site of activity [53]. Recent studies in CHO and HEK293 cell lines that have been stably transfected with human groups IIA and X have shed light on this subject. Following stimulation by fetal bovine serum and IL-1β, both $sPLA_2$-IIA and X are transcribed within the cell nucleus and synthesized in the endoplasmic reticulum prior to packaging in the Golgi apparatus [54] (Fig. 2). This post synthesis packaging most likely results in the perinuclear puncta observed after stimulation, an observation which was previously ascribed to invagination of heparin sulfate proteoglycans (HSPG)-bound $sPLA_2$, a process described later. It is within the Golgi apparatus and later microvesicles, prior to initial secretion, that $sPLA_2$-IIA is primarily active [53]. The reason for this is that most group II $sPLA_2$s as well as $sPLA_2$-IB show a marked preference for anionic phospholipids, such as phosphatidylglycerol, phosphatidylethanolamine and phosphatidylserine, which are generally segregated to the inner leaflet of plasma membranes [55]. In contrast, $sPLA_2$-V and X can hydrolyze both anionic phospholipids and charged-neutral phosphatidylcholines. This difference in phospholipid preference results in secreted groups I and II having decreased abilities to act on the outer layer of plasma membranes [5,53]. Once secreted, $sPLA_2$ isoforms can then 1) metabolize the external plasma membrane, 2) bind to the $sPLA_2$ receptor ($sPLA_2$-R), or 3) be internalized by the HSPG shuttle. Again, each of these actions is governed by isoform and species specificity (Fig. 2).

HSPG Shuttling

Some of the group II subfamily of $sPLA_2$s, including IIA, IID, and V are highly cationic and bind tightly to anionic heparanoids such as heparin and heparin sulfate [56,57] (Fig. 2). Since cell surfaces are usually rich in heparin sulfate proteoglycans (HSPG), significant portions of these $sPLA_2$s are membrane-bound rather than being secreted into the culture media [58,59].

Other $sPLA_2$s (IB, IIC, and X) with neutral to acidic pHs show no, or very low, heparanoid affinity and are secreted into the medium. Some authors suggest that this binding facilitates phospholipid digestion since treatment of stably transfected cells with heparinase, exogenous heparin, and GPI-specific phospholipase C [45,46,60,61], or mutation of the heparin binding domain in $sPLA_2$-IIA [56–58] markedly attenuates $sPLA_2$-IIA-mediated prostaglandin generation. In contrast, one recent study found that treatment of cells with heparin had little or no effect on $sPLA_2$-IIA activity [54]. Also of note, HSPG has been shown to increase following cerebral stab injury with an implicated role in storage, nuclear trafficking, and cell-specific injury responses in CNS wounds [62]. The closely related chondroitin sulfate proteoglycans are also greatly expressed following SCI [63,64].

Receptor Binding Domain

In addition to its enzymatic function, some $sPLA_2$s mediate their biological function through a membrane receptor. Two $sPLA_2$ receptors have been identified to date, the M-type [65–67] and the N-type [68], named for their discovery in muscle and neural tissue respectively. However, mammalian $sPLA_2$s only bind to the M-type which was later discovered to be relatively widespread and not merely confined to muscle [69]. Since only the M-type has the ability to initiate an intracellular signal we will restrict our discussion to its properties and further refer to it as $sPLA_2$-R. The receptor is a type 1 transmembrane glycoprotein with a molecular mass of 180–220 kDa and is a member of the C-type animal lectin family (subgroup VI) [70]. The intracellular region consists of approximately 40 amino acids and contains both a consensus sequence for casein-kinase II phosphorylation [71] and a consensus sequence for coated-pit-mediated endocytosis [70]. Accordingly, the receptor undergoes internalization after $sPLA_2$ binding [72]. Interestingly the isozymes of $sPLA_2$ show varying affinities for the $sPLA_2$-R in different species. For example, the rabbit $sPLA_2$-R is very promiscuous, binding to almost all $sPLA_2$ tested to date. The mouse $sPLA_2$-R on the other hand binds only IB, IIA, and X, while the rat $sPLA_2$-R only binds $sPLA_2$-IB [73] (Fig. 1). Likewise $sPLA_2$-IIA does not seem to bind to the $sPLA_2$-R in humans [73]. In general, $sPLA_2$-IB and X appear to be the predominant ligand of this receptor [74] and most of the research has therefore focused on their effects [71,75,76]. This specificity suggests that the biological function of $sPLA_2$-R could vary wildly among species. However, the generalized functions of the $sPLA_2$-R are far reaching including cell growth [77], proliferation [78], and migration [79]. It has also been suggested that $sPLA_2$-R functions in the clearance of extracellular $sPLA_2$s to protect against enzymatic over activity, particularly $sPLA_2$-X which has potent activity against the extracellular membrane, unlike IB and IIA as stated above [68,80]. Additionally, $sPLA_2$-R knockout mice have significantly reduced levels of TNFα and IL-1β after systemic LPS administration suggesting an inflammatory role [81]. For an excellent review on the role of the $sPLA_2$-R in $sPLA_2$ function, please see [68,82].

Group I –

$sPLA_2$-IB was the first $sPLA_2$ to be discovered and is predominantly present in pancreatic juices [83]. $sPLA_2$-IB lacks a C-terminal extension and is secreted as a catalytically inactive propeptide that is later proteolytically cleaved [84]. Group IB has a unique five amino acid extension termed the pancreatic loop in the middle of the molecule as well as a group specific disulfide bond between Cys^{11} and Cys^{77} [83,84]. $sPLA_2$-IB is almost exclusively secreted into the medium of transfected cells [6]. Interestingly, $sPLA_2$-IB shows low affinity for both heparinoids and phosphatidylcholine (PC) on the external membrane leaflet. Subsequently, it was discovered that $sPLA_2$-IB can only release AA indirectly through the M-type $sPLA_2$ receptor-dependent pathway *via* $cPLA_2\alpha$ activation [71,75,76].

Groups II and V –

The second member of the $sPLA_2$ family, later named group IIA, was first cloned in 1989 [85] and is constitutively expressed in immune tissues, such as the spleen, thymus, tonsils and bone marrow [26,27] as well as the digestive system of some mouse strains [86]. $sPLA_2$-IIA [26,27,85], IID [87,88], and IIE [89,90] are the archetypical group II enzymes. Typically, the enzymes have a C-terminal extension and a disulfide bond linking Cys^{50} with a Cys in the C-terminus. IIC [91] and IIF [92] have minor variations in amino acids and disulfide structure. Similar to IIA, $sPLA_2$-IIE is constitutively expressed in human lung and mouse uterus, brain, heart, liver and testis at low levels [48,87,90]. However, LPS has been shown to induce IIE expression in macrophages, suggesting an inflammatory role as well as [90]. $sPLA_2$-IIF is highly expressed in the mouse embryo, suggesting a roll in development and it is upregulated by LPS as with other group II enzymes [87,93]. Finally, groups IIA, IIC, IID, IIE, and V all utilize the HSPG-shuttling pathway.

Often considered in the same breath with group II enzymes is group V. Group V shows the highest homology with the group II enzymes and is similarly located on human chromosome 1 (1p34–36) [92]. However group V lacks the typical group II C-terminal region, thus justifying its isolation. $sPLA_2$-V functions as the primary mouse $sPLA_2$. This is since mice express group V at higher levels than any of the group II enzymes [94] and since some species of mice have a frame shift mutation resulting in a natural $sPLA_2$-II knock out [86]. However, as with group II, group V is closely linked with inflammation as well as being found in mast cells [94], macrophages [95], and type 2 T helper cells [96]. As with group II, group V is upregulated by LPS [94].

