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OXIDATIVE STRESS IN KAINIC ACID NEUROTOXICITY: IMPLICATIONS FOR THE PATHOGENESIS OF NEUROTRAUMATIC AND NEURODEGENERATIVE DISEASES

Akhlaq A. Farooqui

Department of Molecular and Cellular Biochemistry, The Ohio State University, Columbus, Ohio, USA

4.1 INTRODUCTION

It is well established that neural membranes are composed of glycerophospholipids, sphingolipids, cholesterol, and proteins. The distribution of lipids in two leaflets of lipid bilayer is asymmetric [1, 2]. Glycerophospholipids and sphingolipids contribute to the lipid asymmetry, whereas cholesterol and sphingolipids form lipid microdomains or lipid rafts, which float within the membrane and act as molecular sorting machines and platforms for signal transduction pathways [3, 4]. Thus lipid raft are a unique compartment of the plasma membrane, which not only ensure correct intracellular trafficking of proteins and lipids through protein-protein interactions and concentrate certain proteins in microdomains, while excluding others, but also modulate signal transduction processes associated with neural cell functions. The maintenance of transbilayer lipid asymmetry is a dynamic process, which is necessary for the maintenance of normal neural membrane function. The disruption of asymmetry through receptor activation results in neural cell activation necessary for normal cell function, but overactivation of neural membrane receptors either by agonist or through neural trauma causes neurodegeneration [5, 6].

Kainate (KA) administration in rodents has been used as an animal model to study molecular mechanism of neurodegeneration [7]. KA administration leads to different patterns of neuronal excitation. Thus systemic injection of KA to rats produces selective neuronal vulnerability in the hippocampal hilus, CA1, and CA3 subfields, whereas granule cells in dentate gyrus are resistant to KA-induced neurotoxicity. Systemic administration of KA in adult rats also produces persistent seizures syndrome [8] and triggers acute and delayed neuronal death in the hippocampal CA1 and CA3 regions [7].

KA-mediated neurotoxicity cell death is caused by interactions between KA and KA receptors (KA-R), which are composed of five different subunits (GluR5, GluR6, GluR7, KA1, and KA2) [8, 9]. KA-Rs are classified into low-affinity receptor families (GluK1–GluK3) and high-affinity receptor families (GluK4–GluK5) based on their affinity for the neurotoxin KA. These two families share a 42% sequence identity for the intact receptor but only a 27% sequence identity at the level of extracellular amino terminal domain. These receptors allow the influx of Na^+ and the efflux of K^+. KA-R-mediated influx of Na^+ is accompanied by the passive movement of Cl^- and water molecules into neural cells, resulting not only in the osmotic overloading of water but also in KA-R-mediated membrane depolarization producing increase in intracellular Ca^{2+} [10–12]. Nuclear microscopic high-resolution elemental maps of KA-mediated damaged area show very

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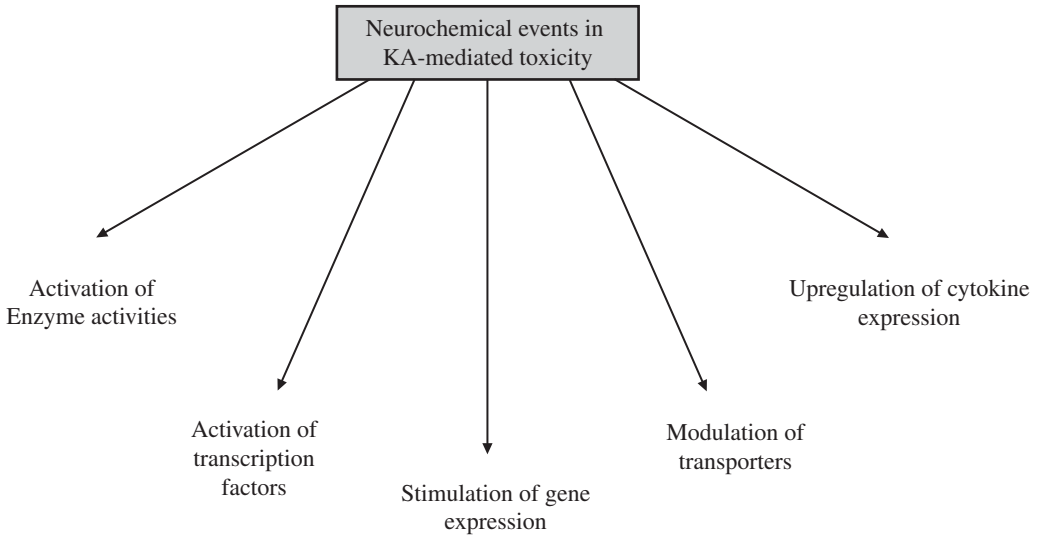

Fig. 4.1 Modulation of neurochemical activities in KA-induced neurotoxicity.

high intracellular calcium concentrations in almost all glial cells, supporting the view that KA-mediated neurotoxicity may be initiated by Ca^{2+} influx through depolarization, maintained by failure of Ca^{2+} extrusion because of ATP depletion, and intensified by increase in intracellular Ca^{2+} leading to mitochondrial dysfunction resulting in further depletion of ATP [7, 8, 13, 14]. These processes also result in the loss of Mg^{2+} blockade of the NMDA receptor, which allows further Ca^{2+} influx through NMDA receptor channel. Thus elevation in intracellular Ca^{2+} is a major component of neural cell death in KA-mediated neurotoxicity [14]. Stimulation of KA receptors results in enrichment of glycerophospholipid, sphingolipid, and cholesterol metabolism through the activation of enzyme activities, modulation of transcription factors, transporters, and gene expression resulting in increase in oxidative stress, inflammation, and neurodegeneration (Fig. 4.1) [7]. At the cellular level KA-induced neurotoxicity results in activation of microglia and astrocytes, which strongly express TNF-α mRNA and protein [15]. Collective evidence suggests that TNF-α derived from KA-activated microglia increases excitotoxic insult to hippocampal neurons, and may be responsible for the induction of neuronal apoptosis in vitro and in vivo.

