Molecular Neurobiology

ISSN0893-7648/05/31(1–3): 3–16/$30.00

Kainic Acid-Mediated Excitotoxicity as a Model for Neurodegeneration

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Abstract

Neuronal excitation involving the excitatory glutamate receptors is recognized as an important underlying mechanism in neurodegenerative disorders. Excitation resulting from stimulation of the ionotropic glutamate receptors is known to cause the increase in intracellular calcium and trigger calcium-dependent pathways that lead to neuronal apoptosis. Kainic acid (KA) is an agonist for a subtype of ionotropic glutamate receptor, and administration of KA has been shown to increase production of reactive oxygen species, mitochondrial dysfunction, and apoptosis in neurons in many regions of the brain, particularly in the hippocampal subregions of CA1 and CA3, and in the hilus of dentate gyrus (DG). Systemic injection of KA to rats also results in activation of glial cells and inflammatory responses typically found in neurodegenerative diseases. KA-induced selective vulnerability in the hippocampal neurons is related to the distribution and selective susceptibility of the AMPA/kainate receptors in the brain. Recent studies have demonstrated ability of KA to alter a number of intracellular activities, including accumulation of lipofuscin-like substances, induction of complement proteins, processing of amyloid precursor protein, and alteration of tau protein expression. These studies suggest that KA-induced excitotoxicity can be used as a model for elucidating mechanisms underlying oxidative stress and inflammation in neurodegenerative diseases. The focus of this review is to summarize studies demonstrating KA-induced excitotoxicity in the central nervous system and possible intervention by anti-oxidants.

Index Entries: Kainic acid; excitotoxicity; oxidative stress; neuronal death; astrocyte; microglia; selective vulnerability; hippocampal neurodegeneration; resveratrol; antioxidant.

Received 6/21/04; Accepted 11/15/04.
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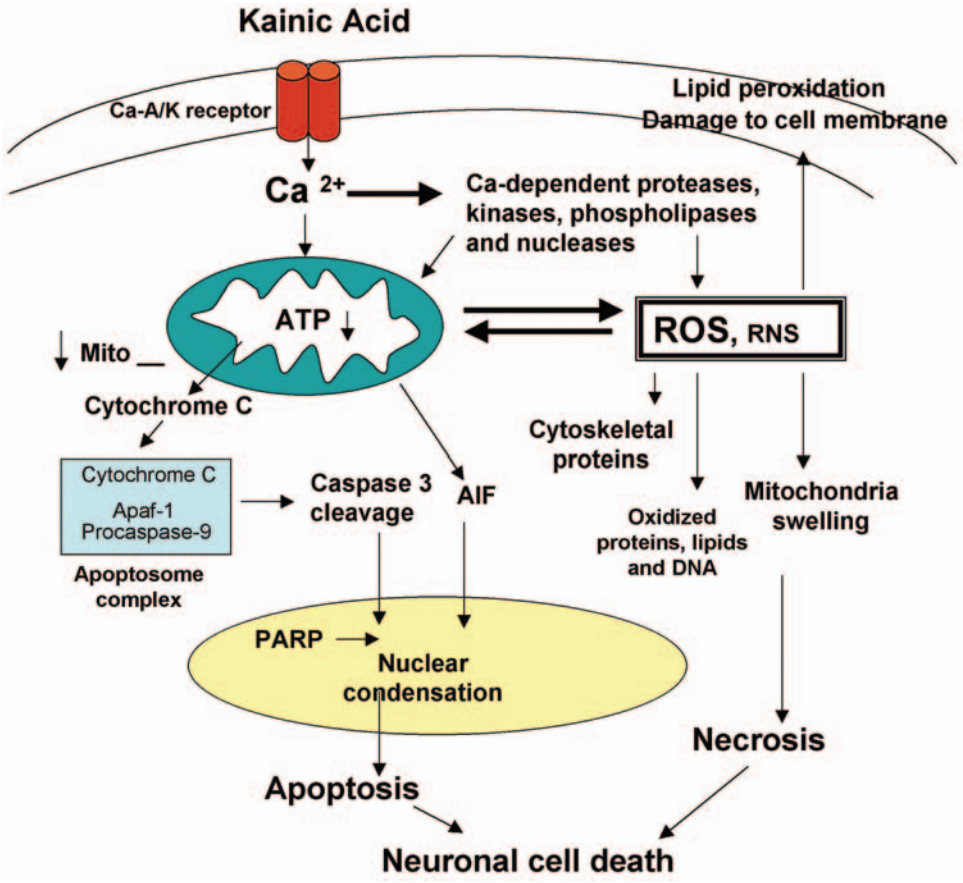