Group X –

Group X possesses characteristics of both group I and group II enzymes. $sPLA_2$-X contains the disulfide bonds of both group I and group II as well as the group II, C-terminal extension [97]. Additionally, like group I, it is secreted as a zymogen with cleavage of the N-terminal propeptide for activation [98]. Like $sPLA_2$-IB, cells transfected with group X, secrete this $sPLA_2$ almost exclusively into the culture medium rather than having it bound to the membrane like group II enzymes [6]. This is not surprising since unlike group II, group X does not readily bind HSPG and shows high activity towards PC, a dominant phospholipid enriched in the outer leaflet of the plasma membrane [19,58,61,74,98]. Typically, $sPLA_2$-X is expressed in the digestive organs such as intestine, colon and stomach and in some immune organs [97]. However, unlike group II, group X is constitutively expressed with little or no change in most tissues [6].

Groups III and XII –

A distinct class of soluble $sPLA_2$s, distantly related to groups I and II, were later discovered in bee and lizard venom and are classified as group III. A mammalian homolog of group III was discovered in 2000 [89]. Groups III and XII only share the Ca^{2+}-binding loop and catalytic site with groups I, II, V, and X. At 55kDa, Group III is considerably large than all the other $sPLA_2$ isozymes. While maintaining all the $sPLA_2$ signature characteristics such as, 10 cystines, the Ca^{2+}-biniding loop, and catalytic site, it additionally has a large N- and C- terminal flanking regions that add to its molecular weight [89]. Within humans, $sPLA_2$-III was found to be in high abundance in heart, skeletal muscle, liver, and kidney, but had only weak expression in the brain [89]. Group-XII is a much smaller enzyme than group III (19 kDa), lacks an N- or C-terminal flanking regions, and has deviations in the Ca^{2+} bind loop, that are inconsistent with other $sPLA_2$s [96,99]. Group XII is expressed in human kidney, heart, and skeletal muscle [89,99]. Relatively little is known about the function of either group III or XII in the mammalian system.

MECHANISM UNDERLYING $sPLA_2$-INDUCED CNS INJURY

The $sPLA_2$ family of enzymes results in CNS damages by multiple injury mechanisms such as attacking cellular membranes, releasing pro-inflammatory mediators, generating free radicals, increasing release of excitotoxic neurotransmitters and enhancing apoptosis [100–102]. These insults in turn exacerbate activation of $sPLA_2$ by a positive feedback loop (Fig. 3). Our discussion will begin by looking at several injury mechanisms common to CNS pathology and how each induces and is exacerbated by $sPLA_2$.

Oxidative Stress

Oxidative injury is a common pathological mechanism in neurological disorders. Free radicals induce not only lipid peroxidation of neural membranes, but also the oxidation of proteins, RNAs, and DNAs. Reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2) and superoxide radicals are produced by a number of cellular oxidative and metabolic processes including metabolism of AA. PLA_2 metabolism of phospholipids is a well-established source of ROS [103,104]. Application of pathophysiological concentrations of free fatty acids has been demonstrated to induce oxidative injury to cultured spinal cord neurons [133]. Microinjections of PLA_2 into the normal spinal cord induced expression of 4-hydroxynonenal, a product of lipid peroxidation and marker for oxygen free radical-mediated membrane injury [105]. Nethery *et al*. [106], found that ROS production in contracting muscle required the presence of $sPLA_2$ but not $cPLA_2$ or $iPLA_2$. In the presence of 15-lipoxygenase the addition of $sPLA_2$-IIA or IB greatly enhances the accumulation of hydroperoxides of oxidized free fatty acids [107]. Finally, it can be assumed that the proinflammatory effects of $sPLA_2$ would result in an immigration of immune cells, which will release copious amounts of ROS [108–113]. For a review of the ROS species produced by the enzymatic effects of $sPLA_2$ please see [104].

While $sPLA_2$ production of ROS is well established, the induction of $sPLA_2$ by ROS is an immerging concept. One study found that mesangial cells treated with H_2O_2 utilized both $cPLA_2$ and $sPLA_2$ during AA release and that elimination of $sPLA_2$ greatly inhibited AA release [114], thus suggesting the first of many positive feedback loops.

Inflammation and Inflammatory Cytokines

Inflammation has been implicated in many CNS neurological disorders including SCI. PLA_2 may serve as a key molecule that controls the biosynthesis of several well-known bioactive mediators of inflammation such as eicosanoids (prostaglandins, thromboxanes, leukotrienes and lipoxins) and PAF in a rate-limiting manner [102,115]. In addition, our recently findings showed that $sPLA_2$ induced expression of cytokines including TNF-α and IL-1β in the injured spinal cord [105]. On the other hand, there is also strong evidence to suggest that cytokines upregulate $sPLA_2$-IIA *in vitro* and *in vivo*. $sPLA_2$-IIA is up-regulated by TNF-α and IL-1α/β after transient focal cerebral ischemia in rats [116]. $sPLA_2$-IIA mRNA is expressed in cultured astrocytes and can be induced in response to proinflammatoy cytokines TNFα, IL-1β, and, INFγ [47,117–119]. Astrocytes isolated from C57Bl/6 mice, which lack the $sPLA_2$-IIA gene, were less responsive to cytokines in the production of PGE_2 than were astrocytes expressing $sPLA_2$-IIA [119]. Additionally, IL-1β and TNFα can also activate COX-2 continuing the proinflammatory pathways [19,45,59,94,120]. $sPLA_2$ also induced AA release and COX-2 expression in cultured neurons independent of other cytokines [121].

Excitatory Neurotoxicity

Elevated levels of excitatory amino acids (EAA) have been implicated in the pathogenesis of neural injury and death in many disorders and evidence suggests that $sPLA_2$ may stimulate the release of EAA following CNS trauma. First, injections of $sPLA_2$ into the brain caused epileptic

seizures and neurotoxicity *in vivo* [122]. Secondly, application of $sPLA_2$ to the rat ischemic cerebral cortex resulted in a significant increase in EAA levels and inhibition with mepacrine, a global PLA_2 inhibitor, significantly decreased the ischemia-evoked efflux of EAA into cortical superfusates [123]. Group IIA $sPLA_2$ stimulates exocytosis and neurotransmitter release in pheochromocytoma-12 cells and cultured rat hippocampal neurons [124]. $sPLA_2$-induced neuronal death was blocked by MK-801, an N-methyl-D-aspartic acid (NMDA) receptor antagonist, both *in vitro* and *in vivo* [125]. Finally, administration of the nonselective PLA_2 inhibitor, 4-bromophenacyl bromide, inhibited glutamate release in the spinal cord [126]. Only one study so far has suggested that excessive EEA concentrations increase $sPLA_2$ levels in the CNS. Interocerebroventricular injection of kainic acid (KA) resulted in an increase in both total PLA_2 and $sPLA_2$ activity and this activity could be blocked by a synthetic short inhibitor peptide for $sPLA_2$-IIA [127].

