Inhibiting glycolysis to reduce seizures: how it might work

Yang Zhong Huang & James O McNamara

An inhibitor of glycolysis is shown to have antiepileptic effects in the rat kindling model, possibly through NADH-dependent regulation of gene expression. This may explain how the 'ketogenic diet' treatment works.

The epilepsies, disorders of recurrent seizures, affect approximately 1% of the population worldwide. About one-third of patients with epilepsy have recurrent seizures despite optimal therapy with available antiepileptic drugs, making it essential to develop new therapies. Almost 100 years ago, researchers discovered that a diet low in carbohydrates and high in fat reduces seizure frequency in some patients with severe epilepsy[1]. This 'ketogenic diet' is still used today as an alternative therapy for drug-resistant epilepsy. How the diet inhibits seizures is uncertain and has been variously attributed to acidosis, dehydration, lipids and changes in energy metabolism within the brain. Because restriction of carbohydrates is a key facet of the diet, and glycolysis is the central pathway of glucose catabolism in mammalian cells, one expected consequence of carbohydrate restriction would be inhibition of glycolysis. Garriga-Canut and colleagues therefore hypothesized that inhibition of glycolysis might be the mechanism by which the ketogenic diet exerts its antiepileptic effects. In this issue, they report[2] that an inhibitor of glycolysis, 2-deoxy-D-glucose (2DG), has powerful antiepileptic effects in rats. These results advance inhibition of glycolysis as an intriguing and plausible mechanism of the ketogenic diet, and suggest that inhibitors of glycolysis may provide new therapies for epilepsy.

To test the effects of 2DG, the authors used the kindling model of epilepsy, in which a brief, low-intensity electrical stimulation is administered through an intracerebral electrode to produce a brief focal seizure. Repeated application of these low-intensity electrical stimulations results in longer and more widely propagated seizures, evident behaviorally as intense contractions of limb and facial muscles similar to those observed in a grand mal seizure in humans. Treatment with 2DG greatly increased the threshold for stimulation-evoked focal seizures and also partially inhibited the progressive severity of epilepsy in this model.

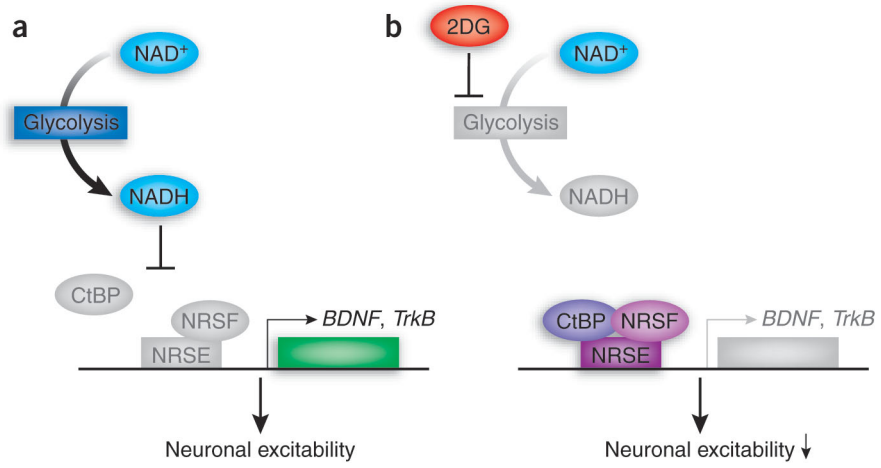

Figure 1 A model for inhibition of kindling progression by 2DG. (**a**) Under normal circumstances, NADH generated by glycolysis destabilizes the interaction of CtBP and NRSF, allowing transcription of NRSF target genes such as *BDNF* and *TrkB* and maintaining normal neuronal excitability. (**b**) Administration of 2DG inhibits glycolysis, reducing NADH concentrations. The co-repressor CtBP is recruited to form the NRSF:CtBP complex on NRSF target genes, reducing their transcription. Lower expression of BDNF and TrkB leads to reduced neuronal excitability, increasing seizure threshold and inhibiting progression of kindling.