Fig. 1. Scheme depicting the neuronal cell death pathway induced by KA. (1) KA stimulates the Ca^{2+}-A/K receptors, leading to rapid Ca^{2+} entry; (2) activation of Ca^{2+}-dependent enzymes and generation of ROS; (3) excessive Ca^{2+} and ROS lead to collapse of mitochondrial membrane potential ($m\Delta\Psi$) and opening of mitochondrial permeability transition pores (MPT); (4) release of mitochondrial factors (e.g., cytochrome-*c* and apoptotic-inducing factor (AIF); (5) cytochrome-*c* binding to Apaf-1 and caspase-9 to form apoptosome complex and activation of caspase-3 pathway; (6) nuclear condensation and DNA fragmentation. Alternatively, intense Ca^{2+} overload could directly cause mitochondrial swelling and damage, decrease in ATP, and increase in ROS, which oxidize protein, lipid, and DNA, causing acute neuronal necrosis.

Introduction

In recent years, different hypotheses have been developed in an attempt to explain the mechanisms of neuronal death in neurodegenerative diseases *(1)*. It is well known that excess release of excitatory neurotransmitters such as glutamate is an important underlying cause of neuronal damage in cerebral ischemia, epilepsy, Parkinson's disease, and Alzheimer's disease *(2,3)*. This type of excitation-induced neuronal damage is frequently accompanied by excess calcium influx and followed by generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which results in damage of intracellular membranes and triggering apoptotic pathways leading to delayed cell death *(4)* (Fig. 1).

Kainic acid (KA) (2-carboxy-4-isopropenyl-pyrrolidin-3-ylacetic acid), also known as alga-*kaininso*, is isolated from *Digenea*, a red alga found in tropical and subtropical waters *(5)*. KA

has been used for centuries as an anthelminthic compound for removal of worms in the gut. Subsequent studies indicated KA as a non-degradable analog of glutamate and it is 30-fold more potent in neurotoxicity than glutamate *(6).* This neuroexcitant can bind to the AMPA/KA receptors, which are subtypes of the ionotropic glutamate receptors in the brain *(6).* Activation of KA receptor has been shown to elicit a number of cellular events, including the increase in intracellular Ca^{2+}, production of ROS, and other biochemical events leading to neuronal cell death *(7–11).* In recent years, neurodegeneration caused by systemic injection of KA has been widely used in studies to investigate mechanisms of excitotoxicity mediated by excitatory neurotransmitter agonists and possible pharmacological intervention against the excitotoxic events *(4,12).* A major focus for this review is to describe cellular effects of KA, especially those related to neuronal damage in the brain. Because KA-induced oxidative stress has been an important mechanism leading to neuron cell death, this review also includes studies describing intervention by antioxidants.

Glutamate Excitotoxicity

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS), with global action on a number of metabotropic and ionotropic receptors. Stimulation of the *N*-methyl-D-aspartate (NMDA) receptor, a subtype of ionotropic glutamate receptors, has been shown to cause membrane depolarization and a large influx of Ca^{2+}, and, in turn, it triggers a train of biochemical reactions, including Ca^{2+}-dependent proteases, protein kinases, phospholipases, and nucleases *(3,13).* These reactions are associated with mitochondrial dysfunction and eventually cell death *(14,15).* There is evidence that excitation-mediated neuronal cell death can also be mediated by non-NMDA receptors *(8,16).* This led to increasing interest on understanding properties and function of the AMPA and kainite receptors.

AMPA and KA receptors belong to the non-NMDA receptors and are channels permeable to Ca^{2+} (Ca-A/K receptors) *(14).* These receptors are present in the dendrites of hippocampal pyramidal neurons, which display considerable molecular heterogeneity in the expression of these channels *(17,18).* The Ca-A/K receptors are heteromers consisting of combination of four subunits (GluR1–4). The Ca^{2+} permeability of the recombinant channels is controlled by the GluR2 subunit, which functions to reduce Ca^{2+} permeability *(6).* KA administration to adult rats is known to cause delayed death of pyramidal neurons in the hippocampal CA1 and CA3 regions and the cell death process is preceded by downregulation of GluR2 mRNA and protein in these same regions. Under these conditions, GluR2 immunolabeling was unchanged in the dentate gyrus (DG) granule cells, which are resistant to KA. These studies demonstrated well the important role of Ca-A/K receptors in the neurotoxicity induced by KA *(19).* CNQX (6-cyano-7-nitroquinoxalone-2, 3-dione), an AMPA receptor antagonist, is able to inhibit neurotoxicity induced by KA *(20).* The ability of KA to induce delayed neuronal cell death *(20)* is consistent with the "GluR2 hypothesis," which relates the reduction of GluR2 subunit expression with an increased Ca^{2+} permeablity of the AMPA receptors and, subsequently, predicts the vulnerability of a subset of pyramidal neurons to KA excitotoxicity *(21,22).* Apparently, the specific distribution of the AMPA/kainate receptors in the brain and their sensitivity to Ca^{2+} permeability are important factors underlying the selective vulnerability of these neurons to neurodegeneration *(23).*

