MEANS, MOTIVE, AND OPPORTUNITY: ESTABLISHING CULPABILITY IN PHARMACORESISTANT EPILEPSY

Inhibition of the Multidrug Transporter P-Glycoprotein Improves Seizure Control in Phenytoin-Treated Chronic Epileptic Rats. van Vliet EA, van Schaik R, Edelbroek PM, Redeker S, Aronica E, Wadman WJ, Marchi N, Vezzani A, Gorter JA. *Epilepsia* 2006;47: 672–680. **PURPOSE:** Overexpression of multidrug transporters such as P-glycoprotein (P-gp) may play a significant role in pharmacoresistance, by preventing antiepileptic drugs (AEDs) from reaching their targets in the brain. Until now, many studies have described increased P-gp expression in epileptic tissue or have shown that several AEDs act as substrates for P-gp. However, definitive proof showing the functional involvement of P-gp in pharmacoresistance is still lacking. Here we tested whether P-gp contributes to pharmacoresistance to phenytoin (PHT) by using a specific P-gp inhibitor in a model of spontaneous seizures in rats. **METHODS:** The effects of PHT on spontaneous seizure activity were investigated in the electrical post-status epilepticus rat model for temporal lobe epilepsy, before and after administration of tariquidar (TQD), a selective inhibitor of P-gp. **RESULTS:** A 7-day treatment with therapeutic doses of PHT suppressed spontaneous seizure activity in rats, but only partially. However, an almost complete control of seizures by PHT ($93\% \pm 7\%$) was obtained in all rats when PHT was coadministered with TQD. This specific P-gp inhibitor was effective in improving the anticonvulsive action of PHT during the first 3–4 days of the treatment. Western blot analysis confirmed P-gp upregulation in epileptic brains (140–200% of control levels), along with approximately 20% reduced PHT brain levels. Inhibition of P-gp by TQD significantly increased PHT brain levels in chronic epileptic rats. **CONCLUSIONS:** These findings show that TQD significantly improves the anticonvulsive action of PHT, thus establishing a proof-of-concept that the administration of AEDs in combination with P-gp inhibitors may be a promising therapeutic strategy in pharmacoresistant patients.

COMMENTARY

The three pillars of guilt will be instantly recognizable to viewers of television cop shows and courtroom dramas. Means, motive, and opportunity often need to be established to secure a successful prosecution in criminal proceedings. A very similar set of criteria can be considered essential in assessing the validity of biological mechanisms that contribute to disease or shape the individual characteristics of that disease. Just like the detective, it is incumbent on the scientist to establish burden of proof and to provide evidence that is beyond any reasonable doubt.

The issue of pharmacoresistance in epilepsy has received considerable attention in recent years, and the search for mechanisms that might explain why 35% of patients fail to respond to current medications continues apace. A number of plausible hypotheses have been proposed, including inadequate penetration of antiepileptic drugs (AEDs) across the blood–brain barrier; acquired alterations to the structure and/or functionality of ion channels and neurotransmitter receptors that represent the principal targets of AEDs; the narrow pharmacological spectrum, and thereby, inadequacy of current agents; and an inher-

ent resistance, governed by genetic variants of proteins involved in the pharmacokinetics and pharmacodynamics of AED action (1). Of these, the so-called transporter hypothesis, which describes the active extrusion of antiepileptic agents from their intended site of action, is the most extensively researched and documented.

Drug-transporter proteins are expressed throughout the body and control the transfer of endogenous and exogenous molecules across biological membranes. They are predominantly expressed in organs with excretory functions, such as the gastrointestinal tract, liver, and kidney, but also protect sensitive tissues, including the brain, placenta, and testes, from potentially toxic xenobiotics (2). The transporter hypothesis of drug-resistant epilepsy is founded on the premise that overexpression of drug transporting proteins at the blood–brain barrier prevents AEDs from reaching the interstitial space and, thereby, exerting their pharmacodynamic effects. There are, of course, several caveats to this proposition, and these have been elegantly summarized in four specific criteria (3), which represent the “means, motive and opportunity” of the drug-transporter hypothesis.