How might 2DG exert these antiepileptic effects? The authors focused on the neurotrophin BDNF and its receptor TrkB, because a conditional deletion of TrkB similarly increases the focal seizure threshold and eliminates the progressive severity of epilepsy in the kindling model[3]. They found that, indeed, 2DG inhibited seizure-induced increases in *BDNF* and *TrkB* mRNA levels *in vivo*. The reduction of *BDNF* and *TrkB* gene expression by 2DG in turn raised the question as to how exactly 2DG affects transcriptional regulation. One possibility would be that a transcriptional repressor complex is recruited to the BDNF and TrkB promoters in a 2DG-dependent manner. There is a master negative regulator of neuronal genes called neuron-restrictive silencing factor (NRSF) or repressor of expression of sodium type II (REST)[4,5]. NRSF functions in neural and non-neural tissue by binding to the neuron-restrictive silencing element (NRSE) and recruiting transcriptional repressor complexes onto chromatin to inhibit gene transcription. Because there is an NRSE sequence in the promoter region of both the *BDNF* and the *TrkB* gene, it seemed plausible that NRSF mediates the 2DG-induced inhibition of BDNF and TrkB gene expression. The authors demonstrated that NRSF does indeed bind to the BDNF NRSE, and that 2DG treatment reduces acetylation and increases methylation of histone H3K9, modifications predicted to reduce BDNF transcription. Moreover, distinct inhibitors of glycolysis increased NRSF-mediated gene repression of a Gal4-responsive reporter in JTC-19 cells and reduced *BDNF* mRNA levels in primary cultures of hippocampal neurons. The effects of 2DG on NRSF function may be mediated by the co-repressor C-terminal binding protein (CtBP), which the authors show to form a complex with NRSF *in vitro* in an NADH-regulated manner. Dual genetic deletion of CtBP1 and CtBP2 in mouse embryonic fibroblast cells, or knockdown of CtBP or NRSF in normal mouse mammary

The authors are at the Department of Neurobiology, Department of Medicine (Neurology) and Center for Translational Neuroscience, Duke University Medical Center, Durham, North Carolina 27710, USA.
e-mail: jmc@neuro.duke.edu

gland cells by small hairpin RNAs, relieved the repression of NRSF-target genes by 2DG, implicating the NRSF:CtBP complex in 2DG-induced changes in gene expression. Together, these findings support a model in which inhibition of glycolysis by 2DG results in an NADH-labile association of the NRSF:CtBP complex with NRSF target genes such as BDNF and TrkB, and their reduced expression results in the 2DG-mediated antiepileptic effects (**Fig. 1**).

The new study by Garriga-Canut *et al.* is of interest for a number of reasons. First, it provides a plausible mechanism for how the ketogenic diet exerts its antiepileptic effects, namely by inhibition of glycolysis. It also provides one potential mechanism by which the inhibition of glycolysis exerts these beneficial effects, namely by reduced expression of BDNF and its receptor, TrkB. Finally, the work provides a plausible mechanism by which the inhibition of glycolysis reduces the expression of BDNF and TrkB, namely by recruitment of the co-repressor CtBP to the promoters. That said, some cautionary notes are in order. Was glycolysis actually inhibited *in vivo* by 2DG under the conditions of this experiment? If so, was inhibition of glycolysis the mechanism by which the 2DG exerted these antiepileptic effects? Given the evidence that glycolysis occurs predominantly in astrocytes, followed by transfer of lactate to neurons to fuel their energy demands[6], might the antiepileptic effects of 2DG be mediated by inhibition of glycolysis in astrocytes? Furthermore, alteration of energy metabolism will almost certainly have pleiotropic effects. For example, modifying dendritic mitochondrial activity affects the number of spines and the plasticity of synapses[7], suggesting that synaptic energy metabolism may locally influence synaptic plasticity. These mechanisms could influence antiepileptic effects detected *in vivo*. It is therefore crucial that future follow-up experiments determine whether the reduced expression of BDNF and TrkB is really sufficient to explain the antiepileptic effects of 2DG.

