Mol Neurobiol (2012) 46:85–95
DOI 10.1007/s12035-012-8261-1

Integrating Cytosolic Phospholipase A_2 with Oxidative/Nitrosative Signaling Pathways in Neurons: A Novel Therapeutic Strategy for AD

Grace Y. Sun · Yan He · Dennis Y. Chuang · James C. Lee · Zezong Gu · Agnes Simonyi · Albert Y. Sun

Received: 2 February 2012 / Accepted: 19 March 2012 / Published online: 3 April 2012

Abstract The pathophysiology of Alzheimer's disease (AD) is comprised of complex metabolic abnormalities in different cell types in the brain. To date, there are not yet effective drugs that can completely inhibit the pathophysiological event, and efforts have been devoted to prevent or minimize the progression of this disease. Much attention has focused on studies to understand aberrant functions of the ionotropic glutamate receptors, perturbation of calcium homeostasis, and toxic effects of oligomeric amyloid beta peptides (Aβ) which results in production of reactive oxygen and nitrogen species and signaling pathways, leading to mitochondrial dysfunction and synaptic impairments. Aberrant phospholipase A_2 (PLA_2) activity has been implicated to play a role in the pathogenesis of many neurodegenerative diseases, including AD. However, mechanisms for their modes of action and their roles in the oxidative and nitrosative signaling pathways have not been firmly established. In this article, we review recent studies providing a metabolic link between cytosolic PLA_2 ($cPLA_2$) and neuronal excitation due to stimulation of ionotropic glutamate receptors and toxic Aβ peptides. The requirements for Ca^{2+} binding together with its posttranslational modifications by protein kinases and possible by the redox-based S-nitrosylation, provide strong support for a dynamic role of $cPLA_2$ in serving multiple functions to neurons and glial cells under abnormal physiological and pathological conditions. Therefore, understanding mechanisms for $cPLA_2$ in the oxidative and nitrosative pathways in neurons will allow the development of novel therapeutic targets to mitigate the detrimental effects of AD.

Keywords Alzheimer's disease · Phospholipase A_2 · Reactive oxygen species (ROS) · Nitric oxide (NO) · NADPH oxidase · Mitochondria

G. Y. Sun (✉) · Y. He · D. Y. Chuang · A. Simonyi
Department of Biochemistry, University of Missouri,
117 Schweitzer Hall,
Columbia, MO 65211, USA
e-mail: sung@missouri.edu

J. C. Lee
Department of Biological Engineering, University of Missouri,
Columbia, MO 65211, USA

Z. Gu · A. Y. Sun
Department of Pathology and Anatomical Sciences,
University of Missouri,
Columbia, MO 65211, USA

Introduction

Alzheimer's disease (AD) is one of the most devastating age-related neurodegenerative diseases affecting more than 5 million people in the USA alone. With the rapid increase in aging population in the next two decades, the number of AD patients is expected to double, causing a huge economic burden to the society. AD pathophysiology is comprised of complex metabolic changes in neurons, glia, and neurovascular cells; besides the increased deposition of amyloid plaques and fibrillary tangles in neurons, other hallmarks for the disease include oxidative stress, glial cell inflammation, cerebrovascular abnormalities and, most importantly, synaptic failure [1]. However, despite these neurochemical and physiological manifestations, the biochemical mechanisms underlying the decline in memory and other cognitive functions associated with the progression of AD remain undetermined. Recent studies have provided strong support for the involvement of aberrant oxidative/nitrosative signaling pathways which result in a progressive damage of the

neuronal circuitry, especially those associated with cholinergic and glutamatergic neurons. Excessive stimulation of ionotropic glutamate receptors, e.g., the *N*-methyl-D-aspartic acid (NMDA) receptors, has been shown to cause disturbance of neuronal calcium homeostasis, leading to activation of proteases, nucleases, and phospholipases, and trigger signaling pathways for production of reactive oxygen/nitrogen species (ROS/RNS). In turn, these events become the basis of mitochondrial dysfunction and neuronal apoptosis.

Phospholipases A_2 (PLA_2s) are enzymes for hydrolysis of fatty acids in membrane phospholipids. Among many types of PLA_2s known to occur in the central nervous system, there is increasing recognition for the role of Ca^{2+}-dependent cytosolic phospholipase A_2 ($cPLA_2$) in the pathophysiology of AD. $cPLA_2$ is present ubiquitously in most brain cells including neurons, astrocytes, and microglial cells. Besides Ca^{2+}, this enzyme is regulated by receptor-mediated signaling pathways including phosphorylation by protein kinases and S-nitrosylation, i.e., covalent reaction of NO with specific protein thiol groups. Earlier studies have demonstrated the role of PLA_2s in neurodegenerative diseases [2], and that their damaging effects are attributed to involvement in oxidative/nitrosative signaling pathways [3]. In particular, attention has been focused on the cytosolic PLA_2 in neurons; its activation linked to glutamate excitotoxicity and in neuronal damage after exposure to cytotoxic beta amyloid peptides (Aβ). Since studies to link $cPLA_2$ to the oxidative/nitrosative pathways in neurons and its role in AD pathology have not been extensive, an important goal for this paper is to gather recent information on glutamate excitation and ascertain the role of $cPLA_2$ in oxidative/nitrosative pathways associated with AD pathology.

Cytotoxic Effects of Beta Amyloid Peptides

While accumulation of amyloid plaques has been regarded as one of the pathophysiological landmarks of AD, the "amyloid hypothesis" has been under challenge because a direct correlation between the amount of amyloid plaques deposition and the severity of the disease has not been firmly established [4]. In fact, amyloid plaques in the brain have been regarded as "tomb stones" without obvious functions. Nevertheless, amyloid plaques are enriched in Aβ peptides which are produced from the amyloid precursor protein (APP) through cleavage by beta and gamma secretases. In recent years, special attention has been placed on studies to investigate the mechanism(s) of aberrant Aβ production from APP and their ability to aggregate and cause toxic effects on neurons, glia, and cerebral endothelial cells. In neurons, toxic Aβ oligomers have been shown to downregulate NMDA receptor trafficking [5], alter neuronal circuitry, and impair synaptic activity [6]. Studies by Selkoe's group detected the release of soluble Aβ oligomers in the culture medium of neurons and hippocampal slices overexpressing human mutant APP, and Aβ oligomers recovered from the conditioned media could increase NMDA-induced Ca^{2+} influx into synaptic spines [7,8]. Oligomeric Aβ has been shown to perturb Ca^{2+} homeostasis in neurons, alter Ca^{2+}-dependent enzymes [9–11], and inhibit hippocampal long-term potentiation (LTP), a form of synaptic plasticity [12]. Studies using antibodies, specifically detecting oligomeric form of Aβ, also support the presence of Aβ oligomers in the AD brain. Furthermore, the abundance of Aβ oligomers in AD brain was positively correlated with the degree of synaptic loss and the severity of cognitive impairment [13]. In fact, numerous studies have successfully used in vitro protocols for the preparation of Aβ oligomers to demonstrate their detrimental effects on neurons [14,15]. Consequently, more studies are needed to better understand aberrant Aβ aggregation in the brain and mechanisms whereby these oligomers impair synaptic functions [16].

NMDA Receptor-Mediated Glutamatergic Signaling Pathways Induce Ca^{2+} Influx And the Generation of RNS/ROS

It is well known that excitatory neurotransmission is necessary for normal development and plasticity of synapses and some forms of learning or memory. However, excessive activation of glutamate receptors has been implicated in neuronal damage in many neurological disorders. Glutamate is the major excitatory neurotransmitter in the brain and is rapidly released (in milliseconds) from nerve terminals in a Ca^{2+}-dependent manner. Released glutamate can diffuse across the synaptic cleft to interact with postsynaptic receptors in adjacent neurons. It is currently thought that the overstimulation of extrasynaptic NMDA receptors can result in neuronal damage, whereas, activation of synaptic NMDA receptor can mediate the survival pathways [17–20]. The NMDA receptor has attracted attention for a long period of time because it has specific properties that set it apart from other ionotropic glutamate receptors, e.g., the (2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA) and kainate receptors. One such characteristic is that the NMDA receptor channels are highly permeable to Ca^{2+}, thus permitting Ca^{2+} entry after ligand binding. Subsequent binding of Ca^{2+} to various intracellular molecules can lead to many significant consequences. In particular, excessive activation of NMDA receptors leads to the production of damaging free radicals (e.g., NO and ROS) and other enzymatic processes contributing to cell death [21].