Oxidative Stress Mediated by KA

There is ample evidence linking oxidative stress to KA-mediated neurotoxicity. Previous studies in our laboratory demonstrated the production of free radicals after KA administration in vivo *(24)* and in neuron cells in vitro *(8).* Our recent studies with primary cortical

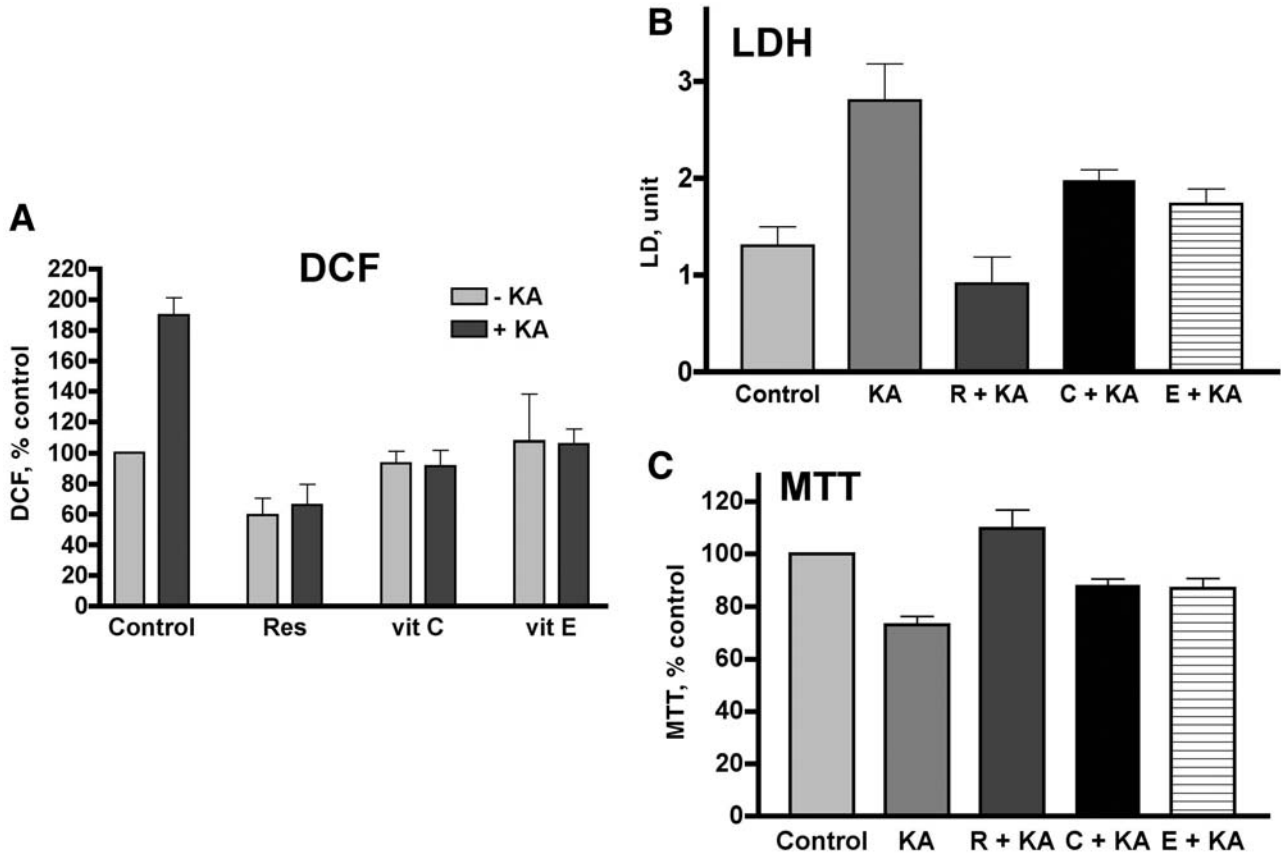

Fig. 2. Effects of KA treatment (100 μ*M*) and protection by resveratrol (res) and vitamins C and E on rat primary cortical neurons: **(A)** the increase of ROS as depicted by DCF, **(B)** increase in LDH, and **(C)** decrease in (MTT).

neurons in culture further indicated the ability of KA to induce an increase in ROS production (using the dichlorofluorescien-acetate [DCF] dye) (Fig. 2A). KA also stimulated the release of lactate dehydrogenase (LDH), an indication of loss of cell membrane integrity (Fig. 2B), and a decrease in 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), suggesting a decrease in mitochondrial function (Fig. 2C). In addition, KA also caused nuclei condensation and fragmentation as shown by staining with 4',6-diamidino-2-phenylindole (DAPI) (Fig. 3). Similar to the excitatory events mediated by the NMDA receptor, many studies also demonstrated the effects of the KA receptor on membrane depolarization, changes in calcium homeostasis, increase in ROS production, and neuronal cell death *(6–11).*

