PS IN THE (CHANNEL) POD ARE NOT ALIKE. . .

Bidirectional Activity-Dependent Regulation of Neuronal Ion Channel Phosphorylation. Misonou H, Menegola M, Mohapatra DP, Guy LK, Park KS, Trimmer JS. *J Neurosci* 2006;26(52):13505–13514. Activity-dependent dephosphorylation of neuronal Kv2.1 channels yields hyperpolarizing shifts in their voltage-dependent activation and homoeostatic suppression of neuronal excitability. We recently identified 16 phosphorylation sites that modulate Kv2.1 function. Here, we show that in mammalian neurons, compared with other regulated sites, such as serine (S)563, phosphorylation at S603 is supersensitive to calcineurin-mediated dephosphorylation in response to kainate-induced seizures *in vivo*, and brief glutamate stimulation of cultured hippocampal neurons. *In vitro* calcineurin digestion shows that supersensitivity of S603 dephosphorylation is an inherent property of Kv2.1. Conversely, suppression of neuronal activity by anesthetic *in vivo* causes hyperphosphorylation at S603 but not S563. Distinct regulation of individual phosphorylation sites allows for graded and bidirectional homeostatic regulation of Kv2.1 function. S603 phosphorylation represents a sensitive bidirectional biosensor of neuronal activity.

COMMENTARY

Excitability governs the relationship between neuronal input and output. Therefore, its tight regulation is critically important for proper neuronal and circuit function. There are two contrasting elements to the optimal regulation of excitability: on one hand, the system should provide enough plasticity to enable it to adapt to constantly changing environments and demands; on the other hand, the system should be constrained within limits that ascertain stability and prevent extreme hyper- or hypoexcitability. Seizures are a clear example of the failure of stabilization mechanisms that are intended to prevent runaway network hyperexcitability. Therefore, uncovering the discrete processes by which neuronal excitability is constrained is important both for an understanding of seizure generation as well as for devising new ways to treat and prevent pathological conditions characterized by seizures, that is, various types of epilepsy.

Stability of neuronal excitability can be attained through processes such as homeostatic scaling of synaptic activity or through mechanisms influencing the intrinsic excitability of a neuron (1). In view of their pivotal role in the determination of excitability, ion channels constitute a key element of regulation of intrinsic neuronal excitability (1). Particularly suitable for this function are ion channels that are active in subthreshold membrane potentials (2); these include the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (3,4), T-type calcium channels (5), and members of the potassium channel family, such as the Kv2.1 delayed-rectifier (6), A-type, and "leak"-type (1,2).

Modulation of ion-channel activity can take place at several levels. For example, whereas transcriptional regulation can influence the number and identity of neuronal ion channels, already translated channels can be regulated by alterations in trafficking, membrane insertion, and internalization, thus controlling their subcellular distribution and surface expression. A more direct type of regulation can occur by modification of surface-expressed channels, which alters their biophysical properties (e.g., through phosphorylation or interaction with accessory proteins). The mechanisms mentioned not only target different aspects of channel regulation but function at different timescales. For instance, transcriptional regulation influences excitability starting within hours of the inciting stimulus and persisting for as long as a lifetime. Posttranslational mechanisms and channel modification, in particular, can occur within seconds and are often of shorter duration than transcriptional changes.

In the paper discussed here, Misonou and colleagues build on previous work and dissect in exquisite detail regulation of the Kv2.1 channel phosphorylation state as a mechanism of activity-induced reduction of neuronal excitability. The Kv2.1 channels are major contributors to the delayed rectifier potassium current at somatodendritic domains of cortical and hippocampal pyramidal neurons (6). The delayed rectifier potassium current regulates excitability at subthreshold potentials, and its activation results in subsequent hyperpolarization and suppression of neuronal activity (7,8). Specifically, Kv2.1 channels contribute to suppression of neuronal activity in response to high-frequency, repetitive input (6,7).