The exact mechanism of $sPLA_2$ action on EAA release remains unknown. It has been suggested that PLA_2 disrupts an artificial planar lipid bilayer in a Ca^{2+}-dependent manner [128]. Matsuzawa demonstrated the role of $sPLA_2$-IIA in EEA release in a set of elegant experiments. First, $sPLA_2$-IIA was detected in purified brain synaptosomes. Secondly, $sPLA_2$-IIA was released upon depolarization of neurons with either high concentrations of potassium or neurotransmitters. Third, addition of $sPLA_2$-IIA to cultures triggered neurotransmitter release. Finally, $sPLA_2$-IIA inhibition suppressed neurotransmitter secretion [8]. The role of $sPLA_2$ in neurotransmitter release is further supported by the fact that exogenously added $sPLA_2$ paralyzed neuromuscular junction preparations by inducing total neurotransmitter release [129]. Interestingly, an equimolar mixture of lysophospholipids and fatty acids closely mimicked the $sPLA_2$ paralysis [129]. These studies further suggest that changes in the local lipid composition within the synaptic buton trigger neurotransmitter release which would lead to excitotoxicity [130]. Inhibitors of total PLA_2 activity such as 4-bromophenacyl bromide, a nonselective PLA_2 inhibitor, 7,7-dimethyleicosadienoic, a $sPLA_2$ specific inhibitor, AACOCF3, a $cPLA_2$ specific inhibitor, and HELSS, an $iPLA_2$ specific inhibitor, all reduced the efflux of both glutamate and aspartate *in vivo* suggesting the involvement of multiple isoforms of PLA_2 in EAA release not merely $sPLA_2$ [131].

Membrane Breakdown and Metabolites

Phospholipids are the main components of the neural cell bi-layer membrane. In addition, they provide the membrane with the necessary environment, fluidity, and ion permeability that are required for the proper function of integral membrane proteins, receptors, and ion channels. PLA_2 directly hydrolyses phospholipids resulting in membrane breakdown. This results in alterations of membrane function such as fluidity and permeability, behavior of transporters and receptors, and ion homeostasis, and can eventually lead to functional failure of excitable membranes [101,102,132].

In addition to the direct effects of membrane breakdown on cell survival, the products of $sPLA_2$ enzymatic activity also exhibit neurotoxic profiles. $sPLA_2$ cleaves phospholipids into the primary metabolites free fatty acid, such as AA, and lysophospholipids such as lysophosphatidyl choline (LPC, a.k.a. lysolechithin) (see insert of Fig. 2 and Fig. 3). Both of these agents have been shown to create injury and cytotoxicity in the CNS. AA induces oxidative injury and death in cultured spinal cord neurons [133]. AA can later form epoxides *via* the cytochrome P450 pathway, leukotrienes *via* the lipoxygenase pathway, or thromboxanes or prostaglandins *via* the COX pathway (see insert of Fig. 2). Many of these products, such as prostaglandin E_2 (PGE_2) can subsequently act as potent chemoattractants increasing endogenous immune responses and subsequent secondary damage. Additionally, the expression of eicosanoids, such as thromboxane A_2 (TXA_2) and PGE_2 following SCI have been linked to trauma induced ischemia [134]. LPC produced by $sPLA_2$ –induced hydrolysis

is also implicated in CNS damage [135]. Interestingly, direct LPC injection has been shown to create a demyelination and infiltration of macrophages in the spinal cord, brain, and peripheral nervous system with a later remyelination by Schwann cells, the myelinating cells of the peripheral nervous system [136–139]. This demyelination and remyelination by Schwann cells mimics that produced by direct injection of $sPLA_2$, however, $sPLA_2$ has the added cost of severe axonopathy and death of oligodendrocytes prior to remyelination [10]. It has been hypothesized that LPC may mediate the demyelinating effects of $sPLA_2$ injections. Additionally, a recent study by Lauber, *et al*. found that LPC, generated by $iPLA_2$, was the main chemoattractant for monocytic cells and primary macrophages released by apoptotic cells thus facilitating the efficient phagocytosis of cellular debris [1].

Apoptosis

In recent years, apoptosis has been identified as an important mechanism of cell death in many neurological disorders including SCI. Cells undergoing apoptosis generally release free fatty acids including AA, which parallels the reduction in cell viability [140,141], suggesting the involvement of PLA_2 in apoptosis. Recently, $sPLA_2$-IB, IIA, and III have all been shown to induce neuronal apoptosis [142–145]. In contrast, recombinant $sPLA_2$ appears to prevent apoptosis of mast cells [140].

ROLE OF $sPLA_2$ IN CNS DISORDERS

Localization within the CNS

$sPLA_2$s are present in all regions of the mammalian brain. The highest $sPLA_2$ activities are found in medulla oblongata, pons, and hippocampus; moderate activities in the hypothalamus, thalamus, and cerebral cortex; and lowest activities in the cerebellum and olfactory bulb [127]. Molloy, *et al*. utilizing RT-PCR found that mRNAs for $sPLA_2$-IIA and IIC were expressed in all regions of normal rat brain, $sPLA_2$-V was found at low levels in most areas of the brain, but at very high levels in the hippocampus, and $sPLA_2$-IB was not detected in the rat brain at all [146]. In contrast, Kolko, *et al*. reported that $sPLA_2$-IB mRNA was detected in the rat and human brain at very high levels as well as in neurons in primary cultures using various detection methods. The distribution of $sPLA_2$-IB seems to be mainly neuronal, with the highest abundance occurring in the cerebral cortex and hippocampus [147]. $sPLA_2$-IIA and V were also detected in the rat cerebellum using immunostaining and *in situ* hybridization methods [148]. $sPLA_2$-IIA is associated with the endoplasmic reticulum in perinuclear regions of Purkinje cell somata and $sPLA_2$-V was localized in Bergmann glia cells [148]. Recently, Kolko, *et al*. found the presence of $sPLA_2$-IIE, V, and X in the rat brain as well as in neurons in primary cultures using RT-PCR, *in situ* hybridization, and immunohistochemistry [149]. The distribution of $sPLA_2$-IIE, V, and X seems to be mainly neuronal, with the highest abundance occurring in the cerebral cortex and hippocampus [149]. In the spinal cord, $sPLA_2$ activity was detected in the normal rat spinal cord homogenate [150]. Western blots revealed that $sPLA_2$-IIA and V are expressed in the normal rat spinal cord [150]. mRNAs of $sPLA_2$s (IB, IIA, IIC and V) are also detected in the normal rat spinal cord with RT-PCR [151].

As stated above, the presence of $sPLA_2$ in the CNS, particularly neurons, appears to be both constitutive (groups IB, IIA, V, and X) and inducible (group IIA). While it is assumed that the inducible $sPLA_2$ expression is associated with inflammation, the normal physiological role of constitutively expressed $sPLA_2$ is believed to play a crucial role in exocytosis of synaptic vesicles [8,152]. Support for this theory arises from studies by Matsuzawa, *et al*., in which differentiated PC12 cells were shown to release $sPLA_2$-IIA after depolarization and that inhibition of IIA resulted in decreased catecholamine secretion [8]. Additionally, snake PLA_2 neurotoxins cause paralysis of neuromuscular junctions by triggering a massive release

of all presynaptic vesicles [129]. Finally, the concentration of group V in the hippocampus and studies by Chabot, *et al*. indicate that $sPLA_2$-V may play a role in the regulation of long-term potentiation and long-term depression [153].

