Mitochondrial Dysfunction and Oxidative Stress in Seizure-Induced Neuronal Cell Death

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Abstract- Epilepsy is considered one of the most common neurological disorders worldwide. The burst firing associated with prolonged epileptic discharges could lead to a large number of changes and cascades of events at the cellular level. From its role as the cellular powerhouse, the mitochondrion is emerging as a key participant in cell death because of its association with an ever-growing list of apoptosis-related proteins. Prolonged seizures may result in the mitochondrial dysfunction and increased production of reactive oxygen species and nitric oxide (NO) precede neuronal cell death and cause subsequent epileptogenesis. Emerging evidences also showed that intrinsic mitochondrial apoptotic pathway may contribute to the neuropathology of human epilepsy, particularly in the hippocampus. Subsequent laboratory studies in the animal model of status epilepticus provide credence to the notion that activation of nuclear factor-κB upregulates NO synthase (NOS) II gene expression with temporal correlation of NOS II derived NO-, superoxide anion- and peroxynitrite-dependent reduction in mitochondrial Complex I activity, leading to apoptotic neuronal cell death in the hippocampus. These results will broaden our understanding on the intimate link between mitochondrial function, oxidative stress and mitochondria-dependent apoptotic signaling triggered by epileptic seizures. It will open a new vista in the development of more effective neuroprotective strategies against seizure-induced brain damage by modification of bioenergetic failure in the mitochondria and in the design of novel treatment perspectives for therapy-resistant forms of epilepsy.

Key Words: Mitochondrial dysfunction, Oxidative stress, Epileptic seizures, Cell death, Hippocampus

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INTRODUCTION

Epilepsy is considered one of the most common neurological disorders worldwide, with a prevalence of 0.5-1% in the general population[1]. It is a chronic dynamic medical problem characterized by recurrent unprovoked seizures that often requires long-term antiepileptic drug therapy[2]. The burst firing associated with prolonged epileptic discharges could lead to a large number of changes and cascades of events at the cellular level, such as activation of glutamate receptors, changes in composition of glutamate and γ-aminobutyric acid

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receptor, cytokine activation, oxidative stress, neoneurogenesis, changes in plasticity or activation of some late cell death pathways[3-5]. Clinical and epidemiological studies suggest that patients with chronic epilepsy undergo progressive brain degeneration that is accompanied by long-term behavioral changes and cognitive declines despite optimal antiepileptic drug therapy[6,7]. Status epilepticus, or the condition of prolonged epileptic seizures, is a major neurological and medical emergency that is associated with significant morbidity and mortality[8]. Status epilepticus in humans[9,10] and animal models[3,11,12] results in significant cerebral damage and increases the risk of subsequent seizures, alongside a characteristic pattern of neuronal cell loss preferentially in the hippocampus. The hippocampus is especially vulnerable with selective neuronal loss in the hilus, CA1 and CA3 regions[11,12]. As a result, there has been considerable interest in defining the molecular pathways involved in seizure-induced neuronal cell death in the vulnerable hippocampus. Oxidative stress and mitochondrial dysfunction could be acute consequences of status epilepticus that are related to the mechanism of seizure-induced neuronal cell death and subsequent epileptogenesis[13]. This article will focus on the potential role of mitochondrial dysfunction and oxidative stress in seizure-associated apoptotic cell death in the hippocampus.

INSIGHTS FROM ANIMAL MODELS

Although animal models have many limitations, studies in animal models have made important contributions to our understanding of seizure-related neuronal injury. Animal studies provide the opportunity to examine anatomical, chemical, cellular, molecular and functional changes after seizures, as well as to identify age- or sex-specific consequences from seizures[14-16]. A wide variety of animal models of epilepsy and status epilepticus had been used, including electrical stimulation models, chemoconvulsant-induced models (e.g. kainic acid; KA, pilocarpine, picrotoxin or bicucullin), physical models (e.g. hyperthermia, or photic or auditory stimulation), genetic models (e.g. mutant, transgenic or knockout) and spontaneous seizure models (e.g. post-kindling or post-chemoconvulsant). In the future, we can anticipate that animal models will move into a new millennium. A new trend in animal models is emerging that promises to offer powerful insights into the cause and effect of seizures. However, extrapolation of conclusion from animal data to human beings must be done with caution, despite apparent neuropathological similarities between experimental and human epilepsies[3,15,16].

