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Low Molecular Weight Phospholipases A_2 in Mammalian Brain and Neural Cells: Roles in Functions and Dysfunctions

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Abstract Several "low molecular weight" or "secretory" phospholipases A_2 isoforms may be expressed in mammalian neural cells. Indeed, mRNAs for GIB, GIIA, GIIE, GIII, GV, GX, and GXII were detected in brain tissues despite different levels. However, only the presence of GIB, GIIA, and GV proteins has been clearly demonstrated in neural cells or in the nervous tissue. Although the roles of GIB and GV in the nervous tissue are still elusive, there is evidence to support the involvement of GIIA in physiological and pathological events, including neurotransmission, long-term potentiation, and neuritogenesis. The neurotoxic effects of an increase in GIIA may be envisaged under pathological conditions associated with the activation of astrocytes during inflammation or through activation of neurons and enzymes due to the stimulation of the NMDA glutamate receptor. In the past, elevation of GIIA expression in many acute and chronic neurological diseases is well known. Although each neurodegenerative disease has a separate etiology, many share similar neurochemical common processes, such as excitotoxicity, oxidative stress, and mitochondrial dysfunction, phenomena where GIIA play an important role.

Keyword Phospholipase A_2 · Brain · Neural cells · Neuritogenesis · Long-term potentiation · Excitotoxicity · Ischemia · Neurodegenerative diseases

Dedicated to Prof. Lloyd A. Horrocks

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Background

Phospholipases A_2 (PLA_2s) catalyze the hydrolysis of the ester bond at the *sn*-2 position of glycerophospholipids producing free fatty acids and 2-lysophospholipids. These enzymes constitute a superfamily that, according to a recent classification based on their catalytical mechanisms [1], are divided into five types of enzymes. The enzymes having low molecular weights (13–19 kDa) are indicated as "small secreted phospholipases A_2" ($sPLA_2$) because they are released into the extracellular fluids by different cell types. However, enzymes belonging to this group are also detected in intracellular compartments where they coexist with isoforms of other PLA_2s, such as the Ca^{2+}-dependent cytosolic ($cPLA_2$), Ca^{2+}-independent ($iPLA_2$) and PAF-acetylhydrolase.

The structural and catalytic properties of ten mammalian $sPLA_2$ have been recently reviewed [2]. Common features of these enzymes are: (1) the presence of 6–8 disulfide bonds, (2) Ca^{2+} requirement for catalysis with a K_{Ca} in the micromolar range, (3) highly conserved catalytical dyad His–Asp and "Ca binding loop", and (4) relatively low selectivity for the fatty acid to be hydrolyzed. The description of structural features of some $sPLA_2$s [3, 4] has permitted to propose molecular mechanisms of catalysis that may be common to different isoforms due to the highly conserved active site. Possible catalytical mechanisms have been recently reviewed [2]. The binding of the enzyme at the interface facilitates the interaction of substrate with the active site and the efficiency of catalysis [5].

Methods for Assaying $sPLA_2$ Activity in Biological Samples

Discriminating the contribution of each type or isoform of PLA_2 that coexists in the same sample, relative to the total

enzyme activity, is a rather difficult task. However, procedures for attributing the measured activity to a single type of PLA_2 (i.e., $iPLA_2$, $cPLA_2$ and $sPLA_2$) with a certain degree of approximation, are available. These procedures are based on their Ca^{2+}-dependence, preference for substrates, presence of activators or inhibitors in the assay systems, and sensitivity to disulfide bond reducing agents. Four specific PLA_2 assays have been proposed for distinguishing between GIVA $cPLA_2$, GVIA $iPLA_2$, GIIA, and GV $sPLA_2$s [6]. More recently, this method has been improved by the use of a combination of specific inhibitors [7]. In these assays, the glycerophospholipid substrates are radiolabelled with fatty acid at the C-2 position and are used as Triton X-100 mixed micelles for assaying GIV or GVI $cPLA_2$ along with smaller unilamellar vesicles for assaying GIIA and GV. Secretory PLA_2s, particularly GIIA, are conveniently assayed using autoclaved *Escherichia coli* labeled with [^{3}H] oleate as substrate and determining the radioactivity of the released fatty acid [8, 9]. The suitability of this method is based on the observation that, under certain conditions, the labeled fatty acid is incorporated essentially into phosphatidylethanolamine (PE) which represents more than 70% of *E. coli* membrane phospholipids and the preferred substrate for GIIA [8]. GV is preferably assayed using phosphatidylcholine liposomes as substrate [10].

Continuous spectrophotometric assay can also be achieved by the use of *sn*-2 thio ester [11] and fluorescent or fluorogenic phospholipid analogs as substrates [12–14]. The use of substrate analogs containing both fluorescent and quenching groups are particularly useful because they show no or low fluorescence at basal conditions, but increase in fluorescence when the quenching group or the fluorophore is removed by the action of PLA_2. An important application with these substrates is the possibility to monitor enzyme activity in real-time and in living cells or organisms by in vivo fluorescence imaging [13, 15, 16]. The substrate, 1-*O*-(6-dabcyl-aminohexanoyl)-2-*O*-(6-[12-BODIPY-dodecanoyl]amino-hexanoyl)-*sn*-3-glycerophosphorylcholine, has been used for continuous assay of purified $sPLA_2$ in vitro and for monitoring PLA_2 activity in living cells by confocal microscopy [16]. However, a limitation of this substrate is that the increase of fluorescence might be the consequence of PLA_1 or PLA_2 activity.

Another fluorogenic substrate (PED6, *N*-((6-(2,4-dinitrophenyl)amino)hexanoyl)-2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoyl)-1-hexadecanoyl-sn-glycero-3-phosphoethanolamine) is suitable for continuous or in vivo assay of PLA_2 activity [13, 15, 17] or for monitoring the enzyme activity using the microplate fluorescence reader [18]. PED6 has also been used recently for monitoring PLA_2 activity in neuronal primary cells by cytofluorimetry [19]. Even if PED6 does not allow discrimination of different PLA_2s present in biological samples, the use of specific incubation conditions and inhibitors might be a strategy for resolving the problem.

Occurrence of $sPLA_2$ in Mammalian Nervous Tissue and Neural Cells

Different $sPLA_2$ have been detected in neural cells or in mammalian nervous tissue by different experimental procedures as summarized in Table 1.

GIB

Originally, GIB was purified and sequenced from pancreas or pancreatic juice of various mammals [20–22]. These three-dimensional crystal structures of bovine [23] and porcine [24] enzymes were determined by X-ray diffraction; however, more recently, the solution structure of the porcine enzyme [PDB (Protein Data Bank) code: 1 PIR] was determined by NMR [25]. GIB has a unique five-amino-acid extension that is located in the middle of the molecule and is identified as the "pancreatic loop".

The presence of transcripts for GIB in undifferentiated PC12 cells was reported [26], but this study failed to detect GIIA mRNA, which was later found in the same cell line. Clear evidence for the presence of GIB in rat and human brain and in primary neuronal cells was provided [27]. Indeed, the presence of GIB mRNA was demonstrated by Northern blotting and by in situ hybridization. The development of a specific antibody against rat GIB [27] revealed the translation of mRNA to the functional protein and allowed the identification of the enzyme. In the rat brain, this isoform is present mainly in neurons of cortex, hippocampus, and cerebellum. mRNA encoding for GIB was also found in rat neuroretina, and gene expression was increased by light [28].

GIIA

GIIA was purified from inflammatory exudates [29, 30] and was subsequently cloned and sequenced [29, 31]. The enzyme, which was purified from human synovial fluid and platelets, has 124 amino acids and about 37% homology with the bovine pancreatic $sPLA_2$. Similar to the other isoforms of group II $sPLA_2$, GIIA has a C-terminal extension that is not present in the pancreatic enzyme [29]. The three-dimensional crystal structure of recombinant human rheumatoid arthritic synovial fluid enzyme (PDB code: 1BBC) [32] revealed the presence of a hydrophobic channel forming a deep pocket in the center of the molecule ending close to the active site.

Table 1 sPL A_2 in nervous tissue and in neural cells

Enzyme	Occurrence	Detection	References
GIB	PC12	RT-PCR	[26]
	Brain (rat and human)	RT-PCR, WB	[27]
	Retina (rat)	NB	[28]
GIIA	Brain (Rat)	RT-PCR	[33]
		Activity	[35]
		NB	[116]
		WB	[9]
	Cerebellum (rat)	IF	[37]
	Astrocytes (rat)	RT-PCR, NB	[176]
		RT-PCR, ISH, NB	[116]
	Oligodendrocytes (rat)	WB, IF	[147]
	Astrocytoma (human)	IF, EM	[9]
	PC12	IF	[9]
	Spinal cord (rat)	Activity, NB, WB,IF, IH	[147]
GIIC	Brain (rat)	RT-PCR	[33]
	PC12	RT-PCR	[26]
GIIE	Brain (human)	RT-PCR	[42]
	Brain (rat)	ISH,real-time-PCR	[47]
	Spinal cord (rat)	IF, IH	[147]
GIIF	Brain (mouse)	RT-PCR	[177]
GIII	DRG neuron (mouse)	RT-PCR	[44]
GV	Brain (rat)	RT-PCR,	[33]
		Activity,IF, WB	[48]
		IH	[47]
	Cerebellum (rat)	IF	[37]
	Astrocytes	RT-PCR	[176]
		IF, IEM	[9]
	PC12	IF	[9]
GX	DRG neuron (mouse)	WB, IF, NB,	[50]
	Brain (rat)	IH	[47]
	PC12	RT-PCR, WB, IF	[50]
GXII	Human brain	RT-PCR, NB	[51]

IEM immuno-electron microscopy, *IF* immunofluorescence, *IH* immunohistochemistry, *ISH* in situ hybridization, *NB* Northern blotting, *RT-PCR* reverse transcription PCR, *WB* Western blotting

In the rat brain, gene encoding for GIIA is ubiquitously expressed in all brain areas with higher levels in the brain stem and midbrain than in the cerebellum and corpus striatum [33]. GIIA mRNA was also detected in human brain [34].

Using specific assays to understand different forms of PLA_2, it was shown that GIIA has major activity in the particulate fraction of rat brain [35]. In the cytosolic fraction of these brain areas, its activity was much lower than that of $iPLA_2$. The activity of particulate GIIA increases 2-fold at 4 weeks after birth and then returns to levels of neonatal animals. The presence of GIIA in the particulate fraction of rat brain was confirmed by Western blotting of mitochondrial proteins [9] using a specific monoclonal antibody raised against rat mitochondrial liver enzyme [36]. Further support for the mitochondrial localization of GIIA in neural cells, e.g., PC12 and U-251 astrocytoma cells, was achieved by confocal immunofluorescence analysis and immune-electron microscopy [9]. The enzyme is associated with mitoplasts whereas $iPLA_2$ is present in the outer mitochondrial membrane.

