CAN REDUCING SUGAR RETARD KINDLING?

2-Deoxy-D-Glucose Reduces Epilepsy Progression by NRSF-CtBP-Dependent Metabolic Regulation of Chromatin Structure. Garriga-Canut M, Schoenike B, Qazi R, Bergendahl K, Daley TJ, Pfender RM, Morrison JF, Ockuly J, Stafstrom C, Sutula T, Roopra A. *Nat Neurosci* 2006;9(11):1382–1387. Temporal lobe epilepsy is a common form of drug-resistant epilepsy that sometimes responds to dietary manipulation such as the 'ketogenic diet'. Here we have investigated the effects of the glycolytic inhibitor 2-deoxy-D-glucose (2DG) in the rat kindling model of temporal lobe epilepsy. We show that 2DG potently reduces the progression of kindling and blocks seizure-induced increases in the expression of brain-derived neurotrophic factor and its receptor, TrkB. This reduced expression is mediated by the transcription factor NRSF, which recruits the NADH-binding co-repressor CtBP to generate a repressive chromatin environment around the BDNF promoter. Our results show that 2DG has anticonvulsant and antiepileptic properties, suggesting that anti-glycolytic compounds may represent a new class of drugs for treating epilepsy. The metabolic regulation of neuronal genes by CtBP will open avenues of therapy for neurological disorders and cancer.

COMMENTARY

Despite over eight decades of clinical experience with the ketogenic diet (KD), the mechanisms accounting for its anticonvulsant action remain unknown. Many theories have been advanced to explain the KD's efficacy, however none has been widely substantiated. Nevertheless, it is becoming increasingly clear that metabolic and biochemical adaptation to the KD is critically linked to its anticonvulsant properties (1). Thus, investigators have sought clues among the various pathways involved in energy metabolism, including glycolysis, fatty acid oxidation, mitochondrial respiration, and more recently, the pentose phosphate shunt (2).

Intriguingly, there are several lines of evidence to suggest that calorie restriction alone may prevent seizure activity. First, the classic KD regimen mandates carbohydrate restriction, which results in mild hypoglycemia, a factor known to reduce seizures. Second, to maintain clinical efficacy (i.e., reduce seizures), the carbohydrate restriction must be strictly enforced; in contrast, patients well controlled on a KD can abruptly lose seizure freedom shortly after carbohydrate ingestion. Third, calorie restriction in a mouse model of stimulus-induced epilepsy produced not only mild hypoglycemia but also retarded epileptogenesis (3). And finally, animals fed a calorie-restricted control diet exhibited diminished neuronal excitability, enhanced paired-pulse inhibition, and elevated electroconvulsive seizure threshold than did ad libitum-fed control rats (4). Taken together, both experimental and clinical observations suggest that inhibition of glycolytic flux might result in anticonvulsant action.

Therefore, it is of considerable interest that Garriga-Canut et al. found that 2-deoxy-D-glucose (2-DG), an inhibitor of phosphoglucose isomerase, reduced seizure progression in the rat kindling model of temporal lobe epilepsy. Administration of 250 mg/kg 2-DG half an hour before kindling stimulation resulted in an increase in the current intensity required to evoke after-discharges during progression to class V seizures. To establish a mechanism for this compelling observation, the authors took note of the recent finding that electrical kindling in mice could be prevented by deleting the gene encoding the neurotrophin, brain-derived neurotrophic factor (BDNF), as well as by eliminating the gene encoding its principal receptor, tyrosine kinase B (TrkB) (5). Accordingly, Garriga-Canut and colleagues hypothesized that 2-DG might block BDNF and/or TrkB expression. Using quantitative real-time PCR of reverse-transcribed RNA (QRT-PCR), they demonstrated that 2-DG-treated rats had significantly decreased hippocampal expression of BDNF and TrkB compared with controls. Garriga-Canut et al. further considered the possibility that 2-DG might affect transcriptional regulation of these genes. Specifically, they demonstrated that the transcription factor, neuron restrictive silencing factor (NRSF), a master negative regulator of neuronal genes (6), recruited the NADH-binding corepressor C-terminal binding protein (CtBP) to establish a repressive chromatin environment around the BDNF promoter.

This study is important and provocative for several reasons. First and foremost, it directly links glycolytic inhibition to a mechanism of transcriptional repression that retards epileptogenesis in the rat kindling model of epilepsy. Second, the work provides compelling evidence that the KD may possess anti-epileptogenic properties, in addition to anticonvulsant actions. Third, there are significant clinical implications, in that 2-DG and possibly related compounds might represent a new class of anticonvulsant medications. As 2-DG has already been demonstrated to be well tolerated in human clinical trials (7), this latter possibility is especially noteworthy, since the traditional KD is difficult to maintain and is fraught with a number of side effects that preclude its use, even in the face of dramatic clinical efficacy.

Epilepsy Currents, Vol. 8, No. 3 (May/June) 2008 pp. 83–84
Blackwell Publishing, Inc.