1. *The mechanism must be detectable in relevant brain tissue:* Initial evidence to support a role for drug-transporter proteins in the causation of refractory epilepsy was

derived from a manuscript by Tishler and colleagues (4) that reported overexpression of the *MDR1* gene, which encodes the drug transporter P-glycoprotein (P-gp), in surgically resected temporal lobe tissue from people with medically intractable epilepsy. Numerous studies have since confirmed this finding and demonstrated an up-regulation of P-gp and related transporters in several other neuropathologies, including focal cortical dysplasia, dysembryoplastic neuroepithelial tumor, and hippocampal sclerosis—all of which are associated with uncontrolled seizures (5). It is interesting to note that the majority of reports suggest that overexpression of transport proteins is confined to the epileptic or lesional area, with relative sparing of adjacent, normal tissue. This observation has important implications for the validity of the hypothesis and would explain why people with non-responsive epilepsy continue to experience CNS-related side effects from their medications.

2. *The mechanism must have appropriate functionality:* If active extrusion by drug-transporter proteins at the blood–brain barrier has functional and clinical relevance to the phenomenon of refractory epilepsy, then the majority of AEDs, if not all of them, must be substrates for one or more transport systems. This fundamental premise has been assessed in a series of experimental investigations employing a variety of techniques and models. Although there remains considerable contention, recent studies in experimental animals appear to suggest that most of the commonly used antiepileptic agents can be transported to some extent by these proteins (6). In the absence of a gold-standard method for the assessment of substrate specificity, there is a common assumption that drug efflux is promiscuous, species-independent, and of sufficient capacity to be nonsaturable at clinically relevant concentrations. On this point, the jury is still out.
3. *The mechanism must be active in drug resistance:* In order to demonstrate the active involvement of transporter proteins in drug responsiveness, it is necessary to establish a link among protein expression, brain drug concentrations, and the efficacy of AEDs. This link has been explored in both experimental seizure models and surgically resected human brain tissue. Up-regulation of P-gp in the rat hippocampus following kainate-induced seizures is associated with a reduction in local phenytoin levels (7), and a direct relationship between the expression of P-gp and concentrations of the primary active metabolite of oxcarbazepine in human brain has been reported (8). These observations are further strengthened by an elegant investigation that demonstrated a correlation between the efficacy of phenobarbital and

levels of P-gp expression in limbic brain regions of animals following status epilepticus induced by electrical stimulation of the basolateral amygdala (9). Not only is the validity of the hypothesis reinforced by these studies, but they also lend support to the notion that seizures can provoke the expression of drug transporters and that this elevated expression, in turn, can restrict brain access of AEDs, completing an apparent vicious cycle.

4. *Overcoming the mechanism counteracts drug resistance:* This final piece of the jigsaw puzzle has been addressed by a handful of experimental and clinical studies (10,11), most notably the recent paper by van Vliet and colleagues, reviewed here. Electrical stimulation of the rat angular bundle was employed to induce an acute status epilepticus and subsequent spontaneous seizures, against which the investigators assessed the efficacy of phenytoin alone and in combination with tariquidar, a novel, selective P-gp inhibitor. When administered alone, phenytoin demonstrated only modest activity, reducing seizure frequency when measured over a 7-day period, without rendering any of the animals seizure-free. In the presence of tariquidar, phenytoin was significantly more effective, with a dramatic reduction in seizure frequency and seizure freedom in five of six animals for up to 4 days. These studies were complemented by Western blot analysis and drug concentration measurements that confirmed elevated expression of P-gp in the ipsilateral hippocampus and entorhinal cortex as well as an increased brain penetration of phenytoin in the presence of tariquidar. When considered together, the multiple findings of this study would appear to offer final and, some might argue, unequivocal confirmation of the multidrug-transporter hypothesis of refractory epilepsy.