Spinal Cord Injury

Damage from acute SCI occurs in two phases, an initial mechanical injury followed by a secondary injury mediated by multiple processes including inflammation, free radical induced cell death, cytokine production, and glutamate excitotoxicity [112,154–156]. Following SCI, inflammatory cells such as polymorphonuclear neutrophils, macrophages, and lymphocytes quickly infiltrate into the traumatized cord, and flood the interstitial tissue with proinflammatory cytokines such as TNF-α and IL-1β, and neurotoxic factors from leukocytes such as nitric oxide, hydrogen peroxide, and myeloperoxidase [108–113]. Free radical generation and lipid peroxidation were also found to be early events subsequent to SCI [155, 157,158]. EAAs such as glutamate and aspartate are released rapidly following SCI and their extracellular concentrations increased to neurotoxic levels within minutes post SCI [159–162]. The interplay between multiple harmful substances likely perpetuates the progressive course of secondary injury, resulting in cell death, axonal destruction, demyelination and functional loss. Evidence suggests that these harmful substances generated in the injured cord might induce $sPLA_2$ expression which in turn exacerbates SCI. This hypothesis is supported by *in vitro* and *in vivo* experiments that indicate that increases in PLA_2 activity and its metabolic products can in turn exacerbate inflammation [4,163], oxidation [4,163], demyelination [10], and neurotoxicity [163,164] suggesting that $sPLA_2$ may serve as a common mediator in the progression of secondary SCI (Fig. 4).

Recently, we showed that PLA_2 activity increased following SCI, peaked at 4 h post injury, and remained elevated for one week. In the same study, we found that $cPLA_2$ expression did not peak until 7 days post injury [105]. *In vitro* experiments showed that both $sPLA_2$ and melittin, an activator of endogenous PLA_2, induced spinal neuronal death in a dose-dependent manner, an effect that could be substantially reversed by mepacrine, a PLA_2 inhibitor [105]. When $sPLA_2$ was directly microinjected into the normal rat spinal cord, it induced tissue damage, demyelination, and sustained impairment in motor function [10]. Such $sPLA_2$-induced demyelination, however, could be effectively attenuated with mepacrine, a PLA_2 inhibitor [105]. Injections of $sPLA_2$ also induced the expression of inflammatory cytokines TNF-α and IL-1β, as well as 4-hydroxynonenal, a product of lipid peroxidation and a marker for oxygen free radical-mediated membrane injury [105]. Indeed, *in vivo* and *in vitro* experiments show that exogenous administration of $sPLA_2$ can induce neuronal death, oligodendrocyte death, and tissue damage [10,105,142–144,164–166]. Importantly, to date no study has directly observed the presence of $sPLA_2$ following SCI, which is a current focus of our lab.

The induction of $sPLA_2$ following SCI is supported by the fact that the substrate of PLA_2 metabolism, phospholipids, decreases acutely following SCI. There is a dramatic loss of membrane phospholipids following CNS trauma. During the first minute of compression trauma to the spinal cord, 10% of the plasmenylethanolamine is reduced with an overall loss of 18% found at 30 min post compression injury [167]. The hydrolysis of membrane phospholipids by PLA_2 is a rate-limiting step for generation of proinflammatory mediators eicosanoids and PAF [102,168].

Additionally, there is an increase in free fatty acids, eicosanoids, lipid peroxides, and lysophospholipids following SCI [169–171]. Severe trauma was associated with biphasic increases in free fatty acids levels, with levels peaking at 15 min and 24 hr post-trauma before declining over the next 6 days [171]. Within the first few minutes of SCI, free fatty acids have increased in the grey matter and later increase within the white matter suggesting acute PLA_2 activity [170–172]. The production of free fatty acid represents a source of potentially

dangerous ROS by initiating lipid peroxidation. Hydroxyl radicals can attack polyunsaturated fatty acids in membrane glycerophospholipids forming peroxyl radicals and propagating the chain reaction of lipid peroxidation products [173,174]. The generation of free fatty acids in SCI is closely associated with increases in free radical formation observed in the lesion of the injured spinal cord [175,176]. Application of pathophysiological concentrations of free fatty acids has been demonstrated *in vitro* to induce oxidative injury in spinal cord cell cultures [133]. The neurotoxic effects of AA have also been observed in hippocampal neurons and cortical neurons [177] as well as oligodendrocytes [178].

Later products of free fatty acid metabolism also increase within the injured spinal cord. COX, also known as prostaglandin G/H synthase, is the rate-limiting step in the production of prostaglandins (see insert of Fig. 2). COX-2 mRNA and protein expression are increased from 2 to 48 hr following SCI and the selective inhibition of COX-2 results in histological and functional sparing as assessed by the Basso, Beattie, and Bresnahan locomotion score [179–182]. COX-1 has also been shown to increase following SCI, persisting for as long as 4 weeks [179]. This upregulation in COX in the presence of free fatty acids, such as AA, logically progresses to an upregulation of eicosanoids. Bioactive eicosanoids, derived from PLA_2-induceds production of AA, have been implicated as mediators of secondary injury *via* a host of mechanisms. The expression of eicosanoids, such as TXA_2 and PGE_2 increased in the injured cord tissue within hours of SCI and their vasoactive properties are thought to create microemboli in addition to PGE_2's well known proinflammatory effects [134,183]. Increased production of TXA_2, PGI_2, LTC_4 and 5-HETE have also been confirmed in experimental SCI [184,185]. $PGF_{2\alpha}$ increases three fold following SCI, and when exogenously added caused significant cell loss, increased hydroxyl radicals, and malondialdehyde - an end product of membrane lipid peroxidation [186].

The effect of lysophospholipids on spinal cord tissue has been extensively studied and lysophospholipids such as LPC and its later metabolites, such as PAF, are metabolically active in the CNS. For over 30 years it was known that injections of LPC into the spinal cord causes demyelination [133,136,138,139] as well as expression of a number of chemokines and cytokines, similar to those produced following SCI [187,188]. While lysophospholipid levels following SCI or traumatic brain injury (TBI) have not been assessed directly, their presence is strongly implied from the generous production of free fatty acids and a decrease in phospholipids. PAF, a metabolite of lysophospholipids, increases 20-fold after SCI induced by stroke [189–193]. Intrathecal administration of PAF leads to reduced spinal cord blood flow and motor deficits, an effect which can be blocked by the PAF receptor antagonist, WEB 2170 [193]. Treatment with WEB 2170 after acute spinal cord contusion resulted in significant increases in white matter sparing as well as decreases in proinflammatory cytokine mRNA levels within the lesion epicenter [192,194]. Treatment with the PAF receptor antagonist BN52021 also improves behavioral function after SCI [195]. *In vitro* experiments showed that low concentrations of PAF resulted in neuronal differentiation and sprouting, while higher concentrations were neurotoxic [196]. PAF-induced death of not only cultured neuronal cells in a concentration-dependent manner [197,198] but also that of oligodendrocytes and astrocytes [194].

Oxidative stress is well established following SCI [186,199–202]. Work by Liu *et al*. has shown that H_2O_2 [199], iron [200], and hydroxyl radicals [200] are formed following SCI. Furthermore pathophysiological doses of these oxidants administered exogenously *in vivo* created significant cell death at 24 hr that could be blocked by a broad spectrum reactive species scavenger [200]. Administration of $PGF_{2\alpha}$ resulted in a 3- fold increase in hydroxyl radicals and a 2-fold increase in malondialdehyde, an end product of membrane lipid peroxidation [186]. It has also been shown that H_2O_2 is toxic to neurons [203–207], astrocytes [208,209], and oligodendrocytes [210–212]. Oxidative stressors, such as H_2O_2 administration, also

increase AA release in neurons and mesanglial cells [114,204]. It has recently been suggested that generation of ROS and polyunsaturated fatty acids, *via* $cPLA_2$, following CNS injury mediates NF-κB translocation from the cytosol to the nucleus where it induces gene expression of $sPLA_2$ and other lipid enzymes, thus potentiating a positive feedback loop [174].