Increased levels of neuronal Ca^{2+}, in conjunction with the Ca^{2+}-binding protein calmodulin, trigger the activation of nNOS and subsequent generation of NO from the amino

acid L-arginine [22,23]. NO is a gaseous radical (thus highly diffusible) and a key molecule that plays a vital role in normal signal transduction, but in excess can lead to neuronal cell damage and death. Three subtypes of NOS have been identified; two constitutive forms, neuronal nNOS and endothelial eNOS, and one inducible form, iNOS. Constitutive and inducible NOSs are also further distinguished by CaM binding: nNOS and eNOS bind calmodulin in a reversible Ca^{2+}-dependent manner. In contrast, iNOS binds calmodulin so tightly at resting intracellular Ca^{2+} concentrations that its activity does not appear to be affected by transient variations in Ca^{2+} concentration. Interestingly, in order to terminate iNOS-mediated NO production, microglia may redistribute iNOS to the aggresome for inactivation [24].

Excitatory Glutamate Receptors Stimulate ROS Production Through NADPH Oxidase

Increase in oxidative/nitrosative stress is an important characteristic feature underlying the pathophysiology of AD [25]. The oxidative/nitrosative hypothesis is supported by detections of increases in markers, such as protein carbonyls, 3-nitrotyrosine, hydroxynonenal, and isoprostanes in mild cognitive impaired (MCI) brains [26–28]. In the brain, neurons in hippocampus are especially susceptible to oxidative stress. Uncontrolled stimulation of ionotropic glutamate receptors, especially the NMDA receptor subtype, is known to cause Ca^{2+} influx and trigger Ca^{2+}-dependent enzymes including protein kinases, proteases, nucleases, and phospholipases, leading to oxidative stress, mitochondrial dysfunction, and apoptosis. In recent studies, oxidative stress in this form of neuronal excitation is linked to ROS production through the activation of NADPH oxidase [29,30].

NADPH oxidase is a redox active enzyme occurring widely in all cell types in the brain [31]. The prototypic NADPH oxidase (NOX2) is comprising of membrane-subunits, i.e., gp91phox and p22phox and cytosolic subunits p47phox, p67phox, p60phox, and rac 1 or rac 2 [32]. Recognition of ROS production from NADPH oxidase in neurons and glial cells in the central nervous system has been an important finding. Due to possible physiological and pathological processes involving ROS in the CNS, there is intense interest to explore receptor signaling pathways linking to NADPH oxidase and inhibitors regulating the ROS production process [33].

A number of NADPH oxidase (NOX) isoforms are present in the mammalian system. Besides NOX2 which is the phagocytic form of NADPH oxidase, other isoforms of NOXs have been identified in the brain, e.g., NOX1, NOX4, and NOX5 [31,34]. NOX1 is shown to play a role in ischemia/reperfusion injury [35] and paraquat-induced oxidative damage in dopaminergic neurons [36]. On the other hand, NOX4 is involved in chronic glutamate toxicity in neurons [37]. The synaptic localization of NOX2 in neurons provides support for the role of this enzyme in mediating physiological functions in the synapse. Recent studies showed an increase in NOX2 expression and activity in the cortex of AD patients [38–40]. The role of NOX isoforms in regulating redox reactions in the brain gives strong indication that these enzymes are important in neurodegenerative diseases including AD, Parkinson's disease (PD), and stroke [41,42].

Nitrosative Stress Regulates Protein Misfolding and Neuronal Cell Death

Oxidative/nitrosative stress can facilitate protein misfolding and aggregation and is thought to play a role as a pathogenic trigger of neurodegenerative diseases. Recent scientific advances, however, support the notion that NO-related species may participate in the process of protein misfolding through protein S-nitrosylation under degenerative conditions.

Early investigations indicated that NO-mediated signaling pathways can regulate broad aspects of brain function, including synaptic plasticity, normal development, and neuronal cell death [43–46]. In general, NO can exert physiological and pathophysiological effects via stimulation of guanylate cyclase to form cyclic guanosine-3′, 5′-monophosphate (cGMP), or through S-nitros(yl)ation of regulatory protein thiol groups [47,48]. S-nitrosylation is the covalent addition of an NO group to a critical cysteine thiol/sulfhydryl (RSH or, more properly, thiolate anion, RS^-) to form an S-nitrosothiol derivative (R-SNO). Over the past decade, accumulating evidence has suggested that S-nitrosylation can regulate the biological activity of a great variety of proteins, in some ways akin to phosphorylation [49–51]. Analyses of mice deficient in either nNOS or iNOS confirmed that NO is an important mediator of cell injury and death after excitotoxic stimulation [52,53]. In addition, inhibition of NOS activity ameliorates the progression of disease pathology in animal models of PD, AD, and amyotrophic lateral sclerosis (ALS), suggesting that excess generation of NO plays a pivotal role in the pathogenesis of several neurodegenerative diseases.

Neuronal ROS Production and Mitochondrial Dysfunction in AD

Besides NADPH oxidase, other redox reactions in subcellular organelles, e.g., mitochondria, can also produce ROS and contribute to synaptic impairments [54]. Studies in our laboratory provided evidence that both NMDA and Aβ can induce rapid production of ROS from NADPH oxidase in neurons [30]. Furthermore, ROS from NADPH oxidase can trigger signaling pathways leading to mitochondrial dysfunction [55].

The role of NADPH oxidase as an initial source of ROS could be demonstrated by pretreatment of neurons with gp91ds-tat, a specific peptide inhibitor for NOX2, which abrogated the NMDA- and Aβ-mediated ROS production and mitochondrial dysfunction [56]. In another study, exposure of Aβ to hippocampal slice culture also induced an increase in mitochondrial superoxide production and a concomitant decrease in long-term potentiation; inhibition of mitochondrial ROS production protected against Aβ-induced hippocampal synaptic damage [57]. Taken together, these results are in line with the notion that excessive neuronal excitation or exposure of neurons to toxic Aβ can induce ROS from both NADPH oxidase and mitochondria; together, these oxidative events form the basis of neuronal apoptosis and cell death, a crucial mechanism underlying the pathophysiology of AD [58].

NADPH oxidase/ROS Signaling Pathway—Activation of Mitogen-Activated Protein Kinases and cPLA$_2$

Production of ROS from NADPH oxidase is known to trigger changes in a number of signaling pathways including activation of mitogen-activated protein kinases (MAPK). In neurons, NMDA and Aβ induce ROS production through NADPH oxidase, and in turn, this leads to activation of MEK1/2 or ERK1/2 and cPLA$_2$ [30]. Cytosolic PLA$_2$ is a 87 kDa protein comprised of a C2 domain with binding sites for intracellular Ca^{2+}, a catalytic domain with three putative phosphorylation sites: Ser505, Ser727, and Ser515 [59], and at least one cysteine residue is receptive for S-nitrosylation by NO [60]. In human epithelial cells, S-nitrosylation of cPLA$_2$ by NO from iNOS led to a sixfold increase in enzyme activity [60]. With these properties, activation of cPLA$_2$ has been linked to a number of cell surface receptors and ion channels, including the G-protein-coupled P2Y$_2$ receptor in astrocytes [61] and the ionotropic glutamate receptors in neurons [30]. Cytosolic PLA$_2$ is known to target membrane phospholipids for the release of arachidonic acid (AA), a lipid mediator serving as a precursor for prostaglandin synthesis. In neurons, AA can also serve as a retrograde messenger, an event implicated in memory functions [62–64]. Studies in vitro indicated that AA could also exert toxic and trophic effects on neurons depending on the concentration presented [65]. Activation of cPLA$_2$ also induces the transient release of lysophospholipids, which because of their detergent-like properties, can perturb membrane microenvironment and alter protein function [66].