The manuscript by van Vliet and colleagues provides support, at least on some level, for all four of the criteria discussed earlier: overexpression of P-gp in a model of epilepsy, evidence to suggest that phenytoin is a substrate for P-gp-mediated extrusion, a demonstration of transporter up-regulation in the causation of drug-resistant seizures, and the reversal of pharmacoresistance by selective inhibition. Of minor concern in this study is the apparently transient effect of P-gp inhibition, the unconvincing efforts to exclude an anticonvulsant effect of tariquidar, the failure to quantify any change in the tolerability profile of phenytoin, and the unusual decision to analyze drug concentrations in the epileptic region alone. Despite these limitations, the experimental evidence offered by van Vliet et al. is relatively compelling. It is perfectly reasonable to conclude that P-gp plays a significant role in mediating resistance to AEDs in animal models of epilepsy and that inhibition of P-gp can

circumvent this mechanism, but whether this phenomenon extends to other drug-transporter proteins or to the clinical arena remains unclear.

Principles of clinical pharmacology dictate that pharmacokinetic barriers to drug action, such as enhanced hepatic metabolism or augmented efflux from the brain, should be surmountable by successive increases in dose. This principle does not apply clinically in the case of intractable epilepsy and, thus far, has not been specifically evaluated in experimental models of refractory seizures. From a clinical standpoint, there is little direct or indirect evidence to support the assertion that AEDs are sufficiently strong substrates for transporter-mediated extrusion from the brain to account for their wholesale lack of efficacy. This premise is supported by data from laboratory studies that suggest compounds, such as phenytoin, are much more effectively transported by rodent P-gp than the human equivalent (12). It is entirely possible that all of this fascinating and at times, convenient evidence applies only to rodents. There is little doubt that the drug-transporter hypothesis of refractory epilepsy has biological plausibility—that is, means, motive, and opportunity have been proven in the laboratory. This assertion is great news for rats with refractory epilepsy but the evidence to suggest that culpability extends to the human species remains largely circumstantial and, as yet, is unlikely to stand up in a court of law.

by Graeme J. Sills, PhD

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A POSSIBLE EXPLANATION FOR THE PARADOX OF HYPEREXCITABILITY AND EPILEPSY IN “LOSS OF FUNCTION” VOLTAGE-GATED SODIUM CHANNEL MUTATIONS

Reduced Sodium Current in GABAergic Interneurons in a Mouse Model of Severe Myoclonic Epilepsy in Infancy. Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA. *Nat Neurosci* 2006;9: 1142–1149. Voltage-gated sodium channels (Na_v) are critical for initiation of action potentials. Heterozygous loss-of-function mutations in Na_v1.1 channels cause severe myoclonic epilepsy in infancy (SMEI). Homozygous null *SCN1A*^{-/-} mice developed ataxia and died on postnatal day (P) 15 but could be sustained to P17.5 with manual feeding. Heterozygous *SCN1A*^{+/-} mice had spontaneous seizures and sporadic deaths beginning after P21, with a notable dependence on genetic background. Loss of Na_v1.1 did not change voltage-dependent activation or inactivation of sodium channels in hippocampal neurons. The sodium current density was, however, substantially reduced in inhibitory interneurons of *SCN1A*^{+/-} and *SCN1A*^{-/-} mice but not in their excitatory pyramidal neurons. An immunocytochemical survey also showed a specific upregulation of Na_v1.3 channels in a subset of hippocampal interneurons. Our results indicate that reduced sodium currents in GABAergic inhibitory interneurons in *SCN1A*^{+/-} heterozygotes may cause the hyperexcitability that leads to epilepsy in patients with SMEI.

COMMENTARY

Voltage-gated sodium channels (Na_v) underlie the generation and propagation of action potentials and are some of the most important proteins contributing to the excitability of neurons. These sodium channels are the primary targets of widely used and efficacious antiepileptic therapies, such as phenytoin, carbamazepine, oxcarbazepine, and lamotrigine. Therefore, it is not surprising that many of the mutations in ion channels associated with human diseases (i.e., channelopathies) involve voltage-gated sodium channels. Considering the complex physiological systems in which they function, particularly in the CNS, the precise mechanisms by which identified Na_v mutations alter neuronal excitability to produce particular disorders, including epilepsy, are not well understood.

