"ONE SWALLOW DOES NOT MAKE A SUMMER" . . . OR DOES IT?

A Single Episode of Neonatal Seizures Permanently Alters Glutamatergic Synapses. Cornejo BJ, Mesches MH, Coultrap S, Browning MD, Benke TA. *Ann Neurol* 2007;61(5):411–426. OBJECTIVE: The contribution of seizures to cognitive changes remains controversial. We tested the hypothesis that a single episode of neonatal seizures (sNS) on rat postnatal day (P) 7 permanently impairs hippocampal-dependent function in mature (P60) rats because of long-lasting changes at the synaptic level. METHODS: sNS was induced with subcutaneously injected kainate on P7. Learning, memory, mossy fiber sprouting, spine density, hippocampal synaptic plasticity, and glutamate receptor expression and subcellular distribution were measured at P60. RESULTS: sNS selectively impaired working memory in a hippocampal-dependent radial arm water-maze task without inducing mossy fiber sprouting or altering spine density. sNS impaired CA1 hippocampal long-term potentiation and enhanced long-term depression. Subcellular fractionation and cross-linking, used to determine whether glutamate receptor trafficking underlies the alterations of memory and synaptic plasticity, demonstrated that sNS induced a selective reduction in the membrane pool of glutamate receptor 1 subunits. sNS induced a decrease in the total amount of *N*-methyl-D-aspartate receptor 2A and an increase in the primary subsynaptic scaffold, PSD-95. INTERPRETATION: These molecular consequences are consistent with the alterations in plasticity and memory caused by sNS at the synaptic level. Our data demonstrate the cognitive impact of sNS and associate memory deficits with specific alterations in glutamatergic synaptic function.

COMMENTARY

The quotation provided in the title is by Aristotle (384 BC–322 BC); in full, it reads: "One swallow does not make a summer, neither does one fine day; similarly one day or brief time of happiness does not make a person entirely happy." The part of the statement regarding happiness is arguable, however if in the first part of the quote, "seizure" is substituted for "swallow" and "epilepsy" for "a summer," one immediately recognizes an old conundrum of both clinical and basic epilepsy research. Much has been written about the "seizures beget seizures" aspect of epileptogenesis, and at last check, the controversies still abound (1). However, there is another, albeit less frequently addressed, potentially damaging outcome to the brain caused by a single seizure, which is a change in the brain's capacity for plasticity at a later time, particularly if the seizure occurs early in development when the brain can best be described as a "plasticity machine" (2). This "metaplasticity" (3), caused by the lone seizure during a critical developmental period, may underlie deficiencies in cognitive function later on in life—which is where the paper by Cornejo et al. weighs in.

In order to induce a single episode of neonatal seizures, the authors subcutaneously administered kainic acid (1–2 mg/kg) to postnatal day 7 (P7) rat pups, a treatment that resulted in "discontinuous behavioral and electrical seizure activity lasting up to 3 hours." It is important to consider whether this type of activity is indeed a "single" seizure or whether it is full-blown status epilepticus. Based on the relatively low (3%) mortality rate in their model and the relatively short duration of ictal bursts (<10 min), the authors argue that the term status epilepticus does not apply to their model. However, in the absence of electrical recordings during the seizures, the authors' claim remains largely unsubstantiated. It is not easy to define status epilepticus in neonates, however "half of the time spent in seizure" is one of the accepted criteria (4,5). In the present study, ictal events of less than 10 min were separated by interictal periods of 5–10 min. Using an average of 7.5 min for each the ictal and interictal periods, during the approximately 3-h period, the total number of ictal events must have been at least 12, equaling about 50% of the total time spent in seizure activity. Considering that in human neonates seizures can go on without any overt physical signs (4,5), behavioral observation alone may not be sufficient to document seizure activity in a neonatal seizure model. The absence of electrical recordings from the brains of the treated animals is one omission in the study. It also would have been extremely useful to know what type of electrical activity was present in the brains of the experimental subjects during the 2–3 months following the neonatal seizures, when the animals were tested in various memory tasks.

Interestingly, the neonatal seizure group performed quite well on water maze memory tasks. Compared with controls,

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there were no differences in their performance on the Morris water maze (MWM), a test of spatial memory. To address subtle changes in episodic-like memory deficits, which can still be present in spite of a normal performance on the MWM, the 2-trial radial arm water maze (RAWM) and the 4-trial RAWM were administered successively to the control and experimental groups. These tests were able to distinguish slight differences between the two groups. On the 2-trial RAWM, the neonatal seizure group performed marginally worse than the controls on the second trial, carried out 4 h after the first. On the 4-trial RAWM, there were slightly more errors produced by the neonatal seizure group than the controls on the first trial, but these animals' learning curve on subsequent trials was indistinguishable from controls. Unfortunately, because of the lack of recordings during seizure induction, no correlations can be drawn between the severity of the neonatal seizures and the amount of memory deficits found in the experimental group. A recent longitudinal study in humans emphasizes the lack of correlation between benign partial epilepsy in infancy and cognitive deficits later on in life (6). Only 4 of 39 children had mild cognitive deficits when assessed about 10 years after the seizures (1 of the 4 patients also was diagnosed with tuberous sclerosis and another with Asperger syndrome).