High levels of EAAs such as glutamate and aspartate in experimental SCI are also an important mechanism inducing secondary injury [112]. Growing evidence indicates that $sPLA_2$ could mediate EAA-induced neuronal death and tissue damage. Marked increases in PLA_2 activity and AA release have been reported after treatments of neuronal cultures with glutamate, NMDA and KA [213,214]. In addition, glutamate release in the spinal cord can be suppressed by PLA_2 inhibitors such as indomethacin by 40%, $AACOCF_3$ by 45%, and 4-bromophenacyl bromide by 36%, suggesting that increased PLA_2-mediated EAA release is part of a positive feedback mechanism [126]. Additionally, application of $sPLA_2$ to the ischemic rat cerebral cortex resulted in a significant increase in EAA levels and a general PLA_2 inhibitor mepacrine significantly decreased the ischemia evoked efflux of EAA into cortical superfusates [123]. Thus, the excessive stimulation of NMDA receptors, as occurs in the spinal cord trauma, may result in stimulation of $sPLA_2$ activity leading to alterations in membrane composition, permeability, and fluidity leading to neuronal and glial cell death.

In summary, $sPLA_2$ can be induced by several key injury mediators such as inflammatory cytokines, free radicals, and EAAs that have been shown to increase following traumatic SCI. Furthermore, this increase in $sPLA_2$ activity can further increase inflammation, oxidation, and EAA release. This indicates that $sPLA_2$ activation may play a central role in a positive feedback loop triggered by traumatic SCI resulting in neuronal and glial cell death, tissue damage, and corresponding behavioral impairments. Thus, $sPLA_2$ may act as a convergence molecule that mediates multiple key mechanisms of secondary spinal cord injury and blocking $sPLA_2$ action may represent a novel and efficient strategy to block multiple injury mechanisms.

Brain Injury

Similar to SCI, TBI also triggers secondary or delayed cell death by multiple injury processes including ischemia, inflammation, generation of free radicals, and glutamate release, all of which have been showed to induce PLA_2 activity [152,215,216]. Like SCI, there is clear evidence that TBI induces PLA_2 activity resulting in membrane phospholipid degradation, generation of proinflammatory mediators, such as eicosanoids and PAF, formation of free radicals, and subsequent lipid peroxidation. Following closed head injury in rats, total PLA_2 activity increased [217]. Additionally, after open traumatic brain injury, free fatty acids, such as AA, were released and membrane phospholipid degradation was found [218–220]. In humans, an increase in free fatty acids in cerebrospinal fluid (CSF) following brain injury has been reported [221].

No report to date has investigated the expression of $sPLA_2$ following traumatic brain injury, however, $cPLA_2$ and 4-hydroxynonenal were expressed in the transected brain [222]. Additional reports have confirmed the presence of down stream metabolites of AA. Pronounced increases in prostaglandin $F_{2\alpha}$, prostaglandin D_2, leukotrienes, and thromboxane B_2 have all been reported to occur in brain tissues after KA injection [223] and increases in PGE_2 following closed head injury [217].

Conditions that increase $sPLA_2$ have been shown following TBI, just as in SCI. Cerebral penetration and contusive injury both increase oxidative stress in the brain [224,225] and blockade of oxidative stress increases learning and histological sparing [225]. Under both experimental and clinical settings, the level of extracellular EAAs such as glutamate and aspartate increased following TBI (Faden *et al*., 1989; Palmer *et al*., 1993; Globus *et al*., 1995; Bullock *et al*., 1998). Additionally, both competitive and noncompetitive NMDA and non-

NMDA receptor antagonists are efficacious in the treatment of experimental brain injury [226]. Several studies showed that glutamate, NMDA, and KA result in a dose-dependent increase in AA release in hippocampal neuronal cultures [214] and PLA_2 activity in neuron enriched spinal cord cultures [213]. *In vivo* intercerebroventricular injections of KA were shown to increase total PLA_2 and $sPLA_2$ activity in the rodent brain [127]. Increased levels of extracellular glutamate following TBI causes overstimulation of glutamate receptors that may result in secondary events such as $sPLA_2$ release, degradation of membrane phospholipids, and accumulation of free fatty acids, leading to neuronal cell death as well as increased levels of eicosanoids and leukotrienes [112,227]. As suggested above oxidative stress, EAA, and cytokines could induce $sPLA_2$ release and abnormal phospholipid metabolism and may represent a common mechanism involved in traumatic spinal cord and brain injuries.

Ischemia

Ischemia is a key mechanism of secondary injury after CNS trauma [228–230]. Posttraumatic ischemia may result in energy failure that initiates a complex series of metabolic events, ultimately causing neuronal death. One such critical metabolic event is the activation of PLA_2 which can result in hydrolysis of membrane phospholipids, release of free fatty acids, generation of oxygen free radicals, and formation of eicosanoids [152,173,231].

In both experimental models of brain [231–234] and spinal cord ischemia [235] significant increases in the level of free fatty acids, indirectly reflecting PLA_2 activity, were found. Significant increases in $sPLA_2$ activities were also reported *in vivo* following brain ischemia [14,143,236] and in astrocytes cultured under ischemic conditions such as oxygen and glucose deprivation [237]. Biphasic increased expression of $sPLA_2$-IIA is observed in ischemic rat forebrain [14]. An early increase in $sPLA_2$-IIA mRNA occurred at 1–6 h post-ischemia and a late phase of greater induction of $sPLA_2$-IIA appeared between 7 and 20 days post-ischemia. Recently, increased expression of $sPLA_2$-IIA has been confirmed at both mRNA and protein levels after brain ischemia [236,237]. Cytokines such as TNF-α and IL-1β have been shown to mediate the ischemia induced PLA_2 activation and $sPLA_2$-IIA expression in transient focal rat cerebral ischemic model [116,236]. Indoxam, a specific $sPLA_2$ inhibitor, was shown to offer protection against the ischemia induced damage [143]. Quinacrine / mepacrine, a non-specific inhibitor of PLA_2 activity, also showed sparing of hippocampal neurons [238] and reduced infarct size following transient focal ischemia [239]. *In vitro* experiments showed that increased $sPLA_2$ activity was associated with ischemia-induced apoptosis [143]. Other studies have shown $cPLA_2$ increases following ischemia [240–244] and other authors suggest that $cPLA_2$ rather than $sPLA_2$ mediates neuronal death in ischemia [245]. In summary, ischemia induces PLA_2 activation which could result in deleterious effects such as the loss of membrane integrity through excessive phospholipids hydrolysis, formation of eicosanoids, cytotoxic products, ROS, and induction of apoptosis of affected cells [216,246].

