A New Approach for Epilepsy

By Ray Dingledine, Ph.D. and Bjørnar Hassel, M.D., Ph.D.

Editor’s Note: About one-third of the 65 million people worldwide affected by epilepsy are treatment-resistant, and the degree to which they suffer from seizures and convulsions can vary widely. Problems occur when nerve cells in the brain fail to communicate properly. A new study has found that inhibiting an enzyme that is critical in metabolic communication has an anti-seizure effect in epileptic mice. These findings, the authors believe, may very well initiate a shift to new therapeutic approaches.
Imagine a doctor telling you that you have to change your diet to one with few carbohydrates in favor of high-fat cheeses, butter-fried steaks, bacon and eggs, and eggnog—all while you snack in between meals on macadamia nuts. Sounds great initially, but most of us would tolerate only a few days of eating this way. Yet many young children with epilepsy, who do not respond to conventional medications, benefit from just such a diet. Strict adherence to the so-called ketogenic diet (i.e., with minimal calories from carbohydrates) can often reduce their seizures enough to allow them to attend school and experience the joys of growing up. The diet was developed to mimic the effects of fasting, which has been known since antiquity to afford some seizure control.

Epilepsy and epileptic seizures affect nearly three million Americans and 65 million people of all ages around the world. According to the International League Against Epilepsy, seizures and epilepsy are not the same: “An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous nerve cell activity in the brain. Epilepsy is a disease characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological, and social consequences of this condition. Translation: a seizure is an event, and epilepsy is the disease involving recurrent unprovoked seizures.”

In fact, “epilepsies” are a group of neurologic disorders. When one or more neural circuits in the brain develop a chronically low seizure threshold, normally innocuous stimuli (external to or within the brain) can trigger a group of nerve cells to fire at once. This abnormal synchronicity is a seizure.

The disease has been known for ages. A very early reference is found on a Babylonian tablet in London’s British Museum dating from approximately 1060 BC, which refers to “the falling disease,” with the subjective aura (an ominous feeling) and the subsequent seizures themselves ascribed to the work of childless demons who viewed humans with envy and spite.\(^1\) Hippocrates argued around 400 BC that epilepsy is a physical disorder of the brain, but he was widely disbelieved.\(^2\) Over the next 2,000 years, seizures were treated by bleeding, exorcism, trepanation (a hole is bored in the skull), and ingestion of silver nitrate or bromides.

Over the past several decades more than 30 anticonvulsant medications have been developed. They pass via the bloodstream into the brain and dampen seizures by reducing the excitability of brain cells. They act on a restricted number of molecular targets in the brain. Some of the drugs act on ion “channels” that allow sodium, calcium, and potassium ions to pass into and out of brain cells.
Others potentiate the major inhibitory system of the brain, which uses the neurotransmitter GABA to dampen nerve cell excitability. Additionally, there are drugs that act on the synaptic vesicle protein SV2A, and the AMPA subtype of glutamate receptor.

The ketogenic diet (KD) was introduced in the 1920s in between the first two modern anti-seizure medications, phenobarbital in 1912 and phenytoin in 1938. Even though some patients did not respond to these drugs and improved with the ketogenic diet, it nonetheless fell into obscurity as more anti-seizure medications were introduced. Then, as now, physicians found it far easier to prescribe a pill than to teach their patients that all that was required was to adhere to a rigid and restricted eating regimen. But even with the plethora of anticonvulsant drugs that are now available for people who live with epilepsy in the developed world, a full one-third of epilepsy patients still do not respond to any medication. This situation led to the creation of a childhood epilepsy center and seizure clinic in the mid-1970s at the Johns Hopkins Department of Neurology/Neurosurgery Hospital Clinic, where the ketogenic diet was resurrected as an option.

A drug that works for everyone, though, remains the goal. Because no magic pill exists to eliminate epilepsy as such, the search for new anti-seizure medications has continued, especially studies of drugs that have novel molecular targets in the brain.

**Hope on the Horizon**

Last year a study reporting an unexpected molecular target that could spawn a new generation of anticonvulsant drugs stirred great interest. Sada et al reported the results from four experiments. The researchers started by looking at the effect in brain slices of bathing nerve cells in a solution that contained beta-hydroxybutyrate (BHB) rather than glucose (sugar) as an energy source. BHB is made in the liver when the body breaks down fat, rather than carbohydrates, for energy. This switch from carbohydrate to fat metabolism is, in fact, what occurs in people when they fast or when they are on the ketogenic diet. The study found that BHB hyperpolarized nerve cells, that is, rendered them less excitable and more stable, and thus less prone to epileptic activity.

When on the ketogenic diet, blood levels of sugar decrease while blood levels of BHB increase to exert its stabilizing influence. Even so, blood glucose remains around one-half of the usual level. When BHB was added to a bathing solution that contained a little rather than no glucose, the cells did not hyperpolarize. Rather, they remained active and prone to epileptic activity. This finding
suggests that glucose might offset the stabilizing effects of BHB.