4.2 GLYCEROPHOSPHOLIPID METABOLISM ALTERATIONS IN KA-INDUCED NEUROTOXICITY

From neural membrane, arachidonic acid (ARA) is released from glycerophospholipids either through the stimulation of phospholipase A_2 (PLA_2) or through the involvement of the phospholipase C (PLC)/diacylglycerol (DAG) lipase pathway [16–18]. Stimulation of PLA_2 and PLC/DAG lipase pathway results in the generation of high levels of eicosanoids (prostaglandins, leukotrienes, and thromboxanes) and reactive oxygen species (ROS). ROS include oxygen radicals, superoxide anions, hydroxyl, alkoxyl, and peroxyl radicals, and hydrogen peroxide. Systemic administration of KA into adult rats increases $cPLA_2$ activity in rat brain homogenate and immunoreactivity in neurons at 1 and 3 days after injection [19] (Fig. 4.2). KA injections increase the $cPLA_2$ immunoreactivity in astrocytes after 1, 2, 4, and 11 weeks. Increased $cPLA_2$ activity in neurons in KA-mediated toxicity may be involved in neurodegeneration, whereas the elevation of $cPLA_2$ immunoreactivity in astrocytes is associated with gliosis [19]. Injections of KA also induce a marked increase in $cPLA_2$ mRNA and protein levels [17, 19, 20], and $cPLA_2$ inhibitors [20] block the increase in PLA_2 activity by inhibiting expression of $cPLA_2$ mRNA. These observations suggest that generation of ARA is a receptor-mediated process. In addition, COX-2 immunoreactivity is also pronouncedly enhanced, particularly in CA3 pyramidal not only in neurons and activated microglial cells but also in activated astrocytes along with the increase in expression of the microglia-specific protein Iba1 and increase in synthesis of prostaglandin E_2 (PGE_2) [19, 21]. Collective evidence suggests that elevated levels of ARA and its metabolites (eicosanoids) produce a variety of detrimental effects on neural membrane structures, activities of membrane enzymes, generation of ROS, and neurotransmitter uptake systems [7, 14].

