In Epilepsy, BAD Is Not Really Bad

Elena Ziviani[1] and Luca Scorrano[1,2,*]
[1]Department of Cell Physiology and Medicine, University of Geneva, 1 Rue M. Servet, 1205 Geneve, Switzerland
[2]Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Via Orus 2, 35129 Padova, Italy
*Correspondence: luca.scorrano@unige.ch
DOI 10.1016/j.neuron.2012.05.003

In this issue of *Neuron*, Giménez-Cassina et al. (2012) show that fuel utilization by neuronal mitochondria, controlled by the Bcl-2 family member BAD, defines response to seizures. Control of K_{ATP} channels by mitochondrial metabolism might be a target for antiepileptic therapies.

Proper neuronal electrical signaling is crucial for coordinated activity of the brain: when this is malfunctioning, epileptic seizures, defined as a "transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain" (Fisher et al., 2005), can arise. However, despite this simple definition, pathogenesis of seizures and of epilepsy is very complex. The early oversimplification that seizures result from a disruption of the equilibrium between neuronal excitation and inhibition has been surpassed by a more integrated view. If we oversimplify our current understanding of how the brain functions, we can say that it results from the integration of multiple cortical networks. Inhibitory neurons, interneuronal synaptic transmission and intrinsic neuronal properties control the continuous oscillation of these networks. Seizures can result from greater spread and neuronal recruitment, caused by the combination of enhanced connectivity and excitatory transmission, reduced inhibitory mechanisms, and changes in intrinsic neuronal properties. Indeed, currently used anticonvulsant drugs remodulate neuronal activity, increasing inhibition, decreasing excitation, or preventing aberrant burst-firing of neurons; ultimately, these drugs prevent excitotoxicity that may lead to brain damage. However, anticonvulsants are not always effective and a cohort of patients is refractory to the current pharmacological treatments. Alternative options range from surgery to diet-dependent glucose limitation (e.g., ketogenic diet) that is recommended for the treatment of pharmacoresistant cases of juvenile epilepsy (Kossoff, 2004).

The efficacy of the dietary therapy in children with epilepsy points to a role for metabolism as a component of the pathogenesis of seizures. Neuronal electrical activity clearly depends on energy metabolism, and therefore on mitochondrial respiration (MacAskill et al., 2010). It is conceivable that administration of alternative metabolic substrates might influence neuronal excitability, although the molecular mechanism of the dependence of activity on metabolic substrates is not fully understood.

Mitochondria are at the crossroad of the most important catabolic pathways, being able to use reducing equivalents from glycolysis, fatty acid beta-oxidation as well as catabolism of amino acids to convert them into ATP. Multiple steps of fine regulation are therefore operative to allow efficient utilization of the different substrates available to the cell. Mitochondria are also crucial in the regulation of diverse cellular responses that ranges from apoptosis (Danial and Korsmeyer, 2004) to autophagy (Gomes et al., 2011). These pathways are often intertwined with the control of metabolism, as exemplified by the function of BAD (BCL-2 associated agonist of cell death), a proapoptotic member of the family of Bcl-2 death regulators, in glucose metabolism and utilization (Danial et al., 2003, 2008). Whether the regulation of neuronal excitability depends on how mitochondria shape intermediate metabolism is however unclear. With this question in mind, Giménez-Cassina et al. (2012) investigated the potential role of BAD in seizures, unraveling in this issue of *Neuron* the existence of a phosphodependent regulatory switch in BAD that reduces neuronal excitability upon kainic acid-induced seizures.

BAD exists in a phosphorylated and dephosphorylated state, which have opposite effects on cell death. Dephosphorylated BAD goes to mitochondria, where it interacts with prosurvival proteins BCL-2 and much more strongly with BCL-XL, sensitizing mitochondria to the action of other BH3-only proapoptotic proteins that can initiate BAX/BAK-dependent apoptosis (Yang et al., 1995). BAD can be specifically phosphorylated on one or multiple specific residues by different protein kinases, including Rsk, PKC, PKB, PKA, and phosphatidylinositol-3-kinase (PI3K). BAD dephosphorylation is also finely tuned by different phosphatases, including PP1, PP2A, and Calcineurin (CnA, also known as PP2B) (Klumpp and Krieglstein, 2002). Phosphorylation of different residues has different effects: for example, phosphorylation of Serine 155 impairs BAD interaction with BCL2/BCL-XL, whereas upon phosphorylation of Serine 112 and Serine 136, binding sites are exposed for its interaction with the cytosolic 14-3-3 proteins. In parallel to and separate from its role in apoptosis, BAD also controls glucose metabolism (Danial et al., 2003). In this respect, BAD phosphorylation does not only prevent initiation of cell death, but it is also required for efficient mitochondrial utilization of glucose in liver, via the scaffolding of a complex containing glucokinase on the surface of the organelle (Danial et al., 2003).