The differential cellular localization of PLA_2s was studied in rat cerebellum using immunolabeling and in situ hybridization [37]. In Purkinje cells, GIIA is associated with the endoplasmic reticuli in the perinuclear regions whereas $cPLA_2\alpha$ is present in the soma and dendrites. Although GIIA $sPLA_2$ mRNA was not detected in primary cultures of dorsal root ganglion, its expression was induced by IL-1β in a time-dependent manner [38].

GIIC

In rats, GIIC seems to be exclusively expressed in brain [33]. However, in mice, this isoform is exclusively expressed in

the testes and particularly in cells undergoing meiosis [39]. In humans, gene encoding for GIIC is not expressed and appears to be a non-functional pseudogene [40]. Thus, the expression of these isoforms is species-specific.

GIID

GIID was cloned from mouse thymus. mRNA for this subtype was detected by Northern blot analysis in different tissues but not in brain [41]. In the human brain, the transcript for this isoform was also not detectable by reverse transcription PCR (RT-PCR) analysis [42].

GIIE

Mouse and human GIIE were cloned by molecular biology technology [42], and the recombinant enzymes were partially purified and characterized after their over-expression in COS-7 cells. The recombinant enzyme consists of 123 amino acids (*Mr*=14,000). The transcript of GIIE was detected in human brain by RT-PCR [42]. However, using a similar experimental procedure in contrast with other reports [27, 34], this study failed to detect transcripts for GIIA and GIB in human brain. Transcript of gene encoding for GIIE was also detected in rat retina [28].

GIIF

Cloned mouse GIIF has a signal peptide of 20 amino acids, and the matured protein (calculated molecular mass 16,800) consists of 148 residues [41]. A peculiar structural feature for this enzyme is a long C-terminal extension of 23 amino acids. Northern blot analysis revealed a very low level of GIIF mRNA in mouse brain. Low expression of gene encoding for GIIF was also detected in rat retina [28].

GIII

Human GIII, having a sequence homology with bee venom enzyme, has been cloned [43]. The recombinant protein is much larger than other $sPLA_2$ (calculated molecular mass 55.3 kDa) and contains a central domain flanked by N- and C-terminal regions. The $sPLA_2$ domain (calculated molecular mass of 16 kDa) is typical of GIII enzymes. Northern blot analysis revealed that GIII mRNA is highly expressed in the human heart, kidney, and liver, but only weakly in the brain.

Recently, it was reported that the expression of GIII in neuronal cells [44] and in PC12 cells can function to facilitate neurite outgrowth and suppresses neuronal death induced by NGF deprivation, suggesting a role in neuronal growth and differentiation.

GV

Another well-characterized form of $sPLA_2$ is GV [45]. The human GV gene is located in chromosome 1 close to the GIIA gene [46]. This enzyme shows a high level of identity with GIIA, but does not possess the C-terminal extension. GV mRNA was found in detectable levels in most rat brain regions, with the exception of the hippocampus, where the transcript for this isoform seems to be rather abundant [33]. In another study, GV mRNA was found in rat hippocampus, and based on immunohistochemistry, the protein was also found in the neurons in cerebral cortex and dentate gyrus [47]. Using immunolabeling and in situ hybridization methods, GV was also found in cerebellum [37], and particularly, in the Bergman glial cells. When assayed by a system supposed to be specific for GV, the activity appeared to be rather low in comparison to $iPLA_2$ and GIIA and was almost equally distributed in all brain areas [35]. Immunofluorescence analysis revealed that GV was highly localized in the nuclei of PC12 and U251 neuroblastoma cells [9]. This finding was confirmed by assaying the activity in neuronal and glial nuclei purified from rat brain, and subsequently identified by Western blot analysis [48]. Immunofluorescence analysis of isolated rat brain nuclei revealed that the enzyme was mainly localized in the nucleoplasm.

GX

Human GX was cloned and expressed on the basis of the expressed sequence tag originally obtained from fetal lung cDNA library [49]. The cDNA clone predicted a mature protein of 123 amino acids and a calculated molecular mass of 13.6 kDa. Contrary to the other $sPLA_2$ enzymes, human GX is very acidic (pI 5.3) and possesses disulfide bridges typical to GIB and GIIA. The crystal structure of hGX revealed that the enzyme is similar to those of other $sPLA_2$ and particularly to the active sites of GIB and GIIA, although the opening of the active site slot is considerably larger [3]. Northern blot analysis detected transcripts for human GX in adult spleen and to a less extent, in peripheral blood leukocytes [49]. This study failed to detect transcripts for hGX, hGIIA, and hGV in fetal or adult human brain. However, the expression of gene encoding for GX was demonstrated in rat brain by real-time PCR and immunohistochemistry, despite of lower levels than most other $sPLA_2$s [47]. GX protein seems to have a neuronal localization, particularly in the neurons in dentate gyrus.

Immunostaining detected expression of this isoform in neuronal fibers of human peripheral tissues and in mouse DRG neurons after exposure to NGF [50]. According to this study, GIIA, GIID, GIIE, and GV were undetectable in these cells.

GXII

Apart from the active site region, cloned human GXII (calculated molecular mass of 18.7 kDa) has a poor sequence homology with other known $sPLA_2$s [51]. Northern blot analysis of tissue distribution of human GXII revealed a major transcript in heart, skeletal muscle, and kidney and lower levels in other tissues including the brain.

Functions of $sPLA_2$ in Neural Cells

Common to other tissues, PLA_2 in neural cells are known to perform a number of functions, including defence against pathogens, membrane remodeling, production of lipid mediators, and removal of oxidized fatty acids. Other functions specific to nervous tissue include participation with and release of neurotransmitters, neuritogenesis, and long-term potentiation [52]. Due to the morphological and functional heterogeneity of the nervous tissue, attribution of specific functions to different PLA_2 isoforms has been difficult, and most data are derived from cellular models, often tumor cells, or based on the use of inhibitors often of doubtful or poor specificity. Considering these limitations, this review is limited to describing those functions where a role of $sPLA_2$ isoforms was demonstrated or hypothesized.

Antibacterial Activity

GIIA is found at high concentration in inflammatory fluids, and it has been recognized as having antibacterial activity against Gram-positive [53] and even some Gram-negative bacteria [54]. Recently, the antibacterial actions of $sPLA_2$s have been reviewed with information showing that human and mouse GIIA are the most potent against Gram-positive bacteria [55, 56]. The high level of GIIA in inflamed tissues is due to its induction by cytokines or bacterial toxins followed by its release in the extracellular space [57, 58]. Thus, the increased expression of GIIA in astrocytes due to their activation by inflammatory cytokines or LPS (Lipopolysaccaride) [59, 60] may represent an innate defence mechanism of the enzyme against brain infections by pathogens.

Production of Lipid Mediators

The specificity for the hydrolysis of fatty acids bound to the C-2 carbon of glycerol moiety of glycerophospholipids that are predominantly unsaturated or polyunsaturated has led to the assumption that the main function of PLA_2 is liberation of substrates for the production of lipid mediators including eicosanoids, docosanoids, platelet-activating factor (PAF), and lysophospholipids. Since $cPLA_2\alpha$ has high specificity for arachidonic acid (AA), this enzyme is believed to be a major contributor to the production of eicosanoids [61]. However, $sPLA_2$ appears to be also involved in the generation of these lipid mediators both by releasing AA from phospholipids and by regulating the expression of COXs. Indeed, exogenous $sPLA_2$ can induce the expression of COX-2 [62], and in COX-$1^{-/-}$ mice, expressions of brain $cPLA_2$, $sPLA_2$, and COX-2 are up-regulated [63]. Similar to other tissues, quantitative contribution of individual types of PLA_2 present in the nervous tissue for the production of lipid mediators is complex and depends on a number of factors. In addition, cross-talks also exist between the pathways regulating their expression and activity [64]. It is likely that both $cPLA_2$ and $sPLA_2$ are also involved in the biosynthesis of PAF by the remodeling pathway producing 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine from membrane 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine in neural cells [65, 66].

Neuritogenesis

Alteration of neuronal phospholipid metabolism during neurite outgrowth is a phenomenon observed by several studies, and it is related to membrane extension and remodeling. Thus, it is not surprising that PLA_2s participate in neuritogenesis. However, the identity of the isoforms involved in this phenomenon is unclear. It is also unclear whether they exert an effect through a receptor-mediated mechanism or through lysophospholipids and free fatty acids, which are the hydrolytic products. Although other types of PLA_2 may be present in the nerve growth cones [67], there is convincing evidence indicating that $sPLA_2$ isoforms are the major enzymes involved in neurite outgrowth. This is in agreement that exogenous bee venom $sPLA_2$ (GIII) can induce neuritogenesis in PC12 cells, and this effect is correlated to its activity rather than to a receptor-mediated mechanism [68]. Snake venoms $sPLA_2$ belonging to GI or GII also can exert neuritogenic effects in the same cells [69]. Mammalian GX, either applied in combination with suboptimal concentration of NGF or over-expressed in PC12 cells, was shown to facilitate neurite outgrowth. However, the endogenous expression of GX protein in these cells was rather modest when compared with that of GIIA [9]. Interestingly, when GX was over-expressed in PC12 cells, the pro-enzyme was released into the medium, and thus activation needs to be activated by proteolytic cleavage [50]. Thus, it is possible that neurite outgrowth is due to production of lysophos-

phatidylcholine (LPC), presumably formed by the hydrolysis of phospholipids in the outer leaflet of plasma membrane after its release. It is also possible that LPC can stimulate neuritogenesis through activation of the G2A receptor, which is a member of the GPCR family [70]. The hypothesis that the neuritogenic effect is exerted by the released GX is supported by the observation that presence of anti-GX antibody in the extracellular medium caused a small but significant reduction of neurite length whereas anti-GIIA antibody exerted no effect. By our experience, unsuccessful inhibition of GIIA-mediated neurite outgrowth may be due to the fact that GIIA antibodies against human or mouse isoforms do not recognize the rat isoform. Treatment of PC12 with NGF induces an increase in endogenous GIIA activity and localization of protein in growth cones and tips of neurites (Fig. 1). Furthermore, a relatively low percentage of GIIA is released into the culture medium (Ferrini et al., unpublished).

GIII has been also proposed to participate in neurite outgrowth in adenoviral expressed in PC12 or dorsal root ganglia neurons through a LPC-mediated mechanism [44].

All together, these observations point out that released or cellular $sPLA_2$s participate in complex mechanisms involved in neuritogenesis. However, it is difficult to completely exclude the role of $cPLA_2$ and $iPLA_2$ because their inhibition also causes a reduction of neuritogenesis in primary cultures of cortical and hippocampal neurons in vitro [71].

