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Moving Metabolism to Make Inroads in a Model of Mitochondrial Epilepsy

Brain Metabolism Modulates Neuronal Excitability in a Mouse Model of Pyruvate Dehydrogenase Deficiency

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Glucose is the ultimate substrate for most brain activities that use carbon, including synthesis of the neurotransmitters glutamate and γ-aminobutyric acid via mitochondrial tricarboxylic acid (TCA) cycle. Brain metabolism and neuronal excitability are thus interdependent. However, the principles that govern their relationship are not always intuitive because heritable defects of brain glucose metabolism are associated with the paradoxical coexistence, in the same individual, of episodic neuronal hyperexcitation (seizures) with reduced basal cerebral electrical activity. One such prototypic disorder is pyruvate dehydrogenase (PDH) deficiency (PDHD). Pyruvate dehydrogenase is central to metabolism because it steers most of the glucose-derived flux into the TCA cycle. To better understand the pathophysiology of PDHD, we generated mice with brain-specific reduced PDH activity that paralleled salient human disease features, including cerebral hypotrophy, decreased amplitude electroencephalogram (EEG), and epilepsy. The mice exhibited reductions in cerebral TCA cycle flux, glutamate content, spontaneous, and electrically evoked in vivo cortical field potentials and γ EEG oscillation amplitude. Episodic decreases in γ oscillations preceded most epileptiform discharges, facilitating their prediction. Fast-spiking neuron excitability was decreased in brain slices, contributing to in vivo action potential burst prolongation after whisker pad stimulation. These features were partially reversed after systemic administration of acetate, which augmented cerebral TCA cycle flux, glutamate-dependent synaptic transmission, inhibition and γ oscillations, and reduced epileptiform discharge duration. Thus, our results suggest that dysfunctional excitability in PDHD is consequent to reduced oxidative flux, which leads to decreased neuronal activation and impaired inhibition, and can be mitigated by an alternative metabolic substrate.

Commentary

Working out the enzymatic reactions that comprise metabolism is one of the hallmark accomplishments of modern biology.[1] However, despite the formulaic description provided in textbooks, the workings of metabolism in vivo, especially in the brain, remain poorly understood. Broadly, adenosine triphosphate (ATP) is generated through 2 groups of reactions: glycolysis and oxidative phosphorylation. Glycolysis refers to the breakdown of glucose into pyruvate in order to generate a relatively limited quantity of ATP. Pyruvate can then be taken up by the mitochondria in order to drive the Krebs cycle forward to generate additional ATP as well as provide the substrates for synthetic reactions.[1] Within the mitochondria, the key enzyme that allows the pyruvate derived from glycolysis to enter the Krebs cycle is pyruvate dehydrogenase (PDH). As a result, PDH plays a central role in regulating both the production of ATP and the synthesis of new molecules whose substrates are derived from the Krebs cycle.[1] In the brain, the Krebs cycle is essential to the production of both glutamate and γ-aminobutyric acid (GABA), which puts PDH in the critical position of linking metabolism to the excitatory–inhibitory balance.[2]

There are many mitochondrial epilepsies whereby a deficit in energy production leads to epilepsy, and Leigh syndrome is one of the most commonly encountered disorders within this category.[3] In addition to PDH deficiency (PDHD), there are many other genetic mutations that lead to the Leigh syndrome phenotype, defined by early onset magnetic resonance imaging changes in the basal ganglia and brainstem in the clinical setting of developmental delay, seizures, intermittent weakness, and optic atrophy.[3] Despite being located on the X chromosome, PDH mutations can affect both males and females depending on the pattern of X inactivation, although how PDH mutations ultimately translate into epilepsy

remains poorly understood. In a recent report by Jakkamsetti et al, their group sought to understand how deficits in PDH translate into epilepsy with an eye toward using first principles from the study of metabolism to try and intervene in the disease.