Similarly to what occurs in liver, Giménez-Cassina et al. (2012) show that also cortical neurons and astrocytes from *Bad*$^{-/-}$ mice display lower glucose utilization for mitochondrial respiration. Intriguingly, cortical neurons and astrocytes from mice bearing a phosphodeficient knockin allele of *Bad* at serine 155 (Bad^{S155A}) harbor the same defect. Conversely, mitochondrial consumption of

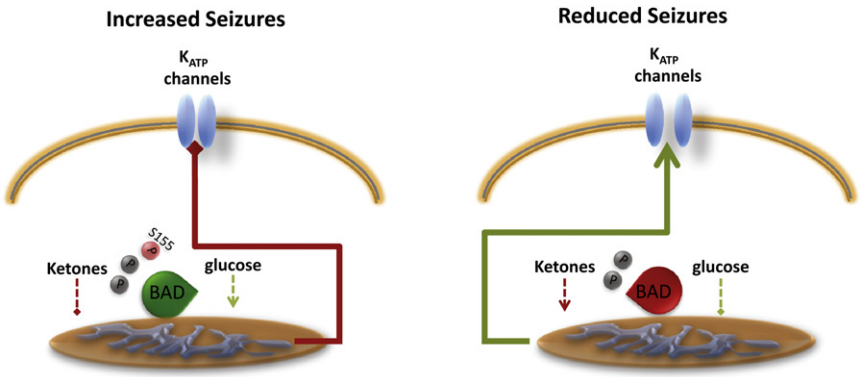

Figure 1. BAD Phosphorylation Controls Mitochondrial Fuel Utilization to Switch Neuronal Excitability

The proapoptotic BH3-only protein BAD can be viewed as a relay: its phosphorylation on Ser 155 switches the preferred source of reducing equivalents for mitochondria from glucose to ketones. Plasma membrane K_{ATP} channels are closed in neurons with "glycolytic" mitochondria, as opposed to the ones where mitochondria use ketone bodies and that are less susceptible to seizures.

the non glucose carbon source β-D-hydroxybutyrate (a ketone body) is increased. Therefore, mitochondria lacking *Bad* selectively switch from glucose to ketone body utilization, whereas BAD phosphorylation on serine 155 favors the opposite switch, from ketone body to glucose. These results suggest an interesting parallelism, at least in terms of fuels used by mitochondria, between mice where BAD is absent or not phosphorylable, and animals on a ketogenic diet that ameliorates seizures. The authors therefore investigated whether BAD might also influence seizure sensitivity in vivo. $Bad^{-/-}$ as well as Bad^{S155A} mice are significantly protected from the proconvulsant drug kainic acid. Decreased sensitivity to seizure response does not result from an impairment of normal brain function in $Bad^{-/-}$ and Bad^{S155A} mice that displayed normal cognitive and motor abilities. Moreover, seizure resistance is specific for BAD and independent from its proapoptotic function, pointing therefore to its role in metabolism.

Neuronal electrical excitability is linked to the activity of ATP-sensitive K^+ (K_{ATP}) channels. K_{ATP} channels are activated following decreased intracellular ATP, in a negative feedback loop that is believed to help neurons to overcome excitotoxicity during seizure. High electrical activity during seizure increases Na^+ influx, which prompts Na^+-K^+ ATPase to actively pump Na^+ outside the cells in a severely endoergonic process. The subsequent decrease in ATP levels opens K_{ATP} channels, tempering excitability during high-activity states (Tanner et al., 2011). Ketogenic diet increases the activity of K_{ATP} channels (Ma et al., 2007), explaining how ketone bodies could ameliorate seizure response. Inspired by this earlier work, Giménez-Cassina et al. (2012) questioned whether K_{ATP} channels played a role in the resistance to seizures of *Bad* mutant mice. Indeed they found that the open probability of single K_{ATP} channels was increased in dentate granule neurons (DGNs) of hippocampal slices from $Bad^{-/-}$ mice. Whole-cell K_{ATP} currents in DGNs were also increased in $Bad^{-/-}$ or Bad^{S155A} mice. In accordance with the hypothesis that *Bad* mutant mice were more resistant to seizure because of the increased activity of K_{ATP} channels, ablation of K_{ATP} channels expression in $Bad^{-/-}$ mice diminished their resistance to seizures (Figure 1).