Exposure of rat brain homogenates to KA can significantly increase the production of malondialdehyde and 4-hydroxy-alkenals, suggesting an increase in lipid peroxidation *(25).* In addition to the increase in lipid peroxidation, systemic administration of KA also caused a decrease in glutathione (GSH) levels in the hippocampus, cerebellum, and amygdala/piriform cortex *(26).* The increase in superoxide production and oxidative DNA damage following KA administration are indications of KA-induced mitochondrial and oxidative damage *(27).* Injection of KA also causes the increase in nitric oxide synthase (NOS) in neurons *(28,29).*

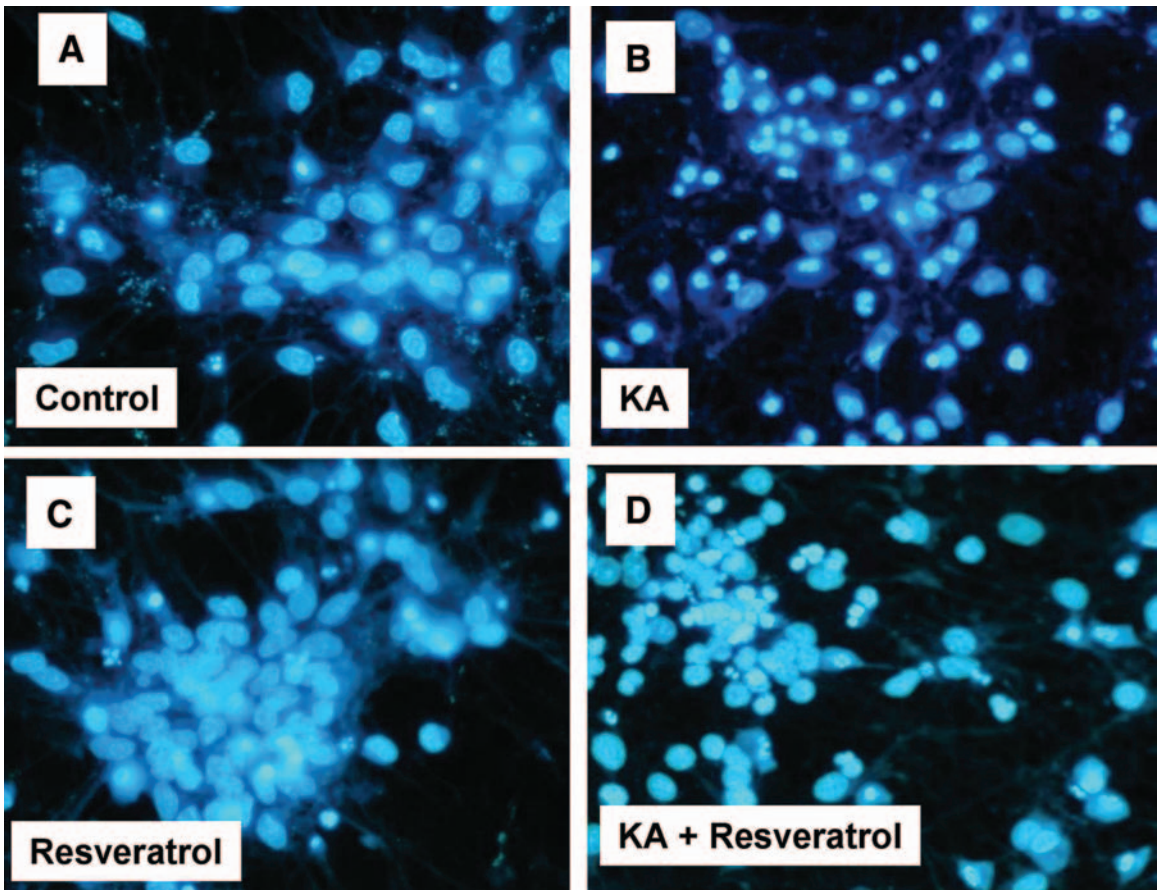

Fig. 3. Effects of KA treatment on nuclei condensation as depicted by DAPI staining: **(A)** control, **(B)** treatment with KA (100 m*M*) for 8 h, **(C)** treatment with resveratrol (10 m*M*), and **(D)** treatment with resveratrol (10 m*M*) 30 min prior to KA (100 m*M*).

NO or its derivatives could cause accumulation of RNS, which, in turn, alters mitochondrial function and triggers apoptosis or necrosis *(30)*.

