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Ketone Bodies as Anti-Seizure Agents

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Abstract

There is growing evidence that ketone bodies (KB)—derived from fatty acid oxidation and produced during fasting or consumption of high-fat diets—can exert broad neuroprotective effects. With respect to epilepsy, KB (such as β-hydroxybutyrate or BHB, acetoacetate and acetone) have been shown to block acutely induced and spontaneous recurrent seizures in various animal models. Although the mechanisms underlying the anti-seizure effects of KB have not been fully elucidated, recent experimental studies have invoked ketone-mediated effects on both inhibitory (e.g., GABAergic, purinergic and ATP-sensitive potassium channels) and excitatory (e.g., vesicular glutamate transporters) neurotransmission, as well as mitochondrial targets (e.g., respiratory chain and mitochondrial permeability transition). Moreover, BHB appears to exert both epigenetic (i.e., inhibition of histone deacetylases or HDACs) and anti-inflammatory (i.e., peripheral modulation of hydroxycarboxylic acid receptor and inhibition of the NOD-like receptor protein 3 or NRLP3 inflammasome) activity. While the latter two effects of BHB have yet to be directly linked to ictogenesis and/or epileptogenesis, parallel lines of evidence indicate that HDAC inhibition and a reduction in neuroinflammation alone or collectively can block seizure activity. Nevertheless, the notion that KB are themselves anti-seizure agents requires clinical validation, as prior studies have not revealed a clear correlation between blood ketone levels and seizure control. Notwithstanding this limitation, there is growing evidence that KB are more than just cellular fuels, and can exert profound biochemical, cellular and epigenetic changes favoring an overall attenuation in brain network excitability.

Keywords

Epilepsy; Ketogenic diet; Ketone bodies; Beta-hydroxybutyrate; Acetoacetate; Neuroprotection

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Introduction

Epilepsy is a common neurological condition characterized by spontaneous recurrent seizures and can occur throughout the age-span [1]. While anti-seizure drugs (ASDs) can effectively control spontaneous recurrent seizures in the small majority of patients, at least one-third of affected individuals continue to exhibit unremitting seizure activity and attendant negative health consequences such as cognitive impairment and co-morbid mental health problems [2, 3]. One effective alternative treatment for medically refractory epilepsy is the high-fat and low-carbohydrate ketogenic diet (KD). The KD was designed in the 1920s to mimic the fasting state, which has been anecdotally reported through the millennia to control seizures [4–6]. The KD is characterized chiefly by systemic ketosis, notably elevations in the concentrations of the ketone bodies (KB), beta-hydroxybutyrate (BHB), acetoacetate (ACA) and acetone.

Although the efficacy of the KD in patients with intractable epilepsy has been clearly demonstrated in controlled prospective clinical trials [5, 7–9], the precise mechanisms responsible for its clinical effects remain unclear. Numerous hypotheses have been proposed over the years, including alterations in neurotransmitter systems (e.g., GABA, glutamate, and adenosine), glycolytic restriction/diversion, improved cellular bioenergetics and mitochondrial function (with subsequent decreases in oxidative stress), direct inhibitory effects of fatty acids, and enhancement of tricarboxylic acid (TCA) cycle function [4, 10].

In this regard, one yet unresolved question is whether ketone bodies are direct mediators of anti-seizure effects or whether they are largely epiphenomena—indicative of fatty acid oxidation and a measure of patient compliance. To date, blood levels of KB have not been shown to correlate well with seizure control [11, 12], and there are assuredly many more mechanisms recruited by the KD that can result in attenuation of seizure activity [10]. Further, the role of KB is most seriously challenged by the low-glycemic index therapy (LGIT) which appears to afford clinical benefits similar to the traditional KD, but is not associated with significant ketonemia [13]. Recently, however, there are growing data indicating that KB can induce a diverse range of physiological effects that individually and collectively result in both functional and structural neuroprotection [5]. These effects are likely to be relevant to the reduction in spontaneous recurrent seizures experienced by the majority of patients treated with the KD and its variants.