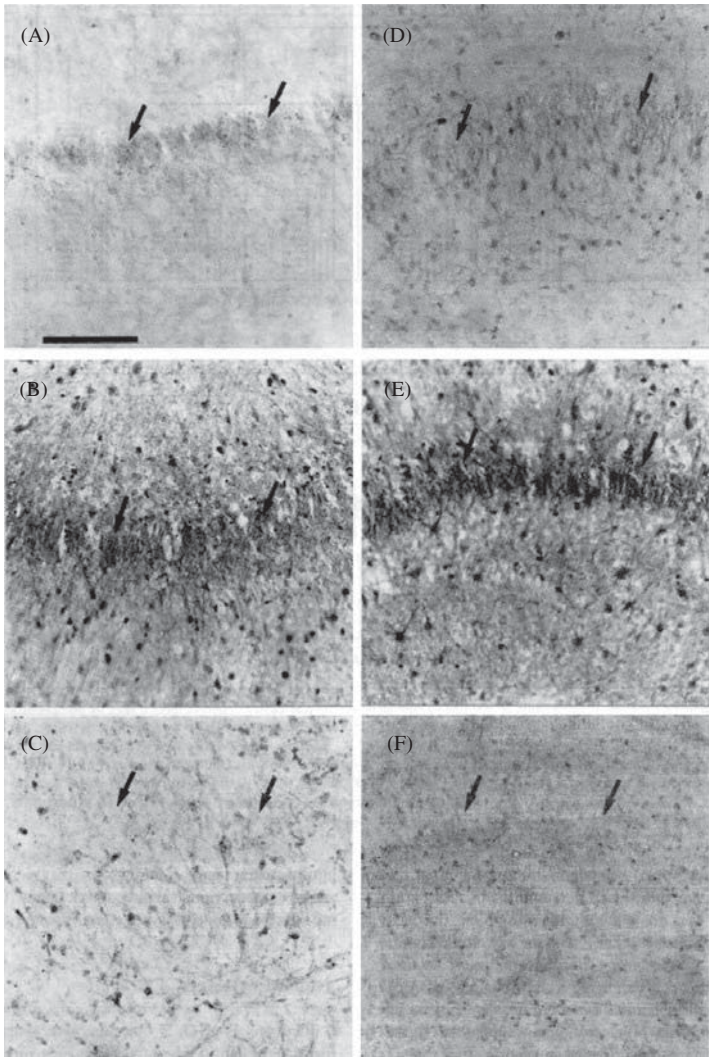

Fig. 4.2 Effect of KA-induced neurotoxicity on $cPLA_2$ and 4-HNE immunoreactivity in CA1 hippocampal subfield. Slices of CA1 subfield of the rat hippocampus are immunostained with monoclonal antibody to $cPLA_2$ (A, B, and C) and 4-HNE monoclonal antibody (D, E, and F). A and D are untreated control slices. B and E are slices that have been stained with 1 mM KA, followed by fixation and immunocytochemical staining 7 days after treatment. C and F are slices that have been treated with 1 mM KA, followed by addition of quinacrine 3 h later and fixation and immunocytochemical staining 7 days later. Arrows indicate hippocampal pyramidal neurons. (A–C) Very little or no staining for $cPLA_2$ is observed in pyramidal neurons in control hippocampal slices (A), while an increase in staining is observed in neurons after KA treatment (B). The increase in $cPLA_2$ immunoreactivity is prevented by treating the slices with quinacrine after the KA application (C). (D–F) Very little or no staining for 4-HNE is observed in pyramidal neurons in the normal hippocampus (D), while an increase in staining is observed in neurons after KA treatment (E). The increase in 4-HNE immunoreactivity is prevented by treating the slices with quinacrine after KA application (F). Scale bar, 160 μm. Reproduced with kind permission from Elsevier [Farooqui et al. *Brain Res Rev* (2001) 38: 61–78].

In KA-mediated neurotoxicity, nonenzymic peroxidation of ARA results in generation of high levels of 4-hydroxynonenal (4-HNE), resulting in increased immunoreactivity in cell bodies of neurons and the neuropil (Fig. 4.2). 4-HNE is a nine-carbon unsaturated aldehyde that not only reacts with lysine, cysteine, and histidine residues in proteins but also binds to free amino acids and deoxyguanosine [7, 22]. 4-HNE causes a number of deleterious effects in cells including inhibition of DNA synthesis, disturbance in calcium homeostasis, and inhibition of mitochondrial respiration [7, 14]. ROS-mediated injury results from the reaction of free radical species with proteins and unsaturated lipids in plasma membrane, leading to chemical cross-linking. This depletion of unsaturated lipids is associated with an alteration in membrane fluidity, which may be responsible for changes in activity of membrane-bound enzymes, ion channels, and receptors [14]. In addition,

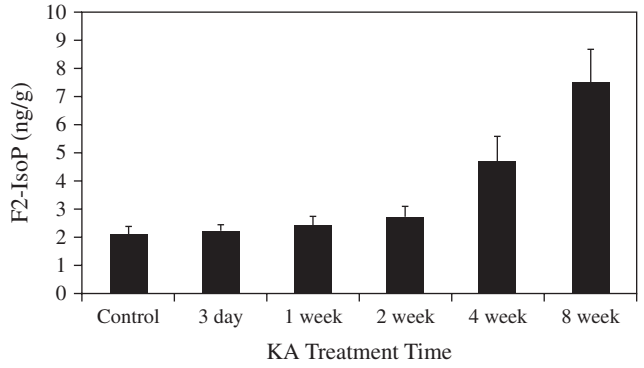

Fig. 4.3 Mean F_2-isoprostane levels in rat hippocampus under control conditions and 3 days, 1 week, 2 weeks, 4 weeks, and 8 weeks after kainate injection. $P < 0.05$ was considered significant. Four rats were used at each time point. Modified from Farooqui et al (2007a). *Brain Res Rev* 56: 443–471.

the C3 position of 4-HNE is a highly reactive site that undergoes a Michael addition reaction with cellular thiols and hence readily forms adducts with glutathione or proteins containing thiol groups. 4-HNE may produce a number of deleterious effects in cells including inhibition of DNA and RNA synthesis, disturbance in calcium homeostasis, and inhibition of mitochondrial respiration. In vivo, nonenzymic peroxidation of ARA also produces isoprostanes, which are prostaglandin-like metabolites that induce their action through isoprostane receptors, which are identical or analogous to thromboxane A_2 receptors [23]. Through a receptor-mediated process, isoprostanes modulate vasoconstriction of retinal vessel and the brain microcirculature [24]. In addition, isoprostanes can be transformed into H_2-isoprostane endoperoxides, which can give rise to cyclopentenone isoprostanes, which are very reactive α,β-unsaturated aldehydes [24]. KA injections produce no changes in F_2-isoPs levels at 3 days, 1 week, and 2 weeks after KA administration; there is a significant increase ($\sim$134%) in F_2-isoP levels at 4 weeks after kainic acid injection compared to controls. At 8 weeks after injection, F_2-isoP levels are increased ($\sim$180%) compared to those in the 4 weeks post-KA injected rats (Fig. 4.3) [25].

Treatment of hippocampal slices with KA also results in a time-dependent increase in nuclear NF-κB levels in CA3 and CA1 areas of hippocampus, but not dentate gyrus, compared with saline-injected controls. ROS, which are generated through KA-induced neurotoxicity, also stimulate NF-κB, a transcription factor that is present in the cytoplasm in a repressed form attached to its inhibitory protein, I-κB (Fig. 4.4) [7]. ROS-mediated stimulation of NF-κB-IκB complex facilitates the release of NF-κB, which is translocated to the nucleus, where it mediates the transcription of more than 150 genes including genes that code for many proinflammatory enzymes, such as $sPLA_2$, COX-2, iNOS, SOD (Fig. 4.5), and matrix metalloproteinases, intracellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), E-selectin, cytokines (TNF-α, IL-β IL-6, c-fos, and protooncogene Bcl-2 [7]. These parameters not only promote the onset of oxidative stress and inflammation but also modulate immune function responses that are closely associated with apoptosis [5]. ROS also promote apoptotic cell death through changes in mitochondrial permeability and poly (ADP-ribose)polymerase activation, which is accompanied by the depletion of NAD. Depletion of NAD causes reduction in ATP, leading to cell death (Fig. 4.4).

