Current Literature

In Basic Science

Sweets Are BAD for Seizures

BAD-Dependent Regulation of Fuel Metabolism and K_{ATP} Channel Activity Confers Resistance to Epileptic Seizures.

Giménez-Cassina A, Martínez-François JR, Fisher JK, Szlyk B, Polak K, Wiwczar J, Tanner GR, Lutas A, Yellen G, Danial NN. *Neuron* 2012;74(4):719–730.

Neuronal excitation can be substantially modulated by alterations in metabolism, as evident from the anticonvulsant effect of diets that reduce glucose utilization and promote ketone body metabolism. We provide genetic evidence that BAD, a protein with dual functions in apoptosis and glucose metabolism, imparts reciprocal effects on metabolism of glucose and ketone bodies in brain cells. These effects involve phosphoregulation of BAD and are independent of its apoptotic function. BAD modifications that reduce glucose metabolism produce a marked increase in the activity of metabolically sensitive K_{ATP} channels in neurons, as well as resistance to behavioral and electrographic seizures in vivo. Seizure resistance is reversed by genetic ablation of the K_{ATP} channel, implicating the BAD- K_{ATP} axis in metabolic control of neuronal excitation and seizure responses.

Commentary

Mitochondria are implicitly linked to neuronal excitability owing to their primary role in cellular ATP generation, maintenance of calcium homeostasis, reactive oxygen species production, control of apoptosis, and biosynthesis of amino acid neurotransmitters (1). It is becoming increasingly apparent that mitochondrial dysfunction is both a cause and a consequence of epileptic seizures (2, 3). Notwithstanding such evidence, the vast majority of studies involving mitochondria have focused on their involvement in cell death processes, and in particular, the consequences of epileptic seizure activity. Few have addressed the role of mitochondria in direct modulation or control of seizure activity (2, 3).

It is well known that mitochondria are dynamic organelles, not only in structure and motility but also in their ability to catabolize different metabolic fuels based on substrate availability and bioenergetic need (4). The importance of metabolic fuels in controlling seizures is underscored by both clinical and experimental observations attesting to the broad-spectrum efficacy of the ketogenic diet (KD). The KD's high-fat/low-carbohydrate composition induces a critical switch in brain utilization of ketones over glucose as a metabolic fuel. While KD is clinically efficacious in controlling diverse types of intractable epilepsies in children and adolescents, its mechanism of action remains obscure (5). Despite a relative lack of mechanistic data, there is renewed interest in the research community to explore ways to modify neuronal excitability by altering meta-

bolic flux, such as through the nonoxidizable glucose analog, 2-deoxyglucose (6).

In this light, Giménez-Cassina and colleagues (2012) provide an intriguing mechanistic link between alternative mitochondrial fuel utilization, apoptotic machinery, and neuronal excitability. These investigators demonstrate that the Bcl-2-associated agonist of cell death (BAD) controls mitochondrial fuel utilization and neuronal excitability via plasmalemmal K_{ATP} channels. The primary rationale of this novel study arose from the recognition that BAD exerts dual effects in cellular function: promoting apoptosis and regulating glucose metabolism (7).

A member of the BH3-only proteins of the Bcl-2 family, BAD exists in either phosphorylated or dephosphorylated states, with resultant opposing effects on cell death. BAD's apoptotic and metabolic roles require (de)phosphorylation of a specific serine 155. Giménez-Cassina and colleagues employed complementary technical approaches to examine the effects of BAD on seizure susceptibility, including BAD mutant mice, mitochondrial functional analysis, cellular electrophysiology, and video-EEG monitoring. Measurement of oxygen consumption rates (OCR) in cultured neurons or astrocytes from BAD knock-out (*Bad*^{−/−}) or phosphodeficient BAD mice engineered with the mutation at serine 155 revealed decreased glucose-driven OCR. Further, neurons or astrocytes from BAD-deficient or *Bad*^{S155A} mutant mice were able to switch to utilization of the primary ketone body β -hydroxybutyrate, but not alternative substrates such as L-glutamine or L-lactate—findings that closely mirror the actions of the KD. However, it is unclear whether the metabolic status of glycolysis in *Bad*^{−/−} and *Bad*^{S155A} mice reflected a global decrease in cellular metabolism or whether glycolytic rates were in fact elevated to compensate for increased OCR. Of importance, *Bad*^{−/−} and *Bad*^{S155A} phospho-mutant mice were resistant

to kainic acid- or pentylenetetrazol-induced seizures, suggesting that the switch in metabolic fuel from glucose to ketones may underlie their resistance to seizure provocation. Further, the authors carefully tease apart the dual apoptotic and metabolic roles of BAD in controlling seizure activity by demonstrating that mice deficient in Bid (BH3-interacting domain death agonist)—another pro-apoptotic gene—do not show seizure resistance.

To establish a causal relationship between fuel regulation by BAD and neuronal hyperexcitability, Giménez-Cassina and colleagues hypothesized that ATP-sensitive potassium (K_{ATP}) channels might be involved. These investigators had appreciated much earlier that K_{ATP} channels represent a compelling mechanistic link between changes in cellular metabolism and neuronal membrane excitability, based on their sensitivity to ATP levels and their ability to dampen excitability during excessive neuronal activation. Specifically, their group had provided strong evidence that ketone bodies increased K_{ATP} channel activity in neurons of both substantia nigra pars reticulata and hippocampus (8, 9).

Using a similar approach, Giménez-Cassina and colleagues found an increased open probability of single K_{ATP} channels in electrophysiological recordings of dentate granule cells in hippocampal slices from *Bad*^{-/-} mice. They also observed increased whole-cell K_{ATP} currents in *Bad*^{-/-} and *Bad*^{S155A} mice. Tolbutamide, a pharmacologic blocker of K_{ATP} channels, reversibly inhibited the increases in both single-channel and whole-cell K_{ATP} currents in *Bad*^{-/-} mice. To establish more directly whether K_{ATP} channels were indeed controlling seizure resistance in BAD-null mice, the authors created *Bad*^{-/-}: *Kir6.2*^{-/-} double mutant mice (*Kir6.2* encodes an inwardly rectifying potassium channel subunit comprising K_{ATP} channels). They found that mutant mice lacking both *Kir6.2* and BAD were resistant to kainic acid-induced seizures, whereas single deletion of *Kir6.2* did not sensitize mice to seizures, confirming the role of K_{ATP} channels in the resistance to seizures exhibited by *Bad*^{-/-} mice.

In their elegant study, Giménez-Cassina et al. piece together mitochondrial fuel regulation by BAD with control of neuronal excitability by K_{ATP} channels and in so doing, help to fill a persistent gap in understanding how a metabolic switch from glycolysis to ketosis can confer an anticonvulsant effect. The renewed quest for elucidating the mechanisms through which the KD controls seizures has led to several intriguing metabolic substrates and enzymes as targets for therapeutic intervention (5). Their study reveals a critical molecular pathway that brings us one step closer towards achieving the lofty

goal of replacing global nonpharmacologic interventions such as diet and exercise with a pharmacologic agent in a “pill” (10). Identification of a phosphorylation-dependent control of fuel choice and seizure control by BAD in mice fed a normal diet suggests a number of pharmacologic approaches, including manipulation of BAD expression/phosphorylation, control of K_{ATP} channels, and utilization of alternative mitochondrial fuels. The latter has recently emerged as a “hot topic” in fields such as aging, cancer, and hormesis (11, 12).

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