Systemic or intracerebral injection of KA, a powerful excitotoxin which stimulates a subtype of the ionotropic receptor of neurotransmitter glutamate, can result in sustained epileptic activity in the hippocampus followed by a selective pattern of brain damage that neuropathological changes are similar to human temporal lobe epilepsy[12,17]. Microinjection of KA into the CA3 subfield of hippocampus in anesthetized rats elicited seizure-like hippocampal electroencephalography (hEEG) activity and simultaneous power spectral changes in hEEG[18,19]. Based on this experimental model, we carried out electrophysiological, biochemical, immunohistochemical, anatomical and electron microscopic investigations on the ipsilateral (injection side for KA) and the contralateral (recording side for hEEG) hippocampal CA3 subfield in our recent studies[19-22]. This allowed us to ascertain that results from those analyses were consequential directly to experimental temporal lobe status epilepticus and not indirectly to KA excitotoxicity.

NEURONAL CELL DEATH IN THE HIPPOCAMPUS FOLLOWING STATUS EPILEPTICUS

Seizure-induced neuronal cell death is no exception to the emerging complexities of the molecular control of neurodegeneration, and there is controversy as to whether cell death occurs in a programmed/controlled (apoptotic) or uncontrolled/passive (necrotic) manner. The nature of hippocampal neuronal cell death following prolonged seizures has been reported as either apoptotic[23-25], necrotic[5,26] or both[27,28]. Necrosis is generally taken as the principal morphological phenotype of dying cells after seizures[11,29], based at least on classical definitions and morphological criteria. However, programmed

cell death mechanisms associated with cellular apoptosis have been shown to be activated by experimental status epilepticus that supports apoptotic cell death plays an important role in seizure-induced brain damages[22-25,30]. Factors such as the variation in duration and severity of seizures, metabolic disturbances, bioenergic failure during or after seizures and age- or genetic- specific factors may all contribute to determining the eventual pathway of cell death[3,22,23]. It is probably that severe or prolonged seizure discharges trigger both forms of neuronal cell death, whereas brief seizures may result in apoptosis in the same or different neuronal population[23]. Apoptotic neuronal cell death is much prominent after seizure-induced excitotoxic injury in immature brain compared with mature brain[3].

Apoptosis emerged as a focus of research on seizure-induced neuronal cell death in the mid-1990s. In the earlier work, researchers detected in situ 'apoptotic' DNA fragmentation (detected by terminal deoxynucleotidyl dUTP nick end labelling; TUNEL) and DNA laddering in tissue samples from the rat brain after prolonged seizures[31]. Based on an animal model of experimental status epilepticus, our recent studies[20-22] revealed that seizure-induced apoptotic cell death was detected in the vulnerable CA1 and CA3 neurons 1-7 days after a low dose of intrahippocampal administration of KA (Fig. 1). We recognize that during a prolonged seizure, neuronal cells may exhibit a temporary drop in adenosine triphosphate (ATP) production[22]. A critical determinant of the eventual cell death fate resides in intracellular ATP concentration, the production of which depends on the structural and functional integrity of the mitochondria. Whereas ATP depletion is associated with necrosis, ATP is required for the development of apoptosis[32]. Our recent research noted that preserved mitochondrial ultrastructural integrity and maintained energy metabolism following experimental status epilepticus is associated specifically with apoptotic, not necrotic, cell death in hippocampal CA3 or CA1 neurons[22]. Since intermediate forms of cell death with both necrotic and apoptotic features have been found after seizures[26-28], further investigations for the detailed mechanisms of different pathways of cell death is needed.

MITOCHONDRIA AND APOPTOTIC SIGNALLING PATHWAYS IN SEIZURE-INDUCED NEURONAL CELL DEATH

Mitochondria are ubiquitous intracellular organelles enclosed by a double membrane-bound structure. The

