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Review

Oxidative Stress, Mitochondrial Dysfunction, and Epilepsy

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Epilepsy is a common and heterogeneous neurological disorder arising from biochemical and molecular events that are incompletely understood. To effectively manage epilepsies, it is important to understand the mechanisms underlying both seizure-induced brain damage as well as seizure initiation. Oxidative stress is emerging as a mechanism that may play an important role in the etiology of seizure-induced neuronal death. Conversely, epileptic seizures are a common occurrence in mitochondrial diseases arising from defects in oxidative phosphorylation. This review focuses on the emerging role of oxidative stress and mitochondrial dysfunction both as a consequence and cause of epileptic seizures.

Keywords: Superoxide; Free radical; Epilepsy; Mitochondria

INTRODUCTION

Epilepsies comprise of a group of clinical syndromes affecting more than 50 million people worldwide. Epileptic seizures are manifested as convulsive or non-convulsive episodes characterized by synchronous paroxymal discharges arising from a group of cerebral neurons. They occur in all age groups and may result from diverse acute or chronic underlying conditions. These include structural abnormalities, hypoxia, and trauma.[1,2] Epileptic seizures are a common manifestation of both childhood diseases, such as mitochondrial diseases,[3] as well age-related neuronal disorders such as stroke and Alzheimer's disease.[4]

Neuronal damage may be an important and detrimental effect of repeated seizures. Although still controversial, increasing evidence suggests that neuronal cell death may be both a cause and consequence of epileptic seizures. The evidence that seizures cause brain injury comes from the demonstration that intense seizure activity associated with status epilepticus is sufficient to cause hippocampal sclerosis in part through excessive activation of glutamate receptors.[5–7] The idea that neuronal death can cause epilepsy is supported by the fact that surgical removal of a sclerotic hippocampus dramatically improves the condition of epilepsy patients.[8] Complicated febrile seizures or status epilepticus in childhood is often followed by the development of temporal lobe epilepsy later in life, perhaps in part due to a collective loss of neurons occurring during episodes of childhood seizures.[9] Furthermore, seizures activate genes encoding neurotophic factors[10] and seizures result in structural rearrangements that could contribute to a life-long state of hyperexcitability.[11] Since there is evidence that seizures can set in motion molecular events that trigger the epileptic condition, i.e. seizures beget seizures, it is important to understand both the biochemical abnormalities that trigger epileptic seizures and mechanisms underlying seizure-induced brain damage. Understanding both mechanisms may lead to therapies that interrupt this vicious cycle of seizures and neuronal damage.

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Oxidative stress is an imbalance that ensues when the oxidant burden, i.e. production of reactive oxygen species (ROS) overwhelms endogenous antioxidant capacity and repair. It is thought to be a key cytotoxic mechanism in the pathogenesis of a variety of acute and chronic disease states. Both ROS and reactive nitrogen species (RNS) are thought to play important roles in diverse nervous system disorders such as stroke, spinal cord injury, Parkinson's Disease, Alzheimer's Disease, Huntington's disease, Freidrich's Ataxia and Amytrophic Lateral Sclerosis.[12] However, the role of ROS/RNS in the epilepsies remains to be defined. Oxidative stress is of particular significance in the nervous system, because both the sources of reactive species and their targets are plentiful in the brain. The brain is uniquely sensitive to oxidative damage due to its high aerobic metabolic demand, high polyunsaturated fatty acid content, poor repair capacity and high iron load.[13] The brain is particularly rich in mitochondria, the principal source of cellular superoxide (O_2^-), formed during respiration.[14] In the brain, O_2^- can also arise from the autooxidation of catecholamines and in the cytoplasm by enzymes such as xanthine oxidase.[15] The plasma membrane of many cell types can also produce O_2^- by the activation of enzymes such as phospholipase A_2[16,17] and NAD(P)H oxidases.[18] Hydrogen peroxide (H_2O_2) is formed wherever O_2^- is generated by its rapid spontaneous conversion to H_2O_2. The cytotoxic mechanisms by which reactive species induce neuronal damage may involve direct oxidative attack on cellular macromolecules (proteins, lipids, DNA and sugars) and/or initiation or propagation of free radical chain reactions that ultimately lead to macromolecular damage.