Other Degenerative Diseases

Beyond neurotrauma, $sPLA_2$ has been suggested as a mediator of neurodegenerative disorders such as Alzheimer's disease (AD) [247], Multiple Sclerosis [248,249], and Parkinson's disease [250]. AD is characterized by an increased deposition of amyloid plaques infiltrated by reactive astrocytes and microglial cells. Aggregated forms of amyloid β (Aβ) peptides, particularly A β 1–42, have been shown to elicit cytotoxic effects resulting in neuron cell death [251]. There is evidence for alterations in phospholipid levels in patients with AD [252]. In two separate studies, a decrease in PLA_2 activity was found in the parietal and temporal cortices [253], as well as in the prefrontal cortex of the AD brain [254]. Contrary to these studies, immunohistochemical experiments showed increases in both $sPLA_2$–IIA [247] and $cPLA_2$ [255] in astrocytes of the AD brain. A recent gene array study in AD patients indicated an increase in $cPLA_2$ and COX-2 expression, as well as upregulation of a number of apoptotic

and proinflammatory genes, but no mention was made of $sPLA_2$ [256]. These findings are in agreement with the increased oxidative and inflammatory responses and presence of reactive astrocytes associated with AD pathology [257]. *In vitro* studies demonstrated the ability of Aβ to enhance the activity of a number of phospholipases [258]. Nicotine, a cholinergic agonist, inhibited an Aβ-induced increase in PLA_2 activation [259]. The ability of PLA_2 inhibitors to attenuate Aβ-induced ROS production could indicate the involvement of PLA_2 in Aβ cytotoxicity [260]. For a more thorough review please see [251].

Evidence also links $sPLA_2$ generation to white matter disorders and their experimental equivalents. An early study by Huterer, *et al*., in the post mortem brains of Multiple Sclerosis patients found no difference in $sPLA_2$-IIA activity and a decrease in $cPLA_2$ activity within white matter lesions [261]. However, more recent studies found that $cPLA_2\alpha$ −/− mice were more resistant to experimental autoimmune encephalomyelitis a rodent model of Multiple Sclerosis. Additionally, $cPLA_2\alpha$ appeared to play a role in both the induction and effector phases as well as increasing inflammation in the white matter lesions [249]. Pinto, *et al*., found that extracellular inhibitors of $sPLA_2$ were able to decrease CNS inflammation, prevent the induction of proinflammatory cytokines and ameliorate experimental autoimmune encephalomyelitis [248]. Finally, in the brains of patients with Krabbe Disease, a demyelinating disease of the CNS, $sPLA_2$ was increased in post mortem human samples, and in twitcher mice, its rodent equivalent. Additionally, the use of a $sPLA_2$ specific inhibitor reduced psychosine-induced oligodendrocyte death *in vitro* [262].

Studies using indirect markers for phospholipid metabolism have also suggested a role for $sPLA_2$ in Parkinson's disease, a degenerative disease of the CNS characterized by bradykinesia and death of dopaminergic neurons in the substantia nigra [263]. More importantly, quinacrine, a nonselective PLA_2 inhibitor, significantly reduced MPTP-induced dopamine loss in an experimental model of Parkinson's disease [250]. Mice deficient in $cPLA_2$ were also shown to exhibit more resistance to MPTP neurotoxicity than wild-type mice, supporting a role for $cPLA_2$ in mediating MPTP neurotoxicity [264].

CONCLUSIONS AND FUTURE DIRECTIONS

Current evidence clearly suggests that $sPLA_2$ is present in the CNS and that its activity and metabolites exacerbate secondary SCI as well as other common CNS pathologies. Secondly, it has been shown that oxidative stress, cytokines, and EAA upregulate $sPLA_2$ and in turn are reciprocally upregulated by $sPLA_2$ following SCI resulting in a pathological positive feedback loop. Thus, $sPLA_2$ clearly represents an important target for developing therapeutic interventions following SCI.

It must be noted that while this review chose to focus on $sPLA_2$, other PLA_2 subfamilies most importantly $cPLA_2$ and $iPLA_2$ seem to play a role in glycerophospholipid metabolism following injury as well [249,265]. While there appears to be a reciprocal accentuation of activity among the PLA_2s this issue merits further study [114]. Additionally, variations in the biological functions and species specificities of various $sPLA_2$ isoforms complicate the issue further. Also the intracellular mechanisms utilized by oxidative stressors, cytokines, and EEA to upregulate $sPLA_2$ after SCI remain obscure. Therefore further studies will need to assess the exact mechanism of $sPLA_2$ –mediated CNS injury and the effect of inhibition on functional recovery following SCI.

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ABBREVIATIONS

AA	Arachidonic acid
AD	Alzheimer’s disease
CNS	Central nervous system
COX	Cyclooxygenase
$cPLA_2$	Cytosolic PLA_2
EAA	Excitatory amino acids
HSPG	Heparan sulfate proteoglycans
IFN-γ	Interferon gamma
IL-1β	Interleukin-1β
$iPLA_2$	Independent PLA_2
KA	Kainic acid
LPC	Lysophosphatidylcholine
LPS	Lipopolysaccharide
LTC_4	Leukotriene C_4
Lyso-PL	Lysophospholipids
NMDA	N-methy-D-aspartate
PAF	Platelet activating factor
PAF-AH	PAF acetylhydrolases
PC	Phosphatidylcholine
PGE_2	Prostaglandin E_2
ROS	Reactive oxygen species
SCI	Spinal cord injury
$sPLA_2$	Secretory phospholipases A_2
TBI	Traumatic btain injury
TNF-α	Tumor necrosis factor-α
TXA_2	Thromboxane A_2

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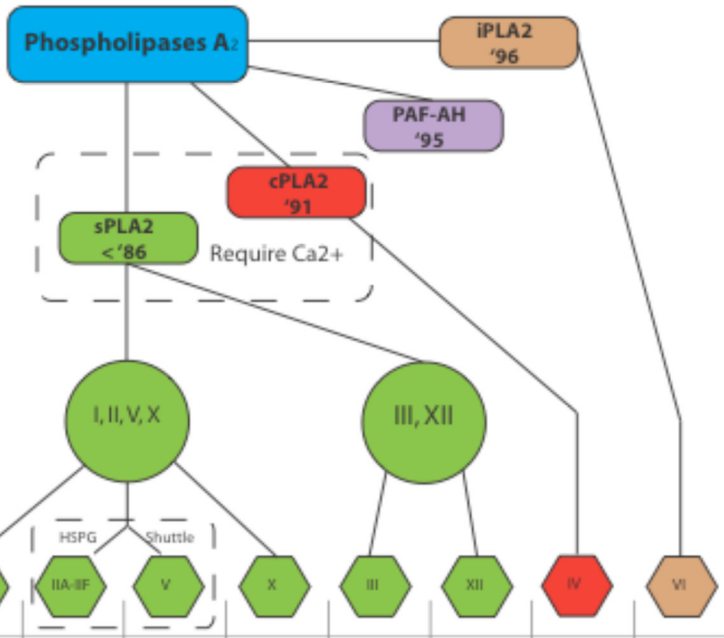

		IB	IIA-IIF	V	X	III	XII	IV	VI
General	Size (kD)	14	14-16	14	14	55	19	60-110	28-146
	Ca^{2+} requirement	mM	mM	mM	mM	mM	mM	μM	None
	Chromosome	12q23-24	1p34-36	1p34-36	16p12-13	22q	4q25	15 & 19	22 & 7
	Propeptide	x			x				
Catalytic Activity	HSPG shuttle		x	x					
	External Membrane		Only IIF	x	x				
	His/Asp dyad	x	x	x	x	x	x		
	Ser/Asp dyad							x	
	Ser/His/Asp triad								x
sPLA2 - R Binding	Rabbit	Yes	Yes			No			
	Mouse	Yes	Yes		Yes	No			
	Rat	Yes	No			No			
	Human	No	No			No			

Fig. (1). Classification of the mammalian PLA_2 isoforms

The top panel shows a branching diagram indicating the relative subdivisions of the PLA_2 subfamily and their years of discovery. The mammalian PLA_2 family of enzymes is grossly divided into the $sPLA_2$, $cPLA_2$, $iPLA_2$, and PAF-AH. The $sPLA_2$ subfamily is further divided into groups IB, group II and V, group X, and group III and XII based on structural and functional differences presented in the table below. HSPG: heparin sulfate proteoglycans.