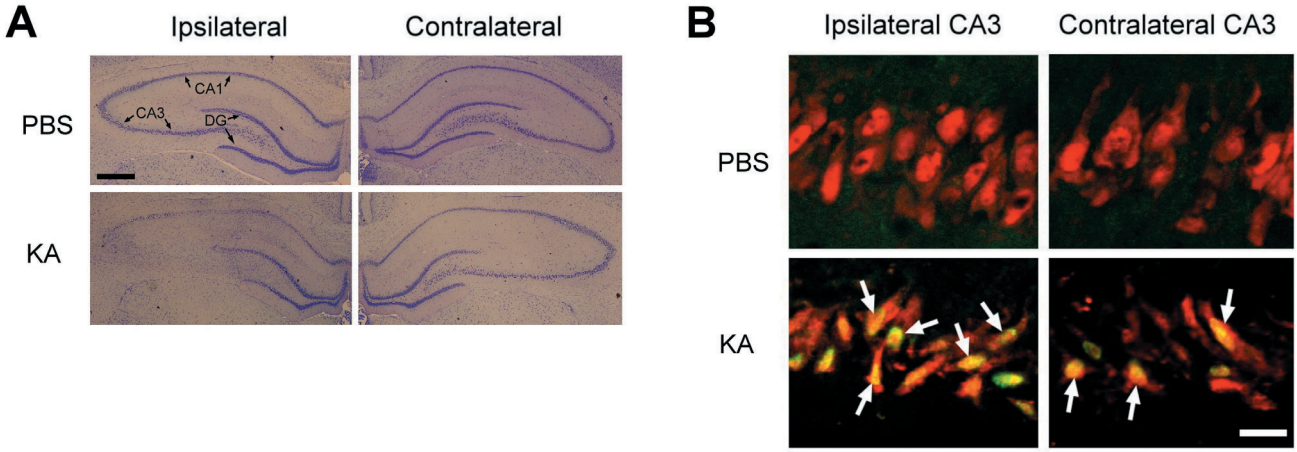

Figure 1. (A) Sections stained with cresyl violet showing neuronal cell loss in the bilateral hippocampal CA3 subfields 7 days after microinjection of kainic acid (KA, 0.5 nmol) into the left hippocampal CA3 subfield in rats. DG: dentate gyrus. Scale bar, 5 mm. (B) Laser scanning confocal microscopic images of bilateral CA3 subfield of hippocampus showing pyramidal cells that were immunoreactive to a neuronal marker, NeuN (red fluorescence), or were additionally stained positively for TUNEL (green fluorescence), 7 days after microinjection of PBS or KA (0.5 nmol) into the left hippocampal CA3 subfield in rats. Note that double-labeled neurons displayed yellow fluorescence and were denoted by arrows. Scale bar, 20 μm.

primary function of mitochondria is the production of cellular energy in the form of ATP by the mitochondrial respiratory chain through oxidative phosphorylation[33]. Mitochondrial oxidative phosphorylation consists of five multienzyme complexes (Complexes I-V) located in the mitochondrial inner membrane. Biochemical evidence suggested that the majority of cerebral ATP consumption is used for operation of the electrogenic activity of neurons[34]. Adequate energy supply by mitochondria is essential for neuronal excitability and neuronal survival.

From its role as the cellular powerhouse, the mitochondrion is emerging as a key participant in cell death because of its association with an ever-growing list of apoptosis-related proteins[35,36]. A variety of key events in apoptosis focuses on mitochondria, including the release of several apoptogenic factors (such as cytochrome c, apoptosis-inducing factor; AIF, endonuclease G, Smac/DIABLO and HtrA2/OMI), changes in electron transport, loss of mitochondrial transmembrane potential, altered cellular oxidation-reduction, and participation of pro- and antiapoptotic Bcl-2 family proteins[35-38]. One of the decisive steps of the apoptotic cascade is related to the mitochondrial permeability transition pore (MPTP)[39]. Transient opening of these non-specific pores in the mitochondrial inner membrane under conditions of cellular stress causes the mitochondrial transmembrane potential to collapse, and triggers the release of cytochrome c and other proapoptotic molecules that initiate the apoptotic cascade. Growing evidence also suggests that cytochrome c participates in mitochondrial pathways of apoptosis by translocation to the cytoplasm where it activates caspase-3 via triggering the caspase-9 pathway[37,39].