Various genes (e.g., *SCN1A–SCN9A*) encode nine isoforms of the main pore-forming sodium channel α subunit. In addition, four genes (*SCN1B–SCN4B*) encode auxiliary β subunits that influence the biophysical properties of the channel. To date, there are nearly 20 known diseases that result from mutations in the sodium channel genes, including neuromuscular disorders and cardiac arrhythmias, with the clinical manifestations reflecting the expression pattern of the gene. For example, *SCN5A* is expressed mostly in cardiac muscle, where it is the major sodium channel of the ventricular conduction system, and mutations in this gene cause congenital long QT syndrome. Four sodium channel genes, *SCN1A*, *2A*, *3A*, and *8A* are highly expressed in the CNS, where they each exhibit a distinct subcellular localization. Accordingly, *SCN1A*, encod-

ing the sodium channel α subunit protein designated Na_v1.1, is expressed on the cell body of neurons throughout the brain (1), while Na_v1.2 appears to be localized to unmyelinated axons and dendrites (2). Since many antiepileptic drugs inhibit Na_vs, there has been speculation that differential expression of the sodium channel α and β subunits on excitatory and inhibitory neurons may lead to a preferential blockade of high-frequency excitatory neurotransmission—but this theory has yet to be substantiated. Still, the heterogeneity of sodium channel expression and localization provides a theoretical basis for understanding how mutations may cause specific diseases and for understanding the mechanisms of action by which drugs used to treat those diseases may have selective effects on subsets of neurons in the brain.

Four epilepsy syndromes, ranging from benign to severe, have been linked to mutations in sodium channels, primarily in *SCN1A*, *SCN2A*, and *SCN1B*. The most grave of these syndromes, severe myoclonic epilepsy in infancy (SMEI), is a familial syndrome characterized by febrile seizures in the first year of life with subsequent progression to medically intractable epilepsy, ataxia, and developmental delay. Most *SCN1A* mutations associated with SMEI introduce frameshifts or premature termination sequences into the coding sequence and, therefore, are predicted to produce nonfunctional channels and haploinsufficiency (3). How does loss of function of Na_v1.1—one of the principal determinants of neuronal excitability—result in hyperexcitability and seizures in the brain, when it would be expected to reduce excitability? Yu and colleagues' recent work offers a possible explanation.

Yu et al. created a deletion of the *SCN1A* gene in mice and examined the phenotype of homozygous and heterozygous offspring. As expected, neurons in homozygous mice expressed no detectable Na_v1.1 and neurons in heterozygotes expressed

approximately one-half the amount of Nav1.1 as wild-type controls. Homozygotes began having seizures on postnatal day 9, the time when Nav1.1 expression normally begins, and died by postnatal day 15, often in status epilepticus. Heterozygotes, which genotypically mimic the human condition in SMEI, began having seizures after 3 weeks of life. Interestingly, this epilepsy phenotype was observed only in mice of one genetic background, but not another, suggesting that currently unknown genetic factors influence the penetrance of the *SCN1A* deletion phenotype. The mice with the epilepsy phenotype were studied further to elucidate the possible underlying mechanism for seizures.

Behavioral and EEG analysis confirmed that the heterozygous mice developed clonic and tonic-clonic seizures. Excitability at the single-cell level was examined in dissociated neurons from the hippocampi of wild-type and mutant mice. When whole-cell sodium currents from wild-type, homozygous, and heterozygous mice were recorded from hippocampal pyramidal neurons, the principal excitatory neurons of this brain region, they were virtually identical. This finding was not wholly unexpected, since it had previously been shown that Nav1.1 expression in the hippocampus was relatively low compared with that of voltage-gated sodium channel 1.2 (4), suggesting that Nav1.2 is the major Nav on pyramidal neurons. However, when hippocampal inhibitory interneurons were examined in the same manner, whole-cell sodium currents were reduced by approximately 50% in the heterozygotes and by 75% in homozygotes. To understand how this selective reduction of sodium channels in interneurons affected their excitability, input-output relationships for the inhibitory neurons were determined. Increasingly depolarizing currents injected into wild-type interneurons produced a linear increase in the number of action potentials; however, this response was blunted in the heterozygotes and even more dramatically in the homozygotes. These results demonstrate that insufficient levels of Nav1.1 expression, as is predicted for the human SMEI mutations, cause diminished excitability. Since this altered excitability occurred selectively in interneurons, the disease-producing *SCN1A* mutations would presumably cause hypofunction of inhibitory circuits and, consequently, hyperexcitability of neuronal networks.