What is surprising in the Cornejo et al. study is the number of electrophysiological and biochemical alterations found in the neonatal seizure group after more than 2 months. In spite of a normal spine density on hippocampal CA1 pyramidal cells, unaltered presynaptic facilitation of glutamate release, and stable synaptic input–output function, long-term potentiation (induced by a 100 Hz train delivered for 1 s) was depressed and long-term depression (induced by 900 paired pulses delivered at 1 Hz at 50-millisecond intervals) was enhanced at P60 in animals that experienced seizures on P7. Large changes in the neonatal seizure group were demonstrated by biochemical studies. A significant, 52% increase was seen in the intracellular pool of the GluR1 subunit of the AMPA receptors; however, this pool constitutes only a small (5–10%) fraction of the total GluR1 subunits, which are mostly located on the plasma membrane. Since the total amount of GluR1 did not change, the membrane fraction must have been reduced by only about 5% in the neonatal seizure group, which is probably why no changes were detected in the synaptic events in electrophysiological studies. Of the NMDA receptor subunits, only the NR2A showed a decrease (28%) in the membrane fraction of the experimental group—a finding that might explain the reduced long-term potentiation found in the animals that experienced neonatal seizures. The scaffolding protein PSD-95 (located at the postsynaptic side of excitatory synapses and known to interact with several receptors, protein kinases, and other scaffold proteins) displayed the largest change among the postsynaptic proteins examined (a 43% increase in the neonatal seizure group). The authors interpret this finding as an increased amount of PSD-95 per synapse, arguing that the number of spines was unaltered. However, their interpretation may not necessarily be the case, as PDS-95 also is abundant at nonspinous synapses, for example, on inhibitory interneurons. An increased excitatory drive onto GABAergic cells secondary to enhanced levels of PSD-95 at excitatory synapses on interneurons may explain the increased paired-pulse inhibition found in a previous extensive study of cognitive, cellular, and synaptic effects of early life seizures (7). Although not considered in the Cornejo et al. study, inhibitory synaptic plasticity may have been altered in more than one way. At P7 in rats, most GABAergic activity is excitatory, because the combined reversal potential for Cl^-/HCO_3 is still depolarizing compared to action potential threshold, which is unlike in human newborns (8). Thus, the model of seizures in P7 rat pups may not correspond particularly well to human and primate neonates, who are born with a hyperpolarizing GABA reversal potential (2,9).

Cornejo et al. did not examine the mechanisms leading to the described alterations or provide any evidence of whether the behavioral, biochemical, and electrophysiological changes following the neonatal seizures are reactive or proactive. Do these changes occur to dampen excitability in order to prevent further seizures or are they simply necessary consequences of a temporary perturbation of excitability in an immature brain? Clearly, many more studies, using both animal models as well as humans, are needed to answer these questions, and to unequivocally determine whether a single swallow can indeed make summer (2). After all, Aristotle was also fond of saying: "It is the mark of an educated mind to be able to entertain a thought without accepting it."

by Istvan Mody, PhD

References

1. Sills GJ. Seizures beget seizures: a lack of experimental evidence and clinical relevance fails to dampen enthusiasm. *Epilepsy Curr* 2007;7:103–104.
2. Holmes GL, Khazipov R, Ben-Ari Y. Seizure-induced damage in the developing human: relevance of experimental models. *Prog Brain Res* 2002;135:321–334.
3. Abraham WC, Bear MF. Metaplasticity: the plasticity of synaptic plasticity. *Trends Neurosci* 1996;19:126–130.
4. Scher MS. Neonatal seizures and brain damage. *Pediatr Neurol* 2003;29:381–390.
5. Silverstein FS, Jensen FE. Neonatal seizures. *Ann Neurol* 2007;62:112–120.
6. Okumura A, Watanabe K, Negoro T, Hayakawa F, Kato T, Maruyama K, Kubota T, Suzuki M. Long-term follow-up

of patients with benign partial epilepsy in infancy. *Epilepsia* 2006;47:181–185.
7. Lynch M, Sayin U, Bownds J, Janumpalli S, Sutula T. Long-term consequences of early postnatal seizures on hippocampal learning and plasticity. *Eur J Neurosci* 2000;12:2252–2264.
8. Sanchez RM, Jensen FE. Maturational aspects of epilepsy mechanisms and consequences for the immature brain. *Epilepsia* 2001;42:577–585.
9. Khazipov R, Esclapez M, Caillard O, Bernard C, Khalilov I, Tyzio R, Hirsch J, Dzhala V, Berger B, Ben-Ari Y. Early development of neuronal activity in the primate hippocampus in utero. *J Neurosci* 2001;21:9770–9781.

Innate Immunity and Inflammation in Temporal Lobe Epilepsy: New Emphasis on the Role of Complement Activation

Complement Activation in Experimental and Human Temporal Lobe Epilepsy. Aronica E, Boer K, van Vliet EA, Redeker S, Baayen JC, Spliet WG, van Rijen PC, Troost D, da Silva FH, Wadman WJ, Gorter JA. *Neurobiol Dis* 2007;26(3):497–511. We investigated the involvement of the complement cascade during epileptogenesis in a rat model of temporal lobe epilepsy (TLE), and in the chronic epileptic phase in both experimental as well as human TLE. Previous rat gene expression analysis using microarrays indicated prominent activation of the classical complement pathway which peaked at 1 week after SE in CA3 and entorhinal cortex. Increased expression of C1q, C3 and C4 was confirmed in CA3 tissue using quantitative PCR at 1 day, 1 week and 3–4 months after status epilepticus (SE). Upregulation of C1q and C3d protein expression was confirmed mainly to be present in microglia and in a few hippocampal neurons. In human TLE with hippocampal sclerosis, astroglial, microglial and neuronal (5/8 cases) expression of C1q, C3c and C3d was observed particularly within regions where neuronal cell loss occurs. The membrane attack protein complex (C5b-C9) was predominantly detected in activated microglial cells. The persistence of complement activation could contribute to a sustained inflammatory response and could destabilize neuronal networks involved.