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Previously, the authors demonstrated a rapid activity-dependent alteration of expression pattern of these channels. Seizure induction in vivo as well as activity enhancement in vitro markedly shifted the channel expression from a clustered pattern to a diffuse one within minutes (9). A similar shift was observed in response to ischemia (8), and in both cases, the underlying mechanism involved elevations of intracellular calcium levels and dephosphorylation of the Kv2.1 channel through a calcineurin-dependent mechanism. It is important to note that these changes in channel expression pattern were coupled to alterations in Kv2.1 channel biophysical properties: the substantial hyperpolarizing shift (>20 mV) of the activation-curve, should increase the channel activity in subthreshold potentials and thus suppress neuronal excitability (8,9). Changes in the expression pattern of Kv2.1 channels were reversible, which was demonstrated by the fact that when excitatory input onto the neurons ceased, there was a gradual recovery of the clustering.

Misonou and colleagues identified the biochemical changes (phosphorylation) of the Kv2.1 channel molecules that mediated the activity-dependent changes in channel clustering and function. They used antibodies that discriminate among different phosphorylated residues in the Kv2.1 molecule and found two phosphorylation sites, serines 563 and 603 (S563 and S603), that are particularly sensitive to activity. Although these two sites are normally in a phosphorylated state, they undergo marked calcineurin-dependent dephosphorylation in response to in vivo kainate-induced seizures and ischemia as well as in response to in vitro glutamate-induced activation. Interestingly, the authors found distinct differences in the regulation of the two sites: the rate and magnitude of the S603 residue dephosphorylation were higher compared with those of the S563. Thus, these phosphorylation sites (Ps) within the Kv2.1 channel molecule are not similar in their response to activity, and differential regulation of the two sites might serve as a delicate sensor that accounts for the graded response of the channel to increased and reduced activity. Finally, the authors propose that this activity-dependent regulation is bidirectional, based on the fact that they demonstrated hyperphosphorylation of the S603 residue in response to suppression of activity by pentobarbital. Taken together, these studies delineate a homeostatic, graded mechanism—namely, bidirectional modulation of the Kv2.1 channel phosphorylation state—that may enable dynamic fine-tuning of intrinsic neuronal excitability in response to altered activity.

The elucidation of the molecular substrates of the mechanism involved in the modulation of the Kv2.1 channel contributes significantly to the understanding of how intrinsic neuronal excitability can be regulated and restricted in the normal brain. It also raises the possibility of failure of this stabilization mechanism within neuronal populations in the epileptic brain. The potential therapeutic value of activity-dependent regulation of KV2.1 channels is intriguing: if technically feasible, augmentation of selective Kv2.1 channel dephosphorylation might help stabilize neuronal excitability and, thus, may serve as a novel therapy for seizures.

by Yoav Noam, MSc, and Tallie Z. Baram, MD, PhD

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The March of Epileptic Activity across Cortex Is Limited (for a While) by the Powerful Forces of Surrounding Inhibition

Modular Propagation of Epileptiform Activity: Evidence for an Inhibitory Veto in Neocortex. Trevelyan AJ, Sussillo D, Watson BO, Yuste R. *J Neurosci* 2006;26(48):12447–12455. What regulates the spread of activity through cortical circuits? We present here data indicating a pivotal role for a vetoing inhibition restraining modules of pyramidal neurons. We combined fast calcium imaging of network activity with whole-cell recordings to examine epileptiform propagation in mouse neocortical slices. Epileptiform activity was induced by washing Mg^{2+} ions out of the slice. Pyramidal cells receive barrages of inhibitory inputs in advance of the epileptiform wave. The inhibitory barrages are effectively nullified at low doses of picrotoxin (2.5–5 μM). When present, however, these inhibitory barrages occlude an intense excitatory synaptic drive that would normally exceed action potential threshold by approximately a factor of 10. Despite this level of excitation, the inhibitory barrages suppress firing, thereby limiting further neuronal recruitment to the ictal event. Pyramidal neurons are recruited to the epileptiform event once the inhibitory restraint fails and are recruited in spatially clustered populations (150–250 μm diameter). The recruitment of the cells within a given module is virtually simultaneous, and thus epileptiform events progress in intermittent (0.5–1 Hz) steps across the cortical network. We propose that the interneurons that supply the vetoing inhibition define these modular circuit territories.

