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How Does the Ketogenic Diet Work? Four Potential Mechanisms

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Abstract

The ketogenic diet and its newer variants are clinically useful in treating epilepsy. They may also have antiepileptogenic properties and may eventually have a role in treating other neurological and non-neurological conditions. Despite being nearly a century old, identifying the molecular underpinnings of the ketogenic diet has been challenging. However, recent studies provide experimental evidence for four distinct mechanisms that may contribute to the anti-seizure and other beneficial effects of these diets. These mechanisms include carbohydrate reduction, activation of adenosine triphosphate (ATP)-sensitive potassium channels by mitochondrial metabolism, inhibition of the mammalian target of rapamycin pathway, and inhibition of glutamatergic excitatory synaptic transmission.

Keywords

ketogenic diet; mechanism of action; glucose; ATP-sensitive K channel; mammalian target of rapamycin; vesicular glutamate transporters

Introduction

Dietary therapies that alter the body's energy source are effective in treating epilepsy and show promise in treating other neurological and non-neurological conditions. These therapies include the classical ketogenic diet, a high fat, low carbohydrate, adequate protein diet, and its variants such as the medium chain triglyceride diet, modified Atkins diet, and low glycemic index treatment. Several randomized, prospective studies have now substantiated earlier observational studies in providing evidence for the efficacy of these

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Conflict of Interest

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diets in treating epilepsy. A recent Cochrane review concluded that the classical ketogenic diet is an efficacious epilepsy therapy.[1] The ketogenic diet has an established role in treating pyruvate dehydrogenase deficiency and glucose transporter deficiency because it can bypass the molecular defects in these conditions.[2] The recently identified cellular and molecular effects of the ketogenic diet have led to studies examining the potential benefits of the ketogenic diet in other conditions.[3] Animal and clinical studies suggest that the ketogenic diet may be useful in treating neurodegenerative diseases such as Alzheimer, Parkinson, and amyotrophic lateral sclerosis. The diet may also limit neuronal dysfunction from traumatic brain injury and stroke. Non-neurological conditions in which the diet may have a therapeutic role include cancer[4] and diabetic nephropathy.[5] These exciting advances result from previous work done examining the mechanisms of action of the ketogenic diet and underscore the need to elucidate them completely.

Since the ketogenic diet has anti-seizure effects, the consumption of a high fat, low carbohydrate diet ketogenic diet must somehow alter neuronal excitability. The underlying molecular mechanisms are not fully understood, but several recent studies suggest elegant mechanisms not shared with other currently used therapies. These studies provide experimentally testable mechanisms by which the known metabolic consequences of the ketogenic diet may alter neuronal excitability. The proposed mechanisms reviewed here involve carbohydrate reduction, adenosine triphosphate (ATP)-sensitive potassium channels and mitochondrial metabolism, the mammalian target of rapamycin pathway, and glutamatergic synaptic transmission.

Carbohydrate Reduction

A fundamental question regarding the ketogenic diet is the degree to which increased fat versus decreased carbohydrate availability contributes to its mechanism of action. All versions of the ketogenic diet including variants such as the modified Atkins diet, low glycemic index treatment, and fasting/calorie restriction limit carbohydrates. Indeed, seizures recur within one hour of an intravenous infusion of glucose in children with well-controlled seizures on a medium chain triglyceride diet.[6] The hypothesis that carbohydrate depletion has anti-seizure effects is experimentally testable by carbohydrate restriction via the ketogenic diet or by inhibiting glycolysis to block carbohydrate metabolism. The glucose analog 2-deoxy-D-glucose partially inhibits glycolysis and exerts a novel anti-seizure profile in animal models.[7] 2-deoxy-D-glucose is generally safe and well tolerated clinically as a tracer in positron emission studies and adjunctive therapy in cancer trials. Importantly, 2-deoxy-D-glucose uptake occurs preferentially and rapidly in brain regions with a high energy demand, as occurs during seizures.[8] These properties led to studies investigating 2-deoxy-D-glucose as a potential anti-seizure agent.