The experimental data raise the question of whether, in the setting of reduced expression of a major brain sodium channel isoform, there is compensatory upregulation of other isoforms. Although no alteration of expression of Nav1.2 or Nav1.6 was observed in the mutant mice, expression of Nav1.3, which is not normally expressed in the hippocampus, was observed on hippocampal interneurons of the heterozygous and homozygous mice. While this abnormal upregulation of another sodium channel appears to be an attempt to compensate for loss of Nav1.1, it clearly is insufficient to restore normal sodium current amplitudes or excitability of these inhibitory neurons, even in the heterozygotic condition.

While SMEI is a rare condition, insights into the disorder provided by this study advance the knowledge of basic mechanisms of epilepsy. First, the Yu et al. study reveals a selective vulnerability of inhibitory versus excitatory neurons to a channelopathy involving a key protein that regulates neuronal excitability. This finding may help explain some of the phenotypic differences between mutations in other channels, such as differences in severity or in whether the mutation causes epilepsy at all. Second, the findings in this study give new promise to the idea of selective antiepilepsy therapies. For example, treatments that selectively block Nav1.2 would be expected to result in relative suppression of excessive excitatory neuronal activity, at least in the hippocampus, while therapies that cause upregulation of Nav1.1 or Nav1.3 may enhance the excitability of inhibitory neurons.

by Gregory C. Mathews, MD, PhD

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BRAIN WATER AND ION FLUXES: A HARD-TO-DIE HYPOTHESIS TO EXPLAIN SEIZURES

Increased Seizure Duration and Slowed Potassium Kinetics in Mice Lacking Aquaporin-4 Water Channels. Binder DK, Yao X, Zador Z, Sick TJ, Verkman AS, Manley GT. *Glia* 2006;53:631–636. The glial water channel aquaporin-4 (AQP4) has been hypothesized to modulate water and potassium fluxes associated with neuronal activity. In this study, we examined the seizure phenotype of AQP4 $-/-$ mice using in vivo electrical stimulation and electroencephalographic (EEG) recording. AQP4 $-/-$ mice were found to have dramatically prolonged stimulation-evoked seizures after hippocampal stimulation compared to wild-type controls (33 ± 2 s vs. 13 ± 2 s). In addition, AQP4 $-/-$ mice were found to have a higher seizure threshold (167 ± 17 A vs. 114 ± 10 A). To assess a potential effect of AQP4 on potassium kinetics, we used in vivo recording with potassium-sensitive microelectrodes after direct cortical stimulation. Although there was no significant difference in baseline or peak $[K^+]_o$, the rise time to peak $[K^+]_o$ ($t_{1/2}$, 2.3 ± 0.5 s) as well as the recovery to baseline $[K^+]_o$ ($t_{1/2}$, 15.6 ± 1.5 s) were slowed in AQP4 $-/-$ mice compared to WT mice ($t_{1/2}$, 0.5 ± 0.1 and 6.6 ± 0.7 s, respectively). These results implicate AQP4 in the expression and termination of seizure activity and support the hypothesis that AQP4 is coupled to potassium homeostasis in vivo.

COMMENTARY

Neuronal action potentials result from rapid influx of sodium or calcium ions and are terminated by a slower potassium efflux from the cell. These events, if not followed by a homeostatic response, lead to extracellular sodium and calcium depletion and potassium accumulation. In the CNS, neuronal ion pumps and transporters cooperate with glial ion channels in extracellular potassium clearance and refurbishment of $[Na^+]_o$ and $[Ca^{2+}]_o$ (1). Epileptic seizures are accompanied by abnormal synchronicity and hyperexcitability, which leads to increased inward sodium/calcium and outward potassium fluxes. Under these conditions, potassium ions reach a plateau in excess of the normal, low millimolar levels under resting or interictal conditions (2). This abnormal potassium accumulation can be explained by: 1) increased release from hyperactive neurons; 2) reduced glial capacity in potassium clearance; and 3) changes in extracellular space favoring potassium accumulation (2,3). The latter is controlled by a number of cellular mechanisms, including blood–brain barrier endothelial cells, production and absorption of CSF, and glial swelling in response to changes in osmolarity.