Feedforward Inhibition Contributes to the Control of Epileptiform Propagation Speed Trevelyan AJ, Sussillo D, Yuste R. *J Neurosci* 2007;27(13):3383–3387. It is still poorly understood how epileptiform events can recruit cortical circuits. Moreover, the speed of propagation of epileptiform discharges *in vivo* and *in vitro* can vary over several orders of magnitude (0.1–100 mm/s), a range difficult to explain by a single mechanism. We previously showed how epileptiform spread in neocortical slices is opposed by a powerful feedforward inhibition ahead of the ictal wave. When this feedforward inhibition is intact, epileptiform spreads very slowly (100 μm/s). We now investigate whether changes in this inhibitory restraint can also explain much faster propagation velocities. We made use of a very characteristic pattern of evolution of ictal activity in the zero magnesium (0 Mg^{2+}) model of epilepsy. With each successive ictal event, the number of preictal inhibitory barrages dropped, and in parallel with this change, the propagation velocity increased. There was a highly significant correlation ($p < 0.001$) between the two measures over a 1,000-fold range of velocities, indicating that feedforward inhibition was the prime determinant of the speed of epileptiform propagation. We propose that the speed of propagation is set by the extent of the recruitment steps, which in turn is set by how successfully the feedforward inhibitory restraint contains the excitatory drive. Thus, a single mechanism could account for the wide range of propagation velocities of epileptiform events observed *in vitro* and *in vivo*.

COMMENTARY

While much is known about how epileptic activity is generated and the ionic and synaptic mechanisms underlying seizure susceptibility, relatively little experimental attention has been paid to the mechanisms by which epileptic activity recruits adjacent neural circuits and spreads across the cortex. Clinically, epileptic activity propagates at widely varying speeds covering several orders of magnitude, from the relatively slow propagation rate in Jacksonian march (1) and in some neonatal seizures (2) to rapidly spreading activity in some secondarily generalized seizures. Numerous factors might govern propagation speed, including the extent and magnitude of local inhibition, the degree of myelination, or the level of maturation of ionic channels. Whatever local physiological factors affect neuronal recruitment and activity spread, abnormally synchronized discharges must overcome local inhibition to recruit adjacent and distant neurons into the hypersynchronous firing pattern. How neuronal circuits manage to counteract an oncoming wave of hyperexcitation is a matter of considerable importance because it might be possible to target propagation mechanisms in future therapeutic development.

In these two papers, Trevelyan et al. used a combination of ingenious and innovative techniques to study the propagation of epileptic activity in slices of mouse occipital neocortex. Their goal was to investigate how inhibition limits the propagation of epileptic activity across the cortex and how the collapse of inhibition allows that activity to continue on its inexorable march. Previous studies using cortical slices in which inhibition was reduced by use of $GABA_A$ receptor antagonists, such as picrotoxin or bicuculline, showed that propagation speed was very rapid (3). In contrast, Trevelyan et al. used the zero-Mg^{2+} model, in which excitation is increased and inhibition remains intact. When Mg^{2+} is omitted from the bathing medium, epileptiform discharges are produced in numerous brain regions, recordable in slices as paroxysmal depolarizations with superimposed rapid spike oscillations (4). In nominally zero Mg^{2+}, epileptiform

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activity is generated with the facilitation of neurotransmitter release and activation of NMDA receptors, as Mg^{2+} blockage is relieved. These discharges spread slowly across the slice (at <0.3 mm/sec, compared with 50–90 mm/sec in slices partially disinhibited by $GABA_A$ receptor antagonists), possibly as a result of preserved inhibition (5).

