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A Metabolic Paradigm for Epilepsy

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There is a resurgence of interest in the role of metabolism in epilepsy. Long considered ancillary and acknowledged only in the context of clinical application of ketogenic diets, metabolic control of epilepsy is gaining momentum and mainstream interest among researchers. A metabolic paradigm for epilepsy rests upon known perturbations in three major interconnected metabolic nodes and therapeutic targets therefrom (i.e., glycolysis, mitochondria, and redox balance).

Regardless of its etiology, epilepsy is widely accepted as a disease of network excitability arising from altered ionic or synaptic transmission. While metabolic and bioenergetic alterations have been noted in diverse epilepsy syndromes, their role has remained ancillary rather than central. The term "metabolism" broadly refers to a set of chemical reactions that maintain the integrity of the organism and therefore its dysregulation is easily inferred in any disorder of the brain. However, metabolic (dys)function is crucial in brain disorders, such as the epilepsies due to their primary characterization by seizures, which result in periodic spikes of energy demand followed by metabolic adaptation. A metabolic paradigm of epilepsy makes it possible to re-think epilepsy as a metabolic disease rather than one solely focused on cell-surface machinery linked to excitation or inhibition. In order to discuss the role of metabolism in any condition, one first needs to distill this broad definition to the key pathways, which are altered in the tissue affected by the condition. In the case of the epilepsies, metabolic alterations can be primarily characterized by alterations in the following two major pathways: energy producing (glycolysis, tricarboxylic acid or TCA cycle and oxidative phosphorylation) and redox balance (ratios of oxidized to reduced nicotinamide adenine dinucleotide phosphate or NAD(P)/NAD(P)H and reactive species). This brief review attempts to explain metabolic perturbations in the three metabolic nodes, glycolysis, mitochondrial energetics, and redox balance in epilepsy.

The Case for Epilepsy as a Metabolic Disease

Although the epilepsies are diverse with varying etiologies ranging from genetic to acquired, and in infants to the elderly, for the most part the metabolic alterations can be attributed to its common features including the initiating event (either genetic or acquired), epileptogenesis, and chronic seizures (ictal and interictal periods). It is important to note that distinct metabolic alterations occur in each of these phases and even within a phase (i.e. ictal vs interictal periods of epilepsy). Furthermore, the term metabolic dysfunction or mitochondrial dysfunction when specific to an organelle can be applied to any change from normal homeostasis. Given this, metabolic dysfunction in epilepsy can occur early as a result of the initiating event, such as status epilepticus (SE), hypoxia-ischemia, genetic mutation, or infection. Additionally, it can occur during epileptogenesis, that is transformation of a normal to hyperexcitable circuit and in established epilepsy when chronic seizures are observed. In addition to the metabolic changes observed in human and experimental epilepsies, a lot has been learned in the field by studying the effects of metabolic dietary therapies that control epilepsies, such as ketogenic diets (KD) (1). Thus, frequent unexpected bursts of excitability in the brain would expectedly alter major energetic pathways, catabolism (conversion of substrates to energy) and anabolism (biosynthesis of glucose, fats and DNA).

Another concept that fosters the metabolic paradigm of epilepsy is that metabolic dysfunction is a cause and consequence of epilepsy. Several known metabolic perturbations are sufficient to initiate acquired and certain genetic epilepsies. Chief among them in acquired epilepsies are hypoxia-ischemia in childhood or stroke in advanced age, traumatic brain injuries, SE, hypoglycemia, hyperoxia, vascular dysfunction, aging, and tumors (2). Genetic causes include mutations in metabolite transporters (GLUT1, SLC135A), enzymes (TRX1 and ALDH5A1), and nuclear/mitochondrial proteins that control mitochondrial function (POLG, MERFF, MELAS) (3). Comorbid diseases associated with epilepsies, such as stroke, Alzheimer's disease, Down's syndrome, and autism as well as processes, such as sleep and cognitive dysfunction are also known to have a metabolic basis (4).

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Glycolysis

The metabolism of glucose, the preferred substrate in the brain, is accomplished in 10 steps of glycolysis to produce pyruvate which has two possible fates, lactate and acetyl CoA normally under anaerobic and aerobic conditions, respectively. Acute seizure activity in naïve or epileptic brain tissue uses glucose to support the increased firing and is predominantly shuttled toward lactate formation (5). When glucose is available as a substrate, glycolytic rates increase during seizure activity (ictal phases) but decrease below baseline between seizure events (i.e., interictal phases). This ictal hypermetabolism and interictal hypometabolism is a signature metabolic change observed in human and experimental epilepsies and useful as a predictive biomarker for epilepsy surgery (6–9). In chronic epilepsy, glucose hypometabolism would likely be more prevalent given that seizures are brief, isolated events (8, 10). Because the ictal metabolism is fueled primarily by glucose (5), this explains the anti-seizure effects of low carbohydrate treatments, such as 2-DG or KD (11, 12). While glycolysis may appear energetically inefficient in yielding ATP compared with mitochondrial oxidative phosphorylation (OXPHOS), a 10- to 30-fold increased glycolytic rate during ictal events can provide instant energy similar to complete oxidation of glucose via mitochondria. Therefore, increasing glucose utilization by neuronal firing can provide sufficient energy for sustaining hyperexcitability on a level comparable to fully functioning mitochondria. This is not to say that mitochondria are not active during seizure activity. In fact, mitochondrial oxygen consumption rates are changed to varying degrees by seizure activity (see below).