Binder et al. have extensively published data obtained from mice lacking the aquaporin water channel, AQP4, which is involved in the regulation of extracellular space and in osmotic brain homeostasis (4). The data show that animals lacking AQP4 have a higher seizure threshold, regardless of the models used. Both chemical (pentylenetetrazol) and electrical stimu-

lation were less effective in inducing epileptiform activity in AQP4-null mice. The other main finding was that in AQP4-null mice the duration of seizures was dramatically reduced. The authors invoke a mechanism involving potassium homeostasis, supported by the fact that $[K^+]_o$ transients lasted longer in AQP-null mice. This finding suggests that rebound excitation may be enhanced by elevated $[K^+]_o$.

Both direct and indirect evidence show that management of extracellular potassium is deficient in epileptic brain (5) or under pathophysiological conditions associated with an increased propensity toward seizures, such as traumatic brain injury (6). The main cause underlying loss of potassium homeostasis seems to be loss of a specific subset of inwardly rectifying potassium (K_{IR}) channels that are also associated with cell cycle (7,8). Although Binder et al. did not find any correlation between either increased seizure duration or decreased seizure threshold and expression of K_{IR} 4.1, they did not evaluate other K_{IR} channels expressed in glial cells, so whether a correlation exists with these channels remains unknown. In addition, it is possible that these channels, while still present in glia, did not work properly because of failed positioning into the membrane or other factors. Further studies will be needed to clarify if a specific channel family is involved in the loss of potassium homeostasis in these mice.

Binder et al. show that water channels are relevant for the control of neuronal excitation, supporting the view that brain homeostasis plays an important role in controlling neuronal firing. These data as well as findings by others (2,3) support the possibility of alternative approaches to epilepsy therapy, such as drugs specifically involved in the reestablishment of brain homeostasis (e.g., diuretics) (9). The link between resistance to an initial seizure and decreased water channel levels may be relevant to the management of elevated risk of acute seizures. Aggressive monitoring and management of intracranial pressure

and brain water content after acute trauma or stroke is common practice. Thus, this work may provide a molecular correlate of an effective medical treatment and further increase our appreciation of how electrolytes and water balance are crucial aspects of CNS function and failure.

Unlike sodium ions, potassium ions are vasoactive and changes in $[K^+]_o$ have a powerful effect on the coupling of cerebral blood flow to neuronal activity (10). The combined effects of altered extracellular space and decreased potassium buffering thus may have many consequences. In addition, K_{IR} channel gating is affected by extracellular potassium. A moderate increase in potassium concentration, in the range of 1–15 mM, enhances potassium efflux through K_{IR} at physiologically relevant potentials. In some cells, including vascular smooth muscle, a moderate increase in extracellular potassium, paradoxically, leads to hyperpolarization and relaxation (11). Further increases in $[K]_o$ (to the plateau values seen during seizures), in contrast, cause vasoconstriction, depriving the brain of the nutrients needed during intense neuronal activity. The combined effects of altered potassium homeostasis and cerebral blood flow may be a crucial aspect of loss of aquaporin signaling.

In conclusion, the results presented support mechanisms of epileptogenesis that encompass nonneuronal cells and concentrate on the regulation of synaptic activity and neuronal synchronization by changes in extracellular ions. The latter impact not only the electrical properties of neurons but also the regulation of cerebral blood flow.