After obtaining whole-cell recordings of layer V pyramidal neurons, Trevelyan et al. induced epileptiform activity by washing out the Mg^{2+}. They found that prior to the onset of massive bursts of depolarization (the epileptiform waves), pyramidal neurons were barraged by bursts of hyperpolarizing currents for several seconds. Then, a transition occurred whereby the inhibitory current bursts were replaced by intense depolarizing current bursts (corresponding to the onset of the epileptiform activity). By recording simultaneously from pairs of nearby neurons, the relative timing of spontaneous neuronal activity could be compared; that is, excitatory bursts appeared in one cell, while inhibitory bursts were still present in its neighbor, which in turn developed excitatory bursts. In addition, utilizing concurrent, fast confocal imaging with the Ca^{2+}-sensing dye Oregon Green 488 Bapta 1 (OGB1), the appearance and propagation of epileptiform activity could be followed over time and space. The epileptiform activity propagated in a discrete, stepwise fashion across the cortex. The stepwise transition from inhibitory to excitatory activity occurred as the inhibitory "restraint" failed at each successive site (confirmed with whole-cell recordings) rather than because of an abrupt increase in excitatory drive. Addition of a small amount of picrotoxin essentially nullified the inhibitory barrages that precede the excitatory bursts. Furthermore, with successive ictal bursts, neurons exhibited progressively less inhibitory barrage, coincident with progressively faster propagation speeds. That is, during epileptiform activity, a given area of cortex is increasingly less able to generate feedforward inhibition and resist the progression of excessive excitation. These findings can account for the varied propagation speeds of epileptic activity seen both in vivo and in vitro.

The authors concluded that the recruitment of neurons into an epileptiform firing pattern and the failure of inhibition occur simultaneously, suggesting that these events are linked mechanistically. In addition, the presence of strong intrinsic inhibition allows the cortical circuit to oppose epileptic spread and withstand the onslaught of extremely powerful excitation until the point at which inhibition finally gives way. The mechanisms of that transition need to be delineated before therapeutic strategies are devised. The stepwise procession of epileptic activity across cortex implies the progressive involvement of modules of cortical circuits and supports the long-held notion of the inhibitory surround as an important restraint on the march of epileptic activity across the neocortex (6).

by Carl E. Stafstrom, MD, PhD

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Calcium Currents Burst Back: A Possible Role for Dendrites in Epileptogenesis

Recruitment of Apical Dendritic T-type Ca^{2+} Channels by Backpropagating Spikes Underlies De Novo Intrinsic Bursting in Hippocampal Epileptogenesis. Yaari Y, Yue C, Su H. *J Physiol* 2007;580(Pt 2):435–450. A single episode of status epilepticus (SE) induced in rodents by the convulsant pilocarpine, produces, after a latent period of 2 weeks, a chronic epileptic condition. During the latent period of epileptogenesis, most CA1 pyramidal cells that normally fire in a regular pattern, acquire low-threshold bursting behaviour, generating high-frequency clusters of 3–5 spikes as their minimal response to depolarizing stimuli. Recruitment of a Ni^{2+}- and amiloride-sensitive T-type Ca^{2+} current (I_{CaT}), shown to be up-regulated after SE, plays a critical role in burst generation in most cases. Several lines of evidence suggest that I_{CaT} driving bursting is located in the apical dendrites. Thus, bursting was suppressed by focally applying Ni^{2+} to the apical dendrites, but not to the soma. It was also suppressed by applying either tetrodotoxin or the K_V7/M-type K^+ channel agonist retigabine to the apical dendrites. Severing the distal apical dendrites 150 μM from the pyramidal layer also abolished this activity. Intradendritic recordings indicated that evoked bursts are associated with local Ni^{2+}-sensitive slow spikes. Blocking persistent Na^+ current did not modify bursting in most cases. We conclude that SE-induced increase in I_{CaT} density in the apical dendrites facilitates their depolarization by the backpropagating somatic spike. The I_{CaT}-driven dendritic depolarization, in turn, spreads towards the soma, initiating another backpropagating spike, and so forth, thereby creating a spike burst. The early appearance and predominance of I_{CaT}-driven low-threshold bursting in CA1 pyramidal cells that experienced SE most probably contribute to the emergence of abnormal network discharges and may also play a role in the circuitry reorganization associated with epileptogenesis.