However, as plausible as the findings of Garriga-Canut et al. may appear, a number of questions and challenges remain. First, does 2-DG treatment induce the same biochemical changes seen with the KD—that is, a shift toward intermediary metabolism (e.g., enhanced fatty acid oxidation)? If so, would the changes from 2-DG treatment be partially reflected in ketosis—even at a modest level comparable to calorie restriction? And if so, could ketone bodies alone explain the effects attributed to 2-DG? It is not known whether ketone bodies alone can retard kindling. Second, although the authors demonstrated that 2-DG elicited transcriptional hallmarks of reduced glycolysis in the hippocampus, was glycolysis actually inhibited in vivo? They did not provide direct data in this regard. Third, is the proposed mechanism also relevant to epileptogenesis in the developing brain? The clinical efficacy of the KD has been most clearly established in the immature brain. Kindling acquisition in immature animals differs in several respects compared with adults. For example, immature rodents are relatively resistant to kindling, suggesting that BDNF and TrkB expression may not play an important role (8). Indeed, BDNF expression is ordinarily quite low in developing regions of the normal central nervous system (9). Fourth, it is understood that the KD exerts anticonvulsant and antiepileptogenic actions throughout the brain (1). If 2-DG decreases BDNF and TrkB expression only in hippocampus, then one could challenge the notion that the finding represents a fundamental mechanism of KD action. Fifth, inhibition of glycolysis in a systemic manner will likely exert widespread and pleiotropic effects in all cell populations—outside the brain as well. Thus, what other actions of 2-DG might account for the antiepileptogenic actions noted herein? For example, reduced glycolytic flux might lead to compensatory mitochondrial biogenesis and an increase in energy reserve (10) initiated in astrocytes, which are the predominant locus of glycolysis in the brain. Also, potentially relevant to 2-DG antiepileptogenic actions, Muller et al. recently reported that 2-DG inhibits protein synthesis in immature rodent brain (11). Finally, 2-DG may have a different profile of activity in conventional seizure models than does the KD (12), suggesting that it is unlikely to truly replace the KD. In this regard, it would be of interest to determine if 2-DG is effective in other experimental models of epilepsy.

Notwithstanding these caveats, the findings of Garriga-Canut et al. are nevertheless thought provoking, and lay novel groundwork in the search of mechanisms responsible for dietary control of epilepsy. Plasma membrane ion channels and transporters have traditionally been considered the major targets for antiepileptic drugs. Now, bioenergetic substrates—including enzymes involved in energy metabolism, mitochondrial proteins, and diverse regulatory factors—also must be considered potential antiepileptic targets. Although this study does not put closure on efforts to determine mechanisms of KD action, it opens the door for additional studies exploring metabolic regulation of seizure activity (2). In the end, bioenergetic modulators may one day represent a novel class of pharmacological agents used to treat seizures resistant to conventional anticonvulsant drugs.

by Jong M. Rho, MD

References

1. Bough KJ, Rho JM. Anticonvulsant mechanisms of the ketogenic diet. *Epilepsia*, 2007;48(1):43–58.
2. Lian XY, Khan FA, Stringer JL. Fructose-1,6-bisphosphate has anticonvulsant activity in models of acute seizures in adult rats. *J Neurosci* 2007;27:12007–12011.
3. Greene AE, Todorova MT, McGowan R, Seyfried TN. Caloric restriction inhibits seizure susceptibility in epileptic el mice by reducing blood glucose. *Epilepsia* 2001;42:1371–1378.
4. Bough KJ, Schwartzkroin PA, Rho JM. Calorie restriction and ketogenic diet diminish neuronal excitability in rat dentate gyrus in vivo. *Epilepsia* 2003;44:752–760.
5. He XP, Kotloski R, Nef S, Luikart BW, Parada LF, McNamara JO. Conditional deletion of TrkB but not BDNF prevents epileptogenesis in the kindling model. *Neuron* 2004;43:31–42.
6. Schoenherr CJ, Anderson DJ. The neuron-restrictive silencer factor (NRSF): a coordinate repressor of multiple neuron-specific genes. *Science* 1995;267:1360–1363.
7. Mohanti BK, Rath GK, Anantha N, Kannan V, Das BS, Chandramouli BA, Banerjee AK, Das S, Jena A, Ravichandran R, Sahi UP, Kumar R, Kapoor N, Kalia VK, Dwarakanath BS, Jain V. Improving cancer radiotherapy with 2-deoxy-D-glucose: phase I/II clinical trials on human cerebral gliomas. *Int J Radiat Oncol Biol Phys* 1996;35:103–111.
8. Haas KZ, Sperber EF, Opanashuk LA, Stanton PK, Moshe SL. Resistance of immature hippocampus to morphologic and physiologic alterations following status epilepticus or kindling. *Hippocampus* 2001;11:615–625.
9. Maisonpierre PC, Belluscio L, Friedman B, Alderson RF, Wiegand SJ, Furth ME, Lindsay RM, Yancopoulos GD. NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. *Neuron* 1990;5:501–509.
10. Bough KJ, Wetherington J, Hassel B, Pare JF, Gawryluk JW, Greene JG, Shaw R, Smith Y, Geiger JD, Dingledine RJ. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. *Ann Neurol* 2006;60:223–235.
11. Muller AP, Rotta LN, Kawano C, Leszczinski DN, Schweigert ID, Londero LG, Gravina FS, da Silveira CK, de Souza CG, Battu CE, Goncalves CA, de Souza DO, Perry ML. Effect of 2-deoxy-D-glucose on aminoacids metabolism in rats' cerebral cortex slices. *Neurochem Res* 2006;31:417–422.
12. Yankura J, French A, Rogawski MA, Hartman A, Gasior M. Glucose analog, 2-deoxyglucose, fails to demonstrate potent anticonvulsant efficacy in rodent seizure tests. *Epilepsia* 2006;47(suppl 4):338–339.