KA Induces Selective Vulnerability in Hippocampal Neurodegeneration

Kainic acid administration to rodents has been used as an animal model to study mechanisms of neurodegenerative pathways induced by excitatory neurotransmitter agonists *(31)*. These studies show that different methods of KA administration can lead to different patterns of neuron excitation. Systemic injection of KA to rats is known to cause selective neuronal vulnerability in neurons in the hippocampal hilus, CA3 and CA1 subfields, whereas granule cells in DG are resistant *(19)*. Pyramidal neurons in hippocampal CA3 region seem to be particularly sensitive to KA-induced neuronal excitation. The molecular basis for this differential susceptibility is not well understood. An important factor may be the result of high levels of KA receptors in this region *(32)*.

Kainic acid-induced neurotoxicity varies depending on the route of administration and animal species. There was no apparent hippocampal neuronal cell death following

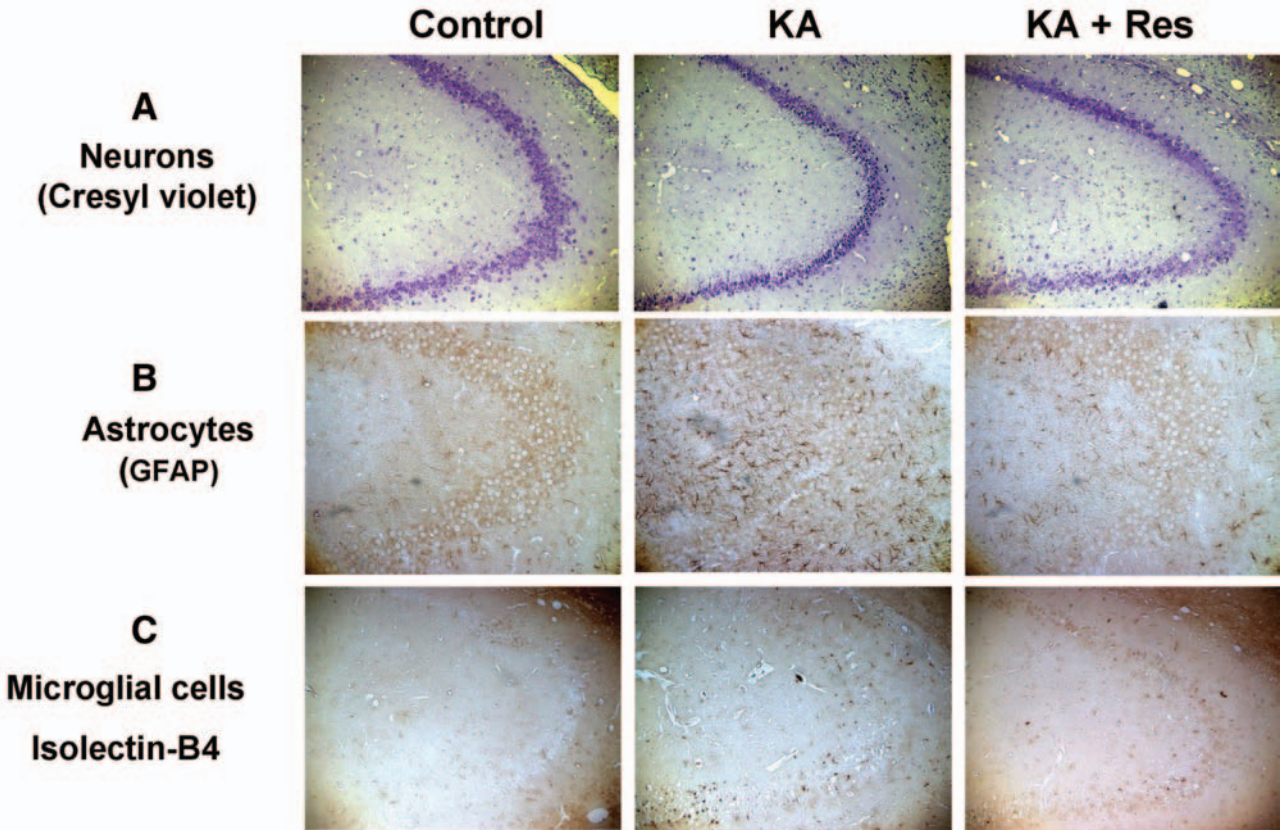

Fig. 4. Representative photomicrographs of neurons, astrocytes, and microglial cells in the CA3 subregion of rat hippocampus as depicted by histochemical or immunohistochemical staining with cresyl violet, GFAP, and isolectin-B4, respectively. Rats were injected intraperitoneally with **(A)** control (0.9% saline), **(B)** KA, 8 mg/kg KA, or **(C)** KA + resveratrol (KA plus 30 mg/kg resveratrol). Resveratrol was injected 30 min prior to KA injection. KA and resveratrol were injected once a day for 5 d. Magnification: ×200. (Data extracted from ref. *26*.)