by Damir Janigro, PhD

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THE KETOGENIC DIET: STOKING THE POWERHOUSE OF THE CELL

Mitochondrial Biogenesis in the Anticonvulsant Mechanism of the Ketogenic Diet. Bough KJ, Wetherington J, Hassel B, Pare JF, Gawryluk JW, Greene JG, Shaw R, Smith Y, Geiger JD, Dingledine RJ. *Ann Neurol* 2006;60:223–235. **OBJECTIVE:** The full anticonvulsant effect of the ketogenic diet (KD) can require weeks to develop in rats, suggesting that altered gene expression is involved. The KD typically is used in pediatric epilepsies, but is effective also in adolescents and adults. Our goal was to use microarray and complementary technologies in adolescent rats to understand its anticonvulsant effect. **METHODS:** Microarrays were used to define patterns of gene expression in the hippocampus of rats fed a KD or control diet for 3 weeks. Hippocampi from control- and KD-fed rats were also compared for the number of mitochondrial profiles in electron micrographs, the levels of selected energy metabolites and enzyme activities, and the effect of low glucose on synaptic transmission. **RESULTS:** Most striking was a coordinated upregulation of all ($n = 34$) differentially regulated transcripts encoding energy metabolism enzymes and 39 of 42 transcripts encoding mitochondrial proteins, which was accompanied by an increased number of mitochondrial profiles, a higher phosphocreatine/creatine ratio, elevated glutamate levels, and decreased glycogen levels. Consistent with increased energy reserves, synaptic transmission in hippocampal slices from KD-fed animals was resistant to low glucose. **CONCLUSION:** These data show that a calorie-restricted KD enhances brain metabolism. We propose an anticonvulsant mechanism of the KD involving mitochondrial biogenesis leading to enhanced alternative energy stores.

COMMENTARY

One of the perplexing mysteries in the field of epilepsy research is how the ketogenic diet (KD) exerts broad therapeutic efficacy against multiple seizure types and epilepsy syndromes, often producing a cessation of seizures—even in patients who have failed to respond to antiepileptic drugs. The KD is a high-fat content diet in which carbohydrates are heavily restricted. In the metabolic state that results from chronic treatment with the KD, high rates of fatty-acid oxidation in mitochondria produce large amounts of acetyl-CoA, which leads to hepatic synthesis of ketone bodies, such as β -hydroxybutyrate and acetoacetate. Ketone bodies then enter the circulation and are used as substrates for fuel by the brain and, in developing mammals, as building blocks for membrane lipids.

Growing appreciation of the diet's efficacy has sparked the dramatic growth of clinical KD programs at epilepsy centers throughout the world. This development, in turn, has stimulated scientific interest in the question of how the KD works. Recently, clinicians have discovered that versions of the diet that have liberalized amounts of carbohydrate and protein still can provide seizure protection but are better tolerated than the classical KD, which consists of 80–90% fat and is often unpalatable and potentially unhealthy (1,2). An understanding of the underlying mechanisms of KD action eventually should enable optimization of dietary therapies for epilepsy and may permit the identification of clinically useful biomarkers that correlate with efficacy.

Multiple theories have been proposed to explain how the KD protects against seizures (2). The presence of high-serum ketone body levels raises the obvious possibility that these metabolites themselves could be endogenous antiepileptic substances. While acetoacetate and acetone (but not β -hydroxybutyrate) have been found to protect against seizures in a variety of animal models (3), a clear demonstration that brain levels of these ketone bodies correlate with treatment success or failure has not yet been forthcoming.

An alternate hypothesis focusing on bioenergetics was first proposed by DeVivo et al. (4), who noted an increased cerebral energy charge in chronically ketotic rats—reflected as an increased ATP/ADP ratio—and proposed that this finding may account for the “increased neuronal stability” required for seizure control. Indirect support for this bioenergetic theory has come from studies showing enhanced mitochondrial biogenesis in skeletal muscle, heart, and liver in response to fatty acids (5). Moreover, a ^{31}P spectroscopic imaging study of patients with intractable epilepsy treated with the KD has provided preliminary confirmation of increased brain energy stores (6); and a recent study of hippocampal gene expression, using cDNA microarrays in mice fed a KD, demonstrated increased expression of genes in mitochondrial metabolic pathways (7).

Gene-expression profiling also was used in the study by Bough et al., highlighted here, to confirm that the KD produces a coordinated upregulation of transcripts for genes encoding proteins involved in energy metabolism in rat hippocampus, including those specific to mitochondria. In addition, using electron microscopy, the authors directly demonstrated an increased number of hippocampal mitochondria, which appeared to be localized primarily to dendrites and axon terminals. The enhanced energy reserves following KD treatment were found to preserve normal synaptic transmission in brain slices for longer

periods of time under conditions of controlled metabolic challenge than in brain slices from rats fed a control diet.