COMMENTARY

The complement system includes a proteolytic cascade of events representing an important component of the human immune response and an essential effector of both humoral and cellular immunity (1). The complement system consists of several fluid-phase and cell-membrane proteins that are divided into three activation pathways (i.e., classical, alternative, and lectin) and the membrane attack complex (MAC), a cytolytic or terminal pathway that results in the formation of a lytic pore-forming complex (1,2). Complement participates in the host defense against pathogens by triggering the formation of the MAC, which damages the phospholipid bilayer to lyse the target cell. In addition to their role in pathogen clearance, complement factors, such as C1q, play an important role in the clearance of apoptotic cells (2). However, activation of the complement system either at inappropriate sites and/or to an inappropriate extent can lead to host tissue damage. To protect against self-damage, host cells express a battery of regulatory proteins (i.e., complement inhibitors) that interfere with complement activation at several steps of the proteolytic cascade. These inhibitors can be associated with the cell membrane or can be soluble and secreted by the cells (e.g., C1q inhibitor and clusterin) (1–3).

In mammals, the liver is the major source of most complement proteins, but many cell types, including monocytes, fibroblasts, and epithelial and endothelial cells, can also synthesize most of the complement components. Since 1987, it has been known that brain cells, including astrocytes, microglia, neurons, and oligodendrocytes (2,4), also synthesize complement components (5). In particular, human astrocytes express and secrete all the components of the three complement pathways. Synthesis of all components is constitutively low but can be enhanced by interferon-γ and inflammatory cytokines, such as IL-1β and TNF-α. Indeed, astrocytes themselves can synthesize cytokines in an inflammatory context, raising the possibility that they may switch on complement biosynthesis in an autocrine manner. Given the high level of expression of membrane regulators in the complement system, human astrocytes appear to be resistant to complement lysis, while oligodendrocytes and neurons are much less resistant in vitro, suggesting that these cells are constantly at risk of complement-mediated damage (6).

Complement has long being thought of as a double-edge sword, with the capacity to harm as well as to heal. Indications of a general role for complement in neurodegenerative processes comes from evidence of chronic complement activation and synthesis in various neuropathological conditions, such as

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multiple sclerosis, stroke, chronic neurodegenerative disorders (e.g., Alzheimer's and Parkinson's disease), as well as in Rasmussen's encephalities (6,7). Cytokines produced in diseased brain tissue may constitute a driving force in stimulating local complement synthesis by resident cells. Furthermore, complement receptors and regulatory proteins allow viruses to enter cells in CNS. Interestingly, HHV6-mediated infection of astrocytes has been demonstrated in a population of patients with mesial TLE and apparently leads to a reduced ability of cells to reuptake glutamate, highlighting the possibility that complement components may play a role in this infection (8).

Aronica and colleagues investigated the complement activation in both experimental and human TLE. Their work is a more extensive evaluation of this inflammatory pathway than previously presented by Rozowski et al. (9) and Xiong et al. (10) in seizure models. Rozowski and colleagues showed increased C1q and C4 mRNA in rat pyramidal neurons after systemic injection of convulsant doses of kainic acid in neuronal layers of limbic areas that are vulnerable to kainic acid-induced neurodegeneration (9); moreover, clusterin and C1q immunoreactivities were observed in both neurons and astrocytes, while increased immunoreactivities (as observed in vivo after seizures) were demonstrated following prolonged exposure of primary cultures of hippocampal neurons to glutamate. One important question is whether activation of the complement cascade could be responsible for increased susceptibility to seizures and neuronal injury. Preliminary, but compelling, evidence, in favor of a detrimental role of complement activation, is provided by Xiong et al., who showed that the sequential infusion into the rat hippocampus of individual proteins of MAC induced both behavioral and electrographic seizures as well as cytotoxicity (10).

Aronica and colleagues reported the increased transcript expression of C1q, C3, and C4 in the CA3 hippocampal layer in rats from day 1 to 4 months after status epilepticus (SE) induced by stimulation of the angular bundle. Their findings clearly show that complement activation occurs chronically after the first damaging event (i.e., SE) but before epilepsy develops, thus during the epileptogenesis phase when no epileptic-like activity is present. Immunohistochemical analysis showed that protein expression was mainly present in microglia, parenchymal and perivascular astrocytes, as well as hippocampal neurons. This pattern of activation persists in chronic epileptic tissue in rats with spontaneous seizures, although immunoreactivity in neurons is scarce compared to day 1 and week 1 following SE, suggesting that the complement-positive neurons in the early phases after SE are damaged and may be destined to die.

Using human sclerotic hippocampi from TLE patients, Aronica et al. show that there are abundant C1q and C3 immunopositive astroglial cells in areas of prominent gliosis and that immunoreactivity in microglia/macrophage lineage cells occurs in regions where cell death predominates (i.e., pyramidal cell layers and hilus). Activation of components of the MAC was mainly observed in microglia/macrophages, rarely in neurons, and not in astrocytes. These components were only barely detectable in control tissue specimens (i.e., in autoptic tissue from patients without history of seizures or other neurological diseases and in nonsclerotic tissue from patients with a focal lesion from a ganglioglioma not involving the hippocampus).

The main finding by Aronica et al. is that there is a prominent activation of the complement cascade during the epileptogenesis phase in the experimental model and in sclerotic hippocampi from rats and human TLE. Interestingly, the expression of CD59, a complement inhibitor of MAC, was increased in microglia/macrophages but only modestly in neurons, suggesting that in this cell population complement activation may be poorly controlled.