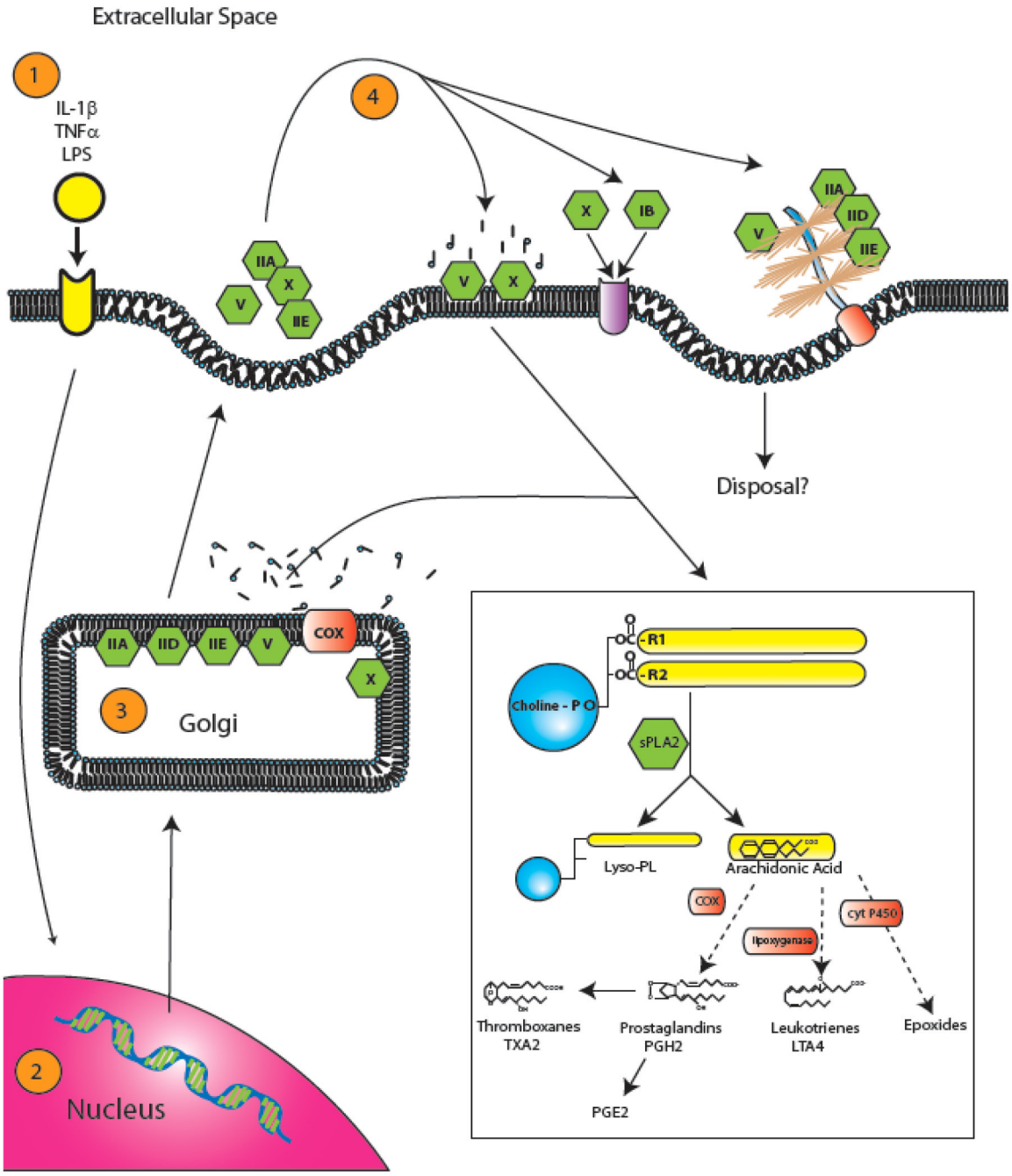

Fig. (2). Intracellular handling and sPLA$_2$ activity

Following stimulation by various cytokines [1], sPLA$_2$ is synthesized in the nucleus [2] and endoplasmic reticulum prior to packaging for secretion in the Golgi apparatus [3]. It is within the Golgi apparatus and later microvesicles that certain isoforms, particularly IIA, are predominantly active. Following secretion [4], sPLA$_2$ can metabolize the extracellular lipid membrane directly, bind to the sPLA$_2$ receptor (sPLA$_2$-R), and/or be endocytosed *via* the heparin sulfate proteoglycan shuttle (HSPG shuttle). Of course, each of these actions is governed by species and isoform specificity. The inset shows the general metabolism of phospholipids by sPLA$_2$. sPLA$_2$ first hydrolyzes the acyl bond at the *sn-2* position of glycerophospholipids to produce free fatty acids (such as arachidonic acid) and

lysophospholipid (Lyso-PL). Arachidonic acid can then be further modified by COX to form prostaglandins, lipoxygenase to form leukotrienes, or cytochrome P450 to form epoxides. Prostaglandins can be further modified to form thromboxanes. These eicosanoids have metabolic activities including proinflammatory and vasoconstrictive functions.