2-deoxy-D-glucose enters cells in an activity-dependent manner and impedes glucose flux through glycolysis at the phosphoglucose isomerase step. This glycolytic inhibition by 2-deoxy-D-glucose results in acute anti-seizure and chronic antiepileptogenic actions in several experimental models including kindling, corneal electroshock, kainic acid, pilocarpine, and audiogenic seizures in Fring's mice.[9–12] 2-deoxy-D-glucose also has anti-seizure effects in hippocampal slices exposed to a variety of convulsants such as elevated extracellular potassium, bicuculline, 4-aminopyridine, and dihydroxyphenylglycol.[10] Although 2-deoxy-D-glucose does not suppress all acute seizures,[10] it is appealing as an anti-seizure agent because it suppresses seizures caused by a variety of distinctive mechanisms, including depolarization by elevated extracellular potassium, blockade of potassium channels, antagonism of γ-aminobutyric acid type A receptors, and antagonism of metabotropic group I glutamate receptors. Thus, 2-deoxy-D-glucose has a broad spectrum of action unlike that of any currently available anti-seizure medication.

The antiepileptogenic action of 2-deoxy-D-glucose involves brain-derived neurotrophic factor and its receptor TrkB.[13] Kindling requires seizure-induced up-regulation of genes for brain-derived neurotrophic factor and TrkB mediated by the transcriptional repressor neuron restrictive silencing factor and its nicotinamide adenine dinucleotide-sensitive co-repressor carboxy-terminal binding protein. Neuron restrictive silencing factor and co-repressor carboxy-terminal binding protein alter chromatin modification at the promoter sites on the brain-derived neurotrophic factor and *trkB* genes. The increased glycolysis and glucose production during a seizure increases the reduced form of nicotinamide adenine dinucleotide that in turn causes co-repressor carboxy-terminal binding protein to dissociate from neuron restrictive silencing factor. The resulting decrease in transcriptional repression and increase in brain-derived neurotrophic factor and *TrkB* expression enhance neuronal excitability. 2-deoxy-D-glucose suppresses this process by inhibiting glycolysis and decreasing the reduced form of nicotinamide adenine dinucleotide. Consequently, the neuron restrictive silencing factor-carboxy-terminal binding protein complex maintains repression of brain-derived neurotrophic factor and *trkB*, and kindling progression is slowed.[9,14] Of note, neuron restrictive silencing factor repression does not appear to be necessary for the antiepileptogenic effect of the ketogenic diet, though both the ketogenic diet and 2-deoxy-D-glucose inhibit glycolysis; in neuron restrictive silencing factor knock out mice, 2-deoxy-D-glucose does not have an antiepileptogenic effect whereas the ketogenic diet inhibits kindling induced epileptogenesis.[15]

The mechanism of the acute anticonvulsant action of 2-deoxy-D-glucose is currently under investigation. 2-deoxy-D-glucose reversibly reduces the increase in burst frequency observed in extracellular recordings from the CA3 area in hippocampal slices exposed to high extracellular potassium.[10] In whole cell patch clamp recordings from hippocampal CA3 neurons, 2-deoxy-D-glucose reduces the frequency and amplitude of spontaneous excitatory postsynaptic currents under elevated but not normal extracellular potassium suggesting an activity-dependent effect.[16] The locus of these 2-deoxy-D-glucose effects appears to be presynaptic because 2-deoxy-D-glucose reduced the frequency but not the amplitude of miniature excitatory postsynaptic currents recorded in high extracellular potassium. Thus, 2-deoxy-D-glucose likely alters synaptic vesicle release.[17]

Further evidence for the clinical promise for 2-deoxy-D-glucose comes from experiments demonstrating that 2-deoxy-D-glucose has disease-modifying actions even when administered after seizures occur. In kindling experiments, the number of afterdischarges to reach the first class V seizure almost doubled regardless of whether 2-deoxy-D-glucose was administered 30 minutes before kindling, or immediately, 10 minutes, or 30 minutes after kindling.[18]

Taken together, these results suggest that 2-deoxy-D-glucose has a unique anticonvulsant and antiepileptogenic profile, unlike that of any currently available drug. Furthermore, 2-deoxy-D-glucose is relatively safe and well-tolerated in rodent behavioral tests including the Morris water maze to investigate hippocampus-based learning and memory and the open field test to study exploration of a novel environment.[19] However, the clinical use of 2-deoxy-D-glucose will require additional safety data because of the association between orally administered 2-deoxy-D-glucose and cardiotoxicity in some models.[20] 2-deoxy-D-glucose modifies disease progression and offers versatility in terms of timing of its administration relative to seizure occurrence. Therefore, 2-deoxy-D-glucose is poised as a novel approach for treatment of seizures and epilepsy, and preclinical studies are underway to support an Investigational New Drug application.

