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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