Emerging evidences suggest that the intrinsic mitochondrial apoptotic pathway plays an important role in neuronal cell death after seizures[21,22,25,40]. Mitochondrial calcium loading has long been known to be related with acute neuronal pathological changes following status epilepticus[41]. Using a rat model of focally evoked status epilepticus we demonstrated that cytochrome c releases from the damaged mitochondria in the hippocampal neurons within 1 day following seizures[20]. Moreover, characteristic biochemical (DNA fragmentation), histochemical (TUNEL or activated caspase-3 staining) or ultrastructural (electron microscopy) features of apoptotic cell death were presented bilaterally in the hippocampus 1-7 days after the elicitation of sustained hippocampal seizure activity by microinjection of KA into the unilateral CA3 subfield[20-22]. Other studies have corroborated that seizures up-regulate caspase-3 within affected neuronal and glial populations in limbic regions such as the entorhinal cortex, amygdala and hippocampus[30,42-44]. Cytochrome c release in the damaged hippocampus following seizures, whereupon it associated with Apaf-1, commensurate with the appearance of activated caspases-9 and -3 and subsequently DNA fragmentation[20,44,45].

In addition to caspase-dependent cell death pathways, mitochondria release proteins also propagate caspase-independent neuronal apoptotic cascade. While AIF is an important mitochondria release protein via caspase-independent cell death pathway, there is evidence that the calpain-mediated release of AIF is important in seizure-induced neuronal cell death[46-48].

Bcl-2 family proteins, like caspases, are involved in regulating seizure-induced neuronal cell death. The evidence of Bcl-2 family involvement in seizure-induced neuronal cell death has also been demonstrated[49-51]. Upstream pro-apoptotic BH3 domain (Bcl-2 homology domain 3)-only members BAD, Bid and Bim, can be activated via calcium-dependent mechanisms and each was found to be activated by seizures[49,50]. In our recent studies (unpublished data), we also noted that translocation of the Bax particle from cytosol to mitochondria in hippocampal CA3 neurons 3 h after experimental status epilepticus, and this coincides with the timing of cytochrome c release. Moreover, the level of serum Bcl-2 significantly increased in patients with uncontrolled epilepsy[52]. In patients with intractable temporal lobe epilepsy, tissues from temporal neocortex expressed raised levels of Bcl-2, Bcl-XL and activated caspase-3[53]. Correlative analysis showed the expression of p53, fas and caspase-3 were positively correlated with seizure frequency in the resected samples of sclerotic hippocampi from patients with mesial temporal sclerosis[54]. These clinical evidences also suggest that intrinsic mitochondrial apoptotic pathway may contribute to the neuropathology of human epilepsy, particularly in the hippocampus.

MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS FOLLOWING EPILEPTIC SEIZURES

Mitochondrial Dysfunction Following Epileptic Seizures

Mitochondrial oxidative phosphorylation provides the major source of ATP in the cortical neurons[34]. Sustained epileptic seizures will change the redox potential and decline the ATP content that may lead to collapse of energy production and supply in the brain[11]. However, whether mitochondrial dysfunction occurs following epileptic seizure is still under debate. There are only limited evidences for mitochondrial dysfunction associated with epilepsy and status epilepticus by both using animal models and human samples (see Table 1 for review)[19,55-61]. In our studies[19], enzyme assay for the key enzymes of mitochondrial respiratory chain revealed significant depression of the activity of nicotinamide adenine dinucleotide cytochrome c reductase (Complex I+III) in the dentate gyrus, CA1 and CA3 subfields of hippocampus following 180 minutes after KA-induced temporal lobe status epilepticus. On the other hand, the activities of succinate cytochrome c reductase (Complex II+III) and cytochrome c oxidase (Complex IV) remained unaltered. After 180 minutes of microinjection of KA into the CA3 subfield, significant mitochondrial ultrastructural injury was observed in hippocampus (Fig. 2). It follows that the prolonged epileptic seizures probably lead to a dysfunction of Complex I in the mitochondrial electron transport chain and mitochondrial ultrastructural injury in the hippocampus.

Complex I is markedly more susceptible to oxidative stress and glutationylation than other respiratory chain complexes[62]. It is a major source of superoxide anion ($O2^{\cdot -}$), making it a candidate for increased mitochondrial reactive oxygen species (ROS) production and redox signaling[62,63]. Dysfunction of Complex I may lead to

Table 1. *Evidences of mitochondrial dysfunction following epileptic seizures from animal and human studies*