Fatty Acid Oxidation and Ketone Body Production

Ingestion of a high-fat, low-carbohydrate diet leads to increased fatty acid oxidation, the end-product being acetyl-CoA, which subsequently enters the TCA cycle. Increased acetyl-CoA production by the liver then initiates ketogenesis, with two acetyl-CoA molecules combining to form acetoacetyl-CoA. Acetoacetyl-CoA condenses with another molecule of acetyl-CoA to form 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) through the action of HMGCoA synthase 2. A molecule each of acetyl-CoA and ACA are then produced through the breakdown of HMG-CoA. ACA can be reduced to BHB through a bidirectional BHB dehydrogenase (BDH1) enzyme which is coupled to nicotinamide adenine dinucleotide (NAD^+). ACA can also be spontaneously decarboxylated to form the volatile ketone body

acetone which can be excreted through the lungs and kidneys. In the blood, BHB and ACA can be transported from the vascular lumen to the brain interstitial space by monocarboxylic acid transporters (MCTs). Of note, MCT1 is the principal carrier at the level of the vascular endothelium. Both ACA and BHB can enter mitochondria within neurons and glia (astrocytes, in particular), where they are converted to acetyl-CoA through several enzymatic steps. The reconstituted acetyl-CoA species are then utilized by the TCA cycle for ATP production [14]. Figure 1 summarizes the key features of KB metabolism and physiology.

In Vivo and In Vitro Studies of Ketone Bodies

The first report of ketone bodies inducing anti-seizure effects was published by Keith in the early 1930s [16–19]. In rabbits, ACA was shown to protect against thujone-induced seizures. Many decades later, this observation was replicated in the Frings audiogenic seizure-susceptible mouse, a model of sensory-evoked reflex seizures [20]. In a separate study, acetone displayed dose-dependent anti-seizure effects in the maximal electroshock, subcutaneous pentylenetetrazol (PTZ), amygdala kindling, and the AY-9944 (an inhibitor of cholesterol biosynthesis, and which evokes atypical absence seizures) models of induced seizures or epileptogenesis [21, 22]. Recently, BHB—administered acutely or chronically—was shown to confer seizure protection in immature rat pups against seizures provoked by flurothyl [23], in the rat betamethasone-NMDA model of infantile spasms [24], and in the mouse *Kcna1*-null model of developmental limbic epilepsy [25]. In contrast, a medium-chain triglyceride KD was ineffective in blocking seizures elicited by maximal electroshock, threshold electroconvulsive shock, threshold pentylenetetrazol and maximal pentylenetetrazol, despite significant elevations in blood ketone levels [26], indicating that KB do not possess acute anti-seizure properties. Collectively, these studies suggest that the anti-seizure properties of KB may be model- and dose regimen-dependent.

Recently, ketone esters have been investigated as potential therapeutic agents that can result in prolonged elevations in blood KB [27]. The R, S-1,3-butanediol acetoacetate diester (BD-AcAc2) resulted in increased blood acetone, ACA and BHB levels in rats and also increased the latency to seizures induced by hyperbaric oxygen. In contrast, administration of the ketone ester 1,3-butanediol did not affect seizure latencies, despite elevations in blood BHB levels. In further support of ketone esters as anti-seizure compounds, single or repeated dosing of BD-AcAc2 has been shown to increase the threshold for PTZ-induced seizures in rats [28, 29]. Taken together, these and other studies support the notion that KB can exert anti-seizure effects, separate from their role in ATP production, and likely through novel mechanisms, the most important of which are described below and depicted in Fig. 2. More specifically, with regard to BHB, research to date suggests that the anti-seizure properties of BHB may be observed only under certain conditions, such as chronic administration in epileptic brain [23–25]. Notwithstanding the somewhat discordant findings, it appears that KB afford broad spectrum anti-seizure activity, and hence the possibility that these substrates contribute to the therapeutic effects of the KD cannot be readily dismissed.

Molecular Targets of Ketone Body Action

Initially, investigators naturally sought to determine whether KB could directly affect ion channels and transporters that are the principal targets of clinically available ASDs. However, as KB had historically been viewed as metabolic substrates and not direct modulators of synaptic function, it was therefore not surprising that investigators found that clinically relevant (i.e., low millimolar) concentrations of BHB and ACA did not affect $GABA_A$ receptors, ionotropic glutamate receptors, or voltage-gated sodium channels in normal rodent hippocampus [30, 31]. From this point, the hunt for novel potential mechanisms commenced and to date has yielded a few very intriguing candidates.