COMMENTARY

The hypothesis that calcium currents play an important role in epileptogenesis was proposed over two decades ago. The early studies supporting this hypothesis analyzed experimentally induced paroxysmal depolarization shifts (PDSs), which were widely viewed as the cellular correlate of the EEG interictal spike. Because an interictal spike has similarities to the spikes that occur during an electrographically recorded seizure, these PDSs also were thought to be an elementary component of seizures. The initial studies focused on PDSs that arise when $GABA_A$ receptor–meditated inhibition is blocked pharmacologically, although numerous other treatments, including ionic manipulations and various chemoconvulsants other than $GABA_A$ receptor antagonists, can create hyperexcitability and bursting in cortical brain slice preparations. One hypothesis was that intrinsic bursting mechanisms in neurons, mainly reliant on voltage-dependent calcium channels, were responsible for the epileptiform activity. Another hypothesis proposed that PDSs are giant glutamate-mediated synaptic potentials, and this view became better accepted than the intrinsic bursting mechanisms hypothesis. Although it was recognized that such synaptic potentials could activate intrinsic membrane currents (including calcium currents) that shaped the epileptiform events, it was thought that these intrinsic currents were not fundamentally involved in the generation of PDSs. In recent years, there has been a resurgence of interest in the view that alterations in calcium currents and other intrinsic mechanisms lead to "epileptic neurons" that are fundamental to epileptogenesis. The present paper adds to data supporting the epileptic neuron hypothesis and proposes a conceptual hypothesis explaining how pathological overexpression of intrinsic ion channels results in bursting.

Previous studies have reported that T-type calcium current, which is not normally prominent in CA1 hippocampal neurons, is increased after experimental status epilepticus (1–5). The present work by Yaari et al. provides evidence that T-type calcium current is increased specifically in apical dendrites. They hypothesize that this phenomenon sets up a situation in which fast sodium spikes in the soma back-propagate into the dendrites, where they detonate bursting. One important methodological concern is the use of relatively nonspecific pharmacological antagonists, including nickel and amiloride, to define T-type calcium current. The reliance on such agents makes it impossible to be certain that T-type calcium channels truly mediate the underlying events that generate the observed bursts. A second issue is the use of current-clamp recording with sharp intracellular electrodes. This technique is well suited for demonstrating differences in spike bursts between control and epileptic animals, but it provides only limited information on biophysical mechanisms and the spatial distribution of channels. Support for the dendritic localization of the relevant T-type calcium channels was provided in the present study by surgically cutting and disconnecting the apical dendrites, which eliminated bursting. However, a more reliable approach, which is now in common use, is dual whole-cell recording from the soma and dendrite. Such dual recording from a single neuron

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requires visualized patch-clamp techniques, which are more feasibly applied to the immature brain and more difficult to use in older, damaged cortex. In the future, dual whole-cell recordings, in addition to on-cell or cell-attached recordings capable of isolating and analyzing single-channel activity, may further clarify the role of dendrites in epileptic activity and should allow a better definition of the contribution of calcium channels. Ultimately, these approaches may provide answers to the critical question of whether dendritic mechanisms involving voltage-gated calcium channels are responsible for interictal spikes and seizure activity in animal models and in humans with epilepsy.

