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The potential role of mitochondrial dysfunction in seizure-associated cell death in the hippocampus and epileptogenesis

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Abstract Epilepsy is considered one of the most common neurological disorders worldwide. The burst firing neurons associated with prolonged epileptic discharges could lead to a large number of changes with events of cascades at the cellular level. From its role as the cellular powerhouse, mitochondria also play a crucial role in the mechanisms of cell death. Emerging evidence has shown that prolonged seizures may result in mitochondrial dysfunction and increase of oxidative and nitrosative stress in the hippocampus that precede neuronal cell death and cause subsequent epileptogenesis. The selective dysfunction of mitochondrial respiratory chain Complex I has been suggested to be a biochemical hallmark of seizure-induced neuronal cell death and epileptogenesis. Therefore, protection of mitochondria from bioenergetic failure and oxidative stress in the hippocampus may open a new vista to the development of effective neuroprotective strategies against seizure-induced brain damage and to the design of novel treatment perspectives against therapy-resistant forms of epilepsy.

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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 (Hauser et al. 1991). The word epilepsy is derived the Greek verb epilambanein (επιλαμϖανειν), which means "to be seized, to be taken hold of, or to be attacked." The medical condition of epilepsy is as old as human existence. It is characterized by recurrent, usually unprovoked epileptic seizures that result from excessive, synchronous, abnormal firing pattern of neurons located predominantly in the cerebral cortex. The burst firing neurons associated with epileptic discharges could lead to a large number of changes with events of cascades at the cellular level, such as activation of glutamate receptors, changes in composition of glutamate and γ-aminobutyric acid receptor, cytokine activation, oxidative stress, neoneurogenesis, changes in plasticity or activation of some late cell death pathways (Haut et al. 2004; Henshall and Simon 2005).

Mitochondria are ubiquitous intracellular organelles enclosed by a double membrane-bound structure. The primary function of mitochondria is the production of cellular energy in the form of adenosine triphosphate (ATP) by the mitochondrial respiratory chain through oxidative phosphorylation. Mitochondrial oxidative phosphorylation consists of five multienzyme complexes (Complexes I–V) located in the mitochondrial inner membrane (Hatefi 1985). Biochemical evidence suggested that the majority of cerebral ATP consumption is used for operation of the electrogenic activity of neurons (Ames 2000). Adequate

energy supply by mitochondria is essential for neuronal excitability and neuronal survival. In addition to the energy production, mitochondria also play a crucial role in the maintenance of intracellular calcium homeostasis, generation of reactive oxygen species (ROS) and mechanisms of cell death. The function of mitochondria has been implicated as important factors in the pathogenesis of many neurodegenerative diseases, such as Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and Alzheimer's disease (Lin and Beal 2006). However, there is relative paucity of data about the role of mitochondria in epilepsy. This minireview will focus on the potential role of mitochondrial dysfunction in seizure-associated cell death in the hippocampus and epileptogenesis.

Mitochondria and neuronal cell death in epilepsy

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 (Fujikawa 2005; Henshall and Simon 2005). Programmed cell death mechanisms associated with cellular apoptosis have been shown in human and animal studies that support apoptotic cell death playing an important role in seizure-induced brain damages (Bengzon et al. 2002; Henshall and Simon 2005). Factors such as variation in duration and severity of seizures, metabolic disturbances, bioenergetic failure during or after seizures and age— or genetic— specific factors may all contribute to determining the eventual pathway of cell death (Haut et al. 2004). 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 (Leist et al. 1997). Our recent research noted that preserved mitochondrial ultrastructural integrity and maintained energy metabolism during a prolonged seizure is associated specifically with apoptotic cell death in hippocampal CA3 or CA1 neurons(Chuang et al. 2009b).

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 (Green and Kroemer 2004; Kroemer 1999). A variety of key events in apoptosis focus 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 (Green and Kroemer 2004; Saelens et al. 2004). One of the decisive steps of the apoptotic cascade is related to the mitochondrial permeability transition pore (Crompton 2000). 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 suggest that cytochrome *c* is released in the damaged hippocampus following seizures, whereupon it is associated with Apaf-1, commensurate with the appearance of activated caspases-9 and -3 and subsequently DNA fragmentation (Crompton 2000; Green and Kroemer 2004; Saelens et al. 2004).