EPILEPTIC SEIZURES CAN CAUSE OXIDATIVE STRESS

A causal role for oxidative mechanisms in seizure-induced neuronal death is supported in part by the following lines of evidence. (1) Experimental seizures increase oxidation of cellular macromolecules (see section below). (2) Certain antioxidants such as superoxide dismutase (SOD) mimetics, vitamin C, spin traps and melatonin prevent seizure-induced pathology.[19–24] (3) Seizure-induced brain damage is inhibited by caloric restriction, a paradigm known to limit free radical formation.[25] (4) Oxidative stress is an important participant in glutamate mediated excitotoxicity,[26–31] which is thought to play a critical role in epileptic brain damage.

SEIZURE-INDUCED OXIDATIVE STRESS

Inactivation of Aconitase, a Sensitive Target of Mitochondrial O_2^-

Direct measurement of steady-state levels of reactive species in biological systems is difficult, given that reactive species are transient, unstable and often localized to the compartments in which they are formed. Proteins, lipids and DNA are sensitive targets of ROS and oxidation of these cellular targets offers a convenient way to detect the presence of oxidative stress in biological systems. The presence of a labile iron motif in the iron–sulfur (Fe–S) center of cytosolic and mitochondrial aconitase(s) renders them sensitive to oxidative attack by O_2^- and related species,[32–34] allowing the measurement of aconitase activity to serve as an index of steady-state O_2^- levels. Measurement of O_2^- production using the endogenous aconitase inactivation as a surrogate marker has several advantages in that it is highly sensitive to inactivation by O_2^-,[33] relatively specific for O_2^-[35] and allows estimation of compartmentalized (mitochondrial or cytosolic) O_2^- production.[23,26] Validation of aconitase inactivation as a marker of mitochondrial superoxide formation comes from the demonstration that brain and heart mitochondrial aconitase activity is drastically diminished in mitochondrial manganese superoxide dismutase (MnSOD) homozygous knockout (*Sod2* $-/-$) mice.[37,38] Liang *et al.*[23] demonstrated that kainate-induced seizures in rats selectively inactivates mitochondrial aconitase, an index of mitochondrial O_2^- production in the rat hippocampus. Maximal inactivation of mitochondrial aconitase occurred in the vulnerable CA3 area of the hippocampus[39] at times preceding overt neuronal death. Kainate-induced mitochondrial aconitase inactivation and hippocampal neuronal loss were attenuated in transgenic mice overexpressing MnSOD or in rats administered manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP), a catalytic antioxidant.[23] These findings suggest a role for mitochondrial superoxide-induced damage in hippocampal pathology produced by experimental seizures.

Lipid Peroxidation

The occurrence of lipid peroxidation following seizure activity has been observed by measuring products such as thiobarbituric acid reactive substances and F_2-isoprostanes (F_2-IsoPs).[21,39] F_2-IsoPs are a novel class of prostaglandin F_2 (PGF_2)-like compounds, produced *in vivo* by a non-cyclooxygenase and free-radical-catalyzed mechanism involving the peroxidation of arachidonic acid.[40] Several attributes render the measurement of F_2-IsoPs as

a sensitive, stable and reliable marker of free radical-induced lipid peroxidation *in vivo*: (1) F_2-IsoPs are specific products of lipid peroxidation; (2) They are detectable in normal biological fluids and increase dramatically in models of oxidant injury; (3) They are modulated by antioxidant status; and (4) They are unaffected by dietary lipids.[41] Experimental seizures result in a large increase in prostaglandin derivatives[42] including prostaglandin-$F_2\alpha$, a precursor of F_2-IsoP.[43] In adult rats, kainate-induced seizures were found to produce a large increase in F_2-IsoP levels in the highly vulnerable CA3, but not CA1 region prior to cell damage in the CA3 area. Interestingly, the dentate gyrus (DG), a region that contains granule neurons which are resistant to kainate-induced neuronal death also showed marked increases in F_2-IsoP levels.[39] This marked subregion-specific increase in F_2-IsoP following kainate administration suggests that oxidative lipid damage results from seizure activity and may play an important role in seizure-induced death of vulnerable neurons. The role of the F_2-IsoP signal in the cell death resistant DG area remains to be resolved.