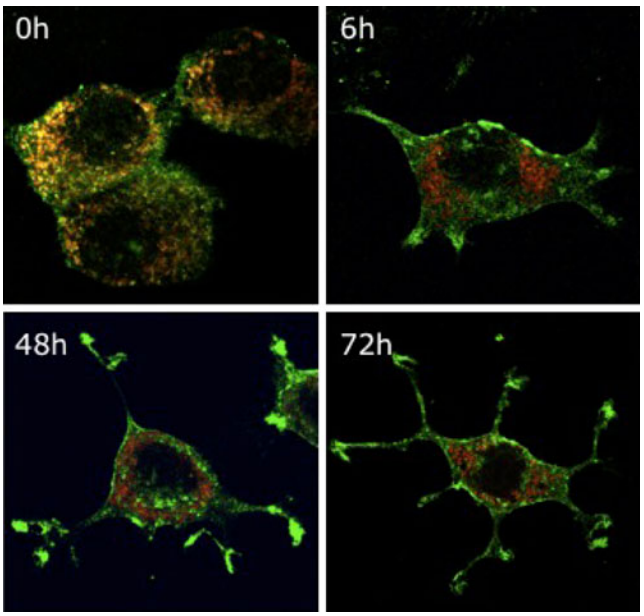

Fig. 1 Effect of NGF on GIIA localization in PC12 cells. PC12 cells were treated with NGF (100 ng/ml) on poly(*L*-lysine)-coated coverglasses in multiwell plates and analyzed after different periods by confocal immunofluorescence using monoclonal antibody against rat GIIA (generous gift from Prof. H. van den Bosch)

Neurotransmission and Long-term Potentiation

Early studies on activity-based subcellular localization of PLA_2 in brain tissue demonstrated the presence of an unidentified enzyme in synaptic vesicles of rat brain, thus suggesting a role in neurotransmitter release [72]. More recently, it was reported that a type II $sPLA_2$ is released from rat brain synaptosomes upon depolarization and from NGF-differentiated PC12 cells upon activation by carbamylcholine [73]. Furthermore, exogenous type II $sPLA_2$ could also cause the release of catecholamines from these cells. Exposure of the same cells to GIIA (obtained either from snake venom or from human synovial fluid) can cause exocytosis and release of neurotransmitters similar to effects observed in hippocampal neurons [74]. Surprisingly, internal application of $sPLA_2$ to PC12 cells was shown to block neurotransmitter release.

The activities of $cPLA_2$ and $sPLA_2$ increased upon exposure of cortical neurons to glutamate [75]. These studies support a functional link between PLA_2 activity and stimulation of glutamate receptors. Furthermore, $sPLA_2$ showed a synergistic effect on the increase of transient Ca^{2+} in hippocampal neurons [76] and release of AA in primary cortical neurons [77] induced by non-toxic and toxic glutamate concentrations. In primary cortical neurons, the combination of exogenous $sPLA_2$ and glutamate also potentiated the release of AA from phosphatidylcholine and phosphatidylethanolamine [78].

There is a large body of evidence for the involvement of glutamate receptors in long-term potentiation (LTP), neuronal plasticity, learning, and memory [79–81], processes where products of PLA_2 may modulate glutamate release, postsynaptic receptor activation, and presynaptic responses [82–89]. However, exact contribution of different PLA_2 isoforms to specific mechanisms of glutamate-mediated synaptic transmission remains to be further elucidated. In addition to lipid mediators (i.e., AA, eicosanoids, and PAF), released $sPLA_2$ from the presynaptic neuron may directly participate in the induction and/or maintenance of LTP through a receptor-mediated mechanism or alternatively through its uptake by the presynaptic neuron [90, 91].

Involvement of $sPLA_2$ in Neurological Diseases

Altered PLA_2 activities have been implicated in acute and chronic brain injury and in other neurological diseases. However, the roles of individual isoforms in inducing or in aggravating these pathologies are still not clearly understood [52, 92, 93].

Excitotoxicity, Oxidative Stress, and Neuronal Cell Death

Excessive activation of glutamate receptors is known to exert a neurotoxic effect accompanied by necrotic or apoptotic neuronal cell death (excitotoxicity) [94, 95]. This type of excitotoxicity is linked to many neurodegenerative disorders. The neurotoxic effect of glutamate is mediated by excessive calcium influx into neurons, which initiates an intracellular cascade of events [96]. These events include, among other effects, the perturbation of mitochondrial Ca^{2+} and disturbance of energy metabolism, activation of Ca^{2+}-dependent enzymes, lipid peroxidation, and production of reactive oxygen and nitrogen species (ROS and RNS). The combination of these events triggers the generation of pro-apoptotic signals leading to neuronal death, which is one of the main mechanisms of cell death in many neurological diseases [97–99]. Several isoforms of PLA_2 may be involved in apoptotic cell death following glutamate excitotoxicity. Indeed, activation of $cPLA_2$ by phosphorylation and the release of AA were observed upon exposure of cortical neurons to NMDA (N-methyl-D-aspartate) [100]. The same treatment induced ROS production by NADPH oxidase. On the other hand, BEL-sensitive $iPLA_2$ was not involved in oxidative stress-induced release of AA from phospholipids of mouse neuronal cells [101].

The involvement of $sPLA_2$ isoforms in the induction of neuronal cell death has been often regarded as an extracellular phenomenon because results were based mainly on addition of exogenous enzymes to the neuronal cultures [102–104]. However, the presence of $sPLA_2$ in intracellular compartments of neural cells [9, 48] suggests that endogenous enzymes might also participate in apoptotic cell death. Indeed, inhibition of GIIA by a cell permeable specific inhibitor could reduce glutamate-induced death of cerebellar granule neurons [105]. In addition, GIIA was also involved in the generation of ROS in peripheral mitochondria. A correlation between apoptotic cell deaths in rat primary neurons with NMDA-induced activation of GIIA has been reported [19]. This study demonstrated the activation of neuronal GIIA by ROS and RNS and the participation of this enzyme to generation of superoxides in mitochondria. Thus, it is conceivable to hypothesize that mitochondrial dysfunction caused by NMDA-mediated overloading of Ca^{2+} in neurons can trigger GIIA activation and generation of ROS by a still-unknown mechanism. The release of GIIA from rat brain mitochondria under energy-deficient conditions seems to lend further support to the hypothesis [9]. Mitochondrial GIIA might bind to the 25 kDa protein, which is present in the same organelle and binds with snake venom GIIA with high affinity [106]. The interaction of GIIA with the outer mitochondrial membrane may contribute to the release of cytochrome c and activation of the caspase cascade. Indeed, exposure of isolated mitochondria to rat recombinant GIIA could induce the release of cytochrome c (Goracci, unpublished). This hypothesis does not exclude the possible contribution of $iPLA_2$ known to be localized in the outer mitochondrial membrane [9]. Indeed, activation of liver mitochondrial $iPLA_2$ was shown to promote spontaneous release of cytochrome c [107].

Neuroinflammation

The involvement of PLA_2s in neuroinflammation caused by traumatic injury or infection has been documented by a number of reports [108]. Particularly, induction of GIIA expression could be observed by treatments of cultured astrocytes with LPS or pro-inflammatory cytokines [59, 109]. The induction of GIIA mRNA by IL-1β is mediated by the activation of ERK1/2 and PI-3 kinase, and the protein is partially released into the medium [110].

Ischemia/Reperfusion

Following the first observation that one of the early biochemical events taking place during brain ischemia is the increase of FFA (free fatty acids) [111], a large number of studies tried to identify the enzymes responsible for this phenomenon and the mechanisms for their activation. This task is particularly difficult due to the anatomical, histological, and physiological complexity of the brain. Furthermore, the variety of animal models utilized for these studies might complicate the interpretation of the results. However, the available data clearly indicate that more than one type of PLA_2 participates in the release of FFA from membrane phospholipids, and their relative contribution may depend on the experimental models, the age of animals, and the time duration of the ischemic insult and reperfusion [52, 93, 112, 113].

Several studies have indicated that $cPLA_2$ and $sPLA_2$, most likely GIIA, are involved in the release of fatty acids during ischemic brain injury [113–117]. Ischemia/reperfusion was shown to cause a biphasic increase of GIIA mRNA, the first increase at 60 min and a second increase after 2 weeks [116]. The increased expression of GIIA was confirmed by in situ hybridization and immunohistochemistry. Confocal microscopy revealed that GIIA was mainly localized in astrocytes but not in microglia. Further support to the involvement of a $sPLA_2$ in brain ischemic injury was derived from the observations that inhibitors of this type of enzymes exert a neuroprotective effect [118–120].

Alzheimer's Disease

An extensive amount of literature supports the involvement of different types of PLA_2 in memory impairment and

neurodegeneration in Alzheimer's disease (AD) [34, 93, 121–123]. However, their role might be different depending on early or late stages of the disease [124]. According to this hypothesis, the decrease of cortical and hippocampal PLA_2 activity observed in the early stage of AD is mainly attributed to $cPLA_2$ and $iPLA_2$ and that these changes may be involved in memory deficits and Aβ production. The decreased activity of these enzymes may be responsible for the down-regulation of glutamatergic and cholinergic receptors observed in the early stage of AD and leading to memory impairment. On the other hand, the increase in PLA_2 activity observed in late stages of AD should be attributed to the involvement of $cPLA_2$ and $sPLA_2$ under inflammation and oxidative stress. Indeed, correlations between Aβ production, oxidative stress, and PLA_2 activation have been documented [100, 125]. The observation that $sPLA_2$ activity increases in human cerebrospinal fluid of AD patients further support the involvement of this isoform in this pathology [126].

Parkinson's Disease

Among different pathogenic mechanisms proposed for Parkinson's disease (PD), mitochondrial dysfunction is considered as a key event [127, 128]. Indeed, a systemic low-grade inhibition of complex I activity was recognized in PD patients. Treatment of animals with inhibitors of this complex, rotenone, or MPTP (1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine), have been successfully used as models for PD. Studies to measure PLA_2 activity in this pathology are limited. A role of $cPLA_2$ in PD was suggested because mice deficient in this enzyme were more resistant to MPTP treatment [129]. Recently, activation of $cPLA_2$ by phosphorylation was demonstrated in vivo and in vitro models of PD [130]. Activation of the enzyme is apparently the consequence of a cascade of events involving the NO/cGMP/PKG pathway. Little information is available on the involvement of specific $sPLA_2$ isoforms and mitochondrial dysfunctions. Further studies should also address mechanisms for the increase in ROS production in PD and possible implication of the oxidative pathway to degeneration of dopaminergic neurons.

Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis

Experimental autoimmune encephalomyelitis (EAE) is considered a model for studying the biochemical events during the demyelination and remyelination processes and is considered an animal model of multiple sclerosis. Early studies demonstrated the increase of PLA_2 activity in rat brain mitochondria during the acute phase of EAE [131]. Interestingly, in a recent study on brains from multiple sclerosis (MS) patients, there was a marked reduction of the transcripts of gene encoding for mitochondrial electron transport proteins and a concomitant decrease in activities of complexes I and III [132].

The use of extracellular inhibitors of PLA_2, including the *N*-derivatized PE linked to polymeric carriers, inhibited the expression and the secretion of $sPLA_2$ from cultured glial cells and reduced the clinical expression of EAE in rats and mice [133]. Another study observed an increase in activity of $sPLA_2$ in urine of EAE rats, suggesting an increase in systemic enzyme activity [134]. These results are in agreement with the finding that urinary activity of $sPLA_2$ was increased in MS patients. Treatment of EAE animals with CHEC-9, an inhibitor of $sPLA_2$, attenuated the increase of urinary enzyme activity between days 8–10 after immunization and abolished the EAE symptoms. The participation of $cPLA_2$ and $iPLA_2$ in the onset and progression of EAE was also proposed, and GIIA was implicated in the remission phase [135, 136].