Activation of ATP-sensitive potassium channels by mitochondrial metabolism

The ketogenic diet and its variants share the property of substantially changing the primary energy source for the body and the brain. The cellular processes involved in mediating these changes in energy homeostasis provide obvious candidate mechanisms by which these diets might exert their beneficial effects. The reduction in glycolysis reviewed above is just one example. The importance of mitochondria to energy homeostasis makes them a potential contributor to the mechanism of action for the dietary therapies considered here. Many studies have shown that the ketogenic diet improves mitochondrial function in part by reducing reactive oxygen species production.[21]

A role for mitochondria in mediating the anti-seizure effects of the ketogenic diet is expected because neuronal excitability depends on energy metabolism. The neuronal ATP-sensitive potassium channel, like the pancreatic β islet cell channel, is an inwardly rectifying potassium channel inhibited by intracellular ATP. The sensitivity of this channel to ATP raises the possibility that it couples energy metabolism to neuronal excitability. Indeed, ATP-sensitive potassium channels mediate the acetoacetate and β-hydroxybutyrate induced decrease in neuronal excitability in γ-aminobutyric acid-ergic neurons in the substantia nigra[22] and dentate gyrus.[23]

These findings led to a search for the molecular link between changes in mitochondrial energy metabolism and ATP-sensitive potassium channel mediated changes in neuronal excitability. One strategy is to identify cell-intrinsic molecular modulators that decrease glucose metabolism while simultaneously increasing ketone utilization in the absence of dietary alterations. BAD (BCL-2-associated Agonist of Cell Death), which is a member of the BCL-2 family of cell death/survival proteins, has these properties.[24] Although BAD has a well-established role in apoptosis, it also modulates glucose metabolism in multiple cell types including hepatocytes, pancreatic β islet cells, and fibroblasts. The phosphorylation state of serine 155 (S155) located within the α helical BCL-2 homology (BH)-3 domain determines BAD function. When S155 is phosphorylated, BAD stimulates mitochondrial oxidation of glucose. When S155 is dephosphorylated, BAD can engage the mitochondrial apoptosis machinery through a BH3 domain-dependent binding and neutralization of the pro-survival BCL-2, BCL-X_L and BCL-w proteins. Dephosphorylated BAD per se is not sufficient to trigger apoptosis, but it plays a permissive role and sensitizes cells having sufficient cellular damage to apoptosis.

BAD fulfils the requirements for being a molecular link between mitochondrial energy metabolism and ATP-sensitive potassium channels in the brain.[25] As in other cell types, BAD stimulates mitochondrial oxidation of glucose in neurons and astrocytes. In addition, BAD reciprocally promotes mitochondrial oxidation of glucose versus ketones because BAD phosphorylation of S155 not only stimulates mitochondrial metabolism of glucose but inhibits ketone body utilization. Consequently, BAD deficient $Bad^{-/-}$ mice or Bad^{S155A} mice, which have a non-phosphorylatable knockin allele of BAD, exhibit diminished glucose utilization and a concomitant metabolic preference for ketone bodies. Moreover, $Bad^{-/-}$ and Bad^{S155A} mice should be resistant to seizures because they metabolically mimic the ketogenic diet without consuming such a diet. As expected, these mutant mice are resistant to acute seizures induced by kainic acid and pentylenetetrazole. Two findings support the hypothesis that increased ATP-sensitive potassium channel activity mediates this resistance to seizures. First, the open probability of ATP-sensitive potassium channels is higher in $Bad^{-/-}$ mice compared to wild type mice. Second, the seizure resistance is lost in double mutant $Bad^{-/-};Kir6.2^{-/-}$ mice, which are deficient in BAD and the Kir6.2 subunit of

the ATP-sensitive potassium channel. The mechanism by which BAD-dependent metabolic changes increase ATP sensitive potassium channel activity is unknown.