DNA Oxidation

The presence of oxidative DNA damage following seizure activity has been demonstrated by using the measurement of 8-hydroxy-2-deoxyguanosine (8-OHdG), an oxidatively modified guanine adduct as an index of oxidative DNA damage.[23,44] The ratio of steady-state levels 8-OHdG to 2-deoxyguanine (2-dG) reflects oxidative DNA damage.[45,46] Kainate administration in adult rats produced a large increase in the ratio of oxidized/non-oxidized bases (8-OHdG:2dG) at times preceding and coinciding overt cell death, suggesting a potential role for oxidative DNA damage in the sequence of events leading to kainate-induced neuronal death. Whereas these studies did not distinguish between nuclear and mitochondrial DNA as the source of increased 8-OHdG levels, the likelihood that mitochondrial DNA contributes to the 8-OHdG signal in kainate treated animals is strengthened by the close proximity of mitochondrial DNA to the major source of O_2^- production, i.e. the electron transport chain and the presence of several fold higher oxidized DNA bases in mitochondrial DNA in comparison to nuclear DNA.[47–49]

SOD Mutant Mice

Whereas oxidized proteins, lipids and DNA are useful surrogate markers for detecting the presence of oxidative stress, it is difficult to pinpoint the identity of the primary reactive species that is important in the oxidative insult. Transgenic/knockout animals of the endogenous antioxidant systems have been useful tools to verify the identity of the ROS that is important in the injury process. The existence of three SODs in the cytoplasm (CuZnSOD or SOD1),[50] mitochondria (MnSOD or SOD2),[51] and extracellular compartment (EC-SOD or SOD3)[52] to maintain low steady-state O_2^- levels underscores the importance of O_2^- in physiological and pathological processes. Kainate excitotoxicity in the hippocampus has been studied in CuZnSOD (SOD1),[53] MnSOD (SOD2)[23] and EC-SOD (SOD3)[54] transgenic mice. These studies show that overexpressing of MnSOD and, to a lesser extent, EC-SOD, protects against kainate-induced hippocampal damage. However, transgenic mice overexpressing CuZnSOD (SOD1) have yielded controversial results,[53,55] which may in part be attributed to the superoxide reductase, oxidase or peroxidase activities of CuZnSOD. On the other hand, transgenic mice with modest overexpression (0.5–2 fold increase in activity) of MnSOD were protected against kainate-induced mitochondrial aconitase inactivation and hippocampal cell death,[23] whereas mice with partial MnSOD deficiency showed exacerbation of kainate-induced hippocampal damage.[56] These studies suggest that mitochondrial O_2^- may play an important role in seizure-induced brain damage.

The ability of EC-SOD overexpressing mice to attenuate kainate-induced hippocampal damage,[54] suggests that the extracellular space may be an additional source of seizure-induced O_2^- production. Activation of the NADPH oxidase complex residing on activated microglial cells may be a likely source of seizure-induced extracellular and/or cytosolic O_2^- (Fig. 1).

Although, transgenic mice overexpressing the SODs are useful tools to verify the importance of a given subcellular compartment as the source of oxidative stress in a disease state, several factors can confound the interpretation of data obtained with these mice. First, depending on the redox environment, an overexpressed SOD may behave as superoxide reductase, superoxide oxidase or peroxidase[57,58] rather than a SOD. This may be especially significant in a subcellular compartment where the ratio of SOD: O_2^- is very high, e.g. cytosol. Secondly, localization of SODs to a given subcellular compartment is not absolute. For example, the cytosolic CuZnSOD has been localized to the mitochondria of mammalian cells.[59] Finally, the background strain of the mice may be important in conferring resistance or sensitivity to excitotoxic cell death.[60]

Seizure-induced Oxidative Cell Death

Together, the studies above demonstrate that intense seizure activity produced in animal models can