Under normal conditions, nitric oxide synthase (NOS) converts arginine into citrulline with the production of nitric oxide (NO). Citrulline is then recycled to arginine by successive actions of argininosuccinate synthetase and argininosuccinate lyase, forming the citrulline-NO cycle. Excessive stimulation of KA-R may also damage neurons through the reaction between NO and superoxide anion and the generation of peroxynitrite ($ONOO^-$). $ONOO^-$ not only interacts with sulfhydryl groups but can hydroxylate the aromatic rings of amino acid residues [7]. In addition, $ONOO^-$ reduces mitochondrial respiration, inhibits membrane pumps, depletes cellular glutathione, and damages DNA, through the activation of poly (ADP-ribose) synthase, an enzyme that leads to cellular energy depletion [26]. Furthermore, $ONOO^-$ not only interferes with key enzymes of the tricarboxylic acid cycle, the mitochondrial respiratory chain, and mitochondrial Ca^{2+} metabolism, but also reacts with lipid, proteins, and DNA [27]. All these processes contribute to neuronal energy deficiency and neurotoxicity caused by KA.

4.3 SPHINGOLIPID METABOLISM ALTERATIONS IN KA-INDUCED NEUROTOXICITY

Intracerebroventricular injections of KA in rats produce a significant increase in ceramide immunoreactivity and levels in the hippocampus at 1 day and 3 days after injection compared to controls. This increase in ceramide may be due to either enhancement in the de novo synthesis of ceramide or increase in hydrolysis of sphingomyelin by sphingomyelinase. Tandem mass spectrometric profiling of lipid extract from KA-injected and control hippocampal tissues indicates significant increase in ceramide with different molecular species including 16:0, 18:0, 20:0, 22:0, and 24:1 fatty acids in KA-injected hippocampus