Inhibition of the mammalian target of rapamycin pathway

The mammalian target of rapamycin pathway has a pathophysiological role in a variety of epilepsy syndromes and animal models of seizures. Mammalian target of rapamycin, a serine-threonine protein kinase, is an important, well characterized intracellular integrator of metabolic signals.[26] This pathway responds indirectly to a variety of metabolic inputs including the insulin receptor, fasting, ATP/adenosine monophosphate (AMP) ratio, and hypoglycemia.[26] In turn, this pathway regulates protein translation, lipid biosynthesis, autophagy, and mitochondrial biogenesis.[26] Mammalian target of rapamycin pathway proteins localize to synapses and inhibition of the mammalian target of rapamycin with rapamycin impairs late-phase long-term potentiation and long-term depression.[27,28] However, excessive activity of this pathway is pathological and leads to human disorders including tuberous sclerosis complex, Cowden syndrome, and some forms of cancer. Most patients with tuberous sclerosis have mutations in hamartin (*Tsc1*) or tuberin (*Tsc2*), proteins that are upstream of the mammalian target of rapamycin.[29]

As expected, inhibiting mammalian target of rapamycin with rapamycin decreases spontaneous seizures in mouse models bearing mutations in proteins in this pathway.[30–33] Rapamycin also decreases seizures in other epilepsy animal models having increased mammalian target of rapamycin activity such as spontaneous seizures after kainic acid- and pilocarpine-induced status epilepticus in rats, hypoxia-induced neonatal seizures in rats, and spasms but not other seizures in the multiple-hit model of infantile spasms.[34–37] Rapamycin does not protect against seizures induced by pilocarpine in mice, despite the inhibition of mossy fiber sprouting in this model, nor in betamethasone/N-methyl-D-aspartate-induced infantile spasms in rats, pentylenetetrazol- and pilocarpine-induced seizures in immature rats, or seizures induced by amygdala kindling in rats.[38–40] As a secondary endpoint, a recent clinical trial showed that everolimus, a rapamycin analog, protected against seizures in patients with tuberous sclerosis.[41] This observation raises the possibility that mammalian target of rapamycin inhibitors may have clinical utility in treating epilepsy.

The exact mechanism of rapamycin's protection against seizures at the synaptic level remains unclear but might be related to when the effect occurs. Seizure protection requires ongoing rapamycin exposure,[30,33,35] which raises the question of whether the anti-seizure effect of rapamycin involves acute or chronic changes in neuronal excitability. Transient seizure protection implies a more acute effect but the 5–6 day latency to onset of seizure protection suggests these changes may not involve direct binding to ion channels, as seen with many current anti-seizure medicines.[34,35] Short-term rapamycin exposure (6 hours) protects only modestly against tonic hindlimb extension in the maximal electroshock threshold test and has no effect against pentylenetetrazol- or kainic acid-induced seizures.[42] The acute seizure test profile of rapamycin *in vivo* is similar to anti-seizure medicines that exert their effect via voltage-gated sodium channels. However, *in vitro* data do not support this finding.[43] Thus, rapamycin has antiepileptogenic but few acute anti-seizure effects. It may alter synaptic function through structural changes involving dendritic spines for example. Alternatively, it may alter neurotransmitter release, ion channel expression, or synaptic protein expression.[44–49]

The findings summarized above suggest that over-activation of mammalian target of rapamycin causes seizures by promoting epileptogenesis. Its central role as an integrator of metabolic signaling raises the possibility that mammalian target of rapamycin inhibition contributes to the anti-seizure and possible antiepileptogenic effects of the ketogenic diet

and other dietary therapies. Accordingly, the ketogenic diet inhibits mammalian target of rapamycin activity.[50] However, rapamycin and the ketogenic diet do not have identical profiles in acute animal models of seizures. For example, rapamycin does not protect against acute seizures in the 6 Hz electroshock test, though the ketogenic diet is highly protective in this model.[42,51] Whether they have similar profiles in models of chronic, spontaneous seizures is unknown. Nevertheless, mammalian target of rapamycin inhibitors may provide a means for mimicking some of the beneficial effects of the ketogenic diet.