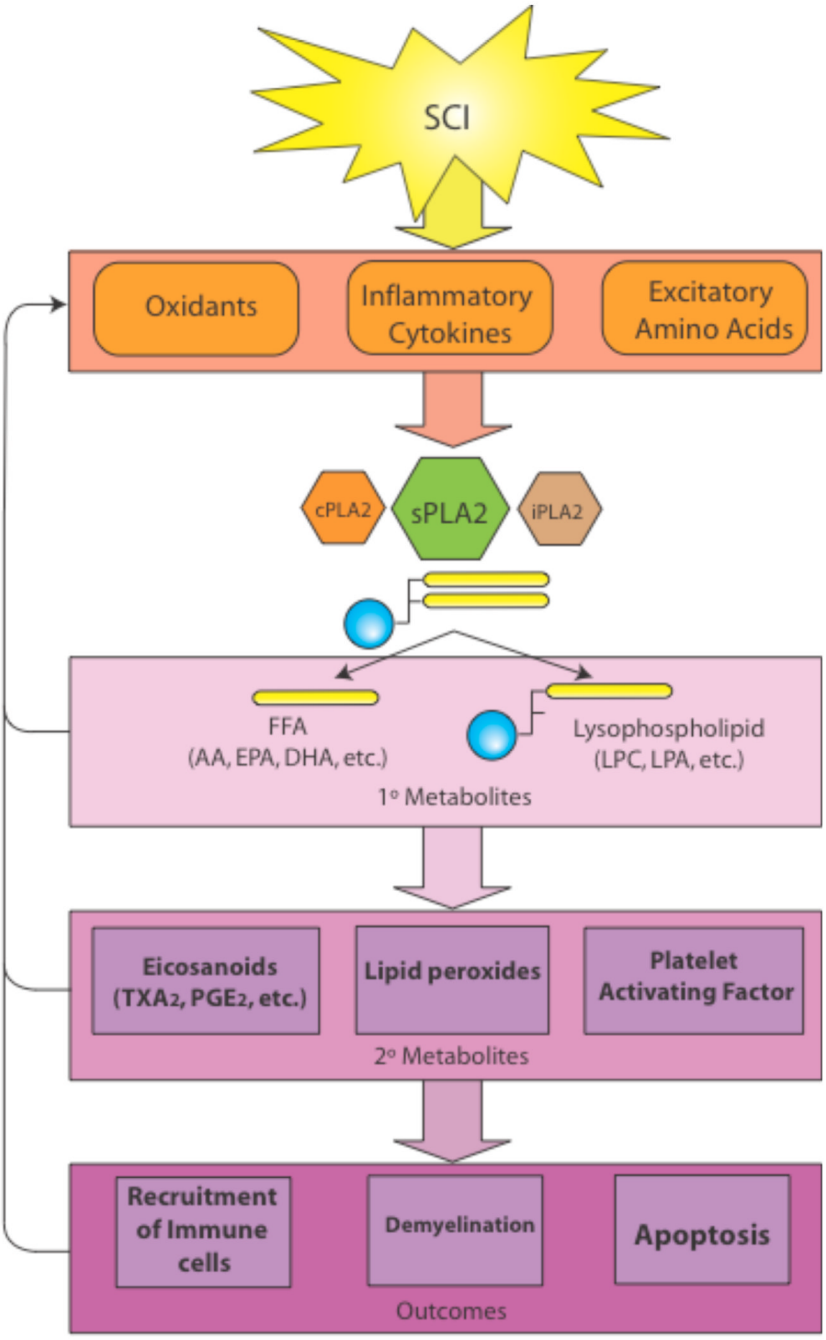

Fig. (3). Overview of $sPLA_2$'s role in spinal cord injury

The toxicity of $sPLA_2$ is compounded by three factors. 1) $sPLA_2$ is upregulated by commonly known neurotoxic mechanisms such as oxidative stress, cytokines, and EAA. 2) Both the primary metabolites of $sPLA_2$ activity, such as free fatty acids and lysophospholipids, and the secondary metabolites, such as eicosanoids and platelet activating factor, are toxic to the CNS. 3) Finally, $sPLA_2$ has been shown to reciprocally upregulate oxidative stress, cytokines, and EAA thus propagating a positive feedback loop resulting in cytotoxicity and secondary SCI. It must also be noted that $sPLA_2$ does not work in isolation from $cPLA_2$ and $iPLA_2$, rather a reciprocal activity is often demonstrated among the PLA_2 subfamilies.

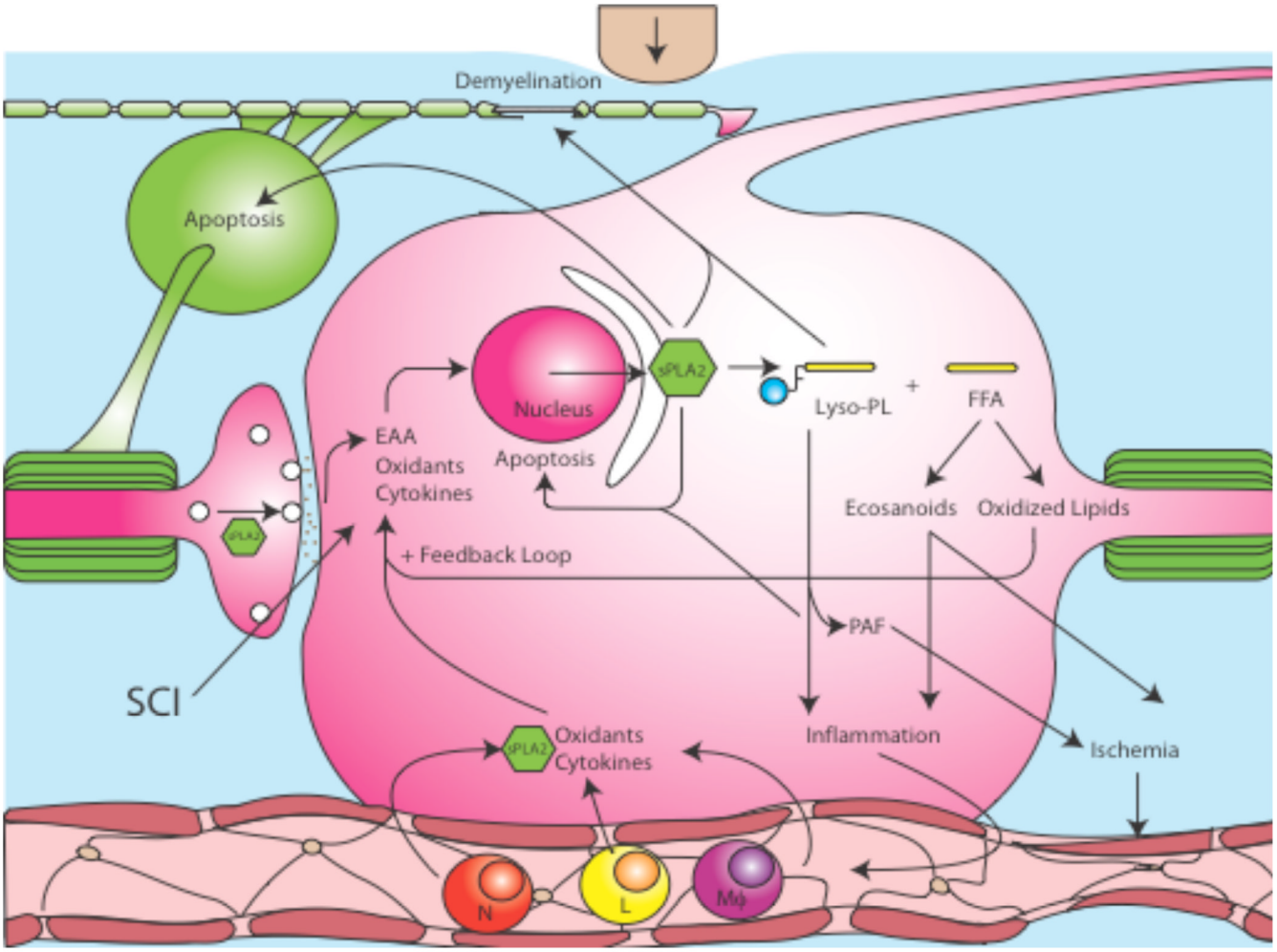

Fig. (4). $sPLA_2$ activity within spinal cord injury

Following SCI, oxidative stress, cytokines, and EAA are upregulated. These toxic factors then upregulate the synthesis of $sPLA_2$. Subsequently $sPLA_2$ mediates the hydrolysis of phospholipids into lysophospholipids (Lyso-PL), such as LPC, and free fatty acids (FFA), such as AA. Independent of other factors, $sPLA_2$ and LPC demyelinate axons in the spinal cord and $sPLA_2$ and AA have been shown to trigger apoptosis in neurons and oligodendrocytes. The metabolism of AA results in increased oxidative stress from lipid peroxidation and increased eicosanoids which have been shown to increase inflammation and ischemia. LPC also increases inflammation while its metabolite, PAF, triggers ischemia. Infiltrating polymorphonuclear neutrophils (N), lymphocytes (L), and macrophages (Mϕ) then flood the CNS with more $sPLA_2$, oxidants, and cytokines thus exacerbating the positive feedback loop, while the upregulation in $sPLA_2$ and LPC trigger the release of EAAs from synaptic terminals.