The parallel validation of experimental and human tissue findings confirms and expands previous evidence indicating the occurrence of a complex, chronic inflammation involving the innate immune system in TLE and in other epilepsies or epileptic syndromes of different etiology (11,12). Increasingly, evidence in experimental models of seizures shows that inflammatory processes may contribute to lower seizure threshold and possibly play a role in epileptogenesis and cell death (13). Further mechanistic insights into these processes and the development of strategies to control their overactivation in diseased conditions may highlight potential new targets for therapeutic intervention.

by Annamaria Vezzani, PhD

References

1. Carroll MC. The complement system in regulation of adaptive immunity. *Nature Immunol* 2004;5:981–986.
2. Speth C, Dierich MP, Gasque P. Neuroinvasion by pathogens: a key role of the complement system. *Mol Immunol* 2001;38:669–679.
3. Morgan BP, Gasque P. Expression of complement in the brain: role in health and disease. *Immunol Today* 1996;10:461–466.
4. Barnum SR. Complement biosynthesis in the central nervous system. *Crit Rev Oral Biol Med* 1995;6:132–146.
5. Levy-Strauss M, Mallat M. Primary cultures of murine astrocytes produce C3 and farctor B, two components of the alternative pathway of complement activation. *J Immunol* 1987;139:2361–2366.
6. Farina C, Aloisi F, Meini E. Astrocytes are active players in cerebral innate immunity. *Trnds Immunol* 2007;28:138–145.
7. Van Beek J, Elward K, Gasque P. Activation of complement in the central nervous system. *Ann NY Acad Sci* 2003;992:56–71.
8. Fotheringham J, Donati D, Akhyani N, Fogdell-Hahn A, Vortmeyer A, Heiss JD, Williams E, Weinstein S, Bruce DA, Gaillard WD, Sato S, Theodore WH, Jacobson S. Association of human herpesvirus-6B with mesial temporal lobe epilepsy. *PLoS Med* 2007;4:e180.

9. Rozowski I, Margan TE, Willoughby DA, Dugich-Djordjevich MM, Pasinetti GM, Johnson SA, Finch CE. Selective expression of clusterin (SGP-2) and complement C1qB and C4 during responses to neurotoxins in vivo and in vitro. *Neuroscience* 1994;62:741–758.
10. Xiong Z-Q, Qian W, Suzuki K, Mcnamara O. Formation of complement membrane attack complex in mammalian cerebral cortex evokes seizures and neurodegeneration. *J Neurosci* 2003;23:955–960.
11. Ravizza T, Boer K, Redeker S, Spliet WGM, van Rijen PC, Troost D, Vezzani A, Aronica E. The IL-1β system in epilepsy associated malformations of cortical development. 2006;24:128–143.
12. Ravizza T, Gagliardi B, Noé F, Boer K, Aronica E, Vezzani A. Innate and adaptive immunity during epileptogenesis and spontaneous seizures: evidence from experimental models and human temporal lobe epilepsy. *Neurobiol Dis* 2007;29:142–160.
13. Vezzani A, Baram TZ. New roles for interleukin-1 beta in the mechanisms of epilepsy. *Epilepsy Curr* 2007;15–19.

Seeing the Forest and the Trees: Dendritic Injury after Status Epilepticus

Kainate Seizures Cause Acute Dendritic Injury and Actin Depolymerization In Vivo. Zeng LH, Xu L, Rensing NR, Sinatra PM, Rothman SM, Wong M. *J Neurosci* 2007;27(43):11604–11613. Seizures may cause brain injury via a variety of mechanisms, potentially contributing to cognitive deficits in epilepsy patients. Although seizures induce neuronal death in some situations, they may also have "nonlethal" pathophysiological effects on neuronal structure and function, such as modifying dendritic morphology. Previous studies involving conventional fixed tissue analysis have demonstrated a chronic loss of dendritic spines after seizures in animal models and human tissue. More recently, in vivo time-lapse imaging methods have been used to monitor acute changes in spines directly during seizures, but documented spine loss only under severe conditions. Here, we examined effects of secondary generalized seizures induced by kainate, on dendritic structure of neocortical neurons using multiphoton imaging in live mice in vivo and investigated molecular mechanisms mediating these structural changes. Higher-stage kainate-induced seizures caused dramatic dendritic beading and loss of spines within minutes, in the absence of neuronal death or changes in systemic oxygenation. Although the dendritic beading improved rapidly after the seizures, the spine loss recovered only partially over a 24 h period. Kainate seizures also resulted in activation of the actin-depolymerizing factor, cofilin, and a corresponding decrease in filamentous actin, indicating that depolymerization of actin may mediate the morphological dendritic changes. Finally, an inhibitor of the calcium-dependent phosphatase, calcineurin, antagonized the effects of seizures on cofilin activation and spine morphology. These dramatic in vivo findings demonstrate that seizures produce acute dendritic injury in neocortical neurons via calcineurin-dependent regulation of the actin cytoskeleton, suggesting novel therapeutic targets for preventing seizure-induced brain injury.

COMMENTARY

Neuronal loss and reorganization of synaptic connectivity are two well-described consequences of chronic epilepsy, both in humans and experimental animal models. This type of neuronal injury can be seen at a macroscopic level, for example, in mesial temporal sclerosis. However, are there more subtle alterations in the structure and function of surviving neurons? The principal neuronal subtype of neocortex and hippocampus, the pyramidal neuron, is an obvious target of investigation, with its striking apical dendrites that arborize over many hundreds of microns, like the branches of an oak tree. The dendrites of pyramidal neurons are the main sites of excitatory synaptic input and comprise greater than 90% of the membrane surface area of the cell. Electrophysiological studies of pyramidal dendrites in the past 10 years or so have disclosed the remarkable signaling occurring in these structures under normal conditions: dendrites support retrograde action potential propagation from the soma, which serves as a signal for synaptic plasticity, and localized activity-induced dendritic calcium transients activate the biochemical machinery of learning and memory (1).