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 (Henshall et al. 2002; Murphy et al. 2010; Shinoda et al. 2004). 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 (Henshall et al. 2002; Shinoda et al. 2004). Moreover, the level of serum Bcl-2 significantly increased in patients with uncontrolled epilepsy (El-Hodhod et al. 2006). In patients with intractable temporal lobe epilepsy, tissues from the temporal neocortex expressed raised levels of Bcl-2, Bcl-XL and activated caspase-3 (Henshall et al. 2000). Correlative analysis showed the expression of p53, Fas and caspase-3 was positively correlated with seizure frequency in the surgically resected samples of sclerotic hippocampi from patients with mesial temporal sclerosis (Xu et al. 2007). These clinical evidences also suggest that the intrinsic mitochondrial apoptotic pathway may contribute to the neuropathology of human epilepsy, particularly in the hippocampus.

Evidences of mitochondrial dysfunction following epileptic seizures

Sustained epileptic seizures will change the redox potential and decrease the ATP content that may lead to collapse of energy production and supply in the brain (Wasterlain et al. 1993). However, whether mitochondrial dysfunction occurs following epileptic seizures is still under debate. There was only limited evidence for mitochondrial dysfunction associated with epilepsy and status epilepticus both in animal models (Chuang et al. 2004; Cock et al. 2002; Folbergrová et al. 2007; Folbergrová et al. 2010; Gibbs et al. 2006; Sleven et al. 2006) and human samples (Kunz et al. 2000).

In our studies (Chuang et al. 2004), enzyme assay for the key enzymes of the 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 the hippocampus following 180 minutes after kainic acid-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. These changes were accompanied by swelling of mitochondrial spaces and membrane disruption the suggested that dysfunction of Complex I in the mitochondrial electron transport chain and mitochondrial ultrastructural injury in the hippocampus were associated with prolonged seizures.

In pilocarpine-treated rats exhibiting spontaneous seizures, a selective decline of the activities of Complex I and IV of the respiratory chain and decreased respiration rates was noted in hippocampal CA1 and CA3 pyramidal subfields (Kudin et al. 2002). These changes were accompanied by a decrease in mitochondrial DNA copy number in the CA3 subfield suggesting that seizure activity downregulates the expression of mitochondrial-encoded enzymes of oxidative phosphorylation. Additionally, corroborating reports of mitochondrial Complex I inhibition after seizures have emerged from other laboratories using different animal models (Folbergrová et al. 2007; Folbergrová et al. 2010; Gibbs et al. 2006; Sleven et al. 2006). This pattern of mitochondrial respiratory chain dysfunction is further strengthened by the finding of patients with refractory temporal lobe epilepsy showing Complex I deficiency in the CA3 subfield (Kunz et al. 2000).

Complex I of the mitochondrial electron transport chain is markedly more susceptible to both oxidative and nitrosative stress than other respiratory chain complexes (Cadenas and Davies 2000). It is considered as one of the major source of superoxide anion ($O_2^{\bullet-}$), making it a candidate target for excessive mitochondrial ROS production and redox signaling. Dysfunction of Complex I may lead to incomplete mitochondrial electron transport and decreased ATP production. Our subsequent laboratory studies (Chuang et al. 2004, 2007, 2009a, 2010) in the animal model of status epilepticus provide credence to the notion that activation of nuclear factor-κB in hippocampal CA3 neurons upregulates the NO synthase (NOS) II gene expression with temporal correlation of NOS II derived nitric oxide (NO)-, $O_2^{\bullet-}$- and peroxynitrite-dependent reduction in mitochondrial respiratory enzyme Complex I activity. This leads to apoptotic neuronal cell death in the hippocampus. Thus, the selective dysfunction of Complex I may be an important biochemical hallmark of seizure-induced neuronal cell death in the hippocampus and play a crucial role in the mechanism of epileptogenesis.