This important study provides insight into a previously unknown signaling pathway, linking BAD phosphorylation and K_{ATP} channels activity to the attenuation of seizures. Thanks to the elegant combination of genetics, bioenergetics, and electrophysiology, Giménez-Cassina et al. (2012) unveil that fuel utilization by neuronal mitochondria is not a "simple" question of thermodynamic efficiency of the cell, but it crucially controls the neuronal excitatory properties. Importantly, the phosphorylation status of a moonlighting protein like BAD, with a day job in apoptosis and a night one in the scaffolding of glycolytic complexes on the surface of mitochondria (Danial and Korsmeyer, 2004), allows this metabolic switch. This finding paves the way to the design of new drugs, which might be able to mimic BAD activity and to stimulate a switch among respiratory substrates in neuronal mitochondria: for example, PKA that phosphorylates Serine 155 of BAD (Lizcano et al., 2000) could be an attractive target to modulate seizure as well as fuel utilization by mitochondria. Interestingly, PKA inhibitors reduce efficiency of mitochondrial ATP production in starved cells by blocking autophagy-induced mitochondrial elongation (Gomes et al., 2011), opening the possibility that the BAD complex is also a hub where morphological and metabolic cues meet.

We are just beginning to unravel the complex loop between mitochondrial metabolism and seizures. For example, although Giménez-Cassina et al. (2012) convincingly show that the activity of K_{ATP} channels is enhanced in $Bad^{-/-}$ and Bad^{S155A} mice, the molecular link between BAD and opening time of K_{ATP} channels is still obscure. In cardiac cells, the intracellular pool of K_{ATP} channels is mobile and can relocate to the sarcolemma after ischemia, increasing their surface density (Bao et al., 2011). Similarly, it is conceivable that in $Bad^{-/-}$ and Bad^{S155A} neurons, the density of K_{ATP} channels on the plasma membrane might be enhanced, following a yet unknown mechanism of BAD-dependent regulation of endocytic recycling. Alternatively, the K_{ATP} channels might be activated by a signal emanating from mitochondria only when they preferentially use fatty acids: a similar "second messenger" has been identified in glutamate, released from beta-cells mitochondria upon glucose stimulation to promote insulin secretion (Maechler and Wollheim, 1999).

In conclusion, the work of Giménez-Cassina et al. (2012) paves the way toward the understanding of the molecular mechanisms of mitochondrial and metabolic control of seizures.

REFERENCES

Bao, L., Hadjiolova, K., Coetzee, W.A., and Rindler, M.J. (2011). Am. J. Physiol. Heart Circ. Physiol. *300*, H262–H270.

Danial, N.N., and Korsmeyer, S.J. (2004). Cell *116*, 205–219.

Danial, N.N., Gramm, C.F., Scorrano, L., Zhang, C.Y., Krauss, S., Ranger, A.M., Datta, S.R., Greenberg, M.E., Licklider, L.J., Lowell, B.B., et al. (2003). Nature *424*, 952–956.

Danial, N.N., Walensky, L.D., Zhang, C.Y., Choi, C.S., Fisher, J.K., Molina, A.J., Datta, S.R., Pitter, K.L., Bird, G.H., Wikstrom, J.D., et al. (2008). Nat. Med. *14*, 144–153.

Fisher, R.S., van Emde Boas, W., Blume, W., Elger, C., Genton, P., Lee, P., and Engel, J., Jr. (2005). Epilepsia *46*, 470–472.

Giménez-Cassina, A., Martínez-François, J.R., Fisher, J.K., Szlyk, B., Polak, K., Wiwczar, J., Tanner, G.R., Lutas, A., Yellen, G., Nika, N., and Danial, N.N. (2012). Neuron *74*, this issue, 719–730.

Gomes, L.C., Di Benedetto, G., and Scorrano, L. (2011). Nat. Cell Biol. *13*, 589–598.

Klumpp, S., and Krieglstein, J. (2002). Curr. Opin. Pharmacol. *2*, 458–462.

Kossoff, E.H. (2004). Lancet Neurol. *3*, 415–420.

Lizcano, J.M., Morrice, N., and Cohen, P. (2000). Biochem. J. *349*, 547–557.

Ma, W., Berg, J., and Yellen, G. (2007). J. Neurosci. *27*, 3618–3625.

MacAskill, A.F., Atkin, T.A., and Kittler, J.T. (2010). Eur. J. Neurosci. *32*, 231–240.

Maechler, P., and Wollheim, C.B. (1999). Nature *402*, 685–689.

Tanner, G.R., Lutas, A., Martínez-François, J.R., and Yellen, G. (2011). J. Neurosci. *31*, 8689–8696.

Yang, E., Zha, J., Jockel, J., Boise, L.H., Thompson, C.B., and Korsmeyer, S.J. (1995). Cell *80*, 285–291.