CURRENT LITERATURE

RADICAL IDEAS ABOUT SEIZURE-INDUCED NEURONAL DAMAGE

Free Radical–Mediated Cell Damage After Experimental Status Epilepticus in Hippocampal Slice Cultures

Kovács R, Schuchmann S, Gabriel S, Kann O, Kardos J, Heinemann U

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Generation of free radicals may have a key role in the nerve cell damage induced by prolonged or frequently recurring convulsions (status epilepticus). Mitochondrial function also may be altered because of production of free radicals during seizures. We therefore studied changes in field potentials (fp) together with measurements of extracellular, intracellular, and intramitochondrial calcium concentration ($[Ca^{2+}]_e$, $[Ca^{2+}]_i$, and $[Ca^{2+}]_m$, respectively), mitochondrial membrane potential ($\Delta\Psi$), nicotinamide adenine dinucleotide phosphate [NAD(P)H] autofluorescence, and dihydroethidium (HEt) fluorescence in hippocampal slice cultures by means of simultaneous electrophysiologic and microfluorimetric measurements. As reported previously, each seizure-like event (SLE) resulted in mitochondrial depolarization associated with a delayed increase in oxidation of HEt to ethidium, presumably indicating reactive oxygen species (ROS) production. We show here that repeated SLEs led to a decline in intracellular and intramitochondrial Ca^{2+} signals despite unaltered Ca^{2+} influx. Mitochondrial depolarization and the NAD(P)H signal became smaller during recurring SLEs. By contrast, the ethidium fluorescence increases remained constant or even increased from SLE to SLE. After about 15 SLEs, activity changed to continuous afterdischarges with steady depolarization of mitochondrial membranes. Staining with a cell-death marker, propidium iodide, indicated widespread cell damage after 2 hours of recurring SLEs. The free radical scavenger, α-tocopherol, protected the slice cultures against this damage and also reduced the ongoing impairment of NAD(P)H production. These findings suggest involvement of an ROS of mitochondrial origin in the epileptic cell damage and that free radical scavenging may prevent status epilepticus–induced cell loss.

COMMENTARY

Every schoolchild learns that mitochondria are the "powerhouse" of the cell, providing energy by oxidative phosphorylation that generates adenosine triphosphate (ATP). However, it is now quite clear that mitochondria subserve other critical functions as well, especially calcium (Ca^{2+}) homeostasis (1). The additional role of mitochondria in cell death through the production of reactive oxygen species (ROS) adds a further dimension to their ability to modulate neuronal excitability. Mitochondrial dysfunction, caused either by an oxidative stress such as a seizure, or by mutation of mitochondrial DNA, impairs the ability of cell to generate sufficient energy and may lead to the cell's demise. Recent studies have shown that mitochondria may be impaired within a seizure focus (but not in nearby uninvolved tissue), in both animal models and from excised tissue from patients from temporal lobe epilepsy (2).

Conventional wisdom holds that excitotoxicity is mediated through voltage-gated or glutamate receptor–induced entry of excess calcium ions into neurons, followed by the activation of a cascade of pathologic processes, many of which are calcium mediated. Neuronal excitation during a seizure (or in the extreme case, status epilepticus) generates excess calcium influx, but the exact mechanisms by which excess calcium is implicated in cell death are unclear, and a controversy currently exists as to whether the mitochondrial contribution to cell death from seizures is necrotic, apoptotic, or both (3,4). In either case, the role of mitochondria in cellular energy production places this organelle in a unique position to mediate pathophysiologic processes in conditions of energy failure (e.g., status epilepticus). There is accumulating evidence that free radicals generated during status epilepticus cause both mitochondrial dysfunction and neuronal cell damage and death (5).

Kovács et al. (6) have long been interested in the role of mitochondria in seizure-induced neuronal death. In previous

work, they showed that "seizure-like events" (SLEs) in explanted hippocampal slice cultures, generated by electrical stimulation of mossy fibers in zero-Mg^{2+} medium, cause intramitochondrial calcium accumulation, mitochondrial depolarization with reduced nicotinamide adenine dinucleotide (NADH) production, and the generation of ROS (6). Even a single SLE was associated with death of hippocampal principal neurons. In the current report, they examine the effects of *recurrent* SLEs (mimicking status epilepticus) on mitochondrial calcium accumulation and cell death.

The authors used simultaneous extracellular electrophysiology (field potential recording) and fluorescent measurement of Ca^{2+} concentrations in various cellular compartments (extracellular, intracellular, mitochondrial) by using specific calcium-sensitive dyes, each of which reflects a change in Ca^{2+} concentration in a particular compartment. During a single SLE, extracellular Ca^{2+} declines, whereas cytosolic and mitochondrial Ca^{2+} concentrations increase (as detected by changes in CaGreen 1 and Rhod-2 fluorescence signals, respectively). The Ca^{2+} influx during a single SLE was associated with mitochondrial depolarization (as measured by Rhod-123). As depolarized mitochondria use oxygen less effectively, it was hypothesized that ROS would be produced. Indeed, using changes in hydroethidine (HEt) fluorescence, there was an increase in ROS during an SLE. NAD(P)H signals also increased during an SLE, as an attempt by the cell to boost ATP production when challenged with a seizure.

With multiple SLEs, each successive seizure caused a less pronounced elevation of cytosolic and mitochondrial Ca^{2+}, whereas there was no change in Ca^{2+} influx into the cell. Furthermore, successive SLE was associated with decreasing NAD(P)H signals. However, with recurrent seizures, mitochondrial depolarization and HEt fluorescence progressively *increased*, suggesting progressive impairment of mitochondrial energy production and increased ROS generation. After 2 hours of repeated SLEs, cell death could be documented, as indicated by the fluorescent marker propidium iodide, which enters damaged cells and binds to RNA and DNA. Therefore during recurrent seizures (SLE), Ca^{2+} enters the cell and mitochondria, resulting in increased NADH production that declines with each subsequent seizure.

The effect of α-tocopherol, a free radical scavenger, is of particular interest in these experiments, because of its potential clinical relevance as a neuroprotective agent. Pretreatment of slice cultures with α-tocopherol prevented the decline of NAD(P)H production, decreased HEt fluorescence (ROS production), and reduced cell death. These results suggest that SLE-induced cell death is associated with free radical production, an effect prevented by free radical scavenging.

These experiments strongly support a role for free radicals in seizure-induced cell death in vitro; there also is evidence for ROS involvement in seizures in whole animals (4). The limitations of in vitro systems must be acknowledged. The experiments described here are technically challenging. It must be assumed that each Ca^{2+}-sensitive dye is specific for the cellular compartment, and that the dye itself or the fluorescence does not cause neurotoxicity. To dye-load tissue effectively, some sort of in vitro system must be used. Both hippocampal slice cultures and standard hippocampal slices may be affected by the inevitable disruption of normal structural architecture, and possibly hypoxia, during their preparation.

Epilepsy is commonplace in disorders of mitochondrial function (2). In such disorders, it is assumed that seizures are associated with "energy failure," as reflected by decreased ATP production. Seizures in mitochondrial disorders respond poorly to standard anticonvulsants, which is not surprising, given their primary mechanisms of action, such as voltage- and ligand-gated ionic channels, etc. Therefore the availability of agents with specific mitochondrial activity would enhance our ability to suppress seizures in these severe disorders. Although it is premature to assign such a role to α-tocopherol, experiments such as those of Kovács et al. provide a potential clinical target to ameliorate seizure-induced cell death.

by Carl E. Stafstrom, M.D., Ph.D.

References

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