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder of the motor neurons in the spinal cord, brainstem, and motor cortex. Riluzole is an anti-glutamatergic agent clinically used for the treatment of ALS. This drug has antioxidant properties and exerts protective effects on cortical neurons. It can also reduce the release of AA from pre-labeled fibroblast cells in culture [137]. The inhibiting effect of riluzole was attributed to $cPLA_2$ because it reduced the activity of partially purified porcine enzyme but had no effect on pancreatic GIB and platelet GIIA in vitro. However, despite the inhibition of AA release, treatment with this drug resulted in an increase in cell death by apoptosis. Despite this paradoxical observation, treatment of ALS patients with riluzole is effective in extending survival and/or time to tracheostomy [138] suggesting that glutamate excitotoxicity and oxidative stress contribute to neurodegeneration in ALS as well as to other neurodegenerative diseases. Although the mechanisms leading to motor neuron degeneration are not known, it has been proposed that corticomotoneurons may mediate anterograde degeneration of anterior horn cells via glutamate-mediated excitotoxicity [139].

Some forms of ALS are caused by mutations of superoxide dismutase-1 gene and transgenic mice expressing human mutant enzyme has been used as a model for ALS. These mice undergo motor neuron degeneration with typical features of mitochondriopathy [140], suggesting the involvement of mitochondrial transition pores [141]. Although there is no available data to date, it is worth contemplating that mitochondrial PLA_2 ($iPLA_2$ and GIIA) contribute to mitochondrial dysfunction in this disease.

Spinal Cord Injury

Complex molecular events are initiated by primary injuries of spinal cord which are followed by a progressive degeneration, known as "secondary injury" that increases the size of the lesion [142]. Evidence for the involvement of apoptotic death of neurons and oligodendrocytes in secondary injury has been reported [143]. Post-injury demyelination continues for several weeks and correlates with the death of oligodendrocytes in white matter. The cascade of events leading to apoptotic cell death includes opening of voltage-dependent ion channels, release of glutamate, activation of NMDA and AMPA receptors, overloading of intracellular Ca^{2+}, production of free-radicals, mitochondrial dysfunction, and caspase activation [144].

Severe injury of rat spinal cord causes a rapid production of prostaglandin $F_{2\alpha}$ and thromboxane and a later accumulation of arachidonic acid indicating a post-injury activation of PLA_2 [145]. The contribution of this class of enzymes in the pathogenesis of spinal cord injury was recently confirmed because total PLA_2 activity was increased with a peak after 4 h [146]. The expression of $cPLA_2$ was also increased but with a peak at 3 and 7 days after spinal cord injury (SCI). Immunofluorescence analysis indicated that the elevation of $cPLA_2$ was mainly localized in neurons of spinal gray matter and in oligodendrocytes close to the damaged area. The increased PLA_2 activity 4 h after SCI was later attributed to the induction of the expression of GIIA and GIIE because the levels of mRNAs and proteins were significantly more elevated with respect to sham-operated rats [147]. The up-regulation of GIIA and GIIE following SCI was confirmed by immunohistochemistry. Confocal immunofluorescence analysis and immunoblotting revealed that the increase of GIIA levels is associated with the membrane fraction of oligodendrocytes. Since the inhibition of GIIA reduces oligodendrocyte cell death induced by H_2O_2 or inflammatory cytokines treatments, it has been proposed that GIIA may represent a target for preventing tissue damage after SCI.

Brain Tumors

Little information is available on the role of $sPLA_2$ isoforms in brain tumors although several studies have demonstrated the increase of their expression in other tissues indicating that these enzymes may represent potential targets for therapies [148, 149]. Particularly, GIIA is considered the isoform mainly involved in the development and progression of tumors in different human organs including breast, pancreas, liver, colon, and prostate. A recent study has shown that GIIA and GV are constitutively expressed in malignant prostate cells (PC-3 and LNCaP) but not in normal human prostate epithelial cells (PrEc) [150]. The expression of GIIA and GV is differentially up-regulated by cytokines. GX is highly expressed in normal PrEc and malignant cells but the expression of this isoform is insensitive to cytokines. GIIA is not expressed in another prostate cancer cell line (DU-145) even when stimulated with IL-1β. This observation has suggested that epigenetic mechanisms may silence the expression of GIIA in this particular cell line.

Exogenous mouse GX stimulates the proliferation of different colon cell lines and activates MAP (Mitrogen Activated Protein) kinase pathway by mechanisms involving its catalytical activity and the generation of lipid mediators [151]. The effect of exogenous GIIA on the proliferation of 1321N1 human astrocytoma cell line was also studied. Contrary to U-251 astrocytoma cells [9], these cells do not express GIIA [152], but their treatment with the human enzyme induces the activation of MAP kinases, phosphorylation of $cPLA_2$, and release of arachidonic acid by a mechanism apparently independent of GIIA activity. The treatment of the same cells with GIIA induces ROS generation triggering Ras and Raf-1 activation and phosphorylation of Akt, p70S6K, and s6 ribosomal protein [153]. The inhibition of these GIIA-mediated effects blocked cell proliferation and did not affect apoptotic program. In addition, TNFα-induced apoptosis is counteracted by the simultaneous treatment with GIIA, and it has been proposed that the effect is mediated by ERK activation [154]. Thus, these observations suggest that extracellular GIIA exerts a dual effect in 1321N1 astrocytoma cells because it induces their proliferation and, at the same time, protects them by pro-apoptotic agents. Although these reported effects are restricted to a particular tumor cell line, it can be supposed that the increased concentration of GIIA or other $sPLA_2$ isoforms, released by activated astrocytes or inflammatory cells in the extracellular environment, may stimulate glial cells to proliferate representing a link between inflammation and development of brain tumors.

Future studies should be addressed to explain why, in certain cells, $sPLA_2$ isoforms exert a mitogenic and anti-apoptotic action and in others, as neurons, the same isoforms are pro-apoptotic.

Inhibitors of $sPLA_2$s and Potential Pharmacological Effects

The identification of molecules capable of inhibiting specific $sPLA_2$ isoforms is of great pharmacological interest as potential drugs for the treatment of pathologies including neurological diseases [155]. However, although some natural compounds have been shown to inhibit specifically $sPLA_2$, none of them appears to be selective. In addition,

several synthetic compounds are potent inhibitors of $sPLA_2$, but some show a certain grade of selectivity towards different isoforms [2, 156–158]. Here, we report a selection of those molecules having potential pharmacological interest for treatment of neurological diseases because they reduce the effects of $sPLA_2$ in the nervous tissue or neural cells.

Indole Derivatives

A large number of indole derivatives [159] have been probed as potential inhibitors of $sPLA_2$ isoforms. The indole derivative LY311727 was reported to be rather selective for GII isoforms but was only poorly effective on reducing AA efflux in rat model of cerebral ischemia [160]. Other indole derivatives, such as indoxam and Me-indoxam, are quite effective competitive inhibitors of different isoforms of human and mouse $sPLA_2$ [157]. Due to their poor membrane permeability, however, these molecules are only suited for studies on extracellular $sPLA_2$. Administration of the prodrug (indoxam methyl ester) 1 h before or 2 h after middle-cerebral artery significantly reduced the infarction of dorsolateral cortex. Indoxam could also significantly decrease the activity of $sPLA_2$ in the affected brain area [103]. The beneficial effect of this treatment was attributed to inhibition of GIIA because indoxam almost completely blocked the death of rat cultured neurons induced by exposure to recombinant human GIIA. However, indoxam is not a specific inhibitor of this isoform because it also reduces the activities of other isoforms including GV present in neuronal and glial nuclei [48].

CHEC-9

CHEC-9, a nonapeptide (CHEASAAQC), is the internal fragment of a human polypeptide (DSEP) with neuroprotective properties [161]. Systemic treatment of CHEC-9 inhibited degeneration of neurons adjacent to brain lesion. This effect was attributed to the uncompetitive inhibition of serum $sPLA_2$. Despite of a yet unknown mechanism, inhibitory effects of CHEC-9 were supported by results from in vitro, ex vivo, and in vivo experiments [162].

Vitamin E

Vitamin E (α-tocopherol) is able to cross blood–brain barrier and may exert a neuroprotective effect by its antioxidant properties [163, 164]. However, it is not clear whether vitamin E exerts effects on different types of PLA_2s. In one study, vitamin E was shown to induce the release of AA through activation of $cPLA_2$ [165]; but in another study, α-tocopherol analogs showed an opposite effect [166]. A non-competitive inhibition of porcine pancreatic GIB by vitamin E was reported, but this effect was attributed more to interaction with the substrate than to the direct effect on the enzyme [167]. GIIA from Russelli's viper venom was also competitively inhibited by vitamin E [168]. Structural analysis of the interaction between α-tocopherol and the crystal structure of the enzyme revealed possible interaction with the active site.

Even if no information is available about direct effects of vitamin E on brain PLA_2 isoforms, it is possible that its action on GIIA and its antioxidant properties might represent a pharmacological intervention for reducing the detrimental effect of inflammation and oxidative stress.

PX-18

PX-18 is a compound indicated as a potential $sPLA_2$ inhibitor [169]. Due to its scarce solubility in water, this compound requires formulation in nano suspensions for in vivo application [170]. Intraperitoneal injection of this drug to gerbils immediately after ischemia/reperfusion was shown to reduce glial cell activation and neuronal apoptotic death [119]. However, no information is available on the specificity of PX-18 on different PLA_2 types.

Native Peptides

A number of pentapeptides corresponding to the sequence 70-74 of the native $sPLA_2$ were developed and tested as potential inhibitors of this type of enzymes [171]. These peptides do not bind to the active site, but can associate with the NH_2-terminal helix known to be important for conferring enzyme activity. Differences in the sequences of the pentapeptides according to the origin of the enzyme may allow the development of selective inhibitors for different $sPLA_2$ isoforms. Molecular modeling and peptide–enzyme interaction analysis have been used to identify a cyclic pentapeptide, c(2NapA)LS(2NapA)R, which binds and inhibits the activity of human GIIA [172]. The same peptide has no effect on porcine GIB and snake venom GIIA. The same peptide inhibits the activity of recombinant rat GIIA, but not that of *Crotalus atrox* GIIA or rat GV when assayed with PED6-containing liposomes as substrate [19].

Commercially available c(2NapA)LS(2NapA)R ($sPLA_2$-IIA inhibitor I) protected cerebellar granule neuron death induced by glutamate and inhibited ROS production in peripheral neuronal mitochondria [105]. A recent study also demonstrated that this pentapeptide could abolish NMDA-induced increase in PLA_2 activity and apoptotic cell death of cortical primary neurons [19]. Because this inhibitor is cell permeable, non-toxic, and appears to be rather specific, it is potentially useful for future studies to test the involvement of $sPLA_2$ isoforms under different pathological conditions.