In order to study PDHD, Jakkamsetti et al created a new mouse line in which PDH was eliminated only in the neurons and astrocytes of the brain. This allowed them to isolate the effect of PDH on central nervous system metabolism without worrying about the effect on peripheral tissues (which are often affected in PDHD in humans). Their new mouse line grossly reproduced some key features of PDHD in humans, and the animals were notably smaller than their wild-type littermates, with a shorter life expectancy and epileptiform activity on their electroencephalogram (EEG). To demonstrate the utility of their animal model, they also performed EEG on human patients with genetically confirmed PDHD. Interestingly, both species with PDHD exhibited significantly less power across the frequency spectrum. Although this finding is not unique to PDHD,[4] it suggests the model recapitulates some key features of the human disease.

Jakkamsetti et al predicted that the loss of PDH would impair the flux of substrates through the Krebs cycle and impair the production of neurotransmitters. Using mass spectrometry, they found that there was significantly less glutamate in the brains of PDHD mice. However, the levels of GABA were unaffected. This led the team to ask: how are the mice epileptic if there is less excitation while inhibition is unchanged? To answer this question, the team performed whole-cell recordings of individual neurons from the brains of PDHD mice. Interestingly, they found that the fast-spiking neurons (which typically represent inhibitory interneurons) fired much less rapidly in the PDHD animals and received less excitatory input. These results suggest that the loss of PDH specifically affected the fast-spiking neurons, and given the high metabolic requirements of these cells, also provides an intuition for why these cells might preferentially be affected in a mitochondrial epilepsy.[5] An important limitation of these experiments was that the team used the activity pattern of the neurons to identify them without a separate readout (such as morphology). Given that the activity patterns were altered in the PDHD model, it's unclear whether or not the differences between groups that Jakkamsetti et al observed were a function of the cells chosen or the experimental model itself.

In a particularly elegant demonstration, Jakkamsetti et al reasoned that the tricarboxylic acid cycle could be rescued by supplementing the PDHD mice with acetate. Acetate bypasses the reaction catalyzed by PDH, and the team reasoned that allowing the Krebs cycle to keep operating might restore the excitatory–inhibitory balance and limit the animal's epilepsy. First, they used ^{13}C labeled acetate to establish that giving the mice acetate would indeed increase the flux through the Krebs cycle. Next, they found that administering a large dose of acetate actually improved the EEG of the PDHD animals such that their spectral pattern more closely resembled that of their wild-type littermates. Finally, they monitored the number and length of seizures in PDHD animals before and after administration of acetate. For a brief period of time after acetate was administered, the animals indeed had shorter seizures, although notably this effect was modest and there was no difference in the number of seizures. This demonstration that acetate supplementation can rapidly rescue the EEG signature and influence epileptiform activity suggests that a working knowledge of brain metabolism could ultimately be used to guide therapies in mitochondrial epilepsies. More generally, this work raises the possibility that despite the fixed developmental lesions that occur in these patients, there might be some therapeutic benefit to metabolic intervention. Interestingly, in one extremely small observational study, there was the suggestion that the ketogenic diet might improve outcomes in patients with genetically confirmed PDHD.[6]

Understanding the metabolism of the brain remains an important problem for basic scientists and clinicians. Even simple questions, such as whether or not neurons metabolize glucose on their own, or preferentially utilize precursors from nearby astrocytes, continues to be debated.[7] Complicating the study of neural metabolism is the unique challenge that neurons face. They are sealed off from the blood (by the astrocytic blood–brain barrier) and their axons are wrapped in myelin.[8] Hence, while there is abundant evidence for important roles played by glial cells in supporting neuronal metabolism, the details remain fuzzy. Studying how ATP is generated in vivo by neurons is a question that requires considering the complex spatial arrangement of neurons, astrocytes, and oligodendrocytes at the same time.[9] Although Jakkamsetti et al were able to show that rescuing the Krebs cycle with acetate limited seizure duration, it remains to be seen if this reductionist approach will bear fruit in the case of human patients, where all cell lines are affected.

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