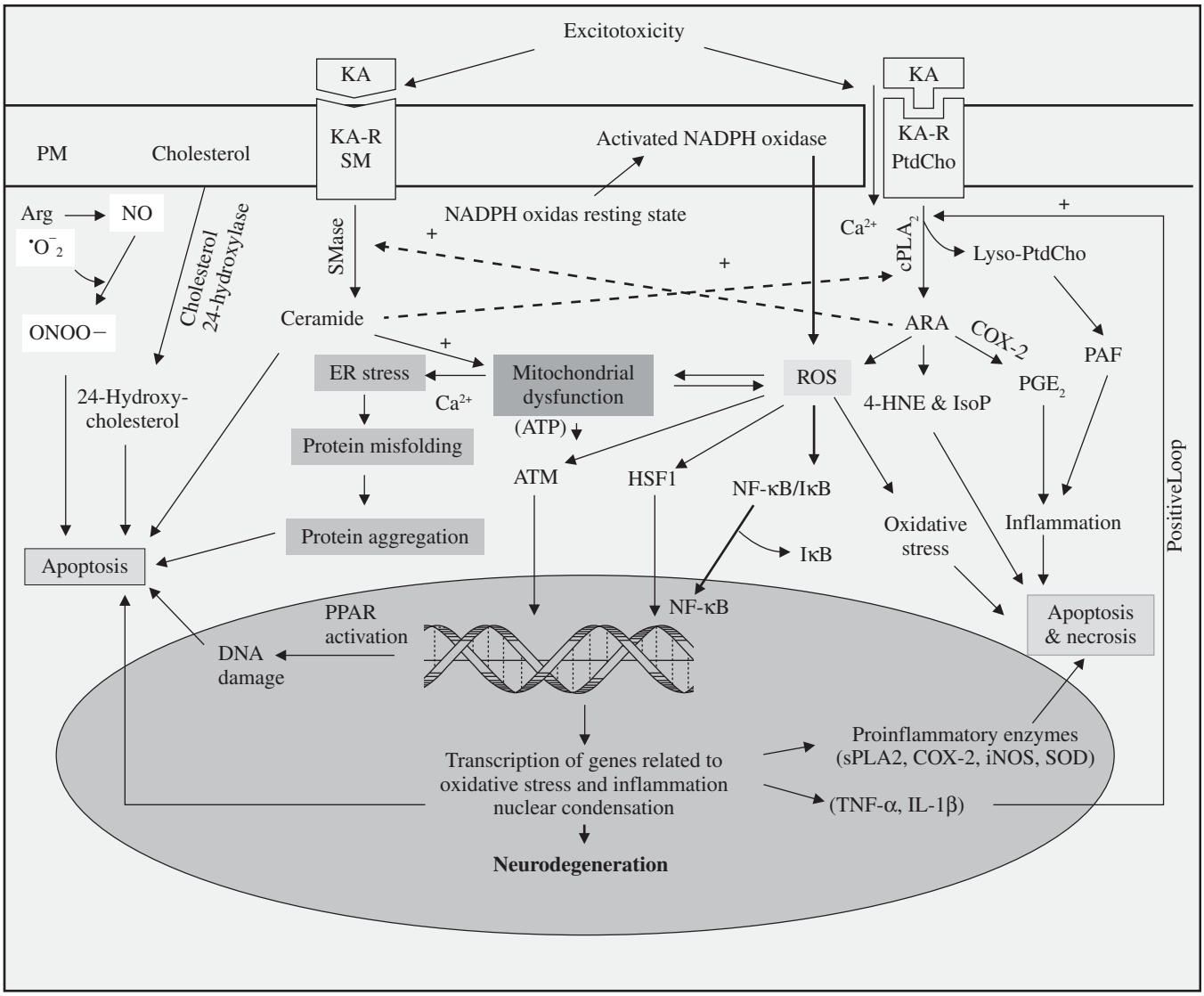

Fig. 4.4 Diagram showing interactions between glycerophospholipid and sphingolipid lipid-derived lipid mediators. KA, kainate; KA-R, kainate receptor; SM, sphingomyelin; SMase, sphingomyelinase; $cPLA_2$, cytosolic phospholipase A_2; PtdCho, phosphatidylcholine; ARA, arachidonic acid; Lyso-PtdCho, lyso-phosphatidylcholine; $sPLA_2$, secretory phospholipase A_2; COX-2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase; ROS, reactive oxygen species; SOD, superoxide dismutase; 4-HNE, 4-hydroxynonenal; PGE_2, prostaglandin E_2; PAF, platelet-activating factor; IsoP, isoprostane; Arg, L-arginine; NO, nitric oxide; $ONOO^{-}$, peroxynitrite; NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; PARP, poly(ADP-ribose) polymerase; ATM, Ataxia-telangectasia mutated; HSF-1, heat shock transcription factor 1. Positive sign (+) indicates stimulation. *(See color insert.)*

[28]. The increase in ceramide levels is associated with increased expression and activity of serine palmitoyltransferase (SPT) after KA injections. Immunohistochemical analyses indicate baseline expression of SPT in neurons and gradual increase in immunoreactivity in astrocytes after KA treatment [29]. The expression of SPT in reactive astrocytes suggests that these cells may be involved in turnover of sphingolipids and generation of ceramide in KA-induced excitotoxic brain injury. In addition, neurons lacking acid sphingomyelinase exhibit decrease in vulnerability to excitotoxicity, which is associated with decreased levels of intracellular calcium and oxyradicals [30]. Accumulating evidence suggests that in KA-mediated neurotoxicity, increase in ceramide may facilitate the opening of the mitochondrial permeability transition pores, which disrupts the transmembrane potential, causing the release of cytochrome *c*, caspase-3 activation, and activation of PLA_2 resulting in apoptotic cell death [7]. Inhibition of SPT by L-cycloserine or myriocin produces a significant neuroprotective effect for a short time after KA-induced toxicity [29]. In addition, studies on temporal changes in sphingosine

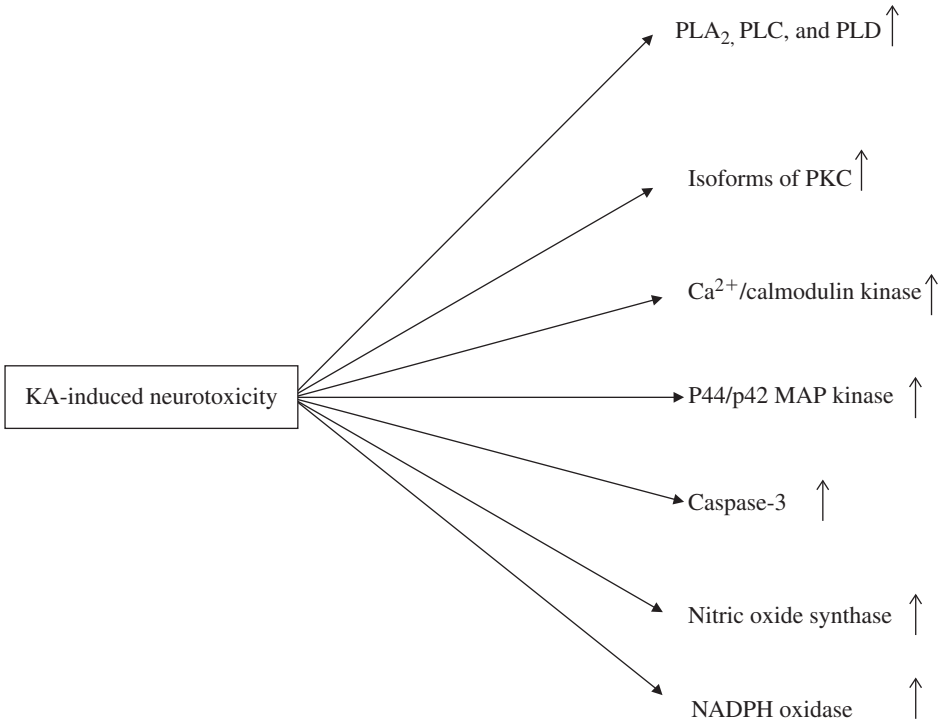

Fig. 4.5 Increase in enzyme activities following KA-induced neurotoxicity.

kinase 1 (SPHK1)/sphingosine 1 phosphate receptor 1 ($S1P_1$) in mouse hippocampus during KA-induced neurotoxicity indicate that the lowest level of SPHK1 protein expression is found 2 h after KA treatment. Six hours after KA treatment, the expression of SPHK1 and $S1P_1$ proteins steadily increases in the hippocampus. Immunohistochemical analysis indicates that SPHK1 and $S1P_1$ are more immunoreactive in astrocytes within the hippocampus of KA-treated mice than in hippocampus of control mice. These results indicate that the SPHK1/$S1P_1$ signaling axis may play an important role in astrocyte proliferation during KA-induced excitotoxicity [31].