Role of cPLA$_2$ in Aβ-Mediated Neuronal Apoptosis

There is increasing evidence suggesting a role for cPLA$_2$ in Aβ-induced neuronal apoptosis. In the study by Pillot's group, Aβ was shown to cause neuronal apoptosis through signaling pathways involving PKC, p38, and MEK/ERK, and subsequently cPLA$_2$ and COX [67]. Our study with primary neurons further showed the ability for oligomeric Aβ to activate NADPH oxidase and MAPK (ERK1/2) prior to cPLA$_2$ activation and release AA (Fig. 1) [30]. In astrocytes, a study by Zhu et al. [68] also showed the ability of oligomeric Aβ to activate NADPH oxidase and decrease mitochondrial membrane potential, a signaling event involving both cPLA$_2$ and iPLA$_2$ [68]. In support of the role of cPLA$_2$ in mediating the toxic events of Aβ, neurons isolated from mice deficient in cPLA$_2$$^{-/-}$ showed resistance to the toxic effects of Aβ [69]. Besides the response to cell injury, cPLA$_2$ was shown to play an important role in mediating neuronal homeostatic processes related to growth cone repulsion and collapse, and neurite outgrowth and differentiation during neurodevelopment [70,71]. In an in vivo study, intracerebroventricular injection of Aβ oligomer to wild-type mice caused the activation of cPLA$_2$, which in turn led to a decline in cognitive function and reduction of synaptic markers [69]. In other studies, activation of cPLA$_2$ in nonneuronal cells can trigger neuroinflammatory responses [72]. In microglial cells, aggregated Aβ was shown to induce superoxide production and stimulate cPLA$_2$ and AA release, and the effects of toxic Aβ were abrogated by cPLA$_2$ inhibitors and antisense oligonucleotides [73].

Evidence for the Role of cPLA$_2$ in AD

In agreement with activation of cPLA$_2$ through the protein kinases, an increase in p-cPLA$_2$ was observed in the hippocampus of transgenic (Tg) mice expressing hAPP as well as in human AD brain [74]. This study further showed that decreasing cPLA$_2$ expression by crossing AD Tg mice with cPLA$_2$-deficient mice resulted in better neurochemical and behavioral outcome as compared with the Tg mice with full copies of cPLA$_2$. Although several cPLA$_2$ isoforms are present in the brain, most studies have demonstrated involvement of cPLA$_2$-alpha. Few studies have investigated the involvement of other cPLA$_2$ isoforms. Besides neurons, whether cPLA$_2$ subgroups contribute to AD pathology in other cell types remain to be further investigated [75].

Despite evidence for increased phosphorylation of cPLA$_2$ in Tg animal models and in AD brain, studies by Gattaz's group demonstrated a reduction in PLA$_2$ activity in human AD brain, and the reduction in activity correlated with memory impairment and neuropathology [71]. Since studies were carried out with brain tissue, it is difficult to differentiate different types of PLA$_2$, and thus the mechanism underlying the decrease in PLA$_2$ activity in AD brain remains elusive. Based on evidence that multiple receptor pathways and ATP-dependent protein kinases are needed for activation of cPLA$_2$, it is possible that the decrease in PLA$_2$ activity is

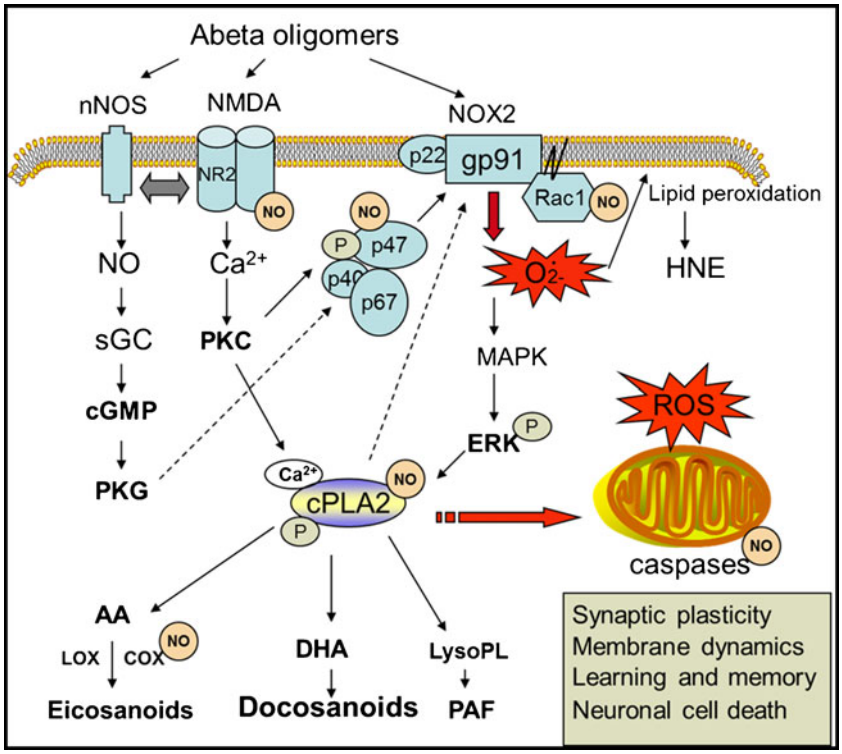

Fig. 1 Aβ oligomers stimulate oxidative/nitrosative pathways involving NMDA receptor, nNOS, and NADPH oxidase leading to production of ROS and activation of $cPLA_2$. Cytosolic PLA_2 has multiple actions; besides the release of AA and synthesis of eicosanoids, it can also produce DHA and lysophospholipid. These products have been shown to disturb mitochondrial function and modulate ROS production by NADPH oxidase. *Dotted arrows* are pathways not yet clarified. Note that besides the NO/cGMP/PKG pathway, many proteins in these pathways can undergo posttranslational modifications through S-nitrosylation

linked to the decline in mitochondrial function and ATP production, which are characteristic features for AD brains [76,77]. Since AD pathology is associated with chronic impairment of neuronal function, it is also possible that a diminished response to neuronal excitation may result in a decrease in the ERK pathway for phosphorylation of $cPLA_2$ (He et al. 2011). Recent lipidomic studies have identified changes in lipids and lipid mediators in the AD brain membranes, including elevation of AA and production of prostaglandins [78]. However, although a change in lipid mediators and lipid membrane environment may explain the alterations of neuron and glial cell function, more studies are needed to further elucidate the mechanism leading to these changes [79]. Taken together, these studies provide strong evidence for the critical role of $cPLA_2$ in mediating physiological and pathological functions in the brain.

Regulation of $cPLA_2$ Through the nNOS Pathways in Neurons

While there is evidence for an upregulation of iNOS in AD brain, the role of eNOS and nNOS in the pathology of this disease remains largely unknown. Study by Masliah's group reported a decrease in nNOS expression in neurons in the entorhinal cortex of AD patients, a finding correlating nNOS in neurons to neurodegeneration [80]. As shown in Fig. 1, a number of proteins in the NMDA receptor pathway, including p47phox, nNOS, COX-2, and caspases, are susceptible to posttranslational modifications through S-nitrosylation. There is further evidence that NMDA-mediated ROS production by NADPH oxidase in neurons is mediated by NO from nNOS [81]. Since NMDA receptor and NADPH oxidase is known to induce ERK1/2, $cPLA_2$, and AA release [30], it is possible that the regulation of ROS by the NO/nNOS pathway may also alter $cPLA_2$/AA and COX-2 in neurons. Interestingly, high levels of COX-2 are found in the postsynaptic neurons, and NMDA receptor-induced nNOS can cause S-nitrosylation and activation of COX-2, as demonstrated by the production of high levels of PGE_2 [82]. In fact, inhibition of COX-2 in neurons can ameliorate the toxic effects of Aβ [67].

