MOTHER'S MILK PROTECTS THE IMMATURE BRAIN FROM SEIZURE-INDUCED CELL DEATH

Mitochondrial Uncoupling Protein-2 Contributes Crucially to the Resistance of Immature Brain to Excitotoxic Neuronal Death

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Excitotoxic cell death is the fundamental process responsible for many human neurodegenerative disorders, yet the basic mechanisms involved are not fully understood. Here we exploited the fact that the immature brain is remarkably resistant to seizure-induced excitotoxic cell death and examined the underlying protective mechanisms. We found that, unlike those in the adult, seizures do not increase the formation of reactive oxygen species or result in mitochondrial dysfunction in neonatal brain, because of high levels of the mitochondrial uncoupling protein (UCP2). UCP2 expression and function were basally increased in neonatal brain by the fat-rich diet of maternal milk, and substituting a low-fat diet reduced UCP2, restored mitochondrial coupling, and permitted seizure-induced neuronal injury. Thus modulation of UCP2 expression and function by dietary fat protects neonatal neurons from excitotoxicity by preventing mitochondrial dysfunction. This mechanism offers novel neuroprotective strategies for individuals, greater than 1% of the world's population, who are affected by seizures.

COMMENTARY

One of the persistent mysteries of pediatric epilepsy is why, at least experimentally, seizures do not cause nearly as much damage in the developing brain as in the adult brain. This observation has seemed at odds with the clinical practice of pediatric neurology, in which children often seem to experience cognitive decline as a result of ongoing epilepsy. However, recent animal studies have shown that seizures of sufficient number, duration, or severity indeed can cause brain damage during early development—especially if "damage" is defined broadly in terms of subsequent behavior, cognitive function, and seizure susceptibility (as opposed to just structural damage or cell death) (1–4). Even in cases in which structural damage has been demonstrated after seizures, the devastation is trivial in the developing brain compared with similar seizures during adulthood.

Although several hypotheses have been proposed, no unifying explanation exists as to why the immature brain is protected from seizure-induced excitotoxic damage (5,6). Possibilities include developmental changes in glutamate and in glycine and γ-aminobutyric acid (GABA) receptors (5,7), differences in calcium-buffering capacity, and age-related metabolic factors. The exciting report by Sullivan and colleagues proposes a novel mechanism for childhood protection from structural damage, and the source may be surprising—mother's milk! Sullivan et al. report on an intriguing set of experiments that examines the relative lack of vulnerability of the immature brain to kainic acid (KA) seizure–induced injury. In the adult rat brain, KA seizures caused widespread, extensive death of neurons in several limbic areas, including perirhinal cortex, piriform cortex, hippocampal CA3, and dentate hilus. Notably, those areas were immune to damage in the immature (P10–11) rat brain.

Seizures, particularly those that are prolonged, strain a neuron's ability to generate energy necessary for cell function. Mitochondria, which subserve this metabolic function, produce reactive oxygen species (ROS) under stressful conditions, such as seizures. It is generally accepted that ROS initiate excitotoxic injury by altering calcium homeostasis and energy production. As a natural protection against ROS-induced damage, a family of mitochondrial uncoupling proteins (UCPs) dissociates (i.e., uncouples) adenosine triphosphate (ATP) production from oxygen consumption by reducing the membrane potential across the mitochondrial membrane. The resultant decrease of membrane potential, in turn, reduces ROS formation. When UCPs are not expressed, ROS damage ensues. Sullivan and coworkers demonstrated that in adult rats, KA seizures led to striking increases in ROS at both 6 and 24 hours after seizures. However, no seizure-induced increase in ROS formation was found in immature mitochondria.

One of the uncoupling proteins, UCP2, is expressed in high concentration in the immature rat brain but not the adult brain, as demonstrated by UCP2 immunocytochemistry. The authors

hypothesized that during early development, neurons are less vulnerable to seizure-induced damage because of the expression of large amounts of UCP2—by increasing uncoupling, UCP2 inhibits ROS production. The investigators next demonstrated that in neuronal mitochondria isolated from immature rats, the basal level of uncoupling increases markedly on exposure to a fatty acid, as assessed by O_2 respiration. In contrast, in adult mitochondria, little UCP2 is available for activation by fatty acids.

Because UCPs are activated by free fatty acid (FFA), and maternal milk is enriched in these fats, the possibility is raised that the high expression of UCP2 in neonatal brain might be related to the high-fat diet of newborn rats, contributing to their relative seizure resistance. Therefore, the authors reduced the fat content of the rats' milk to determine whether UCP2 production would decrease. Indeed, after only 24 hours on a low-fat, isocaloric diet, mitochondria from rat pups had significantly decreased mitochondrial uncoupling capacity and increased ROS production. Most amazingly, fat-restricted rat pups exhibited seizure-induced limbic damage patterns similar to the usual adult pattern! This damage was seen in the piriform cortex but not in the hippocampus, suggesting a regional specificity to the protective effect of UCP2. The duration and severity of KA seizures was not affected by this brief dietary manipulation.

The conversion from a seizure damage–resistant phenotype to a seizure damage–susceptible phenotype by brief dietary manipulation is a remarkable finding. It is important that future studies determine whether this effect is limited to the P10–11 age window or whether a longer period of fat restriction would have a similar detrimental effect. In addition, it would be informative to learn whether such seizure-induced vulnerability could be reversed in the second week of life by resuming a high-fat diet.

The findings of Sullivan et al. have a number of potential implications for our understanding of developmental epilepsy. First, they represent one, although probably not the only, explanation for the unique seizure-damage resistance of the immature brain. Second, the results may be relevant to the mechanism of action of the high-fat ketogenic diet. Finally, this original finding may suggest a novel therapeutic strategy for the treatment of infantile epilepsies and thus prevent their sequelae. *Got milk?*

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