Conclusions

Although mammalian glial and neuronal cells potentially express multiple $sPLA_2$ isoforms, the GIIA enzyme seems to be the major one involved in physiological and pathological mechanisms. Under normal conditions, astrocytes may synthesize and release low levels of GIIA for interaction with *N*-type receptors. Alternatively, it can be up-taken by neuronal cells and participate in modulating neuronal functions such as neurotransmission, neuritogenesis, and in combination with other PLA_2 types, e.g., $cPLA_2$ and $iPLA_2$, for production of lipid mediators. On the other hand, neurons may also produce GIIA, and it seems that the endogenous enzyme is targeted to intracellular compartments such as mitochondria and nerve endings. At the present status of knowledge, it is reasonable to speculate that the mitochondrial enzyme, due to its low specificity, exerts a protective effect by removing oxidized fatty acids, particularly from cardiolipin, which is particularly enriched in polyunsaturated fatty acids. In addition, GIIA may participate in membrane remodeling during mitochondrial fusion and fission, processes important in maintaining functional integrity of these organelles [173].

The presence of $sPLA_2$, most likely GIIA, in synaptic vesicles may indicate its role in the mechanisms for neurotransmitter release. Furthermore, the expression of neuronal GIIA is increased by NGF, and its localization at the neurite tips is a clear evidence for its participation in neuritogenesis.

The involvement of GIIA in neurodegenerative diseases is well-documented. In this case, its participation to mechanisms leading to neuronal cell death is the consequence of its excessive expression in reactive astrocytes and in neurons after stimulation by NMDA. Increased release of GIIA in reactive astrocytes may exert a neurotoxic effect similarly to that reported for exogenous enzymes such as snake venoms. High levels of the enzyme might reach intracellular organelles including mitochondria [106, 174, 175] causing their dysfunction and contributing to the increase of oxidative stress. In the second case, increased intracellular Ca^{2+} concentration in neurons can influence a number of processes including activation of endogenous neuronal GIIA [19]. Together with the exogenous enzyme, this may lead to mitochondrial dysfunctions, alteration of mitochondria dynamics, and apoptosis. Since abnormal mitochondrial dynamics is a mechanism common to several neurodegenerative diseases, future studies should consider how alterations of mitochondrial phospholipid metabolism may play an important role in the fusion and fission processes, where both inner and outer membranes undergo important structural modification.

In conclusion, investigations on the mechanisms regulating expression and activity of GIIA should be encouraged, considering its involvement in important brain functions and in the onset and aggravation of neurodegenerative diseases. Furthermore, development of new molecules exerting a selective inhibition of individual $sPLA_2$ isoforms will be of great importance as potential drugs for prevention and treatment of neurodegenerative disorders.

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References

1. Schaloske RH, Dennis EA (2006) The phospholipase A_2 superfamily and its group numbering system. Biochim Biophys Acta 1761:1246–1259
2. Lambeau G, Gelb MH (2008) Biochemistry and physiology of mammalian secreted phospholipases A_2. Annu Rev Biochem 77:495–520
3. Pan YH, Yu BZ, Singer AG, Ghomashchi F, Lambeau G, Gelb MH, Jain MK, Bahnson BJ (2002) Crystal structure of human group X secreted phospholipase A_2. Electrostatically neutral interfacial surface targets zwitterionic membranes. J Biol Chem 277:29086–29093
4. Sekar K, Yu BZ, Rogers J, Lutton J, Liu X, Chen X, Tsai MD, Jain MK, Sundaralingam M (1997) Phospholipase A_2 engineering. Structural and functional roles of the highly conserved active site residue aspartate-99. Biochemistry 36:3104–3114
5. Jain MK, Berg OG (2006) Coupling of the i-face and the active site of phospholipase A_2 for interfacial activation. Curr Opin Chem Biol 10:473–479
6. Yang HC, Mosior M, Johnson CA, Chen Y, Dennis EA (1999) Group-specific assays that distinguish between the four major types of mammalian phospholipase A_2. Anal Biochem 269:278–288
7. Lucas KK, Dennis EA (2005) Distinguishing phospholipase A_2 types in biological samples by employing group-specific assays in the presence of inhibitors. Prostaglandins Other Lipid Mediat 77:235–248
8. Elsbach P, Weiss J (1991) Utilization of labeled *Escherichia coli* as phospholipase substrate. Methods Enzymol 197:24–31
9. Macchioni L, Corazzi L, Nardicchi V, Mannucci R, Arcuri C, Porcellati S, Sposini T, Donato R, Goracci G (2004) Rat brain cortex mitochondria release group II secretory phospholipase A_2 under reduced membrane potential. J Biol Chem 279:37860–37869
10. Janssen MJ, Vermeulen L, van der Helm HA, Aarsman AJ, Slotboom AJ, Egmond MR (1999) Enzymatic properties of rat group IIA and V phospholipases A_2 compared. Biochim Biophys Acta 1440:59–72
11. Yu L, Dennis EA (1991) Thio-based phospholipase assay. Methods Enzymol 197:65–75
12. Hendrickson HS (1991) Phospholipase A_2 assays with fluorophore-labeled lipid substrates. Methods Enzymol 197:90–94
13. Hendrickson HS, Hendrickson EK, Johnson ID, Farber SA (1999) Intramolecularly quenched BODIPY-labeled phospholipid analogs in phospholipase A_2 and platelet-activating factor acetylhydrolase assays and in vivo fluorescence imaging. Anal Biochem 276:27–35

14. Thuren T, Virtanen JA, Somerharju PJ, Kinnunen PK (1988) Phospholipase A_2 assay using an intramolecularly quenched pyrene-labeled phospholipid analogue as a substrate. Anal Biochem 170:248–255
15. Farber SA, Pack M, Ho SY, Johnson ID, Wagner DS, Dosch R, Mullins MC, Hendrickson HS, Hendrickson EK, Halpern ME (2001) Genetic analysis of digestive physiology using fluorescent phospholipid reporters. Science 292:1385–1388
16. Feng L, Manabe K, Shope JC, Widmer S, Dewald DB, Prestwich GD (2002) A real-time fluorogenic phospholipase A_2 assay for biochemical and cellular activity measurements. Chem Biol 9:795–803
17. Kim YJ, Kim KP, Rhee HJ, Das S, Rafter JD, Oh YS, Cho W (2002) Internalized group V secretory phospholipase A_2 acts on the perinuclear membranes. J Biol Chem 277:9358–9365
18. Boilard E, Bourgoin SG, Bernatchez C, Poubelle PE, Surette ME (2003) Interaction of low molecular weight group IIA phospholipase A_2 with apoptotic human T cells: role of heparan sulfate proteoglycans. FASEB J 17:1068–1080
19. Chiricozzi E, Fernandez-Fernandez S, Nardicchi V, Almeida A, Bolanos JP, Goracci G (2010) Group IIA secretory phospholipase A_2 (GIIA) mediates apoptotic death during NMDA-receptor activation in rat primary cortical neurons. J Neurochem 112:1574–1583
20. de Caro J, Boudouard M, Bonicel J, Guidoni A, Desnuelle P, Rovery M (1981) Porcine pancreatic lipase. Completion of the primary structure. Biochim Biophys Acta 671:129–138
21. Grataroli R, de Caro A, Guy O, Amic J, Figarella C (1981) Isolation and properties of prophospholipase A_2 from human pancreatic juice. Biochimie 63:677–684
22. Verheij HM, Westerman J, Sternby B, de Haas GH (1983) The complete primary structure of phospholipase A_2 from human pancreas. Biochim Biophys Acta 747:93–99
23. Steiner RA, Rozeboom HJ, de Vries A, Kalk KH, Murshudov GN, Wilson KS, Dijkstra BW (2001) X-ray structure of bovine pancreatic phospholipase A_2 at atomic resolution. Acta Crystallogr D Biol Crystallogr 57:516–526
24. Dijkstra BW, Kalk KH, Hol WG, Drenth J (1981) Structure of bovine pancreatic phospholipase $_{A2}$ at 1.7A resolution. J Mol Biol 147:97–123
25. van den Berg B, Tessari M, de Haas GH, Verheij HM, Boelens R, Kaptein R (1995) Solution structure of porcine pancreatic phospholipase A_2. EMBO J 14:4123–4131
26. Ohsawa K, Mori A, Horie S, Saito T, Okuma Y, Nomura Y, Murayama T (2002) Arachidonic acid release and prostaglandin F2alpha formation induced by phenylarsine oxide in PC12 cells: possible involvement of secretory phospholipase A_2 activity. Biochem Pharmacol 64:117–124
27. Kolko M, Christoffersen NR, Varoqui H, Bazan NG (2005) Expression and induction of secretory phospholipase A_2 group IB in brain. Cell Mol Neurobiol 25:1107–1122
28. Kolko M, Christoffersen NR, Barreiro SG, Bazan NG (2004) Expression and location of mRNAs encoding multiple forms of secretory phospholipase A_2 in the rat retina. J Neurosci Res 77:517–524
29. Kramer RM, Hession C, Johansen B, Hayes G, McGray P, Chow EP, Tizard R, Pepinsky RB (1989) Structure and properties of a human non-pancreatic phospholipase A_2. J Biol Chem 264:5768–5775
30. Stefanski E, Pruzanski W, Sternby B, Vadas P (1986) Purification of a soluble phospholipase A_2 from synovial fluid in rheumatoid arthritis. J Biochem (Tokyo) 100:1297–1303
31. Seilhamer JJ, Pruzanski W, Vadas P, Plant S, Miller JA, Kloss J, Johnson LK (1989) Cloning and recombinant expression of phospholipase A_2 present in rheumatoid arthritic synovial fluid. J Biol Chem 264:5335–5338
32. Wery JP, Schevitz RW, Clawson DK, Bobbitt JL, Dow ER, Gamboa G, Goodson T Jr, Hermann RB, Kramer RM, McClure DB, Mihelich ED, Putnam JE, Sharp JD, Stark DH, Teater C, Warrick MW, Jones ND (1991) Structure of recombinant human rheumatoid arthritic synovial fluid phospholipase A_2 at 2.2 A resolution. Nature 352:79–82
33. Molloy GY, Rattray M, Williams RJ (1998) Genes encoding multiple forms of phospholipase A_2 are expressed in rat brain. Neurosci Lett 258:139–142
34. Moses GS, Jensen MD, Lue LF, Walker DG, Sun AY, Simonyi A, Sun GY (2006) Secretory PLA_2-IIA: a new inflammatory factor for Alzheimer's disease. J Neuroinflam 3:28
35. Yang HC, Mosior M, Ni B, Dennis EA (1999) Regional distribution, ontogeny, purification and characterization of the Ca^{2+}-independent phospholipase A_2 from rat brain. J Neurochem 73:1278–1287
36. Van Schaik RH, van den Koeduk CD, Neijs FW, Aarsman AJ, van den Bösch H (1993) Monoclonal antibodies against rat liver mitochondrial phospholipase A_2: epitope analysis and application in western blotting. Int J Biochem 25:433–439
37. Shirai Y, Ito M (2004) Specific differential expression of phospholipase A_2 subtypes in rat cerebellum. J Neurocytol 33:297–307
38. Morioka N, Takeda K, Kumagai K, Hanada T, Ikoma K, Hide I, Inoue A, Nakata Y (2002) Interleukin-1beta-induced substance P release from rat cultured primary afferent neurons driven by two phospholipase A_2 enzymes: secretory type IIA and cytosolic type IV. J Neurochem 80:989–997
39. Chen J, Shao C, Lazar V, Srivastava CH, Lee WH, Tischfield JA (1997) Localization of group IIc low molecular weight phospholipase A_2 mRNA to meiotic cells in the mouse. J Cell Biochem 64:369–375
40. Tischfield JA, Xia YR, Shih DM, Klisak I, Chen J, Engle SJ, Siakotos AN, Winstead MV, Seilhamer JJ, Allamand V, Gyapay G, Lusis AJ (1996) Low-molecular-weight, calcium-dependent phospholipase A_2 genes are linked and map to homologous chromosome regions in mouse and human. Genomics 32:328–333
41. Valentin E, Koduri RS, Scimeca JC, Carle G, Gelb MH, Lazdunski M, Lambeau G (1999) Cloning and recombinant expression of a novel mouse-secreted phospholipase A_2. J Biol Chem 274:19152–19160
42. Suzuki N, Ishizaki J, Yokota Y, Higashino K, Ono T, Ikeda M, Fujii N, Kawamoto K, Hanasaki K (2000) Structures, enzymatic properties and expression of novel human and mouse secretory phospholipase A_2s. J Biol Chem 275:5785–5793
43. Valentin E, Ghomashchi F, Gelb MH, Lazdunski M, Lambeau G (2000) Novel human secreted phospholipase A_2 with homology to the group III bee venom enzyme. J Biol Chem 275:7492–7496
44. Masuda S, Yamamoto K, Hirabayashi T, Ishikawa Y, Ishii T, Kudo I, Murakami M (2008) Human group III secreted phospholipase A_2 promotes neuronal outgrowth and survival. Biochem J 409:429–438
45. Cho W (2000) Structure, function and regulation of group V phospholipase A(2). Biochim Biophys Acta 1488:48–58
46. Chen J, Engle SJ, Seilhamer JJ, Tischfield JA (1994) Cloning and recombinant expression of a novel human low molecular weight Ca(2+)-dependent phospholipase A_2. J Biol Chem 269:2365–2368
47. Kolko M, Christoffersen NR, Barreiro SG, Miller L, Pizza AJ, Bazan NG (2006) Characterization and location of secretory phospholipase A_2 groups IIE, V and X in the rat brain. J Neurosci Res 83:874–882
48. Nardicchi V, Macchioni L, Ferrini M, Goracci G (2007) The presence of a secretory phospholipase A_2 in the nuclei of neuronal and glial cells of rat brain cortex. Biochim Biophys Acta 1771:1345–1352