systemic KA administration in C57BL/6 mice. However, intra-amygdala microinjection of KA to these mice could effectively elicit CA3 pyramidal neuronal death *(33)*. Injection of KA directly into the hippocampus of C57BL/6 mice also showed morphological changes in the pyramidal neurons in hippocampal CA1, CA3, and CA4 regions, and damage in neurons in the CA1 region is more severe than those in the CA3 and CA4 regions *(34)*. Selective neuronal damage in the hippocampal CA3 region was also observed after KA administration to mice through intranasal injection *(35)*. The ability of KA to induce neuronal activity and neuronal cell death appears to differ with different mouse strains. Although both the C57BL/6 and FVB/N mice exhibited similar seizure activity in response to KA administration, C57BL/6 mice were resistant to KA-induced neuronal cell death. The pharmacological basis for this strain difference in hippocampal sensitivity was attributed to the prevalence of the KA receptor in different mouse strains *(36)*. In a recent study by Wang et al. (Figs. 4 and 5) *(26)*, repeated systematic injection of KA to rats for 5 d resulted in severe damage of neurons in the hippocampal CA3 area (Fig. 4A). Furthermore, KA-induced neuronal cell death is accompa-

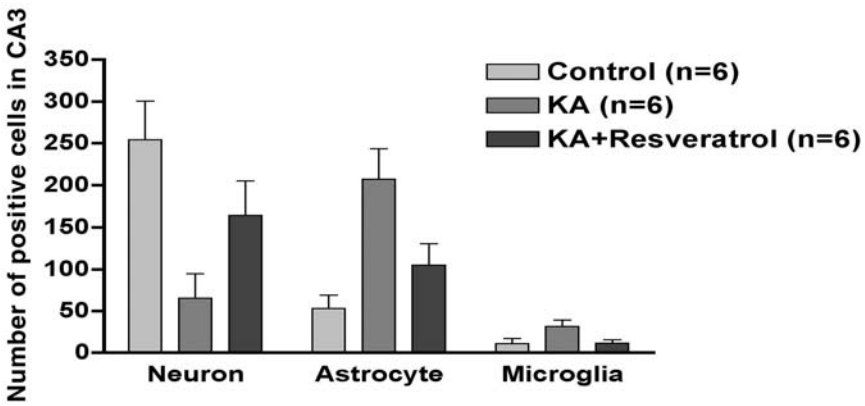

Fig. 5. Effects of resveratrol on neuronal viability, activation of astrocytes, and recruitment of nicroglial cells in the hippocampal CA3 subregion in rats treated with the paradigm as described in Fig 4. Data represent mean ± SEM, with *n* representing the number of rats in each group. Quantitative assessment of histological damage was performed under light microscopy (magnification: ×400), using the Bioquant Image Analysis System. Statistical analysis using one-way analysis of variance, followed with the Newman–Keuls posttest indicated significant differences comparing the KA group with the control group and the KA + resveratrol group with the KA group; $p < 0.05$. (Data extracted from ref *26*.)

nied by increased proliferation of astrocytes (Fig. 4B) as well as microglial cells (Fig. 4C). Data in Fig. 5 show a semiquantitative assessment of neuronal cell death and glial cell activation as a result of KA administration.

KA Induces Apoptotic Neuronal Cell Death

The opening of Ca-A/K channels in the postsynaptic terminal permits rapid Ca^{2+} influx and stimulates oxidative pathways resulting in the generation of ROS. There is sufficient evidence that ROS generation could lead to mitochondrial dysfunction and subsequent apoptotic or necrotic cell death pathways (Fig. 1). Apoptotic pathways can be triggered as a result of the collapse of the mitochondrial membrane potential ($m\Delta\Psi$) and the opening of mitochondrial permeability transition pores (MPT) that allow the release of cytochrome-*c* into the cytoplasm. In turn, cytochrome-*c* in cytoplasm is coupled with the apoptotic-inducing factor (AIF), which subsequently leads to activation of the caspase cascade *(23)* (Fig. 1). At 1–3 d after intraperitoneal injection of KA into rats, many degenerating neurons in the hippocampus were intensely stained with *in situ* nick end labeling (ISNEL) and displayed pathological features suggesting both necrosis and apoptosis *(37).* Intraventricular infusion of KA into adult mouse brain also caused neuronal morphological changes, with condensed nuclei reflecting of apoptosis in the pyramidal layer of the hippocampal formation *(38).* In organotypic hippocampal slice cultures, KA-mediated neuronal damage was associated with a complete reduction of rhodamine 123 fluorescence, an indication of $m\Delta\Psi$ dissipation, and increased levels of cytochrome-*c* and caspase-3 in the cytosol. Cyclosporin A, an inhibitor of MPT opening, partially prevented cytochrome-*c* release, caspase activation, and neuronal death. Inhibition of caspase-3 activity by an inhibitor, z-VAD-fmk, also partially protected neurons from KA-induced cytotoxicity *(39).* It is of interest to note that caspase-3 activation occurred at 30 h following KA treatment in the sensitive strain of mice but not in the resistant strain *(40).*