Returning to the nerve cells in Sada’s experiment, the key question is whether they became more stable from the presence of BHB or the absence of glucose. This question gets at the heart of how the ketogenic diet works, because it is still not clear whether its antiepileptic effect is due to low blood levels of glucose or to the high levels of BHB that occur when the body breaks down fat for energy.\textsuperscript{5,6}

Sada et al. investigated this issue by asking whether the glucose-to-BHB switch acts by reducing the formation of pyruvate and lactate. These organic compounds are produced when glucose is broken down, and each can be converted into the other (interconverted) by the enzyme lactate dehydrogenase (LDH). Through a series of experiments, the researchers found: 1) that lactate could indeed undo the stabilizing effect of BHB by reversing its hyperpolarizing action; 2) that lactate caused this effect after it had been converted into pyruvate by LDH, because 3) inhibition of LDH by the small molecule, oxamate, eliminated the anti-hyperpolarizing (depolarizing) effect of lactate, but not that of pyruvate, which continued to depolarize nerve cells even when LDH was inhibited by oxamate, 4) just exposing the nerve cells to oxamate caused them to become hyperpolarized.

**At a Crossroads**

This led to a crucial question: does pyruvate reverse the hyperpolarizing (stabilizing) effect of oxamate by acting as a nutrient — or does it do something else?

Pyruvate is a “keto” acid, carrying a keto (C=O) group. Sada et al. showed that other keto acids (alpha-ketobutyrate and oxaloacetate, see Figure 1) also reversed the stabilizing effect of oxamate, but that ATP or other energy metabolites derived from pyruvate were ineffective. Together, these findings suggest that the depolarizing effect of pyruvate is related to its ability to scavenge the coenzyme NADH (Figure 1) rather than its role as a nutrient. It is likely that BHB, a hydroxyacid, becomes converted to the ketoacid “acetoacetate” with an accompanying formation of NADH. This is especially likely to be the case when BHB is delivered in large quantities, as in a solution that bathes the nerve cells.
Mechanisms to Consider

Thus, the stabilizing or hyperpolarizing effect of BHB appears not to be related to energy production in the nerve cells, but instead could be caused by its promotion of an NADH-dependent process regulating the excitability of nerve cells. Several candidate mechanisms can be considered to explain how LDH inhibition hyperpolarizes nerve cells; much work remains to pin down the exact series of events.

The next question that Sada et al. asked was how pyruvate, converted from lactate, ends up in nerve cells. They found that it probably is delivered to the nerve cells by another cell type, astrocytes. When glucose enters the brain from circulation, it is partly taken up into astrocytes. Inside these cells, some of the glucose will be converted into lactate, which leaves the astrocytes. Nerve cells then take up the lactate from astrocytes through a specific transport protein, completing an astrocyte-to-neuron lactate shuttle.\(^7\)\(^9\) The Sada group concluded that lactate from astrocytes is an important source of “epileptogenic” (seizure-promoting) pyruvate in nerve cells.

Sada and colleagues had found that bathing nerve cells in the LDH inhibitor oxamate caused the
cells to hyperpolarize and become electrically stable. They followed this finding up by injecting oxamate into the hippocampus of mice that had been rendered epileptic after treatment with kainate (a neuroexcitatory amino acid). Oxamate reduced seizures in this mouse model of epilepsy, suggesting that LDH inhibition could be antiepileptic in vivo too. Now that LDH was identified as a potential target for antiepileptic therapy, Sada et al. looked for antiepileptic drugs (AEDs) that might inhibit LDH. The researchers screened 20 AEDs and found that a little-used AED, stiripentol, did inhibit LDH. Stiripentol is currently used to treat a rare and devastating epileptic condition known as Dravet syndrome or severe myoclonic epilepsy of infancy. They then tested similar molecules for LDH inhibition and found that the structurally simpler compound, isosafrole, was a more potent inhibitor of LDH than stiripentol itself.

**Pyruvate depolarizing nerve cells**

The picture emerging from the work of Sada et al. is that pyruvate facilitates epileptic activity by depolarizing nerve cells. It had been shown earlier that rapid injection of pyruvate could actually cause seizures. Sada and colleagues showed that blocking the enzyme responsible for pyruvate formation from lactate (LDH) has an anti-seizure effect.

This remarkable study helps explain the well-known observation that eating a sugar-laden cookie can quickly promote seizures in a child on the ketogenic diet: when the sugar (glucose) from the cookie is metabolized, it forms pyruvate; the pyruvate then rapidly reverses the stabilizing effect of BHB. Further, the Sada group identified the role of LDH in the glucose effect, and they showed that pharmacological inhibition of LDH exerts marked anti-seizure effects in epilepsy animal models — thus fingering LDH as the first new target for discovering epilepsy drugs since 2004. Important for understanding their results is the recognition that the seizure-promoting mechanism of the glucose metabolites—pyruvate and oxaloacetate—is unrelated to ATP generation but could involve NADH scavenging.