Proinflammatory cytokines can stimulate the production of NO from iNOS in microglial cells [83]. There is increasing evidence for the pleiotropic effects of NO; neuronal degeneration can be resulted from excess NO produced by microglial cells [84,85]. Exogenous NO can cause damage to neurons and trigger intracellular signaling pathways. In a study with PC-12 cells, exposure of cells to sodium nitroprusside, an NO donor, was shown to activate $cPLA_2$ and AA release [86]. Aβ (25–35) can also induce NO release in rat temporal cortex and this event was attributed to memory impairment [87]. Taken together, these studies suggest a synergism among nNOS, $cPLA_2$, and COX-2 in Aβ toxicity in neurons.

Studies with $cPLA_2$ Inhibitors

A number of studies (mainly with immune cells in the peripheral system) have demonstrated a role for PLA_2 or

its metabolic products to regulate ROS production induced by NADPH oxidase [88–93]. However, although our studies with neurons have demonstrated the signaling pathway linking NMDA-induced NADPH oxidase/ROS to $cPLA_2$ through ERK1/2 [30], a retrograde interaction between $cPLA_2$ and/or its metabolic products with NADPH oxidase has not been examined in detail. Nevertheless, studies with PLA_2 inhibitors have demonstrated pleiotropic action of this enzyme in regulating cell functions. In prion disease, the toxic prion-derived peptide (PrP82-146) was attached to the glycosylphosphatidylinositol anchor and localized to the cholesterol-rich lipid raft region of neurons [94]. Treatment with PLA_2 inhibitors such as arachidonyl trifluoromethyl ketone (AACOCF3) or methyl-arachidonoyl-fluoro-phosphonate (MAFP) prevented the entry of the prion peptide to the lipid rafts of cortical neurons. These events were attributed to the production of platelet-activating factor (PAF), a lipid mediator formed as a result of $cPLA_2$ activation and acetylation of the lysophospholipid [95]. There is also an indication that the inhibition of PLA_2 through AACOCF3 can render protection against Aβ-mediated degeneration of prion protein in the synapse [96]. In this regard, PAF receptor antagonists such as ginkgolide B, hexa-PAF, and CV6029 are beneficial and can protect against synaptic degeneration induced by the toxic prion and Aβ peptides [96]. Besides synthesis of PAF, $cPLA_2$-induced production of PGE_2 can also play a role in mediating synaptic degeneration because treatment with the PGE_2 receptor antagonist AH13205 can also protect against synapse degeneration induced by Aβ and prion protein.

Other studies demonstrated defects in neurite outgrowth in cortical neurons upon sustained inhibition of $cPLA_2$ with MAFP [97,98]. Studies with $cPLA_2$ inhibitors further demonstrated the involvement of $cPLA_2$ in mediating secondary effects in spinal cord injury [99] and, in clinical symptoms, in experimental autoimmune encephalomyelitis (EAE), an in vivo model for multiple sclerosis [100,101]. In this latter study, however, the effect of $cPLA_2$ inhibitor was attributed to its ability to block peroxynitrite formation in the spinal cord white matter [102]. In human monocytes, downregulation of $cPLA_2$ (by siRNA) was shown to block the stimulation-induced translocation of p47phox and p67phox, further suggesting a relationship between $cPLA_2$ and NAPDH oxidase [103]. In microglial cells, LPS can induce the upregulation of $cPLA_2$, iNOS, and NADPH oxidase, and pretreatment of AACOCF3 significantly attenuated iNOS induction, NO production, ROS production, and ability for microglial cells to confer cytotoxic effects on oligodendroglial cells [102].

There is evidence for the role of PLA_2 in the modification of cholinergic and glutamatergic pathways during the early stages of AD [104]. Studies in vivo [105] demonstrated the ability of MAFP (by i.c.v. infusion for 3 days) to decrease the Tau protein levels in the frontal cortex and hippocampus. Measurement of physical properties (anisotropy) of brain membranes showed a reduction of flexibility of fatty acyl chains and increased fluidity of the lipid–water interface after MAFP treatment. Therefore, besides the release of AA and synthesis of eicosanoids, PLA_2 itself can also play a role in modulating membrane physical properties, leading to altered neuronal excitation, glucose metabolism, memory, and cognitive function, factors important in the pathophysiology of neurological diseases including AD [106].

Botanical Phenolics Inhibit Glutamate Excitotoxicity and Oxidative/Nitrosative Stress

The ability to link the NMDA- and Aβ-induced ROS production through NADPH oxidase to signaling pathways leading to activation of ERK and $cPLA_2$ provided a strong indication for the involvement of oxidative stress due to multiple actions of $cPLA_2$ [30]. With prolonged exposure, oligomeric Aβ may gradually perturb neurons leading to mitochondrial dysfunction [55]. Besides neurons, Aβ can also stimulate other receptor pathways leading to $cPLA_2$ activation [107]. Results of these and other studies provide a link between NADPH oxidase/ROS and $cPLA_2$ in neuronal damage as elicited by excitotoxic NMDA and cytotoxic Aβ. Although how this pathway is linked to neuronal impairment remains to be further investigated, it is reasonable that efforts to inhibit the oxidative events can be beneficial in retarding the progression of neurodegenerative diseases. Our laboratory has successfully demonstrated protective effects of some botanical polyphenols against neuronal damage due to global cerebral ischemia [108–110]. Neuroprotective effects were obtained after treating animals with resveratrol, curcumin, and apocynin [111–114]. In the AD field, there is special interest towards understanding the protective effects of curcumin which has been shown to possess pleiotropic properties including inhibition of Aβ fibrillation, accumulation of amyloid plagues, and ameliorating behavioral deficits in animal models [115–118]. Besides in vivo studies, other botanicals have been shown to inhibit the induction of iNOS and other inflammatory responses induced by proinflammatory cytokines, LPS, and Aβ in microglial cells [83,119]. In a study with spinal cord neurons, treatment with Ginkgo biloba (EGb761) could inhibit glutamate excitotoxicity and subsequent increase in the phosphorylation of $cPLA_2$ [120].

In recent years, there is substantial interest to investigate protective effects of green tea polyphenols, compounds exhibiting strong antioxidant and iron-chelating properties [121–123]. Many studies have demonstrated the ability for epigallocatechin gallate (EGCG), a major ingredient of the green tea polyphenols, to delay AD progression [124] through a variety of mechanisms including inhibition of Aβ fibrillation [125] and inhibition of Aβ production in

neurons isolated from TgAPPsw mice [126–128], and decrease levels of Aβ oligomers in the hippocampus of senescence-accelerated mouse prone-8 (SAMP8) [129]. EGCG was also shown to inhibit cerebral amyloidosis in TgAPPsw mice through activating α-secretase, the enzyme mediating the non-amyloidogenic pathway [125,127]. Studies from our laboratory as well as others have demonstrated effects of EGCG to mitigate Aβ-induced ROS production, lipid peroxidation, and mitochondrial dysfunction in neurons [55,124,130–132]. Studies have demonstrated effects of EGCG to inhibit Aβ-induced NO production in BV-2 cells and decreased Aβ-induced nitrosative damage in rat hippocampus [133], and ROS production by NADPH oxidase [134,135], and LPS-induced expression of inflammatory proteins, iNOS and COX-2 in vivo [136]. Taken together, studies with botanical phenolic compounds have demonstrated the ability to diminish oxidative/nitrosative stress and glial inflammatory responses in the brain [109,130]. Despite that more studies are needed to understand mechanisms for these effects, there is hope that some compounds may prove to be excellent therapeutic agents to combat neurodegenerative diseases.

Conclusion and Perspective

The pathophysiology of AD is marked by complex molecular mechanism(s) underlying aberrant metabolic pathways in neurons and glial cells. The complexity underscores the difficulty to develop a single drug to effectively cure this disease. There is a general consensus for increased oxidative/nitrosative stress in many neurodegenerative diseases, including AD, and the aberrant oxidative/nitrosative pathways are common basis for glial cell inflammatory responses, neuronal damage, and synaptic impairments. In recent years, NADPH oxidase has been recognized as an important player in ROS production in brain cells, including neurons, astrocytes, microglia, and cerebrovascular endothelial cells. Although emphasis here is on neurons, more studies are needed to unravel the role of this enzyme in the oxidative and inflammatory pathways in other cell types.