Recent work has shown that epilepsy alters dendritic physiology. In the pilocarpine animal model of temporal lobe epilepsy, patch clamp recordings in the dendrites of hippocampal pyramidal neurons demonstrate a loss of A-type potassium channels and a concurrent enhancement of dendritic action potential propagation, both producing a potentially proconvulsant increase in neuronal excitability (2). Similarly, the onset of epilepsy is associated with a loss of dendritic hyperpolarization-activated cation channels, a situation that likewise predisposes to hyperexcitability (3). These studies, among others, suggest

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that in epilepsy, dendrites are a locus of change for the intrinsic excitability properties of pyramidal neurons.

The present study asks whether morphological change in the dendrites also occurs in epilepsy. In some ways, this is a question with a decades-old answer. Observations dating to the 19th century described two main pathologies in dendrites from human epileptic tissue: varicose "swellings" along the dendritic shaft and loss of spines—the sites of excitatory synaptic contacts (4). Similar findings have been replicated in animal models of epilepsy. However, a limitation to these earlier studies is that they were performed in fixed tissue under conditions of chronic epilepsy and represented purely histological observations without investigation of underlying mechanisms. The work of Zeng et al. seeks to address the same issues by observing changes in dendritic structure in living animals mere hours after an episode of status epilepticus (SE). Their findings confirm that dendritic injury occurs on an acute timescale, and they begin to dissect the underlying mechanisms that depend on phosphorylation signaling.

A remarkable feature of the present study is the use of in vivo multiphoton imaging to visualize neocortical pyramidal neurons in a restrained, anesthetized but otherwise intact animal. In brief, a window of skull is removed in a transgenic mouse expressing a fluorescent protein in a subset of pyramidal neurons (mostly layer V cells). A microscope objective is positioned above the cortex, and stimulation with light at infrared wavelengths allows visualization of the most distal 100 μm or so of the dendritic tree. In essence, it is a 21st century glow-in-the-dark version of the Golgi stain, but in a living animal. The investigators were able to visualize dendritic shafts and spines in submicron resolution, first in untreated animals, and then after 30 min of kainate-induced SE.

Using this demanding technique, the authors found that swelling or beading of dendrites occurred within the first hour after SE, accompanied by the apparent obliteration of about 50% of the spines. Some of the micrographs show a dramatic conversion of a spine-studded dendritic shaft to a form resembling a series of blobby pearls on a string, in which the spines could no longer be seen. The pathology was identical to that seen in the classical Golgi studies over 100 years ago. Interestingly, the changes seen in this study occurred only with the most severe (Racine stage 5) examples of SE; stage 4 SE failed to provoke any dendritic changes. The dendritic beading and spine loss partially resolved within a few hours post-SE and persisted to some extent as late as 24 h.

The authors made several key observations about the underlying mechanisms. Reasoning that dendrite and spine structure is dependent on the filamentous, polymerized form of actin (a structural protein), they found that the depolymerized form increased after SE. Cofilin is a protein that depolymerizes actin after it is activated by dephosphorylation. Dephosphorylated cofilin levels were indeed elevated after seizures, and when calcineurin (a key phosphatase) was inhibited, cofilin activation decreased as did acute dendritic pathology. These results suggest that SE sets into a motion a biochemical cascade that alters the phosphorylation status of proteins maintaining dendrite structure. The end result, at least acutely, is a collection of sickly-appearing dendrites.

The findings show that SE causes acute structural changes to dendrites. While the images are graphic testimony to the deleterious effects of prolonged seizures, it is worthwhile considering what is not yet known about the causes and consequences of this dendritic pathology. It appears self-evident that such morphological change is bad for neuronal function in that the presence of fewer synaptic spines probably implies diminished neuronal information processing. However, it actually is not known whether reduced spine number is a cause of the diminished cognitive function seen in human epilepsy (5). Nor for that matter, is it clear that the acute changes in dendritic structure are the same as those seen at chronic time points. Also, the distinction between pathology following SE and that following recurrent seizures must be kept in mind; the changes seen here occurred only after the most severe grade of SE and thus, may be distinct from neuronal pathology observed in animals with epilepsy without antecedent SE.

One finding that coincides with other recent work is the involvement of calcineurin in post-SE pathology. Calcineurin is a recurring theme in other studies examining epileptogenic changes in intrinsic neuronal excitability, suggesting that the existence of a common initial biochemical pathway that leads to diverse cellular alterations (6,7). If so, then there may be reason for optimism that an antiepileptogenic intervention can be found, as calcineurin inhibitors have long been in clinical use as immunosuppressant drugs; in which case, "save the trees" will take on a whole new meaning after a neurological insult.

by Nicholas P. Poolos, MD, PhD

References

1. Hausser M, Spruston N, Stuart GJ. Diversity and dynamics of dendritic signaling. *Science* 2004;290:739–744.
2. Bernard C. Anderson A, Becker A, Poolos NP, Beck H, Johnston D. Acquired dendritic channelopathy in temporal lobe epilepsy. *Science* 2004;305:532–535.
3. Jung S, Jones TD, Lugo J Jr, Sheerin AH, Miller JW, D'Ambrosio R, Anderson AE, Poolos NP. Progressive dendritic HCN channelopathy during epileptogenesis in the rat pilocarpine model of epilepsy. *J. Neurosci* 2007;27:13012–13021.