4.4 CHOLESTEROL METABOLISM ALTERATIONS IN KA-INDUCED NEUROTOXICITY

As stated above, cholesterol is an integral component of neural membranes. It is crucial for the function of neuronal and glial cells in brain [32, 33]. Cholesterol not only modulates the physicochemical properties of neural membranes but also regulates endocytosis, antigen expression, and activities of membrane-bound enzymes, receptors, and ion channels [32, 33]. Although neurons synthesize sufficient quantities of cholesterol to survive and grow, at the time of synaptogenesis cholesterol is synthesized by glial cells and delivered to neurons by cholesterol-transporting proteins, such as apoE and ATP binding cassette proteins (ABCA1 and ABCG1) [33].

In brain, cholesterol is metabolized to oxycholesterols through hydroxylation reactions, and the occurrence of 24-, 27-, and 7-hydroxycholesterol has been well established in brain tissue and CSF samples [34]. Intracerebroventricular injections of KA in rats results in an increase in immunoreactivity to cholesterol in the affected CA fields of the hippocampus. The increase is confirmed by increased filipin staining of cholesterol in adjacent sections from the same animals and in hippocampal slice or neuronal cultures after KA treatment [20]. The increase in cholesterol staining in slice cultures suggests that increase in cholesterol in neurons after KA treatment is not the result of exogenous transfer from the bloodstream. Likewise, increase in cholesterol staining in individual neurons indicates that increase in neuronal cholesterol staining is likely due to either an increase in cholesterol biosynthesis or a defect in cholesterol export in neurons themselves. In neuronal cultures, addition of lovastatin, an inhibitor of cholesterol synthesis, attenuates the increased filipin staining after KA treatment. The increase in brain cholesterol therefore points to a local disturbance in cholesterol homeostasis [20]. This increase in cholesterol level in KA-treated brain is accompanied by an increase in cholesterol oxidation products, such as 7-ketocholesterol and 24-hydroxycholesterol. These metabolites not only produce neurotoxic effects but also induce exocytosis when applied externally to PC12 cells, as

determined by capacitance measurements under patch-clamp conditions and total internal reflection fluorescence microscopy (TIRFM) of labeled vesicles [35]. The effect of 7-ketocholesterol is dependent on the integrity of lipid rafts. Enhanced exocytosis induced by the oxysterol can be abolished by pretreatment of cells with methyl cyclodextrin, which chelates cholesterol and disrupts rafts. Similar effects on enhanced exocytosis are observed after external application of several ceramide species, including C18:0 ceramide, and, as with oxysterols, the effect is raft dependent. These results indicate that increased oxysterol [36] and ceramide [28, 29] levels in the hippocampus following KA-mediated neurotoxicity may enhance neurotransmitter release through exocytosis and further propagate excitotoxic brain injury [29, 36, 38]. Furthermore, 7-ketocholesterol contains a reactive keto group and may form adducts with the amino group of other lipid and protein components of neural membranes. Studies on metabolism of 27-, 25-, and 24-hydroxycholesterol in cultures of rat astrocytes, Schwann cells, and neurons indicate that 27- and 25-hydroxycholesterol, but not 24-hydroxycholesterol, undergo a 7 α-hydroxylation with subsequent oxidation to 7 α-hydroxy-3-oxo-δ4 steroids in all three cell types [37]. All these oxysterols behave as neurotoxic agents toward the human neuroblastoma cell line SH-SY5Y and induce apoptosis, as indicated by DNA-fragmentation, caspase-3 activation, and a decrease of the mitochondrial membrane potential.

The generation of high levels of oxycholesterols (hydroxycholesterols) in KA-mediated neurotoxicity modulates neural cell survival. Oxysterols also exert tight control over neural cell cholesterol trafficking by altering cholesterol influx/efflux [38]. Oxysterols not only regulate Ca^{2+} signals, modulate $cPLA_2$ activity, and block the phosphorylation of endothelial NOS but also interact with lipid metabolites of glycerophospholipid and sphingolipid metabolism [39–41]. In addition, some oxycholesterols trigger the stimulation of NADPH oxidase, generation of superoxide anions, loss of mitochondrial transmembrane potential ($\Delta\Psi_m$), release of cytochrome *c*, and activation of caspase-3. These processes are closely associated with apoptotic cell death [42]. Furthermore, some hydroxycholesterols, such as 7β-hydroxycholesterol not only retard the secretion of soluble amyloid precursor protein (APP) from cultured rat hippocampal H19-7/IGF-IR neuronal cells but also inhibit α-secretase activity [43]. 7β-Hydroxycholesterol also inhibits protein kinase C-α, an enzyme critical in memory consolidation and synaptic plasticity. Low levels of 7-hydroxycholesterol modulate normal APP processing, but high levels of hydroxycholesterol promote the formation of Aβ that may contribute to the oxidative stress and neural cell loss observed in Alzheimer disease (AD) [43]. Levels of cholesterol and oxysterol in KA-induced neurodegeneration in the hippocampus are affected by intraperitoneal injections of a blood-brain barrier-permeant statin, lovastatin. Modulation of excessive increase in cholesterol and oxysterol levels in brain tissue after statin treatment correlates with increased survival of hippocampal pyramidal neurons, suggesting that reduction in oxysterols may be an important mechanism for the neuroprotective effect of statins [28, 29, 44].