49. Cupillard L, Koumanov K, Mattei MG, Lazdunski M, Lambeau G (1997) Cloning, chromosomal mapping and expression of a novel human secretory phospholipase A_2. J Biol Chem 272:15745–15752
50. Masuda S, Murakami M, Takanezawa Y, Aoki J, Arai H, Ishikawa Y, Ishii T, Arioka M, Kudo I (2005) Neuronal expression and neuritogenic action of group X secreted phospholipase A_2. J Biol Chem 280:23203–23214
51. Gelb MH, Valentin E, Ghomashchi F, Lazdunski M, Lambeau G (2000) Cloning and recombinant expression of a structurally novel human secreted phospholipase A_2. J Biol Chem 275:39823–39826
52. Farooqui A A and Horrocks L A (2007) Glycerophospholipids in brain. Phospholipases A_2 in neurological disorders, Springer Science + Business Media, New York.
53. Vadas P, Browning J, Edelson J, Pruzanski W (1993) Extracellular phospholipase A_2 expression and inflammation: the relationship with associated disease states. J Lipid Mediat 8:1–30
54. Dubouix A, Campanac C, Fauvel J, Simon MF, Salles JP, Roques C, Chap H, Marty N (2003) Bactericidal properties of group IIa secreted phospholipase A_2 against *Pseudomonas aeruginosa* clinical isolates. J Med Microbiol 52:1039–1045
55. Boyanovsky BB, Webb NR (2009) Biology of secretory phospholipase A_2. Cardiovasc Drugs Ther 23:61–72
56. Nevalainen TJ, Graham GG, Scott KF (2008) Antibacterial actions of secreted phospholipases A_2. Review. Biochim Biophys Acta 1781:1–9
57. Crowl RM, Stoller TJ, Conroy RR, Stoner CR (1991) Induction of phospholipase A_2 gene expression in human hepatoma cells by mediators of the acute phase response. J Biol Chem 266:2647–2651
58. Pfeilschifter J, Schalkwijk C, Briner VA, van den Bosch H (1993) Cytokine-stimulated secretion of group II phospholipase A_2 by rat mesangial cells. Its contribution to arachidonic acid release and prostaglandin synthesis by cultured rat glomerular cells. J Clin Invest 92:2516–2523
59. Sun GY, Hu ZY (1995) Stimulation of phospholipase A_2 expression in rat cultured astrocytes by LPS, TNF alpha and IL-1 beta. Prog Brain Res 105:231–238
60. Tong W, Hu ZY, Sun GY (1995) Stimulation of group II phospholipase A_2 mRNA expression and release in an immortalized astrocyte cell line (DITNC) by LPS, TNF alpha and IL-1 beta. Interactive effects. Mol Chem Neuropathol 25:1–17
61. Kramer RM, Sharp JD (1997) Structure, function and regulation of Ca^{2+}-sensitive cytosolic phospholipase A_2 (cPLA_2). FEBS Lett 410:49–53
62. Kolko M, Nielsen M, Bazan NG, Diemer NH (2002) Secretory phospholipase A(2) induces delayed neuronal COX-2 expression compared with glutamate. J Neurosci Res 69:169–177
63. Choi SH, Langenbach R, Bosetti F (2006) Cyclooxygenase-1 and -2 enzymes differentially regulate the brain upstream NF-kappa B pathway and downstream enzymes involved in prostaglandin biosynthesis. J Neurochem 98:801–811
64. Han WK, Sapirstein A, Hung CC, Alessandrini A, Bonventre JV (2003) Cross-talk between cytosolic phospholipase A_2 alpha (cPLA_2 alpha) and secretory phospholipase A_2 (sPLA_2) in hydrogen peroxide-induced arachidonic acid release in murine mesangial cells: sPLA_2 regulates cPLA_2 alpha activity that is responsible for arachidonic acid release. J Biol Chem 278:24153–24163
65. Goracci G, Balestrieri ML, Nardicchi V (2009) Metabolism and functions of platelet-activating factor (PAF) in the nervous tissue, in neural lipids pp. 311-352. Springer, New York, USA.
66. Goracci G, Francescangeli E (1991) Properties of PAF-synthesizing phosphocholinetransferase and evidence for lyso-PAF acetyltransferase activity in rat brain. Lipids 26:986–991
67. Negre-Aminou P, Nemenoff RA, Wood MR, de la Houssaye BA, Pfenninger KH (1996) Characterization of phospholipase A_2 activity enriched in the nerve growth cone. J Neurochem 67:2599–2608
68. Nakashima S, Kitamoto K, Arioka M (2004) The catalytic activity, but not receptor binding, of sPLA_2s plays a critical role for neurite outgrowth induction in PC12 cells. Brain Res 1015:207–211
69. Makarova Ya V, Osipov A, Tsetlin VI, Utkin Yu N (2006) Influence of phospholipases A_2 from snake venoms on survival and neurite outhgrowth in pheochromocytoma cell line PC12. Biochemistry (Mosc) 71:678–684
70. Ikeno Y, Konno N, Cheon SH, Bolchi A, Ottonello S, Kitamoto K, Arioka M (2005) Secretory phospholipase A_2 induces neurite outgrowth in PC12 cells through lysophosphatidylcholine generation and activation of G2A receptor. J Biol Chem 280(30):28044–28052
71. Forlenza OV, Mendes CT, Marie SK, Gattaz WF (2007) Inhibition of phospholipase A_2 reduces neurite outgrowth and neuronal viability. Prostaglandins Leukot Essent Fatty Acids 76:47–55
72. Woelk H, Peiler-Ichikawa K, Binaglia L, Goracci G, Porcellati G (1974) Distribution and properties of phospholipases A_1 and A_2 in synaptosomes and subsynaptosomal fractions of rat brain. Hoppe Seylers Z Physiol Chem 355:1535–1542
73. Matsuzawa A, Murakami M, Atsumi G, Imai K, Prados P, Inoue K, Kudo I (1996) Release of secretory phospholipase A_2 from rat neuronal cells and its possible function in the regulation of catecholamine secretion. Biochem J 318(Pt 2):701–709
74. Wei S, Ong WY, Thwin MM, Fong CW, Farooqui AA, Gopalakrishnakone P, Hong W (2003) Group IIA secretory phospholipase A_2 stimulates exocytosis and neurotransmitter release in pheochromocytoma-12 cells and cultured rat hippocampal neurons. Neuroscience 121:891–898
75. Kim DK, Rordorf G, Nemenoff RA, Koroshetz WJ, Bonventre JV (1995) Glutamate stably enhances the activity of two cytosolic forms of phospholipase A_2 in brain cortical cultures. Biochem J 310(Pt 1):83–90
76. DeCoster MA, Lambeau G, Lazdunski M, Bazan NG (2002) Secreted phospholipase A_2 potentiates glutamate-induced calcium increase and cell death in primary neuronal cultures. J Neurosci Res 67:634–645
77. Kolko M, DeCoster MA, de Turco EB, Bazan NG (1996) Synergy by secretory phospholipase A_2 and glutamate on inducing cell death and sustained arachidonic acid metabolic changes in primary cortical neuronal cultures. J Biol Chem 271:32722–32728
78. Rodriguez de Turco EB, Jackson FR, DeCoster MA, Kolko M, Bazan NG (2002) Glutamate signalling and secretory phospholipase A_2 modulate the release of arachidonic acid from neuronal membranes. J Neurosci Res 68:558–567
79. Anwyl R (2009) Metabotropic glutamate receptor-dependent long-term potentiation. Neuropharmacology 56:735–740
80. Macdonald JF, Jackson MF, Beazely MA (2006) Hippocampal long-term synaptic plasticity and signal amplification of NMDA receptors. Crit Rev Neurobiol 18:71–84
81. Medina JH, Izquierdo I (1995) Retrograde messengers, long-term potentiation and memory. Brain Res Brain Res Rev 21:185–194
82. Bazan NG, Zorumski CF, Clark GD (1993) The activation of phospholipase A_2 and release of arachidonic acid and other lipid mediators at the synapse: the role of platelet-activating factor. J Lipid Mediat 6:421–427
83. Dorman RV, Hamm TF, Damron DS, Freeman EJ (1992) Modulation of glutamate release from hippocampal mossy fiber nerve endings by arachidonic acid and eicosanoids. Adv Exp Med Biol 318:121–136