Reference	Model	Samples	Experimental findings
Kunz et al.[55]	KA-treated rats	Hippocampal slices	Increased basal energy turnover with glucose as substrate Higher uncoupled rate of respiration
Kunz et al.[56]	Temporal lobe epilepsy (human)	Hippocampal specimens	Mitochondrial Complex I deficiency and ultrastructural abnor malities of mitochondria in the epileptic focus
Cock et al.[57]	Perforant path stimulation model of rats	Whole brain tissues	Reduction of brain aconitase and α-ketoglutarate dehydro genase activities Decrease in reduced glutathione levels
Kudin et al.[61]	Pilocarpine-treated rats	Hippocampal tissues and slices	Decline of the activities of Complexes I and IV and lower mitochondrial membrane potential in CA1 and CA3 sub fields Decrease in mitochondrial DNA copy number in CA3
Chuang et al.[19]	Microinjection of KA into the hippocampus of rats	Hippocampal tissues	Dysfunction of Complex I in the mitochondrial electron transport chain and mitochondrial ultrastructural injury
Gibbs et al.[60]	Perforant path stimulation model of rats	Hippocampal tissues	Reductions in glutathione, α-ketoglutarate dehydrogenase, aconitase, citrate synthase, and Complex I activities
Gao et al.[59]	Pilocarpine-treated rats	Hippocampal tissues	Depression of mitochondrial- and nuclear-encoded COX activity and COX III expression Mitochondrial ultrastructural damage
Folbergrová et al.[58]	Intracerebroventricular infusion of homocysteic acid in rats	Cerebral cortex	Mitochondrial Complex I inhibition

KA: kainic acid, COX: cytochrome oxidase

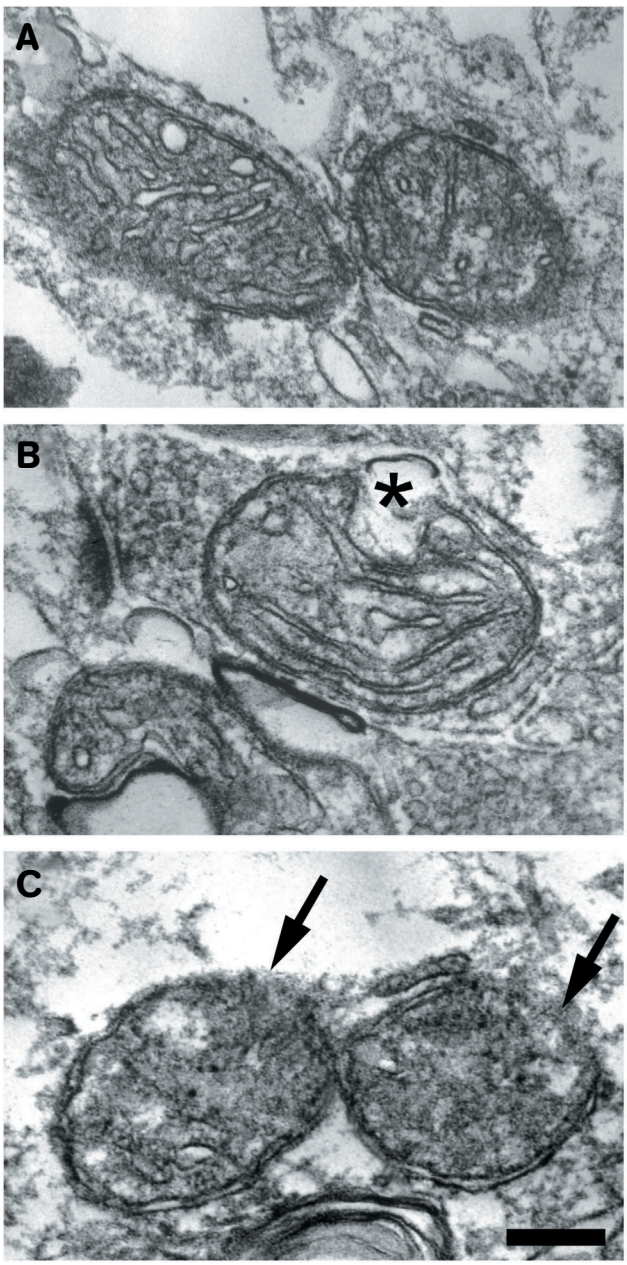

Figure 2. Representative electron photomicrographs of mitochondrial ultrastructure in hippocampal CA3 subfield. (A) Intact mitochondrial ultrastructure 30 min after microinjection of kainic acid (KA) into the hippocampus. (B) Mild mitochondrial damage 180 min after microinjection of KA into the hippocampus. Note swelling of all mitochondrial spaces, particular in cristae (asterisk). (C) Severe mitochondrial damage 180 min after hippocampal application of KA. Note severe mitochondrial swelling accompanied by a disruption in membrane integrity (arrows). Scale bar: 200 nm.