In addition to intrinsic cellular mechanisms, it is well accepted that recurrent excitatory circuits also play an important role in synchronous bursting. Perhaps the best example is in the hippocampal CA3 area, where modeling and experimental studies have shown that all-or-none epileptiform bursts after blockade of $GABA_A$ receptors involve recurrent excitation, although the intrinsic burst-generation properties of CA3 neurons also play a role (6). The relative lack of both intrinsic bursting and recurrent excitation in the normal CA1 area may explain why CA1 generates comparatively weaker bursts, which are graded with the intensity of afferent stimulation. However, CA1 pyramidal cells form recurrent excitatory circuits during epileptogenesis (e.g., after status epilepticus), and the present study further supports the hypothesis that CA1 neurons also undergo intrinsic changes that promote network bursting.

The concept that an increase in T-type calcium current in CA1 neurons is involved in the epileptic process in the status epilepticus model would be strengthened if it could be shown that there is a temporal correlation between the cellular electrophysiological changes and the development of electrographic and behavioral seizures. Yaari et al. made their measurements in the second and third week after pilocarpine-induced status epilepticus. This time window was chosen with the view that it represents the latent period prior to the onset of spontaneous seizures in this model. However, quantitative comparisons between changes in calcium current–mediated burst generation and both the development of interictal spikes and epileptic seizures are necessary to clarify the relevancy of the proposed alteration of T-type calcium current.

A critical question to be answered is whether dendritic bursting and changes in calcium current actually precede the onset of seizures (in which case, it could be argued that they are responsible for the epileptogenic process) or whether they coincide with the seizures (in which case, they may mediate the seizures but may not be responsible for the alterations in cellular properties that occur in the latent period). A well-accepted pharmacological fact, which would appear to contradict the conclusions of this study, highlights the dichotomy. Ethosuximide is known to inhibit T-type calcium current, and this is the proposed mechanism by which it suppresses absence seizures. However, ethosuximide is not an effective treatment for partial seizures of the kind that occurs in the status epilepticus model. The differing efficacy suggests that T-type calcium current is not critical to the generation of these seizures. Perhaps this paradox would be resolved if the increase in dendritic T-type calcium current contributed not to spontaneous seizures, but rather to the generation of subclinical interictal activity during the latent period in the initial weeks after the epileptogenic insult. The T-type calcium current–dependent activity would then hypothetically trigger an epileptogenic process leading to a distinct type of mature interictal activity and also to spontaneous behavioral seizures, which, again hypothetically, would be dependent on a different set of channels (or even new circuit connections) for their expression. This concept provides a testable hypothesis: administration of T-type calcium current blockers, like ethosuximide (and more specific agents, which are being identified by pharmaceutical companies), would be predicted to prevent the development of spontaneous seizures, if administered during a critical period of epileptogenesis after an insult. It will be of interest to test this hypothesis, which could lead to disease-modifying epilepsy treatments.

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SUBSTANTIA(TING) KETONE BODY EFFECTS ON NEURONAL EXCITABILITY

Ketogenic Diet Metabolites Reduce Firing in Central Neurons by Opening K_{ATP} Channels Ma W, Berg J, Yellen G. *J Neurosci* 2007;27(14):3618–3625. A low-carbohydrate ketogenic diet remains one of the most effective (but mysterious) treatments for severe pharmacoresistant epilepsy. We have tested for an acute effect of physiological ketone bodies on neuronal firing rates and excitability, to discover possible therapeutic mechanisms of the ketogenic diet. Physiological concentrations of ketone bodies (β-hydroxybutyrate or acetoacetate) reduced the spontaneous firing rate of neurons in slices from rat or mouse substantia nigra pars reticulata. This region is thought to act as a "seizure gate," controlling seizure generalization. Consistent with an anticonvulsant role, the ketone body effect is larger for cells that fire more rapidly. The effect of ketone bodies was abolished by eliminating the metabolically sensitive K_{ATP} channels pharmacologically or by gene knock-out. We propose that ketone bodies or glycolytic restriction treat epilepsy by augmenting a natural activity-limiting function served by K_{ATP} channels in neurons.