4. Swann JW, Al-Noori S, Jiang M, Lee CL. Spine loss and other dendritic abnormalities in epilepsy. *Hippocampus* 2000 10:617–625.
5. Dodrill CB. Progressive cognitive decline in adolescents and adults with epilepsy. *Prog Brain Res* 2002;135:399–407.
6. Misonou H, Menegola M, Mohapatra DP, Guy LK, Park KS, Trimmer JS. Bidirectional activity-dependent regulation of neuronal ion channel phosphorylation. *J Neurosci* 2006;26:13505–13514.
7. Sanchez RM, Dai W, Levada RE, Lippman JJ, Jensen FE. AMPA/kainate receptor-mediated downregulation of GABAergic synaptic transmission by calcineurin after seizures in the developing rat brain. *J Neurosci* 2005;25:3442–3451.

In Search of a New and Improved Target for Antiepileptic Drugs: Sialic Acid?

Role of Extracellular Sialic Acid in Regulation of Neuronal and Network Excitability in the Rat Hippocampus. Isaev D, Isaeva E, Shatskih T, Zhao Q, Smits NC, Shworak NW, Khazipov R, Holmes GL. *J Neurosci* 2007;27(43):11587–11594. The extracellular membrane surface contains a substantial amount of negatively charged sialic acid residues. Some of the sialic acids are located close to the pore of voltage-gated channel, substantially influencing their gating properties. However, the role of sialylation of the extracellular membrane in modulation of neuronal and network activity remains primarily unknown. The level of sialylation is controlled by neuraminidase (NEU), the key enzyme that cleaves sialic acids. Here we show that NEU treatment causes a large depolarizing shift of voltage-gated sodium channel activation/inactivation and action potential (AP) threshold without any change in the resting membrane potential of hippocampal CA3 pyramidal neurons. Cleavage of sialic acids by NEU also reduced sensitivity of sodium channel gating and AP threshold to extracellular calcium. At the network level, exogenous NEU exerted powerful anticonvulsive action both in vitro and in acute and chronic in vivo models of epilepsy. In contrast, a NEU blocker (*N*-acetyl-2,3-dehydro-2-deoxyneuraminic acid) dramatically reduced seizure threshold and aggravated hippocampal seizures. Thus, sialylation appears to be a powerful mechanism to control neuronal and network excitability. We propose that decreasing the amount of extracellular sialic acid residues can be a useful approach to reduce neuronal excitability and serve as a novel therapeutic approach in the treatment of seizures.

COMMENTARY

It is well known that over the last 20 years, the availability of several new antiepileptic drugs (AEDs) has not greatly reduced the percentage of patients who are considered intractable or pharmacoresistant (1). A central tenet in the search for new AEDs is that novel targets for selectively blocking epileptic seizures are needed in order to provide better treatment for people with epilepsy. The study by Isaev and coworkers aims to provide evidence that neuraminidase (NEU), which is a bacterial enzyme that cleaves sialic acid residues, may reveal potential new strategy for blocking seizures. The authors offer a rigorous and comprehensive analysis of the effects of NEU on sodium currents, action potential threshold, and in vitro and in vivo seizures, using the high-potassium and kindling models. The key finding is that NEU caused a depolarizing shift in action potential threshold without altering resting membrane potential, which in turn, elevated seizure threshold, blocking the occurrence of stimulated seizures in both the in vitro and in vivo models tested.

One of the strengths of the report by Isaev and colleagues is the degree to which it is designed to identify a new molecular target. The work essentially starts with an assessment of the effects of NEU on sodium currents and ends with an analysis of drug effects on kindled seizures. Although NEU effectively suppresses sodium currents and blocks these experimental seizures, the findings leave open the question of whether NEU or a related molecule will be effective for chronic epileptic seizures. The kindling model has been used to study the progressive component of epileptogenesis, but kindled seizures still are electrically evoked seizures—possibly quite different from the spontaneous seizures observed in an appropriate animal model or in human studies of chronic epilepsy. Virtually all of their trials involved acute dosing, which does not address the issue of whether chronic dosing would be effective at suppressing spontaneous seizures. Furthermore, in the in vitro experiments, the brain slices were bathed for 2 h in the NEU solution, and the in vivo experiments involved NEU injections 12 h prior to stimulation. Thus, NEU itself is not going to be a clinical treatment for epilepsy, but sialic acid could conceivably be a target

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for a new AED. Unfortunately, a great deal of work will be necessary to move from an enzyme that cleaves sialic acid residues to a small molecule that might perturb sialic acid residues but probably will not cleave them.

The findings of this study introduce the possibility that NEU molecular interactions with sialic acid might be more effective than the traditional sodium-channel blocker AEDs, such as phenytoin and carbamazepine. Interestingly, the traditional AEDs are thought to block seizures by acting on sodium-channel inactivation, thus causing a use-dependent block that reduces high-frequency repetitive firing without greatly altering the threshold for sodium-mediated action potentials or low-frequency repetitive firing. The rationale underlying use of traditional sodium-channel blocker AEDs is that seizures involve particularly large depolarizations with abnormal high-frequency firing of action potentials—one that is different from sodium-channel function during normal behavior. Thus, drugs that act on this high-frequency firing mechanism should theoretically be less apt to have nonspecific effects on other normal neural activity. Accordingly, will elevating sodium-current threshold with NEU actually block seizures without having effects on other functions of these neural circuits or on normal behaviors? Intuitively, it seems likely that drugs that preferentially block high-frequency, repetitive firing versus ones that raise threshold for action potentials would more selectively block seizures without affecting behavior, but this assumption has not yet been tested.

A key concept in the development of potential new AEDs is to study behavioral toxicity early in the screening process as the agent is assessed for efficacy in suppressing seizures. Dose-dependent toxicity is obviously an important issue in screening of any AED (2,3), and a drug with substantial toxicity at doses that suppress seizures is unacceptable. Thus, the dose-response between establishing antiseizure actions is only meaningful in relationship to the negative effects on normal behaviors (e.g., cognitive tests). Thus, these studies raise the issue of whether potential new AEDs should not only block mechanisms that are known to be active during seizures, but also not affect those that are likely to be active during normal brain function. The question is whether new AEDs should target the threshold for voltage-gated sodium current (and thus, action potential threshold) as a mechanism to block seizures, since virtually all neurophysiological mechanisms underlying normal behaviors engage sodium-mediated action potentials. Future research undoubtedly will need to target this and related questions.