Glial Cell Activation After KA-Induced Injury

Activation or recruitment of glial cells (astrocytes and microglial cells) is a common event associated with neuronal injury. Astrocytes comprised the major cell type in the brain and they are known to play multiple functional roles in support of neurons *(41,42).* Both astrocytes and microglial cells are immune active and become activated under pathological conditions. Reactive astrogliosis and microgliosis are intimately associated with many neurodegeneration processes and contribute to the increase in pro-inflammatory factors and ROS. Our earlier studies have demonstrated the ability of astrocytes to respond to pro-inflammatory cytokines, which stimulate transcription factors and cause induction of a number of genes, including inducible nitric oxide synthase (iNOS) *(43–45),* cyclooxygenase-2 (COX-2) *(46),* and secretory phospholipase A_2 ($sPLA_2$) *(43,47,48).* Increase in PLA_2 has been regarded as an important factor underlying a number of neurodegenerative diseases, and PLA_2 inhibitors have been shown to protect against neurotoxicity induced by oxidative stressors *(49–52).* Systemic injection of KA to rats was shown to enhance cytosolic PLA_2 immunoreactivity in the hippocampal area *(53).* In our study, systematic injection of low levels of KA into rats for 5 d resulted not only in neuronal cell death in the hippocampal area but also a large increase in reactive astrocytes and microglial cells (Figs. 4 and 5) *(26).* These results further demonstrate the intimate relation between glial cell activation and neuron cell death in excitotoxic injury in the brain.

KA Enhances Aging and Neurodegenerative Processes

Excitotoxicity and oxidative stress have been shown to play an important role in advancing aging processes and in the progression of a number of neurodegenerative diseases *(31,54).* Therefore, KA-induced hippocampal excitotoxicity and injury has been used as a model for studying the human aging process and neurodegenerative disorders *(10,23,26,31).* Oxidative stress markers, as demonstrated by formation of malondialdehyde (MDA) and protein carbonyls, increased significantly in the hippocampus at 4 h or 2 d after KA administration, a time prior to neuronal cell death. This increase was more pronounced in senile-prone animals *(55).*

Lipofuscin-like substances or age pigments are known to accumulate during the aging process *(56).* Kim and colleagues *(55)* observed an increase in fluorescent lipofuscin-like substances and lipofuscin granules 7 d after KA administration to the senescence-accelerated mice *(55).* Their results suggest that the aging-prone mice are more susceptible to KA-induced oxidative damage than normal mice *(55).* Dietary supplementation of vitamin E significantly lowered the accumulation of lipofuscin-like substances in the aging rat heart *(57).*

There is evidence linking the excitotoxicity mediated by KA to the progression of Alzheimer's disease (AD), one of the most prominent age-associated neurodegenerative disorders. AD is characterized by the impairment of cognitive function, neuronal cell loss, and deposition of senile plaques and tangles *(58).* Amyloid-β peptides (Aβ) with 40–42 amino acids are important components of the senile plaques and they are produced by processing of amyloid precursor protein (APP) with β- and γ-secretases. On the other hand, neurofibrillary tangles are intracellular deposits in neurons resulting from hyperphosphorylation of tau protein *(59).* Both Aβ and tau are important in the pathogenesis of AD.

There is some evidence linking excitotoxicity of KA and altered expression of APP in rat brain. KA administration was shown to stimulate synthesis of APP770, the major APP isoform in glial cell, and this effect was associated with increased astrogliosis and neuronal cell loss *(60).* On the other hand, KA administration was shown to decrease the neuronal form

of APP in rats *(61).* Increased Aβ immunoreactivity in the hippocampus of rats was observed after KA administration, and synergistic increase in neuronal damage was found in the hippocampus after coinjection with Aβ and KA *(62).* CNQX, a KA receptor antagonist, suppressed the neuronal loss induced by the Aβ/KA coinjection. CNQX also blocked neurotoxicity elicited by Aβ (1–42) in embryonic chick retina *(63).* These results suggest that neuronal excitation may exacerbate Aβ toxicity in rat hippocampus *(64).*

Less is known about KA-mediated excitotoxicity on neurofibrillary proteins. In human temporal lobe slices, tau phosphorylation was altered in a dose-dependent manner by the phosphatase inhibitor okadaic acid but not by NMDA or KA *(65).* However, glutamate toxicity elicited by NMDA and AMPA/KA could enhance tau gene expression in neuronal cultures *(66).* Neuronal loss was found in affected regions of the AD brain *(67).* Apoptotic neurons induced by glutamate showed a decrease in tau mRNA expression, whereas neurons resistant to apoptosis display either stable or increased tau mRNA levels *(68).* Therefore, experimental models of neuronal apoptosis can help to understand biochemical processes underlying cellular degeneration and pathogenesis in AD *(69).*