A shift toward glycolysis during seizure activity is prominent as noted extensively by the increased production of lactate (13). Increased glycolytic rates and lactate production by seizure activity in the presence of oxygen, that is aerobic glycolysis, is reminiscent of the Warburg Effect seen in pathologic conditions like cancer cells (14). That epileptic circuits follow a similar metabolic pattern of energy usage as cancer cells suggests that high-energy demands in the brain may be met by this mechanism under pathologic conditions. While cancer cells are thought to selectively use glycolysis to create biomass and/or upregulate glucose-dependent signaling it is unclear what impact a periodic glucose overutilization has in epileptic networks. One long-term consequence of ictal glucose metabolism may be reductive biosynthesis via NADPH and the inadvertent creation of biomass in the epileptic brain, such as gliosis, axon spouting, neurogenesis, and protein synthesis. Another consequence of utilization of glycolysis during ictal periods is glucose-regulated signal transduction for which there is plenty of evidence in epileptic tissue (15, 16). In addition to epilepsies resulting from glucose transporter deficiency, disrupted glucose metabolism is observed in models of acquired (17, 18) and genetic epilepsies (19, 20). Another purpose for increased glucose utilization by hyperexcitable circuits is the formation of NAD^+ in the pyruvate to lactate step catalyzed by lactate dehydrogenase (LDH), which sustains glycolysis. Increased formation of lactate from glycolytic pyruvate via LDH typically occurs in overactive tissues, such as muscle or fermentation by tumor cells when oxygen availability is limited. In hyperexcitable brain networks, this is most likely also the case as brain tissue pO_2 is lowered by continuous seizure activity (21, 22) and lactate production is increased. Recently the anti-seizure effect of LDH inhibitors was demonstrated due to the accumulation of pyruvate (23). However, LDH inhibition may also break the glycolytic cycle by limiting the NAD^+ availability and/or coax the complete oxidation of pyruvate to CO_2 and H_2O via mitochondrial metabolism. The other glycolytic fork leading to increased formation of acetyl-CoA can alter histone acetylation and epigenetic regulation, which are also critically linked with neuronal excitability (but not discussed here for the sake of brevity).

Mitochondria

Mitochondria integrate energy requirements of neuronal cells and circuits with nutrient, ionic, and redox status. Mitochondria are highly dynamic cellular organelles with functional and structural flexibility, and participate in a variety of important cellular functions, including ATP production for maintenance of ionic gradients across cell membranes, neurotransmitter synthesis, regulation of calcium homeostasis, cell death machinery, and redox homeostasis. Trafficking of mitochondria provides additional versatility to meet local energy demands particularly to presynaptic terminals when neuronal excitability is increased (24). Consequently, this can have implications for a variety of brain functions thereby leading to the pathogenesis of neurologic diseases, such as epilepsies associated with periods of high energy utilization (ictal state) interrupted by periods of apparent "rest" (interictal period). Human epileptogenic foci show decreases in N-acetyl-aspartate, a mitochondria-specific signature that has been linked to human temporal lobe epilepsy (TLE) and chronically epileptic mouse hippocampus (25, 26). Severe metabolic dysfunction characterized by biphasic abnormal NAD(P)H fluorescence transients and changes in mitochondrial membrane potential have been observed in ex vivo preparations from both chronically epileptic rats and human TLE subjects (27, 28). Among the enzymes preceding and within the TCA cycle, several have been shown to alter activities (typically decreased) after SE or during chronic epilepsy including citrate transport (SLC13A5) (29), pyruvate dehydrogenase (17, 30), alpha ketoglutarate dehydrogenase, 2-oxoglutarate dehydrogenase (18), and aconitase (31). This is accompanied by evidence of an overall slowing of the TCA cycle in the epileptic brain (26). In terms of deficiencies in OXPHOS enzyme complexes, the most notable is complex I impairment in human TLE tissue (32) and in animal models of epilepsy (33, 34). While alterations in these individual mitochondrial functions may suggest "dysfunctional" mitochondria, direct assessment of the physiologic function(s) of mitochondria in generating ATP via OXPHOS is necessary to truly demonstrate mitochondrial "dysfunction" and assign its role in disease pathophysiology (35). This has been achieved by high-resolution respirometry (36) and extracellular flux analysis (37). Oxygen consumption rates typically increase within minutes of SE onset, decrease after approximately 24 hours (primarily due to increased reactive oxygen species [ROS]), return to control values during the seizure-free latent period and decrease during chronic epilepsy (37).