incomplete mitochondrial electron transport and decreased ATP production[63]. The selective loss of Complex I activity contributes to neurodegenerative diseases such as Parkinson's disease and Huntington's disease[64]. Based on this animal model, we have demonstrate that the time-dependent selective dysfunction in activity of Complex I. Whereas the pilocarpine-treated rats with spontaneous seizures exhibited a selective decline of the activities of Complexes I and IV in the hippocampal CA1 and CA3 subfields[61], corroborating reports of mitochondrial Complex I inhibition after seizures have since emerged from other laboratories[58,60]. This pattern of mitochondrial respiratory chain dysfunction is strengthened by the finding of patients with refractory temporal lobe epilepsy showing Complex I deficiency in the CA3 subfield[56]. Thus, we proposed that the selective dysfunction of Complex I may be linked to seizure-induced neuronal cell death in the hippocampus and play an important role in the intrinsic mitochondrial apoptotic pathway.

Oxidative Stress Following Epileptic Seizures

ROS and reactive nitrogen species has been implicated in neuronal cell death in both acute and chronic neurological diseases such as stroke, trauma, spinal cord injury, Parkinson disease, Alzheimer disease, Huntington disease, Freidrich ataxia, and amyotrophic lateral sclerosis[64,65]. Oxidative stress is thought to be an important consequence of glutamate receptor activation and excitotoxicity[13,65], which play a critical role in epileptic brain damage[13,65]. Data from animal studies suggested that prolonged seizure activity might result in the increased production of ROS and generation of nitric oxide (NO) and peroxynitrite preceded neuronal cell death in vulnerable brain regions[20,21,66-69].

Under normal physiological conditions, 1-2% of molecular oxygen consumed by mammalian cells is metabolized to ROS via electron leakage from mitochondrial electron transport chain. Therefore, the mitochondrial respiratory chain, in particular Complex I, is a primary site for leakage of electrons from the transport chain, leading to an increase in ROS generation[70,71]. Since mitochondria are a major source of ROS production, impaired mitochondrial respiratory chain function

and calcium-dependent depolarization of mitochondrial membrane potential may further lead to incomplete O2 consumption, reduced production of ATP and exacerbated overproduction of ROS[13,63]. Free radicals can damage all cell structures, including lipids, proteins, DNA and mitochondrial membrane structure[63]. As inhibition of mitochondrial respiratory chain by prolonged seizures results in excess free radical production, and free radicals themselves are direct inhibitors of the mitochondrial respiratory chain, this can result in a vicious cycle that leads to oxidative cell damage[13,63,72].

Nitric oxide (NO) is a free radical that is widely regarded as a molecular messenger that participates in diverse physiological processes in the central nervous system (CNS), including brain development, pain perception, neuronal plasticity, memory and behavior[73]. A pathological role for NO in neurological diseases, including epilepsy, has also been described[20,21,73-75]. The functional significance of NO in epileptic seizures, however, remains controversial. Hippocampal neuronal damage caused by excessive formation of NO has been implicated in experimental epilepsy models using acetylcholinesterase inhibitors[74] or glutamatergic receptor agonists[69,75-77] as the seizure-inducing agents. However, the roles of NO synthase (NOS) isoforms in the seizure-induced neuronal cell death are also unsettled. The mechanism that triggers limbic seizures and delayed excitotoxic damage in the hippocampus is generally attributed to NO generated by neuronal NOS (NOS I or nNOS)[74-76,78]. The role of inducible NOS (NOS II or iNOS) or endothelial NOS (NOS III or eNOS) in seizure-induced neuronal cell death is unclear. Based on the experimental model of status epilepticus, our recent studies provided credence to the notion that NO-, $O2^{\bullet-}$ and peroxynitrite-activated mitochondrial apoptotic signaling underlies neuronal damage induced by status epilepticus[20,21]. The repertoire of cellular events elicited by sustained seizure activity in the hippocampal CA3 subfield entails overproduction in NOS II-derived NO, increase in $O2^{\bullet-}$ -production, formation of peroxynitrite and depression of Complex I in the mitochondrial respiratory chain, followed by translocation of cytochrome c from mitochondria to the cytosol and activation of caspase-3, leading to apoptotic cell death[19-21]. More recently, we noted that transcriptional upregulation of NOS II gene by nuclear factor-κB (NF-κB) promotes apoptotic neuronal cell death in hippocampal CA3 neurons (unpublished data).