84. Grassi S, Francescangeli E, Goracci G, Pettorossi VE (1998) Role of platelet-activating factor in long-term potentiation of the rat medial vestibular nuclei. J Neurophysiol 79:3266–3271
85. Kato K, Clark GD, Bazan NG, Zorumski CF (1994) Platelet-activating factor as a potential retrograde messenger in CA1 hippocampal long-term potentiation. Nature 367:175–179
86. Lazarewicz JW, Wroblewski JT, Palmer ME, Costa E (1988) Activation of *N*-methyl-D-aspartate-sensitive glutamate receptors stimulates arachidonic acid release in primary cultures of cerebellar granule cells. Neuropharmacology 27:765–769
87. Massicotte G, Oliver MW, Lynch G, Baudry M (1990) Effect of bromophenacyl bromide, a phospholipase A_2 inhibitor, on the induction and maintenance of LTP in hippocampal slices. Brain Res 537:49–53
88. Miller B, Sarantis M, Traynelis SF, Attwell D (1992) Potentiation of NMDA receptor currents by arachidonic acid. Nature 355 (6362):722–725
89. Pellerin L, Wolfe LS (1991) Release of arachidonic acid by NMDA-receptor activation in the rat hippocampus. Neurochem Res 16:983–989
90. Lambeau G, Lazdunski M (1999) Receptors for a growing family of secreted phospholipases A_2. Trends Pharmacol Sci 20:162–170
91. Praznikar ZJ, Kovacic L, Rowan EG, Romih R, Rusmini P, Poletti A, Krizaj I, Pungercar J (2008) A presynaptically toxic secreted phospholipase A_2 is internalized into motoneuron-like cells where it is rapidly translocated into the cytosol. Biochim Biophys Acta 1783:1129–1139
92. Adibhatla RM, Hatcher JF (2008) Phospholipase A_2, reactive oxygen species and lipid peroxidation in CNS pathologies. BMB Rep 41:560–567
93. Sun GY, Xu J, Jensen MD, Simonyi A (2004) Phospholipase A_2 in the central nervous system: implications for neurodegenerative diseases. J Lipid Res 45:205–213
94. Ankarcrona M (1998) Glutamate induced cell death: apoptosis or necrosis? Prog Brain Res 116:265–272
95. Nicotera P, Ankarcrona M, Bonfoco E, Orrenius S, Lipton SA (1997) Neuronal necrosis and apoptosis: two distinct events induced by exposure to glutamate or oxidative stress. Adv Neurol 72:95–101
96. Mattson MP (2007) Calcium and neurodegeneration. Aging Cell 6:337–350
97. Adibhatla RM, Hatcher JF, Dempsey RJ (2003) Phospholipase A_2, hydroxyl radicals and lipid peroxidation in transient cerebral ischemia. Antioxid Redox Signal 5:647–654
98. Moncada S, Bolanos JP (2006) Nitric oxide, cell bioenergetics and neurodegeneration. J Neurochem 97:1676–1689
99. Murphy AN, Fiskum G, Beal MF (1999) Mitochondria in neurodegeneration: bioenergetic function in cell life and death [Review]. J Cereb Blood Flow Metab 19:231–245
100. Shelat PB, Chalimoniuk M, Wang JH, Strosznajder JB, Lee JC, Sun AY, Simonyi A, Sun GY (2008) Amyloid beta peptide and NMDA induce ROS from NADPH oxidase and AA release from cytosolic phospholipase A_2 in cortical neurons. J Neurochem 106:45–55
101. Peterson B, Stovall K, Monian P, Franklin JL, Cummings BS (2008) Alterations in phospholipid and fatty acid lipid profiles in primary neocortical cells during oxidant-induced cell injury. Chem Biol Interact 174:163–176
102. Kolko M, Bruhn T, Christensen T, Lazdunski M, Lambeau G, Bazan NG, Diemer NH (1999) Secretory phospholipase A_2 potentiates glutamate-induced rat striatal neuronal cell death in vivo. Neurosci Lett 274:167–170
103. Yagami T, Ueda K, Asakura K, Hata S, Kuroda T, Sakaeda T, Takasu N, Tanaka K, Gemba T, Hori Y (2002) Human group IIA secretory phospholipase A_2 induces neuronal cell death via apoptosis. Mol Pharmacol 61:114–126
104. Yagami T, Ueda K, Asakura K, Hayasaki-Kajiwara Y, Nakazato H, Sakaeda T, Hata S, Kuroda T, Takasu N, Hori Y (2002) Group IB secretory phospholipase A_2 induces neuronal cell death via apoptosis. J Neurochem 81:449–461
105. Mathisen GH, Thorkildsen IH, Paulsen RE (2007) Secretory PLA_2-IIA and ROS generation in peripheral mitochondria are critical for neuronal death. Brain Res 1153:43–51
106. Sribar J, Copic A, Poljsak-Prijatelj M, Kuret J, Logonder U, Gubensek F, Krizaj I (2003) R25 is an intracellular membrane receptor for a snake venom secretory phospholipase A_2. FEBS Lett 553:309–314
107. Gadd ME, Broekemeier KM, Crouser ED, Kumar J, Graff G, Pfeiffer DR (2006) Mitochondrial iPLA_2 activity modulates the release of cytochrome c from mitochondria and influences the permeability transition. J Biol Chem 281:6931–6939
108. Farooqui AA, Horrocks LA, Farooqui T (2007) Modulation of inflammation in brain: a matter of fat. J Neurochem 101:577–599
109. Oka S, Arita H (1991) Inflammatory factors stimulate expression of group II phospholipase A_2 in rat cultured astrocytes. Two distinct pathways of the gene expression. J Biol Chem 266:9956–9960
110. Jensen MD, Sheng W, Simonyi A, Johnson GS, Sun AY, Sun GY (2009) Involvement of oxidative pathways in cytokine-induced secretory phospholipase A_2-IIA in astrocytes. Neurochem Int 55:362–368
111. Bazan NG Jr (1970) Effects of ischemia and electroconvulsive shock on free fatty acid pool in the brain. Biochim Biophys Acta 218:1–10
112. Muralikrishna AR, Hatcher JF (2006) Phospholipase A_2, reactive oxygen species and lipid peroxidation in cerebral ischemia. Free Radic Biol Med 40:376–387
113. Phillis JW, O'Regan MH (2004) A potentially critical role of phospholipases in central nervous system ischemic, traumatic and neurodegenerative disorders. Brain Res Brain Res Rev 44:13–47
114. Adibhatla RM, Hatcher JF (2007) Secretory phospholipase A_2 IIA is up-regulated by TNF-alpha and IL-1alpha/beta after transient focal cerebral ischemia in rat. Brain Res 1134:199–205
115. Kramer RM, Stephenson DT, Roberts EF, Clemens JA (1996) Cytosolic phospholipase A_2 (cPLA_2) and lipid mediator release in the brain. J Lipid Mediat Cell Signal 14:3–7
116. Lin TN, Wang Q, Simonyi A, Chen JJ, Cheung WM, He YY, Xu J, Sun AY, Hsu CY, Sun GY (2004) Induction of secretory phospholipase A_2 in reactive astrocytes in response to transient focal cerebral ischemia in the rat brain. J Neurochem 90:637–645
117. Sapirstein A, Bonventre JV (2000) Phospholipases A_2 in ischemic and toxic brain injury. Neurochem Res 25:745–753
118. Hoda MN, Singh I, Singh AK, Khan M (2009) Reduction of lipoxidative load by secretory phospholipase A_2 inhibition protects against neurovascular injury following experimental stroke in rat. J Neuroinflammation 6:21
119. Wang Q, Sun AY, Pardeike J, Muller RH, Simonyi A, Sun GY (2009) Neuroprotective effects of a nanocrystal formulation of sPLA_2 inhibitor PX-18 in cerebral ischemia/reperfusion in gerbils. Brain Res 1285:188–195
120. Yagami T, Ueda K, Hata S, Kuroda T, Itoh N, Sakaguchi G, Okamura N, Sakaeda T, Fujimoto M (2005) S-2474, a novel nonsteroidal anti-inflammatory drug, rescues cortical neurons from human group IIA secretory phospholipase A(2)-induced apoptosis. Neuropharmacology 49:174–184
121. Farooqui AA, Horrocks LA (1998) Plasmalogen-selective phospholipase A_2 and its involvement in Alzheimer's disease. Biochem Soc Trans 26:243–246
122. Farooqui AA, Horrocks LA (2006) Phospholipase A_2-generated lipid mediators in the brain: the good, the bad and the ugly. Neuroscientist 12:245–260