The role of mitochondrial dysfunction in epileptogenesis

As impairment of mitochondrial function and increased ROS have recently been observed in the seizure focus of human and experimental epilepsy (Kudin et al. 2009; Patel 2004), the crucial question is whether seizure-induced free radical production and mitochondrial dysfunction result 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

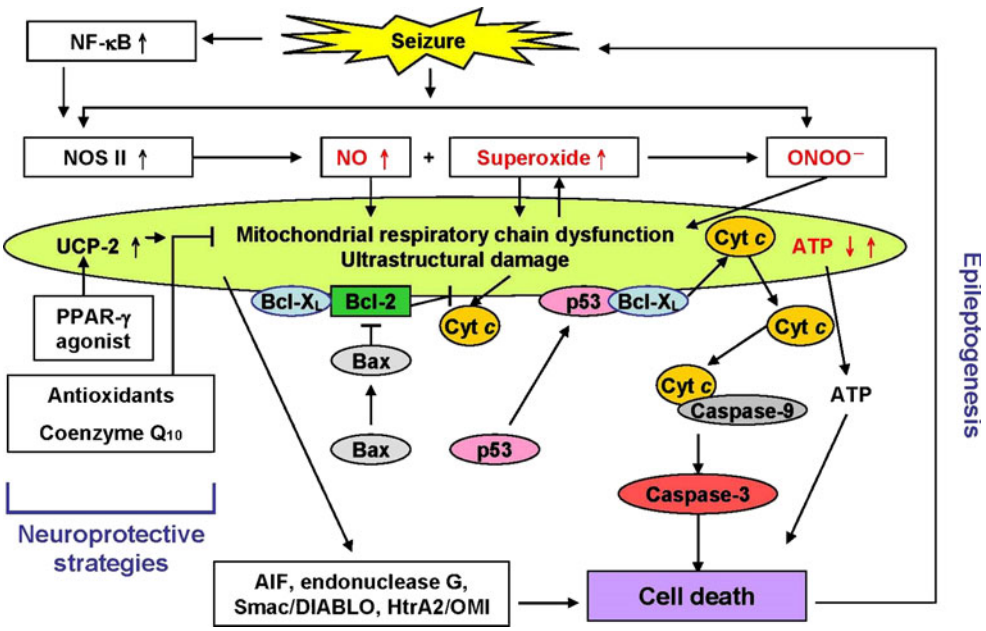

Fig. 1 The proposed mechanisms of mitochondrial dysfunction in seizure-associated cell death and epileptogenesis. Protection of mitochondria from bioenergetic failure and oxidative and nitrosative stress in the hippocampus may be considered as a target for potential neuroprotective strategies in epilepsy (for example, administration with coenzyme Q_{10} or antioxidants, or enhancement of mitochondrial uncoupling protein 2, UCP-2). NF-κB, nuclear factor-κB; NOS II, nitric oxide synthase II; NO, nitric oxide; $ONOO^-$, peroxynitrite; PPARγ, peroxisome proliferator-activated receptors γ; Cyt *c*, cytochrome *c*; AIF, apoptosis-inducing factor

mitochondrial diseases arising from mutations in mitochondrial DNA (mtDNA) or nuclear DNA (Kudin et al. 2009). Defect in the process of oxidative phosphorylation in the CNS is a characteristic sign of mitochondrial encephalomyopathies. A well known mitochondrial disorder with generalized seizures which is linked to point mutations in the mitochondrial $tRNA^{Lys}$ gene (Shoffner et al. 1990) 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 (Canafoglia et al. 2001; DiMauro et al. 1999). In addition, systemic administration of mitochondrial toxins, such as 3-nitropropionic acid (Urbanska et al. 1998) and cyanide (Yamamoto 1995), inhibits the functions of the 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 (Hauser and Annegers 1991). In fact, increased oxidative stress and mitochondrial dysfunction are the common cellular events underlie 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 (Patel 2004). Increased oxidative mtDNA damage, mitochondrial H_2O_2 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. This suggested that mitochondrial oxidative stress and mitochondrial injury may contribute to epileptogenesis (Jarrett et al. 2008).

The mechanisms of mitochondrial dysfunction during epileptogenesis remain unclear. 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 (Kudin et al. 2009; Patel 2004). Further studies are mandatory in the future to confirm this implication.

Summary

Mitochondrial dysfunctions occur as a consequence of prolonged epileptic seizures and promote seizure-induced neuronal cell death. Moreover, mitochondrial dysfunction and chronic oxidative stress can render the brain more susceptible to epileptic seizures that contribute to epileptogenesis (summary in Fig. 1). Therefore, mitochondria can be considered as a target for potential neuroprotective strategies in epilepsy. Protection of the mitochondria from bioenergetic failure and oxidative and nitrosative stress in the hippocampus may open a new vista to the development of more effective neuroprotective strategies against seizure-induced brain damage and to the design of novel treatment perspectives against therapy-resistant forms of epilepsy.

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