4.5 CONSEQUENCES OF INTERACTIONS AMONG GLYCEROPHOSPHOLIPID-, SPHINGOLIPID-, AND CHOLESTEROL-DERIVED LIPID MEDIATORS IN KA-MEDIATED NEUROTOXICITY

As stated above, biophysical properties of neural membranes are maintained by a specific physiological glycerophospholipid, sphingolipid, and cholesterol composition [41]. KA-mediated neurotoxicity results not only in the enrichment of glycerophospholipid, sphingolipid, and cholesterol metabolism but also in generation of high levels of glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators (Table 4.1) [7]. As

TABLE 4.1 Alterations in levels of glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators in KA-induced neurotoxicity and ischemia

Lipid Mediator	KA-Mediated Toxicity	Neurotraumatic Diseases	Neurodegenerative Diseases	Reference
Fatty acids (arachidonic acid)	Increased	Increased	Increased	14, 46
Prostaglandins	Increased	Increased	Increased	14, 46
4-HNE	Increased	Increased	Increased	6, 14, 60
Isoprostanes	Increased	Increased	Increased	61, 62
Ceramide	Increased	Increased	Increased	28, 63–67
7-β-Hydroxy-cholesterol	Increased	—	Increased	20, 43, 68
7-Keto-cholesterol	Increased	—	Increased	44, 69, 70
24-Hydroxy-cholesterol	Increased	No effect	Increased	44, 69, 70
22(*R*)-hydroxycholesterol	–	–	Decreased	71

mentioned above, KA neurotoxicity not only induces the generation of elevated levels of enzymic and nonenzymic lipid mediators of phospholipid metabolism but also increases the synthesis of ceramide, which may induce a spontaneous formation of large ceramide-enriched membrane platforms. These large ceramide-enriched membrane platforms may facilitate the increased production of ROS [45]. In neural membranes, both ceramide and its metabolites (ceramide-1-phosphate and sphingosine-1-phosphate) stimulate the generation of ARA, ARA-derived lipid mediators, and ROS through the stimulation of PLA_2 isoforms and arachidonic acid cascade [5, 41, 46]. Similarly, ARA stimulates sphingomyelinase activity and generates more ceramide. Simultaneous intensification of these processes following KA-mediated toxicity not only increases the interplay (cross talk) among lipid mediators of phospholipid and sphingolipid metabolism but may also lower the levels of essential glycerophospholipid and ceramide molecular species in neural membranes, a process that may be closely associated with neurodegeneration [7]. Like ceramide-metabolizing enzymes, mechanisms associated with generation and release of ROS are also localized in membrane rafts. It is suggested that optimal integrity of ceramide-enriched rafts may be required for continuous cellular ROS release during KA-mediated neurotoxicity [7, 47]. Furthermore, KA stimulates the hydrolysis of plasmalogens, the vinylether-containing glycerophospholipids, by plasmalogen-selective-PLA_2 (PlsEtn-PLA_2) in a dose- and time-dependent manner [48], and ceramide decreases the levels of plasmalogens by inhibiting PlsEtn-PLA_2 in rat brain slices [49]. The decrease in plasmalogen levels by ceramide can be blocked by quinacrine, ganglioside, and bromoenol lactone. These compounds inhibit PlsEtn-PLA_2 activity. Thus it is likely that interplay between plasmalogen and sphingomyelin-derived lipid mediators may modulate inflammation and oxidative stress. These processes are closely associated with KA neurotoxicity [48, 49]. Collective evidence suggests that high levels of glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators in KA-mediated neurotoxicity may disturb normal signal transduction homeostasis and threaten neural cell survival due to increased intensity of cross talk among glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators [7].

4.6 KA-INDUCED NEUROTOXICITY AND ITS IMPLICATION FOR NEUROTRAUMATIC AND NEURODEGENERATIVE DISEASES

Involvement of excitotoxicity, oxidative stress, and neuroinflammation in neurotraumatic [stroke, spinal cord injury (SCI), and traumatic brain injury (TBI)] and neurodegenerative diseases [AD, Parkinson disease (PD), and amyotrophic lateral sclerosis (ALS)] has gained increasing acceptance, but the underlying mechanisms through which excitotoxicity, oxidative stress, and neuroinflammation mediate neurodegeneration still remain elusive [50]. In addition, recent data from human studies highlight the association of KA-Rs in certain psychiatric diseases, such as schizophrenia and major depression, and a recent association of KA-R gene variants with response to antidepressants has brought considerable interest in developing a clearer understanding of KA-R action in the brain [51]. It is reported that exposure to stress and stress hormone administration can produce contrasting changes in KA-R subunit expression in the rat hippocampus, suggesting that a modification of hippocampal KA-Rs by stress may be a mechanism for predisposing individuals to stress-related psychiatric diseases [51]. Enhanced rate of interplay (cross talk) among excitotoxicity, oxidative stress, and neuroinflammation through lipid mediators may be associated with the increased vulnerability of neurons in neurotraumatic, neurodegenerative, and neuropsychiatric diseases [5, 46].