Evidence that mitochondria are not quiescent bystanders despite major alterations in glycolysis during seizure activity comes from the demonstration that mitochondrial dysfunction

is sufficient to cause seizures and epilepsy. Several rare genetic epilepsies can be caused by mutations in mitochondrial proteins. These include the mitochondrial encephalopathies, pyruvate dehydrogenase, and IDH3A (30, 38, 39). In other cases, genetic epilepsies caused by cytosolic or membrane proteins have been shown to result in mitochondrial dysfunction. These include genes implicated in Dravet Syndrome, such as citrate transporters, potassium channels, and most notably, sodium channels (20, 29, 40, 41). Direct pharmacologic inhibition of mitochondria by fluorcitrate at the TCA cycle level or electron transport chain (ETC) at individual complexes can elicit seizures (42, 43). Validation of TCA cycle disruption in epilepsy comes from the observed efficacy of anapleurotic therapies, such as triheptanoin diet (44) and mitochondrial permeability transition pore inhibition (45).

Redox Balance

The brain is particularly vulnerable to oxidant production due to the abundance of mitochondria, high oxygen demand, poor repair capacity, and the enrichment of polyunsaturated fatty acids (46). Reactive oxygen/nitrogen species (superoxide radicals or O_2, hydrogen peroxide or H_2O_2, nitric oxide and peroxynitrite) and lipid peroxidation end products (malondialdehyde, isoprostanes, 4-hydroxynonenal) are all known to be generated by seizure activity (47). While low concentrations of specific ROS, such as H_2O_2, can function in redox signaling, most other oxidants at high steady-state levels have deleterious roles resulting from oxidative damage (48). The lack of high specificity, non-autooxizable and reliable fluorescent probes have made detailed measurement of the temporal and spatial generation of reactive species in animal and cell models difficult (49). Reliable measurement of reactive species in vivo is possible by redox modification of vulnerable macromolecules, interconvertible redox couples measured by mass spectrometry or high-performance liquid chromatography, spin trapping, or in the case of lipid mediators, mass spectrometry coupled with gas chromatography. At least two sources of SE-induced O_2 (mitochondrial and plasma membrane Nox2) have been revealed by a combination of analytical techniques with genetic mutant animals lacking or overexpressing compartment-specific antioxidants (superoxide dismutases or Sod1 and Sod2) (31, 50, 51). Several mechanisms could contribute to seizure-induced mitochondrial O_2 and H_2O_2 production. These include higher substrate utilization and electron transfer to oxygen during interictal periods, calcium overload, and inhibition of electron transport chain (ETC) complexes downstream causing partial reduction of complexes and transfer of electrons to oxygen. Other mechanisms of increased O_2^- and H_2O_2 production following seizures may involve hyperacetylation and inactivation of the mitochondrial antioxidant Sod2 via decreased activity of Sirtuin-3 or altered activities of enzymes in the peroxide detoxification pathways. Additionally, vicious cycles created by generation of ROS by mitochondrial or nonmitochondrial sources can result in inhibition of the ETC complexes, such as complex I leading to ROS-induced ROS production. When ROS overwhelm endogenous antioxidant defenses, oxidative stress ensues that results in oxidative damage to cellular macromolecules. Oxidative damage has been implicated both as a cause and consequence of prolonged epileptic seizures (52). Highly reactive products arising from oxidative stress, that is reactive aldehydes, hydroxyl radicals, and redox active iron can induce damage to mtDNA, lipids, and proteins leading to seizure-induced neuronal death (53, 54). Depletion of low molecular weight antioxidants, such as glutathione and formation of their oxidized form, glutathione disulfide (GSSG) has been shown to occur in human epilepsy and several animal models of acquired epilepsy (26, 55–57). Validation of reactive species as a pathogenic factor in acquired epilepsy comes from the demonstration that the KD improves the mitochondrial redox status and activates a pleiotropic electrophile gene response that involves activation of Nrf2 (58). This pathway has recently been shown to be therapeutic in epilepsy models (59–61).

Although, genetic inactivation of antioxidant genes, such as *Sod2* and glutathione peroxidase-4 in animals is sufficient to cause epilepsy, mutations in these common antioxidant genes have not been identified in human epilepsies (62, 63). Two notable exceptions exist. First, Kudin and colleagues (64) reported on a mutation in thioredoxin reductase 1 (*TXNRD1*) and its association with genetic generalized epilepsy. Another study, through exome sequencing in patients with seizures and infantile-onset neurodegeneration uncovered a homozygous stop mutation in thioredoxin (*TXN2*) (65). These human studies suggest that epilepsy can arise due to reduced activity of the thioredoxin system. Further support of the importance of this antioxidant system in epilepsy comes from the reciprocal association between selenium-based enzymes, glutathione peroxidases, or thioredoxin reductases and seizures (66, 67).

Conclusion

There is now sufficient knowledge that critical signatures of energy metabolism (i.e. glycolysis, mitochondria, and redox) are altered in diverse epilepsies. These include ictal glucose metabolism, interictal hypometabolism, increased N-acetylaspartate, increased formation of ROS, and dysfunction of mitochondrial TCA cycle and OXPHOS. While KDs have long provided validation of an overall involvement of metabolic dysfunction in epilepsy, newer studies show that specifically targeting glycolysis, mitochondria, and redox pathways have also begun to fuel a metabolic paradigm of epilepsy. In summary, metabolism is poised to take a center stage in epilepsy research.

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