Studies with neurons support an aberrant excitatory neurotransmitter signaling pathway leading to excessive activation of NADPH oxidase and ROS production. Activation of this pool of ROS further triggers downstream pathways leading to activation of protein kinases and phosphorylation of $cPLA_2$. Interestingly, toxic Aβ oligomers can also enhance ROS production in neurons through NADPH oxidase. Although the exact mechanism remains to be further investigated, these studies support the deleterious effects of protein misfolding and aggregation in mediating toxic effects to neurons and causing synaptic impairments. ROS produced by NADPH oxidase is linked to activation of ERK1/2, which in turn, leads to phosphorylation and activation of $cPLA_2$. Cytosolic PLA_2 has pleiotropic properties, and besides the release of AA for synthesis of eicosanoids, lysophospholipids may play a role in modulating membrane physical properties, and serving as substrate for synthesis of platelet-activating factor. Thus, integrating PLA_2 into the oxidative signaling pathways underlying glutamate excitotoxicity and Aβ toxicity will have a great impact on understanding the pathogenesis of AD and will allow the development of novel therapeutic targets to mitigate the damaging effects of Aβ.

Activation of NMDA receptor is also linked to NO production through nNOS, and in turn, NO triggers downstream pathway for production of cGMP, a second messenger. NO has pleiotropic effects, and in addition to reacting with ROS to form peroxynitrite (ONOO-), it can also be the source of S-nitrosylation, a mechanism for posttranslational protein modification. There is increasing recognition about proteins associated with the NMDA receptor, and signaling pathways are S-nitrosylated (including $cPLA_2$). Although the mechanism is not yet understood, there is increasing evidence that the NMDA/nNOS pathway may impact the NMDA/NADPH oxidase pathway and that $cPLA_2$ plays a role in mediating both pathways. These studies further demonstrate the importance for developing specific inhibitors for $cPLA_2$ to suppress the detrimental effects resulting from excitotoxic and oxidative/nitrosative pathways common to AD. Since AD is developed over a long period of time, and the complexity of the disease progression prevail the use of single drugs, there is a strong quest for a preventative action through dietary supplementations, e.g., using botanical antioxidants. Therefore, future studies should target on understanding factors causing Aβ to aggregate and become toxic in AD brain, better understanding of the toxic effects of these protein aggregates on neurons and glial cells, and developing novel strategies to suppress deleterious effects due to excessive oxidative/nitrosative stress in the brain. Since many botanical polyphenols have been shown to exhibit antioxidant and anti-inflammatory properties, future studies should focus on discovering novel botanicals to suppress oxidative/nitrosative stress in the degenerating brain.

Acknowledgments This study was supported by grants NIH P02 AG018357 NIA and 1P50 AT006273 from NCCAM.

References

1. Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. Science 298:789–791
2. Sun GY, Xu J, Jensen MD, Simonyi A (2004) Phospholipase A2 in the central nervous system: implications for neurodegenerative diseases. J Lipid Res 45(2):205–213
3. Sun GY, Horrocks LA, Farooqui AA (2007) The roles of NADPH oxidase and phospholipases A2 in oxidative and inflammatory

responses in neurodegenerative diseases. J Neurochem 103(1):1–16
4. Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R (1991) Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 30(4):572–580
5. Snyder EM, Nong Y, Almeida CG et al (2005) Regulation of NMDA receptor trafficking by amyloid-beta. Nat Neurosci 8 (8):1051–1058
6. Palop JJ, Mucke L (2010) Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. Nat Neurosci 13812–818
7. Shankar GM, Bloodgood BL, Townsend M, Walsh DM, Selkoe DJ, Sabatini BL (2007) Natural oligomers of the Alzheimer amyloid-beta protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. J Neurosci 27(11):2866–2875
8. Shankar GM, Li S, Mehta TH et al (2008) Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med 14:837–842
9. Alberdi E, Sanchez-Gomez MV, Cavaliere F, Perez-Samartin A, Zugaza JL, Trullas R, Domercq M, Matute C (2010) Amyloid beta oligomers induce Ca2+ dysregulation and neuronal death through activation of ionotropic glutamate receptors. Cell Calcium 47 (3):264–272
10. Demuro A, Parker I, Stutzmann GE (2010) Calcium signaling and amyloid toxicity in Alzheimer disease. J Biol Chem 285:12463–12468
11. Paul S, Connor JA (2010) NR2B-NMDA receptor-mediated increases in intracellular Ca2+ concentration regulate the tyrosine phosphatase, STEP, and ERK MAP kinase signaling. J Neurochem 114:1107–1118
12. Ondrejcak T, Klyubin I, Hu NW, Barry AE, Cullen WK, Rowan MJ (2010) Alzheimer's disease amyloid beta-protein and synaptic function. Neuromolecular Medicine 12:13–26
13. Pham E, Crews L, Ubhi K et al (2010) Progressive accumulation of amyloid-beta oligomers in Alzheimer's disease and in amyloid precursor protein transgenic mice is accompanied by selective alterations in synaptic scaffold proteins. FEBS J 277:3051–3067
14. Stine WB Jr, Dahlgren KN, Krafft GA, LaDu MJ (2003) In vitro characterization of conditions for amyloid-beta peptide oligomerization and fibrillogenesis. J Biol Chem 278(13):11612–11622
15. Stine WB, Jungbauer L, Yu C, LaDu MJ (2010) Preparing synthetic Aβ in different aggregation states. Methods Mol Biol 670:13–32
16. Wilcox KC, Lacor PN, Pitt J, Klein WL (2011) Aβ oligomer-induced synapse degeneration in Alzheimer's disease. Cell Mol Neurobiol 31(6):939–948
17. Hardingham GE, Fukunaga Y, Bading H (2002) Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. Nat Neurosci 5:405–414
18. Okamoto SI, Pouladi MA, Talantova M et al (2009) Balance between synaptic versus extrasynaptic NMDA receptor activity influences inclusions and neurotoxicity of mutant huntingtin. Nat Med 15:1407–1413
19. Papadia S, Soriano FX, Leveille F et al (2008) Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. Nat Neurosci 11:476–487
20. Papadia S, Stevenson P, Hardingham NR, Bading H, Hardingham GE (2005) Nuclear Ca2+ and the cAMP response element-binding protein family mediate a late phase of activity-dependent neuroprotection. J Neurosci 25:4279–4287
21. Lipton SA, Rosenberg PA (1994) Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med 330:613–622
22. Abu-Soud HM, Stuehr DJ (1993) Nitric oxide synthases reveal a role for calmodulin in controlling electron transfer. Proc Natl Acad Sci U S A 90:10769–10772
23. Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH (1991) Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. Nature 351:714–718
24. Kolodziejska KE, Burns AR, Moore RH, Stenoien DL, Eissa NT (2005) Regulation of inducible nitric oxide synthase by aggresome formation. Proc Natl Acad Sci U S A 102:4854–4859
25. Butterfield A, Perluigi M, Reed T, Muharib T, Hughes CP, Robinson RA, Sultana R (2011) Redox proteomics in selected neurodegenerative disorders: from its infancy to future applications. Antioxid Redox Signal. doi:10.1089/ars.2011.4109
26. Keller JN, Schmitt FA, Scheff SW, Ding Q, Chen Q, Butterfield DA, Markesbery WR (2005) Evidence of increased oxidative damage in subjects with mild cognitive impairment. Neurology 64:1152–1156
27. Mattson MP (2009) Roles of the lipid peroxidation product 4-hydroxynonenal in obesity, the metabolic syndrome, and associated vascular and neurodegenerative disorders. Exp Gerontol 44:625–633
28. Sultana R, Butterfield DA (2010) Role of oxidative stress in the progression of Alzheimer's disease. J Alzheimers Dis 19:341–353
29. Brennan AM, Suh SW, Won SJ, Narasimhan P, Kauppinen TM, Lee H, Edling Y, Chan PH, Swanson RA (2009) NADPH oxidase is the primary source of superoxide induced by NMDA receptor activation. Nat Neurosci 12:857–863
30. 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 A2 in cortical neurons. J Neurochem 106(1):45–55
31. Sorce S, Krause KH (2009) NOX enzymes in the central nervous system: from signaling to disease. Antioxid Redox Signal 11 (10):2481–2504
32. Bedard K, Krause KH (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 87:245–313
33. Jaquet V, Scapozza L, Clark RA, Krause KH, Lambeth JD (2009) Small-molecule NOX inhibitors: ROS-generating NADPH oxidases as therapeutic targets. Antioxid Redox Signal 11:2535–2552
34. Brown DI, Griendling KK (2009) Nox proteins in signal transduction. Free Radic Biol Med 47:1239–1253
35. Kahles T, Kohnen A, Heumueller S et al (2010) NADPH oxidase Nox1 contributes to ischemic injury in experimental stroke in mice. Neurobiol Dis 40:185–192
36. Cristovao AC, Choi DH, Baltazar G, Beal MF, Kim YS (2009) The role of NADPH oxidase 1-derived reactive oxygen species in paraquat-mediated dopaminergic cell death. Antioxid Redox Signal 11:2105–2118
37. Ha JS, Lee JE, Lee JR, Lee CS, Maeng JS, Bae YS, Kwon KS, Park SS (2010) Nox4-dependent H2O2 production contributes to chronic glutamate toxicity in primary cortical neurons. Exp Cell Res 316:1651–1661
38. Ansari MA, Scheff SW (2011) NADPH-oxidase activation and cognition in Alzheimer disease progression. Free Radic Biol Med 51:171–178
39. Bruce-Keller AJ, Gupta S, Parrino TE, Knight AG, Ebenezer PJ, Weidner AM, LeVine H 3rd, Keller JN, Markesbery WR (2010) NOX activity is increased in mild cognitive impairment. Antioxid Redox Signal 12:1371–1382
40. Shimohama S, Tanino H, Kawakami N et al (2000) Activation of NADPH oxidase in Alzheimer's disease brains. Biochem Biophys Res Commun 273(1):5–9
41. Yoshioka H, Niizuma K, Katsu M, Okami N, SakataH KGS, Narasimhan P, Chan PH (2011) NADPH oxidase mediates striatal neuronal injury after transient global cerebral ischemia. J Cereb Blood Flow Metab 31(3):868–880
42. Zekry D, Epperson TK, Krause KH (2003) A role for NOX NADPH oxidases in Alzheimer's disease and other types of dementia? IUBMB life 55(6):307–313