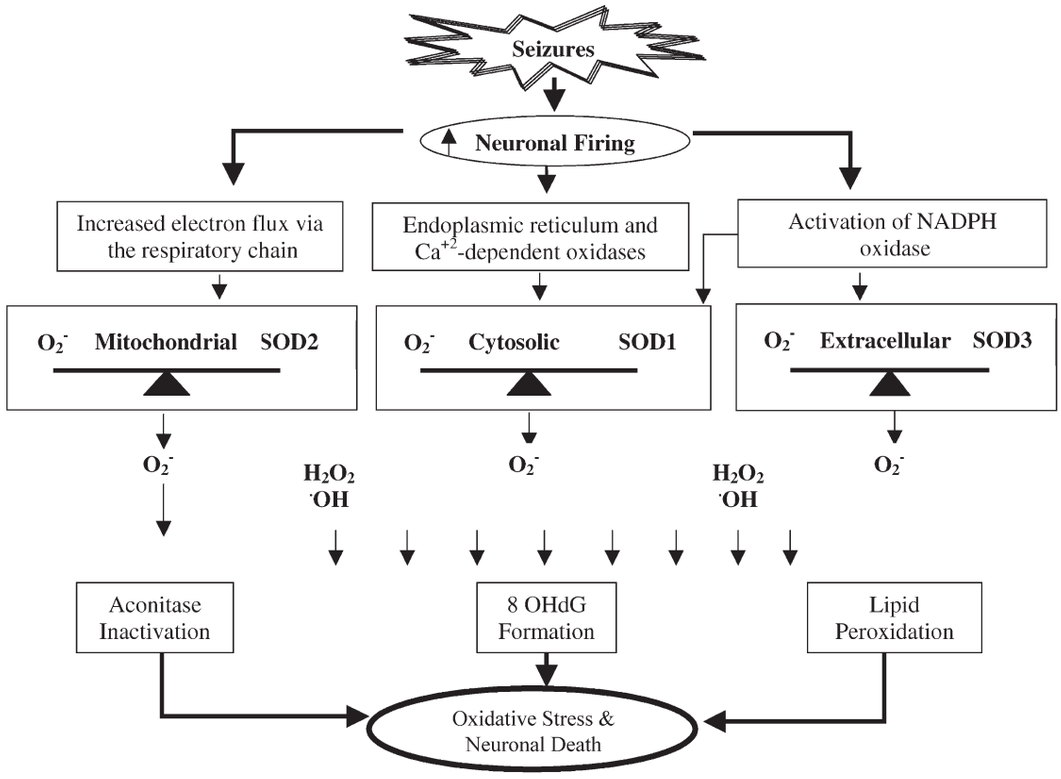

FIGURE 1 A schematic outlining three possible sources of O_2^- production following intense seizure activity. Net O_2^- is the balance between O_2^- production and SOD, which may be tipped in favor of O_2^- production following seizures. O_2^- and related ROS from one or more of these three primary sources could drive the oxidation of sensitive targets (proteins, DNA and lipids) and contribute to oxidative cell death.

result in oxidative stress in vulnerable brain regions. A major source of seizure-induced oxidative stress may arise from the mitochondria (Fig. 1), adding epilepsy to the emerging list of neuronal disorders in which mitochondrial oxidative stress and dysfunction plays a central role. The precise source(s) of seizure-induced O_2^- generation as well as the oxidative mechanisms leading to seizure-induced neuronal loss are speculated in Fig. 1 but remain areas of future research. It may be speculated that prolonged seizures result in sufficient O_2^- production to overwhelm the endogenous mitochondrial antioxidant defenses by a cascade of events initiated by increased neuronal firing, excessive glutamate release, *N*-methyl-D-aspartate (NMDA) receptor activation, cytosolic and mitochondrial calcium influx and increased ATP consumption. The demands on cellular energy could lead to a dramatic flux of electrons through the electron transfer chain resulting in increased O_2^- production. The release of cytochrome C may also be an important source of seizure-induced O_2^- production. Excessive O_2^- produced in this manner could oxidize the $[4Fe–4S]^{2+}$ cluster of aconitase, resulting in a $[3Fe–4S]^{+}$ cluster concomitant with the release of Fe^{2+} and subsequent hydroxyl radical formation by Fenton chemistry. Hydroxyl radicals can oxidize mitochondrial proteins, DNA and lipids thereby amplifying O_2^--initiated oxidative damage.