The Isaev et al. report is best viewed as an initial study on sialic acid and NEU, with intriguing observations that deserve further investigation. All of the technical and conceptual issues aside, the study reminds the neuroscience community about the importance of glycoproteins in the modulation of neuronal and network excitability. For investigators interested in developing treatments for epilepsy, sialic acid might be a new target to explore. It is very unlikely that NEU, itself, could be an agent, as it would be difficult to administer over the long term, and it is unclear how it could be delivered to the epileptogenic zone or seizure focus. However, simple molecules that are activated when orally administered and that interact with sialic acid in some way to reduce neuronal excitability might be worth evaluating. Whether this approach would have the same therapeutic effect when applied globally rather than through focal infusion also will need to be evaluated. For the moment, the study provides reason to consider indirect approaches to the modulation of neuronal excitability, rather than the full frontal assault of direct channel agonists or antagonists. Ultimately, it may be that a combination of several indirect approaches will yield greater benefits in selectively suppressing seizures in epilepsy.

by F. Edward Dudek, PhD, and Edward H. Bertram, MD

References

1. Stables JP, Bertram E, Dudek FE, Holmes G, Mathern G, Pitkanen A, White HS. Therapy discovery for pharmacoresistant epilepsy and for disease-modifying therapeutics: summary of the NIH/NINDS/AES models II workshop. *Epilepsia* 2003;44:1472–1478.
2. White HS. Clinical significance of animal seizure models and mechanism of action studies of potential antiepileptic drugs. *Epilepsia* 1997;38(suppl 1):S9–17.
3. White HS. Preclinical development of antiepileptic drugs: past, present, and future directions. *Epilepsia* 2003;44(suppl 7):2–8.

Traffic Jam at the Sodium Channel

Modulatory Proteins Can Rescue a Trafficking Defective Epileptogenic Nav1.1 Na^+ Channel Mutant. Rusconi R, Scalmani P, Cassulini RR, Giunti G, Gambardella A, Franceschetti S, Annesi G, Wanke E, Mantegazza M. *J Neurosci* 2007;27(41):11037–11046. Familial epilepsies are often caused by mutations of voltage-gated Na^+ channels, but correlation genotype–phenotype is not yet clear. In particular, the cause of phenotypic variability observed in some epileptic families is unclear. We studied $Na_v1.1$ (SCN1A) Na^+ channel subunit M1841T mutation, identified in a family characterized by a particularly large phenotypic spectrum. The mutant is a loss of function because when expressed alone, the current was no greater than background. Function was restored by incubation at temperature <30°C, showing that the mutant is trafficking defective, thus far the first case among neuronal Na^+ channels. Importantly, also molecular interactions with modulatory proteins or drugs were able to rescue the mutant. Protein–protein interactions may modulate the effect of the mutation in vivo and thus phenotype; variability in their strength may be one of the causes of phenotypic variability in familial epilepsy. Interacting drugs may be used to rescue the mutant in vivo.

COMMENTARY

Neuronal sodium channels subserve action potential generation and are critical for membrane excitability. Sodium channels are large transmembrane proteins comprised of α and β subunits. The α subunit consists of four homologous domains with intracellular amino- and carboxy-terminus tails (1) and forms the voltage-sensing region of the channel as well as the pore through which sodium ions diffuse. Each α subunit is associated with one or more β subunits that modulate the voltage dependence, kinetics, and expression of the sodium channel (2). More than 150 mutations of genes coding for α or β subunits have been reported in patients with epilepsy; these mutations are found throughout the protein structure. Nevertheless, despite the growing recognition of the role of sodium channel mutations in epilepsy, much remains to be discovered regarding the correlation of genotype with phenotype.

The gene *SCN1A* codes for the neuronal sodium channel α protein $Na_v1.1$. Mutations in *SCN1A* have been documented in a spectrum of clinical epilepsy syndromes, ranging from relatively benign generalized epilepsy with febrile seizures plus (GEFS+) to intractable epilepsy with mental retardation and ataxia seen in severe myoclonic epilepsy of infancy (SMEI, or Dravet syndrome). GEFS+ is mainly familial, occurring in large pedigrees, within which a wide range of epilepsy severity exists; most GEFS+ mutations are missense. SMEI usually arises from a de novo truncation mutation, though some inherited cases have been reported. The overlap between these syndromes is yielding important information for the elucidation of genotype/phenotype mechanisms.

The paper by Rusconi and colleagues examines the cellular functional consequences of a specific *SCN1A* mutation, M1841T, in which methionine is replaced by threonine at the amino acid position 1841; the mutation is located on the C-terminus tail region of the $Na_v1.1$ α subunit. This missense mutation was previously found in an Italian family exhibiting a variety of epilepsy phenotypes, ranging from simple febrile seizures to SMEI (3). Here, Rusconi et al. show that the M1841T mutation results in loss of function of the sodium channel. When the mutated protein is transfected into human embryonic kidney cells, the cells harboring mutant channels pass almost no sodium current. There was no difference in biophysical characteristics of sodium channels in wild type and mutants, such as activation, inactivation, recovery, and persistent Na^+ current (I_{NaP}) (4).