43. Bredt DS, Snyder SH (1994) Nitric oxide: a physiologic messenger molecule. Annu Rev Biochem 63:175–195
44. Dawson VL, Dawson TM, London ED, Bredt DS, Snyder SH (1991) Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. Proc Natl Acad Sci U S A 88:6368–6371
45. O'Dell TJ, Hawkins RD, Kandel ER, Arancio O (1991) Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. Proc Natl Acad Sci U S A 88:11285–11289
46. Schuman EM, Madison DV (1994) Locally distributed synaptic potentiation in the hippocampus. Science 263:532–536
47. Lei SZ, Pan ZH, Aggarwal SK, Chen HS, Hartman J, Sucher NJ, Lipton SA (1992) Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex. Neuron 8:1087–1099
48. Stamler JS, Lamas S, Fang FC (2001) Nitrosylation. the prototypic redox-based signaling mechanism. Cell 106(6):675–683
49. Gu Z, Kaul M, Yan B, Kridel SJ, Cui J, Strongin A, Smith JW, Liddington RC, Lipton SA (2002) S-nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. Science 297:1186–1190
50. Uehara T, Nakamura T, Yao D, Shi ZQ, Gu Z, Ma Y, Masliah E, Nomura Y, Lipton SA (2006) S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. Nature 441(7092):513–517
51. Yao D, Gu Z, Nakamura T et al (2004) Nitrosative stress linked to sporadic Parkinson's disease: S-nitrosylation of parkin regulates its E3 ubiquitin ligase activity. Proc Natl Acad Sci U S A 101 (29):10810–10814
52. Huang Z, Huang PL, Panahian N, Dalkara T, Fishman MC, Moskowitz MA (1994) Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. Science 265:1883–1885
53. Iadecola C, Zhang F, Casey R, Nagayama M, Ross ME (1997) Delayed reduction of ischemic brain injury and neurological deficits in mice lacking the inducible nitric oxide synthase gene. J Neurosci 17:9157–9164
54. Pagani L, Eckert A (2011) Amyloid-beta interaction with mitochondria. Int J Alzheimer's Dis 2011:925050
55. He Y, Cui J, Lee JC et al (2011) Prolonged exposure of cortical neurons to oligomeric amyloid-β impairs NMDA receptor function via NADPH oxidase-mediated ROS production: protective effect of green tea (-)-epigallocatechin-3-gallate. ASN Neuro 3: e00050
56. He Y, Cui J, Lee JC et al (2011) Prolonged exposure of cortical neurons to oligomeric amyloid-beta impairs NMDA receptor function via NADPH oxidase-mediated ROS production: protective effect of green tea (-)-epigallocatechin-3-gallate. ASN Neuro 3(1):e00050
57. Ma T, Hoeffer CA, Wong H, Massaad CA, Zhou P, Iadecola C, Murphy MP, Pautler RG, Klann E (2011) Amyloid β-induced impairments in hippocampal synaptic plasticity are rescued by decreasing mitochondrial superoxide. J Neurosci 31:5589–5595
58. Simonyi A, He Y, Sheng W, Sun AY, Wood WG, Weisman GA, Sun GY (2010) Targeting NADPH oxidase and phospholipases A2 in Alzheimer's disease. Mol Neurobiol 41(2–3):73–86
59. Murakami M, Taketomi Y, Miki Y, Sato H, Hirabayashi T, Yamamoto K (2011) Recent progress in phospholipase A research: from cells to animals to humans. Progress in Lipid Research 50:152–192
60. Xu L, Han C, Lim K, Wu T (2008) Activation of cytosolic phospholipase A2alpha through nitric oxide-induced S-nitrosylation. Involvement of inducible nitric-oxide synthase and cyclooxygenase-2. J Biol Chem 283(6):3077–3087
61. Xu J, Weng YI, Simonyi A, Krugh BW, Liao Z, Weisman GA, Sun GY (2002) Role of PKC and MAPK in cytosolic PLA_2 phosphorylation and arachadonic acid release in primary murine astrocytes. J Neurochem 83(2):259–270
62. Bolshakov VY, Siegelbaum SA (1995) Hippocampal long-term depression: arachidonic acid as a potential retrograde messenger. Neuropharmacology 34:1581–1587
63. Leu BH, Schmidt JT (2008) Arachidonic acid as a retrograde signal controlling growth and dynamics of retinotectal arbors. Dev Neurobiol 68:18–30
64. Rapoport SI (2008) Arachidonic acid and the brain. J Nutr 138:2515–2520
65. Okuda S, Saito H, Katsuki H (1994) Arachidonic acid: toxic and trophic effects on cultured hippocampal neurons. Neuroscience 63:691–699
66. Lee JC, Simonyi A, Sun AY, Sun GY (2010) Phospholipases A2 and neural membrane dynamics: implications for Alzheimer's disease. J Neurochem 116(5):813–819
67. Kriem B, Sponne I, Fifre A et al (2005) Cytosolic phospholipase A2 mediates neuronal apoptosis induced by soluble oligomers of the amyloid-beta peptide. FASEB J 19(1):85–87
68. Zhu D, Lai Y, Shelat PB, Hu C, Sun GY, Lee JC (2006) Phospholipases A2 mediate amyloid-beta peptide-induced mitochondrial dysfunction. J Neurosci 26(43):11111–11119
69. Desbene C, Malaplate-Armand C, Youssef I et al (2011) Critical role of cPLA(2) in Aβ oligomer-induced neurodegeneration and memory deficit. Neurobiol Aging. doi:10.1016/j.neurobiolaging.2011.11.008
70. Sanford SD, Yun BG, Leslie CC, Murphy RC, Pfenninger KH (2012) Group IVA phospholipase A(2) is necessary for growth cone repulsion and collapse. J Neurochem 120(6):974–984
71. Schaeffer EL, Forlenza OV, Gattaz WF (2009) Phospholipase A2 activation as a therapeutic approach for cognitive enhancement in early-stage Alzheimer disease. Psychopharmacology 202(1–3):37–51
72. Sundaram JR, Chan ES, Poore CP et al (2012) Cdk5/p25-induced cytosolic PLA_2-mediated lysophosphatidylcholine production regulates neuroinflammation and triggers neurodegeneration. J Neurosci 32(3):1020–1034
73. Szaingurten-Solodkin I, Hadad N, Levy R (2009) Regulatory role of cytosolic phospholipase A2alpha in NADPH oxidase activity and in inducible nitric oxide synthase induction by aggregated Abeta1-42 in microglia. Glia 57(16):1727–1740
74. Sanchez-Mejia RO, Newman JW, Toh S et al (2008) Phospholipase A2 reduction ameliorates cognitive deficits in a mouse model of Alzheimer's disease. Nat Neurosci 11:1311–1318
75. Schaeffer EL, da Silva ER, Novaes Bde A, Skaf HD, Gattaz WF (2010) Differential roles of phospholipases A2 in neuronal death and neurogenesis: implications for Alzheimer disease. Prog Neuropsychopharmacol Biol Psychiatry 34(8):1381–1389
76. Hirai K, Aliev G, Nunomura A et al (2001) Mitochondrial abnormalities in Alzheimer's disease. J Neurosci 21:3017–3023
77. Moreira PI, Carvalho C, Zhu X, Smith MA, Perry G (2010) Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology. Biochim Biophys Acta 1802:2–10
78. Igarashi M, Ma K, Gao F, Kim HW, Rapoport SI, Rao JS (2010) Disturbed choline plasmalogen and phospholipid fatty acid concentrations in Alzheimer's disease prefrontal cortex. J Alzheimers Dis 24(3):507–517
79. Sanchez-Mejia RO, Mucke L (2010) Phospholipase A2 and arachidonic acid in Alzheimer's disease. Biochim Biophys Acta 1801:784–790
80. Thorns V, Hansen L, Masliah E (1998) nNOS expressing neurons in the entorhinal cortex and hippocampus are affected in patients with Alzheimer's disease. Exp Neurol 150(1):14–20
81. Girouard H, Wang G, Gallo EF, Anrather J, Zhou P, Pickel VM, Iadecola C (2009) NMDA receptor activation increases free radical production through nitric oxide and NOX2. J Neurosci 29 (8):2545–2552