COMMENTARY

Ever since its inception in the early 1920s, the anticonvulsant ketogenic diet (KD) has provoked curiosity and speculation regarding the underlying mechanism(s) of action. Investigators initially surmised that ketone bodies (i.e., β-hydroxybutyrate [BHB], acetoacetate [ACA], and acetone) might act directly as anticonvulsant compounds. This hypothesis seemed plausible given the striking ketosis associated with the KD. Indeed, in 1933, Keith demonstrated that an intraperitoneal injection of ACA in rabbits was protective against seizures induced by thujone, a convulsant constituent found in many essential oils and an antagonist of $GABA_A$ receptors (1).

Over the ensuing years, clinical observations in many patients have shown that seizure control gradually improves within the first few weeks of KD initiation, as serum ketone levels steadily increase, while seizure control is abruptly lost when ketosis is broken, usually through ingestion of carbohydrates. Blood BHB levels also appeared to correlate directly with seizure control in children placed on a KD. However, a strong correlation between blood ketone levels and seizure control in patients with epilepsy has not been consistently found. Similarly, in animal studies, even in the presence of prominent ketosis (i.e., >4 mM), the KD does not always protect against acutely induced seizures (2). However, in support of Keith's initial findings, other recent studies have demonstrated that both ACA and acetone exert broad anticonvulsant activity in multiple animal seizure models (3,4). Collectively, clinical and laboratory studies suggest a direct role for ketone bodies in limiting seizure activity, but this notion is not firmly established.

Once in vivo efficacy of an anticonvulsant compound is established, traditionally the goal is to identify underlying mechanisms, typically using in vitro cellular electrophysiological techniques. In a thorough electrophysiological study examining the effects of ketone bodies on neuronal excitability, Thio and colleagues showed that acute application of BHB and ACA (at low millimolar concentrations) did not affect (i) EPSPs and population spikes in CA1 pyramidal neurons after Schaffer collateral stimulation; (ii) spontaneous epileptiform activity in the hippocampal–entorhinal cortex slice seizure model; or (iii) whole-cell currents evoked by glutamate, kainate, and GABA in cultured hippocampal neurons (5). According to these findings, it appears that ketone bodies do not interact with the usual molecular targets of anticonvulsant medications, nor do they affect standard parameters of synaptic transmission, at least not in the hippocampus. However, there are several limitations to the study: (i) ketones were infused acutely, not chronically; (ii) experiments were conducted in normal, not epileptic, brain; and (iii) both culture and perfusion media contained glucose, which theoretically could counter a ketotic environment. Thus, the study by Thio et al. did not conclusively put closure on the ketone body hypothesis of KD action. A simple alternative possibility is that ketone bodies may affect molecular targets in brain regions outside the hippocampus.

Shortly after the resurgence of interest in the KD in the mid-1990s, a potential mechanism linking changes in bioenergetic substrates and neuronal excitability was proposed (6). ATP-sensitive potassium (K_{ATP}) channels were noted to be excellent candidates for mediating metabolic control of cellular membrane excitability. K_{ATP} channels represent a type of inwardly rectifying potassium channel (Kir6) that is activated when intracellular ATP levels fall. Although these channels were originally described in pancreatic beta-cells and are best known for regulating insulin release, K_{ATP} channels also appear to be widely expressed in central neurons, especially within the substantia nigra.