The investigators showed that several manipulations could rescue the M1841T loss of function. That is, the sodium current could be restored to more than 50% of the amplitude seen in wild type control cells by decreasing temperature or adding $\beta 1$ (or other β subunits), calmodulin, or phenytoin. How do these disparate compounds rescue the mutant? Interestingly, each one binds to the intracellular tail of the C-terminus region of the α subunit, near the M1841T mutated region. It has been shown previously that the C-terminus region subserves interactions between sodium channel α and β subunits, suggesting that the M1841T mutation disrupts channel function by altering that interaction.

In particular, the ability of decreased temperature (in this case, a permissive temperature of 27°C) to rescue channel function suggests that the mutation causes a defect in protein trafficking from the endoplasmic reticulum to the plasma membrane. Improperly folded proteins cannot pass from the endoplasmic reticulum to the plasma membrane. Lowering temperature allows a greater proportion of misfolded protein to bypass endoplasmic reticulum quality control mechanisms and reach the target location (5). Thus, a mutation in protein trafficking would prevent translocation of the abnormally folded sodium channel α subunits from reaching its final destination in the

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plasma membrane, where it can exert its excitability function. Such a loss of excitability might seem paradoxical to the development of epilepsy, but the exact pathophysiological consequences depend on a number of factors, including the specific cells and specific brain regions that harbor the mutation. For example, it recently has been shown that *SCN1A* mutations cause epilepsy in $Na_v1.1$ knockout mice by decreasing the excitability of inhibitory GABA interneurons (6).

The authors speculate that in vivo variability of protein–protein interactions may underlie phenotypic variation in some epileptic families and that loss of function of $Na_v1.1$ function, as a result of a M1841T, facilitates development of SMEI—similar to loss of function truncation mutations. Whether novel therapeutics could be developed by targeting protein trafficking is uncertain. An intrinsic problem would need to be overcome: the therapy would need to be able to rescue the mutant proteins but not alter the rescued channels already extant in the plasma membrane. Since phenytoin, a therapeutic agent already in widespread use as an antiepileptic, partially rescues mutant channel function, these findings widen the possible mechanisms by which this drug reduces excitability. However, caution is raised because phenytoin acts by blocking activated sodium channels and other sodium channel blockers (carbamazepine and lamotrigine) seem to be contraindicated in SMEI (7).

This paper provides an intriguing potential mechanism for a specific *SCN1A* sodium channel mutation—a defect in protein trafficking. Aberrant protein trafficking has already been demonstrated for several GABA-receptor subunits including $\gamma 2$ (8) and $\alpha 1$ (9). For example, mutations of the $GABA_A$-receptor $\gamma 2$ subunit, which mediates receptor trafficking, show temperature dependence, with temperature increases causing rapid trafficking impairment and receptor dysfunction, possibly contributing to genetic susceptibility to febrile seizures (8).

Therefore, these studies raise the intriguing possibility that genetic mutations causing defective protein trafficking comprise a common motif for genetic epilepsies, especially channelopathies. Protein folding and trafficking defects are well described in other disorders of excitability regulation, including long QT syndromes (involving mutations of the *SCN5A* sodium channel gene causing inherited arrhythmias (10)) and cystic fibrosis, in which a mutation of the transmembrane conductance regulator (CFTR) results in abnormal chloride secretion (11). Protein trafficking defects now join a multitude of other pathogenetic mechanisms underlying hyperexcitability caused by *SCN1A* mutations suggest that manifold opportunities for novel therapeutic interventions exist. The challenge is now to unravel the traffic jam at the sodium channel.

by Carl E. Stafstrom, MD, PhD

References

1. Meisler MH, Kearney JA. Sodium channel mutations in epilepsy and other neurological disorders. *J Clin Invest* 2005;115:2010–2017.
2. Isom LL. Sodium channel beta subunits: anything but auxiliary. *Neuroscientist* 2001;7:42–54.
3. Annesi G, Gambardella A, Carrideo S, Incorpora G, Labate A, Aurora Pasqua A, Civitelli D, Polizzi A, Annesi F, Spadafora P, Tarantino P, Ciro Candiano IC, Romeo N, DeMarco EV, Ventura P, LePiane E, Zappia M, Aguglia U, Pavone L, Quattrone A. Two novel SCN1A missense mutations in generalized epilepsy with febrile seizures plus. *Epilepsia* 2003;44:1257–1258.
4. Stafstrom CE. Persistent sodium current and its role in epilepsy. *Epilepsy Curr* 2007;7:15–22.
5. Bernier V, Lagace M, Bichet DG, Bouvier M. Pharmacological chaperones: potential treatment for conformational diseases. *Trends Endocrinol Metab* 2004;15:222–228.
6. Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat Neurosci* 2006;9:1142–1149.
7. Dravet C, Bureau M, Oguni H, Fukuyama Y, Cokar O. Severe myoclonic epilepsy in infancy: Dravet syndrome. *Adv Neurol* 2005;95:71–102.
8. Kang JQ, Shen W, Macdonald RL. Why does fever trigger febrile seizures? $GABA_A$ receptor gamma2 subunit mutations associated with idiopathic generalized epilepsies have temperature-dependent trafficking deficiencies. *J Neurosci* 2006;26:2590–2597.
9. Gallagher MJ, Ding L, Maheshwari A, Macdonald RL. The $GABA_A$ receptor alpha1 subunit epilepsy mutation A322D inhibits transmembrane helix formation and causes proteasomal degradation. *Proc Natl Acad Sci U S A* 2007;104:12999–13004.
10. Delisle BP, Anson BD, Rajamani S, January CT. Biology of cardiac arrhythmias: ion channel protein trafficking. *Circ Res* 2004;94:1418–1428.
11. Bertrand CA, Frizzell RA. The role of regulated CFTR trafficking in epithelial secretion. *Am J Physiol Cell Physiol* 2003;285:C1–C18.

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.

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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.