82. Tian J, Kim SF, Hester L, Snyder SH (2008) S-nitrosylation/activation of COX-2 mediates NMDA neurotoxicity. Proc Natl Acad Sci U S A 105(30):10537–10540
83. Sheng W, Zong Y, Mohammad A et al (2011) Pro-inflammatory cytokines and lipopolysaccharide induce changes in cell morphology, and upregulation of ERK1/2, iNOS and sPLA$_2$-IIA expression in astrocytes and microglia. J Neuroinflammation 8:121
84. Brown GC (2010) Nitric oxide and neuronal death. Nitric Oxide 23:153–165
85. Brown GC, Neher JJ (2010) Inflammatory neurodegeneration and mechanisms of microglial killing of neurons. Mol Neurobiol 41:242–247
86. Chalimoniuk M, Stolecka A, Cakala M et al (2007) Amyloid beta enhances cytosolic phospholipase A$_2$ level and arachidonic acid release via nitric oxide in APP-transfected PC12 cells. Acta Biochimica Polonica 54:611–623
87. Limon ID, Diaz A, Mendieta L, Chamorro G, Espinosa B, Zenteno E, Guevara J (2009) Amyloid-beta(25-35) impairs memory and increases NO in the temporal cortex of rats. Neurosci Res 63:129–137
88. Chatterjee S, Feinstein SI, Dodia C, Sorokina E, Lien YC, Nguyen S, Debolt K, Speicher D, Fisher AB (2011) Peroxiredoxin 6 phosphorylation and subsequent phospholipase A$_2$ activity are required for agonist-mediated activation of NADPH oxidase in mouse pulmonary microvascular endothelium and alveolar macrophages. J Biol Chem 286:11696–11706
89. Chenevier-Gobeaux C, Simonneau C, Therond P, Bonnefont-Rousselot D, Poiraudeau S, Ekindjian OG, Borderie D (2007) Implication of cytosolic phospholipase A$_2$ (cPLA$_2$) in the regulation of human synoviocyte NADPH oxidase (Nox2) activity. Life Sci 81:1050–1058
90. Dana R, Malech HL, Levy R (1994) The requirement for phospholipase A$_2$ for activation of the assembled NADPH oxidase in human neutrophils. Biochem J 297(Pt 1):217–223
91. Henderson LM, Chappell JB, Jones OT (1989) Superoxide generation is inhibited by phospholipase A2 inhibitors. Role for phospholipase A2 in the activation of the NADPH oxidase. Biochem J 264:249–255
92. Levy R, Lowenthal A, Dana R (2000) Cytosolic phospholipase A2 is required for the activation of the NADPH oxidase associated H+channel in phagocyte-like cells. Adv Exp Med Biol 479:125–135
93. Shmelzer Z, Haddad N, Admon E, Pessach I, Leto TL, Eitan-Hazan Z, Hershfinkel M, Levy R (2003) Unique targeting of cytosolic phospholipase A2 to plasma membranes mediated by the NADPH oxidase in phagocytes. J Cell Biol 162(4):683–692
94. Bate C, Ingham V, Williams A (2011) Inhibition of phospholipase A$_2$ increased the removal of the prion derived peptide PrP82-146 from cultured neurons. Neuropharmacology 60:365–372
95. Bate C, Tayebi M, Williams A (2010) A glycosylphosphatidylinositol analogue reduced prion-derived peptide mediated activation of cytoplasmic phospholipase A$_2$, synapse degeneration and neuronal death. Neuropharmacology 59:93–99
96. Bate C, Tayebi M, Williams A (2010) Phospholipase A$_2$ inhibitors protect against prion and Aβ mediated synapse degeneration. Mol Neurodegener 5:13
97. Forlenza OV, Mendes CT, Marie SK, Gattaz WF (2007) Inhibition of phospholipase A2 reduces neurite outgrowth and neuronal viability. Prostaglandins, Leukotrienes, and Essential Fatty Acids 76(1):47–55
98. Mendes CT, Gattaz WF, Schaeffer EL, Forlenza OV (2005) Modulation of phospholipase A2 activity in primary cultures of rat cortical neurons. J Neural Transm 112:1297–1308
99. 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
100. Marusic S, Thakker P, Pelker JW et al (2008) Blockade of cytosolic phospholipase A2 alpha prevents experimental autoimmune encephalomyelitis and diminishes development of Th1 and Th17 responses. J Neuroimmunol 204(1–2):29–37
101. Vana AC, Li S, Ribeiro R, TchantchouF ZY (2011) Arachidonyl trifluoromethyl ketone ameliorates experimental autoimmune encephalomyelitis via blocking peroxynitrite formation in mouse spinal cord white matter. Exp Neurol 231(1):45–55
102. Li S, Vana AC, Ribeiro R, Zhang Y (2011) Distinct role of nitric oxide and peroxynitrite in mediating oligodendrocyte toxicity in culture and in experimental autoimmune encephalomyelitis. Neuroscience 184:107–119
103. Zhao X, Bey EA, Wientjes FB, Cathcart MK (2002) Cytosolic phospholipase A$_2$ (cPLA$_2$) regulation of human monocyte NADPH oxidase activity. cPLA$_2$ affects translocation but not phosphorylation of p67(phox) and p47(phox). J Biol Chem 277(28):25385–25392
104. Schaeffer EL, Gattaz WF (2008) Cholinergic and glutamatergic alterations beginning at the early stages of Alzheimer disease: participation of the phospholipase A2 enzyme. Psychopharmacology 198(1):1–27
105. Schaeffer EL, De-Paula VJ, da Silva ER, de Novaes AB, Skaf HD, Forlenza OV, Gattaz WF (2011) Inhibition of phospholipase A(2) in rat brain decreases the levels of total Tau protein. J Neural Transm 118(9):1273–1279
106. Schaeffer EL, Skaf HD, Novaes Bde A, da Silva ER, Martins BA, Joaquim HD, Gattaz WF (2011) Inhibition of phospholipase A$_2$ in rat brain modifies different membrane fluidity parameters in opposite ways. Prog Neuropsychopharmacol Biol Psychiatry 35 (7):1612–1617
107. Askarova S, Yang X, Sheng W, Sun GY, Lee JC (2011) Role of Aβ-receptor for advanced glycation endproducts interaction in oxidative stress and cytosolic phospholipase A$_2$ activation in astrocytes and cerebral endothelial cells. Neuroscience 199:375–385
108. Simonyi A, Wang Q, Miller RL, Yusof M, Shelat PB, Sun AY, Sun GY (2005) Polyphenols in cerebral ischemia: novel targets for neuroprotection. Mol Neurobiol 31(1–3):135–147
109. Sun AY, Wang Q, Simonyi A, Sun GY (2008) Botanical phenolics and brain health. Neuromolecular Med 10(4):259–274
110. Sun AY, Wang Q, Simonyi A, Sun GY (2010) Resveratrol as a therapeutic agent for neurodegenerative diseases. Mol Neurobiol 41(2–3):375–383
111. Simonyi A, Serfozo P, Lehmidi TM, Cui J, Gu Z, Lubahn DB, Sun AY, Sun GY (2012) The neuroprotective effects of apocynin. Front Biosci 4:2183–2193
112. Wang Q, Simonyi A, Li W, Sisk BA, Miller RL, Macdonald RS, Lubahn DE, Sun GY, Sun AY (2005) Dietary grape supplement ameliorates cerebral ischemia-induced neuronal death in gerbils. Mol Nutr Food Res 49(5):443–451
113. Wang Q, Sun AY, Simonyi A et al (2005) Neuroprotective mechanisms of curcumin against cerebral ischemia-induced neuronal apoptosis and behavioral deficits. J Neurosci Res 82(1):138–148
114. Wang Q, Tompkins KD, Simonyi A, Korthuis RJ, Sun AY, Sun GY (2006) Apocynin protects against global cerebral ischemia-reperfusion-induced oxidative stress and injury in the gerbil hippocampus. Brain Res 1090(1):182–189
115. Begum AN, Jones MR, Lim GP et al (2008) Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease. J Pharmacol Exp Ther 326:196–208
116. Cole GM, Teter B, Frautschy SA (2007) Neuroprotective effects of curcumin. Adv Exp Med Biol 595:197–212
117. Frautschy SA, Cole GM (2010) Why pleiotropic interventions are needed for Alzheimer's disease. Mol Neurobiol 41:392–409