Much work is need to examine and establish a role for oxidative stress in human epilepsies. The likelihood of finding a link between epilepsy and oxidative stress is strengthened by the finding that some patients with temporal lobe epilepsy show mitochondrial Complex 1 deficiency, which is the leading cause of increased O_2^- production in patients with Parkinson's Disease.[61]

ROLE OF NITRIC OXIDE IN EPILEPSY

The role of nitric oxide (NO) and other RNS in seizure susceptibility and seizure-induced neuronal death has been examined with tools that inhibit or increase the formation of NO. However, these studies have yielded somewhat controversial data.[62–67] This is not entirely surprising given that NO subserves both useful and harmful roles in living systems and its formation by NO synthases is highly compartmentalized within the cell. The precise physiological and/or pathological roles of RNS in seizure disorders remains to be defined.

EPILEPSY AND MITOCHONDRIAL DISEASE

Thus far the role of oxidative stress as a consequence of epileptic seizures was discussed. The section below, briefly reviews the role of mitochondrial dysfunction arising from mitochondrial DNA (mtDNA) mutation/deletion as the cause of certain epilepsies. Epileptic seizures are a common phenotype of inherited mitochondrial diseases. The best characterized of these diseases is myoclonic epilepsy with ragged red fibers (MERRF), the first epilepsy in which a molecular defect was identified and linked with the epilepsy syndrome.[68] The molecular defect in MERRF arises from a single mutation of the $tRNA^{lys}$ resulting in a disorder consisting of myoclonic epilepsy and a characteristic myopathy with ragged red fibers.[69]

Several neuronal disorders have been linked to mutations in mitochondrial genes encoded by either the nuclear or mitochondrial genome.[3,70] Mitochondrial diseases arising from mtDNA mutations can result from rearrangements or base substitutions. mtDNA nucleotide substitutions can be missense mutations or tRNA mutations and include Leber's hereditary optic neuropathy (Complex I), MERRF ($tRNA^{lys}$), mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS; $tRNA^{Lue}$), Leigh's syndrome (ATP synthase) and neuropathy, ataxia, and retinitis pigmentosa (NARP; ATPas 6). The mtDNA rearrangements consist of deletions or duplications and include conditions such as Chronic Progressive External Opthalmoplegia (CPEO), Kearns-Sayre Syndrome (KSS) and Pearson Syndrome (PS). Epileptic seizures are more prevalent in mitochondrial disorders associated with nucleotide substitutions (e.g. MERRF, MELAS, Leigh's syndrome and NARP) and less so with the mtDNA rearrangement disorders (e.g. KSS and PS). However, notable exceptions to this general rule apply. For example, seizures are often observed in certain disorders arising as a result of mtDNA depletion, e.g. Alper's Syndrome.[71] The variable distribution of epilepsy among mitochondrial diseases underscores the importance of defining the biochemical triggers of epileptic seizures in these disorders.

MITOCHONDRIAL DYSFUNCTION AS A CAUSE OF EPILEPSY

The biochemical basis of epilepsy in mtDNA disorders remains unclear. Several of the mtDNA mutation/deletion syndromes mentioned above result in defects in mitochondrial oxidative phosphorylation and therefore have a major impact on energy production and mitochondrial function. For example, the $tRNA^{lys}$ mutation in MERRF results in defects in complex I and IV of oxidative phosphorylation.[69] Mitochondria have several important functions that include cellular ATP production, ROS formation, control of apoptotic/necrotic cell death and fatty acid metabolism. Each of these vital interrelated mitochondrial functions is crucial for normal brain function and a defect in one or more of these can be a likely cause of seizures. Mitochondrial dysfunction can therefore have a major impact on epilepsies associated with mtDNA disorders. However, which of these factors contributes to the seizures associated with these syndromes remains to be deciphered. Whereas epilepsies arising from mtDNA mutations are rare, epilepsies arising from pathological insults, e.g. hypoxia or trauma that can increase oxidative stress and mitochondrial dysfunction are common. Therefore, understanding the role of mitochondria in seizure initiation can provide insight into the mechanisms by which both rare mtDNA mutations and common metabolic insults trigger epileptic seizures. The incidence of seizures in disorders arising from nuclear DNA mutations encoding mitochondrial proteins further highlights the central role of mitochondrial dysfunction in the pathogenesis of certain epilepsies.