Key steps remain in the quest to develop novel anti-seizure drugs based on reduced formation of pyruvate. First, we might identify additional anti-seizure targets by identifying the mechanism involved in oxaloacetate-induced depolarization. A good next step for this would be systematic testing of other Kreb’s cycle metabolites, especially NADH, for their ability to reverse oxamate-induced hyperpolarization. Second, there are multiple, cell-specific but functionally similar forms of
LDH; further drug screening might allow glycolytic inhibition that is restricted to nerve cells, which could reduce side effects. Third, their unexpected finding that inhibitory interneurons are not hyperpolarized (stabilized) by LDH inhibitors, although fortunate for epilepsy therapy, is worth following up.

Finally, inhibition of glucose metabolism to treat epilepsy has been reported previously, using 2-deoxyglucose (2-DG), and it would be interesting to determine if 2-DG and isosafrole block one another’s action or are synergistic. The answer could inform whether reduced glycolysis or some unrelated action is responsible for the anti-seizure effect of 2-DG. If the two are synergistic, combination therapy could be warranted. While not quite “epilepsy diet in a pill”, the study by Sada et al. points the way to a “starvation in a pill” strategy—i.e., pharmacologically simulating fasting—for seizure control. Anti-seizure drugs have been repeatedly repurposed for other neuropsychiatric disorders such as bipolar disorder and neuropathic pain. If chronic treatment with 2-DG and an LDH inhibitor prove safe in people, might we add another repurposed clinical use to this list—weight control?

Bios

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ability to communicate. He explores the use of sensors for physiological parameters (pulse, plasma glucose, etc.) as a means by which they can communicate their needs and their degree of well-being. His preclinical research centers on cerebral energy metabolism, including how it is affected by anti-epileptic drugs.

References

1. Wilson JV, Reynolds EH. (1990). Texts and documents: translation and analysis of a cuneiform text forming part of a Babylonian treatise on epilepsy. Med. Hist. 34:185–198.
2. Todman D. (2008). Epilepsy in the Graeco-Roman world: Hippocratic medicine and Asklepiean temple medicine compared. J. Hist. Neurosci. 17:435–441.
3. Sada N, Lee S, Katsu T, Otsuki T, Inoue T. (2015) Epilepsy treatment. Targeting LDH enzymes with a stiripentol analog to treat epilepsy. Science. 347:1362-1367.
4. Klepper J, Leiendecker B, Bredahl R, Athanassopoulos S, Heinen F, Gertsen E, Flörcken A, Metz A, Voit T. (2002) Introduction of a ketogenic diet in young infants. J Inherit Metab Dis. 25:449-460.
5. Muzykewicz DA, Lyczkowski DA, Memon N, Conant KD, Pfeifer HH, Thiele EA.(2009) Efficacy, safety, and tolerability of the low glycemic index treatment in pediatric epilepsy. Epilepsia. 50:1118-1126.
6. Wu YJ, Zhang LM, Chai YM, Wang J, Yu LF, Li WH, Zhou YF, Zhou SZ. (2016) Six-month efficacy of the Ketogenic diet is predicted after 3 months and is unrelated to clinical variables. Epilepsy Behav. 55:165-9.
7. Mächler P, Wyss MT, Elsayed M, Stobart J, Gutierrez R, von Faber-Castell A, Kaelin V, Zund M, San Martín A, Romero-Gómez I, Baeza-Lehnert F, Lengacher S, Schneider BL, Aebischer P, Magistretti PJ, Barros LF, Weber B. (2016) In Vivo Evidence for a Lactate Gradient from Astrocytes to Neurons. Cell Metab. 23:94-102.
8. Tsacopoulos M, Magistretti PJ. (1996) Metabolic coupling between glia and neurons. J. Neurosci. 16:877-885.
9. Bélanger M, Allaman I, Magistretti PJ. (2011) Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. Cell Metab. 14:724-738.
10. Gonzalez SV, Nguyen NH, Rise F, Hassel B. (2005) Brain metabolism of exogenous pyruvate. J Neurochem. 95:284-93.
11. Huttenlocher PR. (1976) Ketonemia and seizures: metabolic and anticonvulsant effects of two ketogenic diets in childhood epilepsy. Pediatr Res. 10:536-540.

12. Lynch BA, Lambeng N, Nocka K, Kensel-Hammes P, Bajjalieh SM, Matagne A, Fuks B. (2004) The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. Proc Nat Acad Sci USA 101:9861-9866.

13. Garriga-Canut M, Schoenike B, Qazi R, Bergendahl K, Daley TJ, Pfender RM, Morrison JF, Ockuly J, Stafstrom C, Sutula T, Roopra A. (2006) 2-Deoxy-D-glucose reduces epilepsy progression by NRSF-CtBP-dependent metabolic regulation of chromatin structure. Nat. Neurosci. 9:1382–1387.