The major implication of this study is that the KD results in adaptive changes in energy metabolism, including a striking enhancement in mitochondrial biogenesis that leads to an increase in energy stores. This effect on cellular bioenergetics presumably enables neurons to better withstand metabolic challenges in the face of increased energy demand. Whether the change in bioenergetics accounts for the seizure protection conferred by the KD remains unclear. There is little evidence that the triggering of seizures under ordinary conditions is related to energy failure, although it is well recognized that there is hypometabolism interictally in epileptic foci. Nevertheless, it is possible that a greater reserve pool of bioenergetic substrates could enhance the ability of neurons to maintain the activity of energy-requiring membrane transporters, most notably the Na^+/K^+ -ATPase, thus biasing against aberrant epileptic depolarization. Along similar lines, there is increasing evidence that the KD may have beneficial neuroprotective and disease-modifying actions in a variety of neurological conditions believed to be associated with metabolic stress (3,8), and it is plausible that the effect of the KD on bioenergetics could contribute to these types of actions.

One way in which cellular energy stores could be linked to neuronal excitability is through ATP-sensitive potassium (K_{ATP}) channels, which represent a type of inwardly rectifying potassium channel (Kir6) that is activated when intracellular ATP levels fall. However, the ATP sensitivity of these channels is opposite to that required for dampening neuronal excitability, since enhanced ATP levels result in closure of these channels, which would tend to cause depolarization and destabilize neurons. Thus, a requirement for seizure control would be that the relevant K_{ATP} channels be present in inhibitory neurons. At least some inhibitory GABAergic neurons (e.g., in the substantia nigra) do contain K_{ATP} channels, and the activity of these neurons can be affected by ketone bodies (9).

Although the prevailing view is that the KD enhances brain energy stores, it is possible that some dietary manipulations may influence neuronal excitability through negative effects on cellular energetics. It has long been recognized that the KD is associated with modestly reduced glucose levels. Moreover, calorie restriction alone, even without high-fat ingestion, confers seizure protection (10). The reduced ATP levels that might occur under such situations could lead to less robust activity of membrane transporters, which in turn would limit the ability of neurons to reach (and maintain) high levels of activity necessary for seizures. Of course, low glucose and ATP also may activate K_{ATP} channels, which also could reduce excitability. Although carbohydrate restriction might be therapeutically useful, from a practical standpoint, such a diet is difficult to maintain, given the ubiquity of carbohydrates in contemporary

diets. Therefore, it is of interest that Stafstrom et al. (11) found that 2-deoxyglucose (2-DG), which blocks glucose metabolism by inhibiting phosphoglucose isomerase, inhibits epileptiform bursting in a brain slice model and also elevates the seizure threshold in perforant-path kindled rats. However, it is unclear if 2-DG mimics the KD in animal seizure models (12), and it is unlikely to represent a true replacement for the diet. Given the findings of Bough et al., it will be interesting to determine whether carbohydrate restriction or 2-DG can induce mitochondrial biogenesis.

In conclusion, the study of Bough et al. provides compelling evidence that the KD induces profound changes in brain energy metabolism. Indeed, the mitochondrion, the powerhouse of the cell, certainly appears to be “stoked” by the KD. A major issue that remains is whether the epileptic brain would respond in the same manner to the KD as the normal brain studied by Bough et al. Mitochondria from epileptic brain may be functionally impaired (13). Would these “epileptic mitochondria” also have an impaired bioenergetic response compared with those in normal brain, or would there perhaps be an even more robust response? The answer is presently unknown. In any case, the effects of the KD on energy metabolism may certainly contribute to the neuroprotective and disease-modifying effects of the diet (3,8). Whether such effects confer seizure protection, as do antiepileptic drugs, remains to be determined. Plasma membrane ion channels and transporters conventionally have been considered the major targets for antiepileptic drugs. Now, cellular elements implicated in bioenergetics—including enzymes and substrates involved in energy metabolism, mitochondrial proteins, and diverse regulatory factors—also must be considered potential antiepileptic targets. Perhaps, bioenergetic modulators will one day come to represent a new class of pharmacological agents useful to treat seizures resistant to conventional antiepileptic drugs.

by Jong M. Rho, MD, and Michael A. Rogawski, MD, PhD

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