KA-induced neurotoxicity, excessive activation of KA type of glutamate receptors leads to a number of deleterious consequences, including depolarization-mediated increase in Ca^{2+} influx, stimulation of Ca^{2+}-dependent enzymes (PLA_2 isoforms, NOS, calpains, caspases, and protein kinases), increased expression of proinflammatory cytokines, depletion of ATP, increase in lipid peroxidation products (4-HNE, isoprostanes, ROS), activation of the mitochondrial permeability transition, and loss of glutathione [5, 7, 14]. Like KA-induced neurotoxicity, neurodegeneration in neurotraumatic diseases is accompanied by enhanced degradation of neural membrane glycerophospholipid, activation of PLA_2, NOS, calpains, caspases, and protein kinases, increased expression of cytokines, and production of ROS and lipid hydroperoxides. Generation of 4-HNE, isoprostanes, and ROS induces oxidative stress, whereas formation of high levels of prostaglandins, leukotrienes, thromboxanes, and platelet-activating factor contributes to neuroinflammation [5, 46, 50, 52]. A major source for vascular and neuronal ROS is a family of nonphagocytic NADPH oxidases, including the prototypic Nox2 homolog-based NADPH oxidase, as well as other NADPH oxidases, such as Nox1 and Nox4 [53]. Other possible sources include mitochondrial electron transport enzymes, xanthine oxidase, cyclooxygenase, lipoxygenase, and uncoupled NOS. NADPH oxidase-derived ROS plays a physiological role in the regulation of neural and endothelial function. Although the pathophysiological importance of glutamate-mediated glycerophospholipid degradation in neurotraumatic diseases is

not fully understood, it is proposed that enhanced catabolism of glycerophospholipids in neurotraumatic diseases may be an earliest event [46].

As stated above, neurodegeneration in KA-induced neurotoxicity is accompanied by excitotoxicity-mediated enhanced degradation of neural membrane glycerophospholipid, activation of PLA_2, NOS, calpains, caspases, and protein kinases, increased expression of cytokines, and production of 4-HNE, isoprostanes, and ROS and lipid hydroperoxides [5, 46, 50, 52]. In contrast, neurodegenerative diseases are accompanied by neurodegeneration caused by many different factors, including but not limited to genetic abnormalities, accumulation of abnormal extracellular and intracellular deposits (β-amyloid, α-synuclein, huntingtin, etc.) in specific populations of neurons in specific areas of the brain, changes in neural membrane composition due to increase in activities of PLA_2, COX-2, iNOS, caspases, and calpains, neurotransmitters, and their receptors, alterations in cerebral blood flow and blood-brain barrier, and problems in the immune system [52]. The most important risk factors for sporadic neurodegenerative diseases are old age, positive family history, unhealthy lifestyle, endogenous factors, and exposure to a toxic environment [5, 46, 54]. Neurodegenerative diseases commence late in life and are accompanied by the loss of synapses and accumulation of misfolded protein aggregates [5, 52]. The chemical nature of the misfolded protein aggregate is different in each neurodegenerative disease. For example, β-amyloid peptide and Tau protein aggregate and accumulate in plaques and tangles of AD patients; α-synuclein and perkin accumulate in Lewy bodies of PD patients, huntingtin accumulates as nuclear inclusions in Huntington disease (HD) patients, and mutation in Cu/Zn superoxide dismutase occurs in some inherited forms of ALS. In some neurodegenerative diseases alterations in glutamate homeostasis may also contribute to neurodegeneration. For example, in AD levels of glutamate are not altered, but a marked reduction in the expression of NR2A and NR2B subunit mRNA has been reported in the hippocampus and entorhinal cortex in the brain of AD patients. In addition, alterations in activities of glutamate transporters have been observed in the brain of AD patients [55]. This may induce changes in glutamate homeostasis in AD, causing a major disturbance in Ca^{2+} homeostasis [7] and inducing neurochemical changes similar, but not identical, to KA-induced neurotoxicity. Similarly, involvement of excitotoxicity in the pathogenesis of ALS is supported by the decrease in RNA editing of AMPA receptor subunit GluR2 at the Q/R site in the motor neurons and reduction in glial glutamate transporter GLT-1in brain tissue from sporadic ALS patients [56–58]. Involvement of the NMDA-type of glutamate receptors in pathogenesis of HD in animal models has also been reported [59].

In neurotraumatic diseases, because of the faster rate of upregulation of interplay among excitotoxicity, oxidative stress, and neuroinflammation, neurons die rapidly in a matter of hours to days following the sudden lack of oxygen, decreased ATP level, and sudden collapse of ion gradients. In contrast, in neurodegenerative diseases, oxygen, nutrients, and ATP continue to be available to the nerve cells and ionic homeostasis is maintained to a limited extent. The interplay among excitotoxicity, oxidative stress, and neuroinflammation occurs at a slow rate, resulting in a neurodegenerative process that takes several years to develop [7, 46, 52].

4.7 CONCLUSION

Neural membranes are composed of glycerophospholipids, sphingolipids, cholesterol, and proteins. KA-mediated toxicity causes enrichment of glycerophospholipid, sphingolipid, and cholesterol metabolism that results in elevations of glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators. The increased interaction among glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators intensifies degradation of neural membrane components and loss of essential glycerophospholipids, sphingolipids, and cholesterol, causing changes in neural membrane fluidity and permeability. These processes allow a sustained Ca^{2+} influx and produces stimulation of Ca^{2+}-dependent enzymes including PLA_2, PLC, PLD, NOS, calpains, and endonucleases resulting in stimulation of lipolysis and proteolysis, production of lipid peroxides, and loss of glutathione. Stimulation of these enzymes along with mitochondrial dysfunction, alteration in cellular redox, induction of cytokines, and decrease in ATP levels may be responsible for neural cell death in KA-mediated neurotoxicity.

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