One intriguing possibility is that mitochondrial free radical production and resultant dysfunction may contribute to the epileptic seizures associated with mitochondrial diseases. Several key studies support the idea that mitochondrial oxidative stress and/or dysfunction can cause epileptic seizures. First, defects in oxidative phosphorylation complexes, can result in increased O_2^- production. Secondly, seizure activity can be induced by paradigms which can increase the mitochondrial free radical load: e.g. increased oxygen tension, i.e. hyperbaric hyperoxia,[72] local infusion of redox-active iron salts[73] or mitochondrial toxins,[74] age-related neuronal disorders, e.g. stroke[4] and brief periods of neonatal hypoxia.[1,75] Whereas each of these paradigms can result in increased mitochondrial free radical production, experimental evidence linking mitochondrial oxidative stress with the occurrence of seizures is lacking. Finally, synaptic NMDA receptor activation which results in increased O_2^- production is a necessary component of seizures. Proof that the overproduction of mitochondrial O_2^- results in seizure activity comes from studies using *Sod2* − /− mice. *Sod2* − /− mice have severe mitochondrial disease and behavioral manifestations including seizures.[37] Heterozygous knockout mice for *Sod2* + /− have 50% brain MnSOD activity, appear biochemically and phenotypically normal at birth but develop spontaneous and environmental seizures as they age.[76] The seizure incidence and severity in the *Sod2* + /− mice correlated with mitochondrial aconitase inactivation and advancing age.

Depletion of ATP may be an important factor contributing to seizure activity associated with mitochondrial dysfunction due in part to the dependence of neurotransmitter and ion uptake systems on ATP. However, ATP depletion alone cannot account for epilepsy, since seizure incidence in KSS is low despite ATP depletion. Given the diverse clinical features of mitochondrial diseases, it is difficult to pinpoint the precise biochemical and molecular events that can explain the frequent incidence of epileptic seizures in many, but not all mtDNA diseases. The type and levels of mutant mtDNA, localization of the mutant mtDNA within seizure-prone neuronal populations and the presence of vascular involvement are some of the factors that may contribute to the epileptic phenotype. These factors together with the extent of mitochondrial dysfunction may ultimately trigger the seizures associated with many mitochondrial diseases. A useful approach to understanding the basis of epilepsy in mitochondrial diseases is the development of mouse models in which mitochondrial dysfunction arises from mtDNA mutations/deletions or nuclear DNA mutations. For example, brain specific inactivation of the nuclear mitochondrial transcription factor A gene, which encodes a protein necessary for the transcription and replication of mtDNA results in mice with mitochondrial late-onset neurodegeneration. These mice show severe deficiency in electron transport chain function, massive neurodegeneration in hippocampal and neocortical areas and increased susceptibility to kainate-induced brain damage.[77]

CONCLUSION

Oxidative stress and mitochondrial dysfunction occur as a consequence of prolonged epileptic seizures and may play an important role in seizure-induced brain damage. Mitochondrial dysfunction may be an important biochemical trigger of epileptic seizures arising from mtDNA or nuclear DNA mutations. Understanding the role of oxidative stress and mitochondrial dysfunction as causes and/or consequence of seizures may suggest novel therapeutic approaches for the treatment of mitochondrial epilepsies and shed light on the mechanisms by which common metabolic insults result in epilepsy. The remarkable progress made in two independent fields of research, i.e. free radical biology and mtDNA diseases is poised to shed light on the field of epilepsy.

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