These caveats notwithstanding, the authors should be congratulated for providing a strong and original rationale for how inhibition of glycolysis could reduce neuronal excitability *in vivo*. Moreover, this work advances small-molecule inhibitors of glycolysis as a new and potentially powerful pharmacological approach for the treatment of drug-resistant epilepsy.

1. Engel, J. & Pedley, T. in *Epilepsy: A Comprehensive Textbook* 1339–1342 (Lippincott-Raven, Philadelphia, 1997).
2. Garriga-Canut, M. *et al. Nat. Neurosci.* **9**, 1382–1387 (2006).
3. He, X. *et al. Neuron* **43**, 31–42 (2004).
4. Chong, J.A. *et al. Cell* **80**, 949–957 (1995).
5. Schoenherr, C.J. & Anderson, D.J. *Science* **267**, 1360–1363 (1995).
6. Magistretti, P.J. *J. Exp. Biol.* **209**, 2304–2311 (2006).
7. Li, Z. *et al.* Cell **119**, 873–887 (2004).

Long-distance signaling via presynaptic glutamate transporters

Jacques I Wadiche & Henrique von Gersdorff

Glutamate transporters have long been thought to help terminate the synaptic response through neurotransmitter binding and reuptake, but a new report in this issue identifies a role for their anionic current in information transmission in the retina.

Glutamate is the most common excitatory neurotransmitter in the brain, crucial for communication throughout the nervous system. However, rapid removal of glutamate following its release at the synapse is essential, because it is not subject to extracellular enzymatic degradation, and so without alternative mechanisms for its removal, it would be impossible to control the specificity of its effects. The canonical view of glutamate transporters is that they mediate the reuptake of this transmitter, thereby limiting the potentially damaging effects of lingering glutamate. However, careful study of these transport proteins has suggested that they also mediate an anion conductance that is uncoupled from uptake[1–3]. Since its discovery, the anion current through glutamate transporters has been used as an assay for transport activity, but its physiological function in the cerebellum[4,5] and in the retina[6–8] has remained under debate. A new study by Veruki and colleagues in this issue identifies a novel role for this anion current in information transmission in the retina[9].

To date, five excitatory amino acids transporters (EAATs) have been molecularly isolated. EAATs 1 and 2 are found in glial membranes, whereas EAATs 3–5 are found in neurons. The significance of neuronal uptake is not obvious, as the majority of glutamate uptake is the domain of glial transporters. Unlike that of other neurotransmitters, recycling of vesicular glutamate by neurons is thought to occur through a baroque glutamate-glutamine cycle that uses glial glutamate uptake and glutamine release followed by neuronal glutamine uptake[10]. It is thought that neuronal transporters use their density and location to terminate glutamate signaling.

Besides differences in their location, the fraction of the transporter-mediated current carried by anions also varies among EAAT proteins. EAATs 4 and 5 possess the largest anion conductance among this subfamily, but the functional relevance of this observation remains unclear. The retina-specific EAAT5 is expressed on photoreceptor and bipolar cell terminals, and this strategic location could be revealing because glutamate is released continuously in the dark. However, expression of EAAT5 in heterologous systems is associated with a substantial anion conductance but only modest glutamate uptake[3]. Supporting this notion, transporters from salamander photoreceptors and goldfish bipolar cell terminals boast an anion conductance equivalent to that of small ion channels[6,7,11]. Thus, it has been proposed that the function of EAAT5 transporters lies in their ion channel properties rather than their conventional glutamate transporter activity[7,11].

The tour-de-force study by Veruki *et al.*[9] augments the functional repertoire of glutamate transporters. With a masterful use of paired

Jacques I. Wadiche is in the Department of Neurobiology, University of Alabama at Birmingham, Birmingham, Alabama 35294, USA. Henrique von Gersdorff is at the Vollum Institute, Oregon Health and Science University, Portland, Oregon 97239, USA.
e-mail: jwadiche@nrc.uab.edu or vongersd@ohsu.edu