118. Ringman JM, Frautschy SA, Cole GM, Masterman DL, Cummings JL (2005) A potential role of the curry spice curcumin in Alzheimer's disease. Current Alzheimer Research 2:131–136
119. Capiralla H, Vingtdeux V, Zhao H, Sankowski R, Al-Abed Y, Davies P, Marambaud P (2012) Resveratrol mitigates lipopolysaccharide- and Abeta-mediated microglial inflammation by inhibiting the TLR4/NF-kappaB/STAT signaling cascade. J Neurochem 120:461–472
120. Zhao Z, Liu N, Huang J, Lu PH, Xu XM (2011) Inhibition of $cPLA_2$ activation by Ginkgo biloba extract protects spinal cord neurons from glutamate excitotoxicity and oxidative stress-induced cell death. J Neurochem 116(6):1057–1065
121. Cabrera C, Artacho R, Gimenez R (2006) Beneficial effects of green tea—a review. J Am Coll Nutr 25:79–99
122. Higdon JV, Frei B (2003) Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Crit Rev Food Sci Nutr 43:89–143
123. Weinreb O, Mandel S, Amit T, Youdim MB (2004) Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. J Nutr Biochem 15(9):506–516
124. Dragicevic N, Smith A, Lin X et al (2011) Green tea epigallocatechin-3-gallate (EGCG) and other flavonoids reduce Alzheimer's amyloid-induced mitochondrial dysfunction. J Alzheimers Dis 26(3):507–521
125. Lee JW, Lee YK, Ban JO, Ha TY, Yun YP, Han SB, Oh KW, Hong JT (2009) Green tea (-)-epigallocatechin-3-gallate inhibits beta-amyloid-induced cognitive dysfunction through modification of secretase activity via inhibition of ERK and NF-kappaB pathways in mice. J Nutr 139:1987–1993
126. Obregon DF, Rezai-Zadeh K, Bai Y et al (2006) ADAM10 activation is required for green tea (-)-epigallocatechin-3-gallate-induced alpha-secretase cleavage of amyloid precursor protein. J Biol Chem 281:16419–16427
127. Rezai-Zadeh K, Shytle D, Sun N et al (2005) Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. J Neurosci 25(38):8807–8814
128. Smith A, Giunta B, Bickford PC, Fountain M, Tan J, Shytle RD (2010) Nanolipidic particles improve the bioavailability and alpha-secretase inducing ability of epigallocatechin-3-gallate (EGCG) for the treatment of Alzheimer's disease. Int J Pharm 389(1–2):207–212
129. Li Q, Zhao HF, Zhang ZF, Liu ZG, Pei XR, Wang JB, Li Y (2009) Long-term green tea catechin administration prevents spatial learning and memory impairment in senescence-accelerated mouse prone-8 mice by decreasing Abeta1-42 oligomers and upregulating synaptic plasticity-related proteins in the hippocampus. Neuroscience 163:741–749
130. Choi DY, Lee YJ, Hong JT, Lee HJ (2011) Antioxidant properties of natural polyphenols and their therapeutic potentials for Alzheimer's disease. Brain Res Bull 87(2–3):144–153
131. Choi YT, Jung CH, Lee SR, Bae JH, Baek WK, Suh MH, Park J, Park CW, Suh SI (2001) The green tea polyphenol (-)-epigallocatechin gallate attenuates beta-amyloid-induced neurotoxicity in cultured hippocampal neurons. Life Sci 70:603–614
132. Shimmyo Y, Kihara T, Akaike A, Niidome T, Sugimoto H (2008) Epigallocatechin-3-gallate and curcumin suppress amyloid beta-induced beta-site APP cleaving enzyme-1 upregulation. Neuroreport 19:1329–1333
133. Kim CY, Lee C, Park GH, Jang JH (2009) Neuroprotective effect of epigallocatechin-3-gallate against beta-amyloid-induced oxidative and nitrosative cell death via augmentation of antioxidant defense capacity. Arch Pharm Res 32:869–881
134. Nishikawa H, Wakano K, Kitani S (2007) Inhibition of NADPH oxidase subunits translocation by tea catechin EGCG in mast cell. Biochem Biophys Res Commun 362:504–509
135. Steffen Y, Gruber C, Schewe T, Sies H (2008) Mono-O-methylated flavanols and other flavonoids as inhibitors of endothelial NADPH oxidase. Arch Biochem Biophys 469(2):209–219
136. Lee YK, Yuk DY, Lee JW, Lee SY, Ha TY, Oh KW, Yun YP, Hong JT (2009) (-)-Epigallocatechin-3-gallate prevents lipopolysaccharide-induced elevation of beta-amyloid generation and memory deficiency. Brain Res 1250:164–174