Inhibition of glutamatergic synaptic transmission

A longstanding issue regarding the ketogenic diet is the role of ketones in mediating the anti-seizure and other beneficial effects. If ketones contribute to the anti-seizure effect of the diet, then the degree of seizure control might correlate with the serum ketone concentration while on a ketogenic diet. One clinical study showed better seizure control with higher serum β-hydroxybutyrate levels,[52] but most other clinical reports found no or only a transient correlation between serum β-hydroxybutyrate or acetoacetate concentrations and seizure control.[53–59] Similarly, some animal studies have found a correlation between serum β-hydroxybutyrate levels and seizure control,[60,61] and others have not.[62–64] Together, the clinical studies and animal data indicate that the relationship between ketones and seizure control is unclear.

Another approach to this question is to examine whether ketones have any anti-seizure effects *in vivo* or *in vitro* seizure models. Acetoacetate has anti-seizure effects in the thujone rabbit model of convulsive seizures.[65] Acetone has anti-seizure properties in several animal models.[66,67] However, acute applications of β-hydroxybutyrate and acetoacetate have not exhibited strong anti-seizure effects *in vitro*.[68,69] When applied acutely, these two ketones do not alter fast excitatory synaptic transmission mediated by glutamate receptors or fast inhibitory synaptic transmission mediated by γ-aminobutyric acid type A receptors. Acute applications of these ketones also do not alter α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, N-methyl-D-aspartate, γ-aminobutyric acid type A, kainic acid, or glycine receptor ion channel function.

Although the *in vitro* studies indicate that an acute ketone application does not have an anti-seizure effect and does not alter fast excitatory or inhibitory synaptic transmission, these findings do not exclude the possibility that prolonged ketone applications do have such effects. Moreover, the targets for these effects may be any one of the many steps involved in synaptic transmission. Accordingly, acetoacetate and β-hydroxybutyrate inhibit vesicular glutamate transporters, which load glutamate into synaptic vesicles at glutamatergic synapses.[70] The filling of glutamatergic synaptic vesicles by vesicular glutamate transporters depends on membrane potential and requires the presence of chloride ions outside the vesicle. Both ketones block glutamate transport into synaptic vesicles by allosterically modulating the chloride dependence of the transporters. In the presence of ketones, the chloride dependence of the transporters shifts to higher chloride concentrations in a competitive fashion. Of the two ketones, acetoacetate is more potent, with a half maximal inhibitory concentration less than 1 mM in physiological chloride concentrations, compared to 1–10 mM for β-hydroxybutyrate.

The experiments demonstrating that acetoacetate and β-hydroxybutyrate block vesicular glutamate transporters used purified proteins, but additional studies showed that this inhibition can affect glutamatergic synaptic transmission.[70] Acetoacetate inhibits glutamate release from cultured rat hippocampal neurons but not cultured astrocytes. More importantly, incubating acute mouse hippocampal slices in acetoacetate for at least two hours reduced the amplitude and frequency of miniature excitatory postsynaptic currents

recorded in CA1 pyramidal neurons without altering paired-pulse modulation. This observation is consistent with acetoacetate reducing quantal size as expected from inhibiting the transporters. Acetoacetate had no effect on γ-aminobutyric acid type A receptor mediated miniature inhibitory postsynaptic currents. Furthermore, the results from *in vivo* experiments underscore the potential clinical significance of vesicular glutamate transporter inhibition by ketones. Acetoacetate reduced 4-aminopyridine induced seizures in adult rats and glutamate release during the seizures.

Conclusions

The studies summarized here demonstrate the substantial progress made recently to elucidate the mechanisms by which a ketone-producing, high fat, low carbohydrate diet can have anti-seizure and anti-epileptogenic effects and reveal the multifaceted metabolic mechanisms by which the ketogenic diet works. Emerging data indicate that the beneficial effects of ketogenic diets on epilepsy may result from a variety of different mechanisms including carbohydrate reduction, activation of ATP-sensitive potassium channels by mitochondrial metabolism, inhibition of the mammalian target of rapamycin pathway, and inhibition of glutamatergic excitatory synaptic transmission. These mechanisms depend on ketones binding directly to proteins involved in synaptic transmission and changes in metabolic and cell signaling pathways. The insights gained from these mechanistic studies will continue to provide a better understanding of the pathophysiology of epilepsy and will lead to novel therapies for epilepsy and other neurological disorders.

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