In addition to Aβ and tau protein, KA can alter other proteins that modulate amyloid fibril formation and senile plaque accumulation. In particular, the heparan sulfate proteoglycans (HSPGs) have been suggested to play an important role in the formation and persistence of senile plaques and neurofibrillary tangles. The basement membrane-associated HSPG, agrin, is widely expressed in senile plaques, neurofibrillary tangles, and cerebral blood vessels, whereas expression of perlecan, another basement membrane-associated HSPG, is restricted to the cerebral vasculature and is lacking in senile plaques and neurofibrillary tangles *(70–72).* During the first 3 d following KA injection, there was an increase in perlecan immunoreactivity in degenerating neurons in mouse hippocampus. This event was followed by glial cell activation, which was detectable from 5 d to 4 wk *(73).* This latter event was associated with formation of dense bundles of glial filaments similar to those found in reactive astrocytes. These results suggest that perlecan might play a role in the formation of senile plaques in AD *(73).*

Immunological complements have been postulated to contribute to inflammatory reactions associated with the neuropathology of AD *(74).* Microglial cells are the major CNS resident cells and are implicated to play an important role in the pathogenesis of AD. Microglial cells are major cells for biosynthesis of C1q, an initial component of the complement cascade *(75),* and immunohistochemical analysis showed the increase in C1q protein in senile plaques in the AD brain *(76).* In the AD brain, fibrillar Aβ was shown to bind C1 via C1q, the recognition component of the classical complement pathway, and thereby initiated an inflammatory cascade in the brain. On the other hand, C1q could enhance phagocytic activities of microglia, which could benefit in clearance of apoptotic cells or cellular debris *(77).* Inhibition of C1q–Aβ binding was shown to protect hippocampal cells against complement-dependent Aβ toxicity *(78).* C1q content was increased 20-fold in rat brain 3 d after systemic injection of KA. In parallel with these responses, KA induced a fivefold increase of C1q bioactivity *(79).* Based on these results, induction of C1q as a result of KA administration seems to offer a good model to explore the neural inflammatory responses in AD.

Resveratrol Can Protect Against KA-Induced Damage in the Hippocampus

Resveratrol, a polyphenolic compound enriched in grape and red wine, has attracted much attention lately because of its antioxidant and anti-inflammatory properties *(80–82).* Our in vitro studies with rat pheochromocytoma (PC-12) cells showed the ability of resveratrol

to ameliorate oxidative stress induced by *t*-butyl hydroperoxide *(83),* oxidized lipoproteins *(84,85),* and ethanol *(86–88).* Resveratrol also inhibited KA-induced ROS production in primary cortical neuron culture (Fig. 2A). Studies by others have also demonstrated the ability of resveratrol to ameliorate nitric oxide-related toxicity in cultured hippocampal neurons *(89)* and hydrogen peroxide-induced apoptosis in PC-12 cells *(90).* Previous studies have demonstrated the free-radical scavenging properties of resveratrol and the ability to suppress mitochondria-induced production of ROS in rat brain *(91).* Resveratrol also inhibited lipid peroxidation *(83,92)* and protected against neuronal injury in stroke-prone spontaneously hypertensive rats *(93)* and oxidative DNA damage in vitro *(94).* As a result of its amphipathic property, resveratrol can efficiently cross the blood–brain barrier and exert neuroprotective effects on the brain *(95).* Resveratrol also protected neuronal cells against KA-induced excitotoxic damage and suppressed KA-induced glial cell activation (Figs. 4 and 5) *(26).* Because glutamate excitotoxicity has been implicated in the progression of many neurodegenerative diseases, compounds such as resveratrol could offer beneficial effects in retarding or preventing the progression of these diseases.

Summary

This review provides information about the abundance of the ionotropic Ca-A/K receptors in neurons in specific brain regions and their contribution to the vulnerability of these neurons to excitotoxicity. Stimulation of the affected neurons by KA results in excessive Ca^{2+} influx and triggers a train of activities that lead to the activation of Ca^{2+}-dependent proteases, kinases, and nucleases and the generation of ROS. In turn, these biochemical changes result in alteration of mitochondrial function and lead to the increase in apoptotic and necrotic cell death pathways. Consequently, KA-mediated excitotoxicity can be used as a model to unveil mechanisms of neurodegenerative pathways and to shed light on our understanding of age-related neurodegenerative diseases.

Acknowledgments

This work was supported in part by DHHS grants 5 P01 ES10535 and 1P01 AG 18357 from NIH. Thanks are owed to Ms. Alisa Nettles-Strong for help in preparation of the manuscript.

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