The mitochondrial respiratory chain is sensitive to both NO- and peroxynitrite-mediated mitochondrial damage[79]. NO is known to depress mitochondrial respiratory functions, and its transnitrosylation product, S-nitrosothil, depresses Complex I activity[79,80]. In addition, peroxynitrite can inhibit mitochondrial Complex I by tyrosine nitration[81]. Blockade of NOS II activity or reduction of peroxynitrite in the hippocampal CA3 subfield, which antagonized the reduced mitochondrial Complex I activity, also blunted apoptotic cell death induced by experimental temporal lobe status epilepticus[20,21]. Complex I of the respiratory chain has also been suggested to be a constituent of the MPTP[82], the opening of which purportedly causes the mitochondrial transmembrane potential to collapse, and triggers the mitochondrial pathways of apoptosis by releasing cytochrome c to the cytoplasm where it activates caspase dependent death pathway[83]. It is thus reasonable to speculate that inhibition of mitochondrial respiratory Complex I by NO and peroxynitrite may trigger apoptosis following prolonged seizures by eliciting a reduction in mitochondrial transmembrane potential as a consequence of MPTP opening.

Mitochondrial production of $O2^{\bullet-}$ is a known contributor to apoptosis[70], and $O2^{\bullet-}$-dependent mitochondrial signaling in apoptotic cell death after brain damage has been reported[84]. We also demonstrated recently that $O2^{\bullet-}$ production in the hippocampal CA3 subfield manifested a significant elevation 3-24 h following induction of experimental temporal lobe status epilepticus[21]. Compounds with $O2^{\bullet-}$ scavenger, Tempol, or an electron carrier protein, coenzyme Q10 ameliorated seizure-induced $O2^{\bullet-}$ production and the depression of Complex I, and prevent apoptotic cell death in the hippocampus further suggest an association between mitochondrial respiratory activities and the generation of $O2^{\bullet-}$ in the hippocampus during experimental status epilepticus[21]. In addition, NO augments the generation of reactive oxy-

gen species through interaction with components of the mitochondrial electron transport chain, thereby triggering mechanisms of cell death[85].

THE ROLE OF MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS IN EPILEPTOGENESIS

Because neuronal cell death may be an important factor contributing to epileptogenesis, mechanisms that influence neuronal viability may also play a role in the process of epileptogenesis. As impairment of mitochondrial function and increased ROS have recently been observed in the seizure focus of human and experimental epilepsy[13,86], the crucial question is whether seizure-induced free radical production and mitochondrial dysfunction results in chronic redox alterations in neurons that increase seizure susceptibility and lead to the development of subsequent epilepsy. The most prominent example of mitochondrial dysfunction causing epilepsy is the occurrence of epileptic seizures in mitochondrial diseases arising from mutations in mitochondrial DNA (mtDNA) or nuclear DNA[86-90]. Defects in the process of oxidative phosphorylation in the CNS are a characteristic sign of mitochondrial encephalomyopathies. The most common mitochondrial disorders presenting with an epileptic phenotype has been well reviewed by Kudin et al[86]. A well known mitochondrial disorder with generalized seizures which is linked to point mutations in the mitochondrial $tRNA^{Leu}$ gene[90] is the myoclonus epilepsy with ragged red fibers (MERRF) syndrome. Partial seizures are frequently noticed in mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS) syndrome, which is associated with mutations in the mitochondrial $tRNA^{Leu}$ gene[88,89]. In addition, systemic administration of mitochondrial toxins, such as 3-nitropropionic acid[91] and cyanide[92], inhibits the functions of mitochondrial respiratory chain that can compromise cellular energy metabolism and induce seizures in animal models. These accumulating evidences implicated that both mtDNA mutations and exogenous mitochondrial toxins cause mitochondrial respiratory chain dysfunction which is associated with at least some of the mechanisms of epileptogenesis.