123. Stephenson DT, Lemere CA, Selkoe DJ, Clemens JA (1996) Cytosolic phospholipase A_2 (cPLA_2) immunoreactivity is elevated in Alzheimer's disease brain. Neurobiol Dis 3:51–63
124. Schaeffer EL, Gattaz WF (2008) Cholinergic and glutamatergic alterations beginning at the early stages of Alzheimer disease: participation of the phospholipase A_2 enzyme. Psychopharmacology (Berl) 198:1–27
125. Sun GY, Horrocks LA, Farooqui AA (2007) The roles of NADPH oxidase and phospholipases A_2 in oxidative and inflammatory responses in neurodegenerative diseases. J Neurochem 103:1–16
126. Chalbot S, Zetterberg H, Blennow K, Fladby T, Grundke-Iqbal I, Iqbal K (2009) Cerebrospinal fluid secretory Ca^{2+}-dependent phospholipase A_2 activity is increased in Alzheimer disease. Clin Chem 55:2171–2179
127. Bueler H (2009) Impaired mitochondrial dynamics and function in the pathogenesis of Parkinson's disease. Exp Neurol 218:235–246
128. Schapira AH (2008) Mitochondria in the aetiology and pathogenesis of Parkinson's disease. Lancet Neurol 7:97–109
129. Klivenyi P, Beal MF, Ferrante RJ, Andreassen OA, Wermer M, Chin MR, Bonventre JV (1998) Mice deficient in group IV cytosolic phospholipase A_2 are resistant to MPTP neurotoxicity. J Neurochem 71:2634–2637
130. Chalimoniuk M, Stolecka A, Zieminska E, Stepien A, Langfort J, Strosznajder JB (2009) Involvement of multiple protein kinases in cPLA_2 phosphorylation, arachidonic acid release and cell death in in vivo and in vitro models of 1-methyl-4-phenylpyridinium-induced parkinsonism—the possible key role of PKG. J Neurochem 110:307–317
131. Woelk H, Kanig K, Peiler-Ichikawa K (1974) Phospholipid metabolism in experimental allergic encephalomyelitis: activity of mitochondrial phospholipase A_2 of rat brain towards specifically labelled 1, 2-diacyl-, 1-alk-1'-enyl-2-acyl- and 1-alkyl-2-acyl-sn-glycero-3-phosphorylcholine. J Neurochem 23:745–750
132. Dutta R, McDonough J, Yin X, Peterson J, Chang A, Torres T, Gudz T, Macklin WB, Lewis DA, Fox RJ, Rudick R, Mirnics K, Trapp BD (2006) Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. Ann Neurol 59:478–489
133. Pinto F, Brenner T, Dan P, Krimsky M, Yedgar S (2003) Extracellular phospholipase A_2 inhibitors suppress central nervous system inflammation. Glia 44:275–282
134. Cunningham TJ, Yao L, Oetinger M, Cort L, Blankenhorn EP, Greenstein JI (2006) Secreted phospholipase A_2 activity in experimental autoimmune encephalomyelitis and multiple sclerosis. J Neuroinflammation 3:26
135. Kalyvas A, Baskakis C, Magrioti V, Constantinou-Kokotou V, Stephens D, Lopez-Vales R, Lu JQ, Yong VW, Dennis EA, Kokotos G, David S (2009) Differing roles for members of the phospholipase A_2 superfamily in experimental autoimmune encephalomyelitis. Brain 132:1221–1235
136. Kalyvas A, David S (2004) Cytosolic phospholipase A_2 plays a key role in the pathogenesis of multiple sclerosis-like disease. Neuron 41:323–335
137. Koh JY, Kim DK, Hwang JY, Kim YH, Seo JH (1999) Antioxidative and proapoptotic effects of riluzole on cultured cortical neurons. J Neurochem 72:716–723
138. Cudkowicz M E, Katz J, Moore D H, O'neill G, Glass J. D, Mitsumoto H, Appel S, Ravina B, Kieburtz K, Shoulson I, Kaufmann P, Khan J, Simpson E, Shefner J, Levin B, Cwik V, Schoenfeld D, Aggarwal S, McDermott M P and Miller R G (2009) Toward more efficient clinical trials for amyotrophic lateral sclerosis. Amyotroph Lateral Scler. Dec 4
139. Vucic S, Cheah BC, Kiernan MC (2009) Defining the mechanisms that underlie cortical hyperexcitability in amyotrophic lateral sclerosis. Exp Neurol 220:177–182
140. Martin LJ, Liu Z, Chen K, Price AC, Pan Y, Swaby JA, Golden WC (2007) Motor neuron degeneration in amyotrophic lateral sclerosis mutant superoxide dismutase-1 transgenic mice: mechanisms of mitochondriopathy and cell death. J Comp Neurol 500:20–46
141. Martin LJ (2010) The mitochondrial permeability transition pore: a molecular target for amyotrophic lateral sclerosis therapy. Biochim Biophys Acta 1802:186–197
142. Klussmann S, Martin-Villalba A (2005) Molecular targets in spinal cord injury. J Mol Med 83:657–671
143. Beattie MS, Farooqui AA, Bresnahan JC (2000) Review of current evidence for apoptosis after spinal cord injury. J Neurotrauma 17:915–925
144. Hall ED, Springer JE (2004) Neuroprotection and acute spinal cord injury: a reappraisal. NeuroRx 1:80–100
145. Murphy EJ, Behrmann D, Bates CM, Horrocks LA (1994) Lipid alterations following impact spinal cord injury in the rat. Mol Chem Neuropathol 23:13–26
146. Liu NK, Zhang YP, Titsworth WL, Jiang X, Han S, Lu PH, Shields CB, Xu XM (2006) A novel role of phospholipase A2 in mediating spinal cord secondary injury. Ann Neurol 59:606–619
147. Titsworth WL, Cheng X, Ke Y, Deng L, Burckardt KA, Pendleton C, Liu NK, Shao H, Cao QL, Xu XM (2009) Differential expression of sPLA2 following spinal cord injury and a functional role for sPLA2-IIA in mediating oligodendrocyte death. Glia 57:1521–1537
148. Cummings BS (2007) Phospholipase A2 as targets for anticancer drugs. Biochem Pharmacol 74:949–959
149. Laye JP, Gill JH (2003) Phospholipase A2 expression in tumours: a target for therapeutic intervention? Drug Discov Today 8:710–716
150. Menschikowski M, Hagelgans A, Gussakovsky E, Kostka H, Paley EL, Siegert G (2008) Differential expression of secretory phospholipases A2 in normal and malignant prostate cell lines: regulation by cytokines, cell signaling pathways, and epigenetic mechanisms. Neoplasia 10:279–286
151. Surrel F, Jemel I, Boilard E, Bollinger JG, Payre C, Mounier CM, Talvinen KA, Laine VJ, Nevalainen TJ, Gelb MH, Lambeau G (2009) Group X phospholipase A2 stimulates the proliferation of colon cancer cells by producing various lipid mediators. Mol Pharmacol 76:778–790
152. Hernandez M, Burillo SL, Crespo MS, Nieto ML (1998) Secretory phospholipase A2 activates the cascade of mitogen-activated protein kinases and cytosolic phospholipase A2 in the human astrocytoma cell line 1321N1. J Biol Chem 273:606–612
153. Martin R, Hernandez M, Ibeas E, Fuentes L, Salicio V, Arnes M, Nieto ML (2009) Secreted phospholipase A2-IIA modulates key regulators of proliferation on astrocytoma cells. J Neurochem 111:988–999
154. Ibeas E, Fuentes L, Martin R, Hernandez M, Nieto ML (2009) Secreted phospholipase A2 type IIA as a mediator connecting innate and adaptive immunity: new role in atherosclerosis. Cardiovasc Res 81:54–63
155. Farooqui AA, Ong WY, Horrocks LA (2006) Inhibitors of brain phospholipase A_2 activity: their neuropharmacological effects and therapeutic importance for the treatment of neurologic disorders. Pharmacol Rev 58:591–620
156. Farooqui AA, Litsky ML, Farooqui T, Horrocks LA (1999) Inhibitors of intracellular phospholipase A_2 activity: their neurochemical effects and therapeutical importance for neurological disorders. Brain Res Bull 49:139–153
157. Oslund RC, Cermak N, Gelb MH (2008) Highly specific and broadly potent inhibitors of mammalian secreted phospholipases A_2. J Med Chem 51:4708–4714
158. Singer AG, Ghomashchi F, Le Calvez C, Bollinger J, Bezzine S, Rouault M, Sadilek M, Nguyen E, Lazdunski M, Lambeau G,

Gelb MH (2002) Interfacial kinetic and binding properties of the complete set of human and mouse groups I, II, V, X and XII secreted phospholipases A_2. J Biol Chem 277:48535–48549
159. Schevitz RW, Bach NJ, Carlson DG, Chirgadze NY, Clawson DK, Dillard RD, Draheim SE, Hartley LW, Jones ND, Mihelich ED et al (1995) Structure-based design of the first potent and selective inhibitor of human non-pancreatic secretory phospholipase A_2. Nat Struct Biol 2:458–465
160. Pilitsis JG, Diaz FG, O'Regan MH, Phillis JW (2002) Differential effects of phospholipase inhibitors on free fatty acid efflux in rat cerebral cortex during ischemia-reperfusion injury. Brain Res 951:96–106
161. Cunningham TJ, Souayah N, Jameson B, Mitchell J, Yao L (2004) Systemic treatment of cerebral cortex lesions in rats with a new secreted phospholipase A_2 inhibitor. J Neurotrauma 21:1683–1691
162. Cunningham TJ, Maciejewski J, Yao L (2006) Inhibition of secreted phospholipase A_2 by neuron survival and anti-inflammatory peptide CHEC-9. J Neuroinflammation 3:25
163. Asha DS (2009) Aging brain: prevention of oxidative stress by vitamin E and exercise. Sci World Journal 9:366–372
164. Butterfield DA, Castegna A, Drake J, Scapagnini G, Calabrese V (2002) Vitamin E and neurodegenerative disorders associated with oxidative stress. Nutr Neurosci 5:229–239
165. Tran K, Wong JT, Lee E, Chan AC, Choy PC (1996) Vitamin E potentiates arachidonate release and phospholipase A_2 activity in rat heart myoblastic cells. Biochem J 319:385–391
166. Pentland AP, Morrison AR, Jacobs SC, Hruza LL, Hebert JS, Packer L (1992) Tocopherol analogs suppress arachidonic acid metabolism via phospholipase inhibition. J Biol Chem 267:15578–15584
167. Grau A, Ortiz A (1998) Dissimilar protection of tocopherol isomers against membrane hydrolysis by phospholipase A_2. Chem Phys Lipids 91:109–118
168. Chandra V, Jasti J, Kaur P, Betzel C, Srinivasan A, Singh TP (2002) First structural evidence of a specific inhibition of phospholipase A_2 by alpha-tocopherol (vitamin E) and its implications in inflammation: crystal structure of the complex formed between phospholipase A_2 and alpha-tocopherol at 1.8 A resolution. J Mol Biol 320:215–222
169. Rastogi P, Beckett CS, Mchowat J (2007) Prostaglandin production in human coronary artery endothelial cells is modulated differentially by selective phospholipase A_2 inhibitors. Prostaglandins Leukot Essent Fatty Acids 76:205–212
170. Domoki F, Zimmermann A, Lenti L, Toth-Szuki V, Pardeike J, Muller RH, Bari F (2009) Secretory phospholipase A_2 inhibitor PX-18 preserves microvascular reactivity after cerebral ischemia in piglets. Microvasc Res 78:212–217
171. Tseng A, Inglis AS, Scott KF (1996) Native peptide inhibition. Specific inhibition of type II phospholipases A_2 by synthetic peptides derived from the primary sequence. J Biol Chem 271:23992–23998
172. Church WB, Inglis AS, Tseng A, Duell R, Lei PW, Bryant KJ, Scott KF (2001) A novel approach to the design of inhibitors of human secreted phospholipase A_2 based on native peptide inhibition. J Biol Chem 276:33156–33164
173. Chen H, Chan DC (2009) Mitochondrial dynamics—fusion, fission, movement and mitophagy—in neurodegenerative diseases. Hum Mol Genet 18(R2):R169–R176
174. Rigoni M, Paoli M, Milanesi E, Caccin P, Rasola A, Bernardi P, Montecucco C (2008) Snake phospholipase A_2 neurotoxins enter neurons, bind specifically to mitochondria and open their transition pores. J Biol Chem 283:34013–34020
175. Logonder U, Jenko-Praznikar Z, Scott-Davey T, Pungercar J, Krizaj I, Harris JB (2009) Ultrastructural evidence for the uptake of a neurotoxic snake venom phospholipase A_2 into mammalian motor nerve terminals. Exp Neurol 219:591–594
176. Thomas G, Bertrand F, Saunier B (2000) The differential regulation of group IIA and group V low molecular weight phospholipases A_2 in cultured rat astrocytes. J Biol Chem 275:10876–10886
177. Murakami M, Yoshihara K, Shimbara S, Lambeau G, Gelb MH, Singer AG, Sawada M, Inagaki N, Nagai H, Ishihara M, Ishikawa Y, Ishii T, Kudo I (2002) Cellular arachidonate-releasing function and inflammation-associated expression of group IIF secretory phospholipase A_2. J Biol Chem 277:19145–19155