It is against this backdrop that Ma et al. asked whether BHB and ACA could affect spontaneous discharge of neurons in the immature (P13–15) rodent substantia nigra pars reticulata (SNr). Intriguingly, they found that both BHB and ACA, at physiological concentrations, attenuated the spontaneous firing rate of these GABAergic neurons and that the degree of

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inhibition increased as the firing rate increased—that is, they demonstrated the phenomenon of use dependence that occurs with several standard anticonvulsant agents. Moreover, these investigators found that the slowing of spontaneous discharges within the SNr by BHB was stereoselective; the nonphysiological isomer was ineffective in blocking spontaneous firing. Furthermore, they demonstrated that the ketone body effect required plasmalemmal K_{ATP} channels. First, blockade of the K_{ATP} channels with sulfonylurea inhibitors prevented, but did not mirror, the effect of ketone bodies, suggesting that the channels might be critically involved in inhibiting SNr discharges. Next, deletion of the gene encoding the Kir6.2 subunit (which comprises part of the octameric K_{ATP} channel–sulfonylurea receptor complex) also resulted in elimination of a ketone body effect. Finally, activation of metabotropic $GABA_B$ receptors was required; specifically, the $GABA_B$-selective blocker CGP55845 blocked the slowing of firing rate by ketone bodies—an effect that was shown to be dependent on K_{ATP} channels. Collectively, their data indicated that the physiologic ketone bodies reduced the firing of GABAergic neurons by opening K_{ATP} channels localized to the SNr.

While intriguing, are these findings actually relevant to the mechanism of action of the KD? The SNr is not generally considered a seizure-prone region of the brain. However, for many years, the SNr has been considered a "seizure gate" involved in subcortical modulation of hyperexcitability (7). Certainly, the argument could be made that the SNr is a potent regulator of seizure susceptibility, but it remains unclear whether such a small (i.e., 10%–20%) reduction in SNr neuron firing actually results in an attenuation of seizure activity in vivo. However, the demonstration that $GABA_B$ receptors are required for a ketone body effect in SNr may be clinically relevant. It is well known that activation and blockade of $GABA_B$ receptors in immature rats can produce anticonvulsant and proconvulsant effects, respectively, but does not affect seizure threshold in adult rats (8). This finding is consistent with the notion that the KD is believed (though not substantiated) to be more effective in infants and children than older patients.

In evaluating a major role for K_{ATP} channels in KD action, a central concern is that the KD is known to increase levels of ATP and other bioenergetic substrates (9) and to enhance mitochondrial biogenesis (10). It is important to note that ketone bodies themselves can enhance mitochondrial respiration (11). Since high ATP levels block K_{ATP} channel activity, how can opening of these channels be achieved by infusion of ketone bodies in the SNr? Ma et al. addressed this concern, suggesting that ATP levels may actually vary in different cellular subcompartments. Under conditions of excessive neuronal firing, the area subjacent to K_{ATP} channels may exhibit lower ATP levels than other cellular regions—despite increases in ketone-mediated respiration resulting from enhanced activity of the Na^+–K^+–ATPase (which would lead to increased local ATP utilization). Put another way, metabolism of ketone bodies raises global ATP levels but also reduces glycolysis and glycolytic ATP synthesis. This reduction in glycolytic ATP may occur near the plasma membrane (where these K_{ATP} channels are localized) and, as such, K_{ATP} channels could be recruited to dampen neuronal excitability. While this is a potentially elegant solution to the dilemma posed, there are yet no data directly supporting this hypothesis.

Furthermore, in animal models, BHB has not been observed to exert a direct anticonvulsant effect. Why then do ACA and acetone possess anticonvulsant activity, but not BHB? ACA and BHB are rapidly interconverted by the enzyme β-hydroxybutyrate dehydrogenase, so providing one or the other ketone should result in a similar metabolic effect. Does a lack of an in vivo anticonvulsant effect of BHB potentially negate the relevance of the study by Ma et al.? The answer is unclear, but what is certain is that there remain many hidden pieces of the metabolic puzzle posed by the KD. In conclusion, the present study expands a growing body of research into the metabolic regulation of seizure control. The possibility that ketone bodies could serve as anticonvulsants has once again been tantalizingly raised. If substantiated, studies such as the present one by Ma et al. would be of practical importance to future development of ketone formulations and/or analogues that would retain biological activity without the risk of adverse effects ordinarily encountered with the KD.

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