Several common neurological conditions such as hypoxia, stroke, traumatic brain injury, aging and neurodegenerative diseases render the brain susceptible to epileptic seizures[13,93]. In fact, increased oxidative stress and mitochondrial dysfunction is the common cellular events under these neuropathologic conditions. Mice with partial deficiency of the mitochondrial superoxide dismutase show increased incidence of spontaneous and handling-induced seizures that correlates with chronic mitochondrial oxidative stress[13]. Increased oxidative mtDNA damage, mitochondrial H2O2 production and alterations in the mitochondrial base excision repair pathway have been noted in the rat hippocampus after a period of 3 months following status epilepticus. The data provide evidence for mitochondrial oxidative stress in epilepsy and suggest that mitochondrial injury may contribute to epileptogenesis[94]. These evidences raise an intriguing possibility that mitochondrial dysfunction initiated by free radical production could increase susceptibility of seizure[13].

The mechanisms of mitochondrial dysfunction and oxidative stress during epileptogenesis remain unclear. Since mitochondrial oxidative phosphorylation provides the major source of ATP in neurons and mitochondria participate in intracellular calcium homeostasis, their dysfunction strongly affects neuronal excitability and synaptic transmission[13,86]. Thus, decreased intracellular ATP levels and alterations of neuronal calcium homeostasis may be potential factors contributing to increased susceptibility of epileptic seizures associated with mitochondrial dysfunction. These changes strongly affect neuronal excitability and synaptic transmission, which is proposed to be highly relevant for seizure generation[13,86]. Further studies are mandatory in the future to confirm this implication.

CONCLUSION

Oxidative stress and mitochondrial dysfunction occur as a consequence of prolonged epileptic seizures and contribute seizure-induced neuronal cell death. Subsequent laboratory studies[19-22] in the animal model of

status epilepticus provide credence to the notion that activation of NF-κB in hippocampal CA3 neurons upregulates NOS II gene expression with temporal correlation of NOS II derived NO-, $O_2^{\cdot-}$- and peroxynitrite-dependent reduction in mitochondrial respiratory enzyme Complex I activity, leading to apoptotic neuronal cell death in the hippocampus (summary in Fig. 3). Recent studies suggested that mitochondrial dysfunction and chronic oxidative stress can render the brain more susceptible to epileptic seizures[13,94,95]. Therefore, both of mitochondrial dysfunction and oxidative stress are important causes and consequences of prolonged seizures. In keeping with the role of mitochondrial dysfunction and oxidative stress in the pathogenesis of apoptosis, protection of the mitochondrion from NO-, $O_2^{\cdot-}$- or peroxynitrite-promoted neuronal stress in the hippocampus may therefore become a novel target for therapeutic strategy against seizure-induced brain damage and epileptogenesis[13,37,83,85].

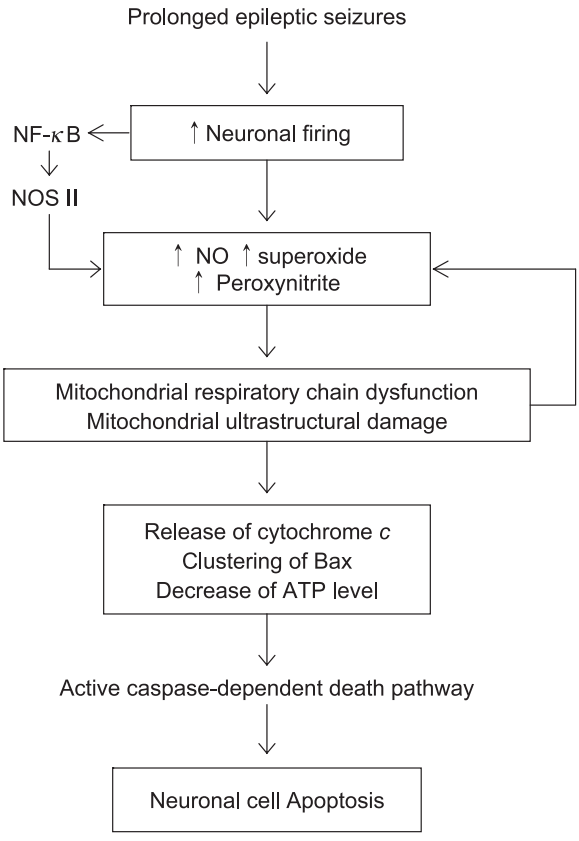

Figure 3. A schematic outline of the proposed role of mitochondrial dysfunction and oxidative stress in seizure-induced neuronal cell death from subsequent laboratory studies in the animal model of status epilepticus.

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