Elevations in GABA Synthesis

The first mechanistic insight into KB action in the brain arose from neurochemical experiments demonstrating that these metabolites altered glutamate metabolism in a manner that increased synthesis of the inhibitory neurotransmitter, γ-aminobutyric acid (GABA), in synaptosomal fractions isolated from rat forebrain and in cultured astrocytes [32–34]. In the ketotic state, there are major shifts in brain amino acid handling, notably the reduction of aspartate relative to glutamate via a change in the equilibrium of the aspartate aminotransferase reaction. Specifically, the rate of glutamate transamination to aspartate is decreased, and the rate of glutamate decarboxylation to GABA is increased [33, 34]. Elevations in synaptic levels of GABA would then dampen seizure activity, principally through activation of inhibitory post-synaptic $GABA_A$ receptors. However, as intuitive as this reasoning appears, the clinical and experimental evidence to date are inconsistent or are reflective of changes outside seizure-prone areas of the brain such as hippocampus, thalamus and neocortex [35–39]. Further, it remains unclear why the KD is effective in stopping seizures in patients who have failed to respond to GABAergic drugs [6].

Activation of K_{ATP} Channels

It has been proposed that activation of ATP-sensitive potassium (K_{ATP}) channels may underlie the anti-seizure effects of the KD [40]. K_{ATP} channels are inwardly rectifying potassium channels (Kir6) that are activated when intracellular ATP levels fall, and have long been considered a fundamental link between metabolic changes and cellular membrane excitability. In electrophysiological recordings of brain slices, BHB and ACA reduced the spontaneous firing rate of GABAergic neurons in the substantia nigra pars reticulata, a putative subcortical seizure gate, and this action was dependent on K_{ATP} and $GABA_B$ receptors. Further, the same group has shown that BHB enhances the open probability of K_{ATP} channels in the hippocampus in vitro [41]. As compelling as these observations may be, they need to be reconciled with the fact that the KD increases levels of ATP through enhanced mitochondrial respiration and biogenesis [42–44]. Elevations in ATP concentration would tend to close K_{ATP} channels and enhance, instead of inhibit, neuronal excitability.

There is, however, an alternative hypothesis for the effects of KD and KB on K_{ATP} channels. Using patch-clamp electrophysiological techniques, Kawamura et al. [45] found that low-glucose conditions favor the opening of pannexin channels which are resident on cellular

membranes of hippocampal CA3 pyramidal cells. Increased intracellular concentrations of ATP led to ATP efflux via pannexin channels, subsequent breakdown to adenosine by ectonucleotidases in the extracellular space, and activation of adenosine A1 inhibitory receptors coupled to plasmalemmal K_{ATP} channels via G-protein coupled second messenger signaling. Thus, BHB-induced increases in ATP production could recruit a form of metabolic autocrine regulation that dampens neuronal excitability.

Vesicular Glutamate Transporters

There is further evidence that KB may block seizures at the synaptic level. Vesicular glutamate transporters are comprised of three homologous proteins (VLUT1-3) localized to functionally distinct populations of glutamatergic neurons [46]. VGLUTs utilize a proton electrochemical gradient to carry glutamate into synaptic vesicles, and the efficiency of this inward transport is maximized by low concentrations of the chloride anion [47]. Juge et al. [48] demonstrated that BHB and ACA can suppress neuronal excitability by inhibiting the presynaptic release of glutamate, directly competing with Cl^- for allosteric modulation of VGLUTs. They also showed that in vivo administration of 4-aminopyridine, a non-selective potassium channel blocker, evoked seizures with concomitant release of glutamate, but that these effects could be reversed by ACA. This was the first demonstration that metabolic substrates such as KB can directly modulate excitatory neurotransmission, and in so doing, block seizure activity.

mPT Inhibition

Over the past decade, investigators have increasingly reported a multitude of alterations induced by the KD on brain mitochondrial structure and function [5, 42, 44]. Among the many actions described for the KD and/or its substrates, BHB has been shown to augment mitochondrial respiration, increase NADH oxidation, limit reactive oxygen species (ROS) production, and enhance ATP production [42, 49, 50]. Such effects would be predicted to prevent mitochondrial permeability transition (mPT), a phenomenon that results in the collapse of the mitochondrial membrane potential, shutdown of ATP production (through uncoupling of electron transport from ATP synthase activity), mitochondrial swelling, ROS formation, release of calcium and pro-apoptotic factors, ultimately triggering cell death [51, 52]. In this context, it has recently been shown that BHB blocks spontaneous recurrent seizures in epileptic *Kcna1*-null mice by raising the threshold for mPT [25], through an indirect modulation of cyclophilin D, a regulatory component that is the target of the clinical immunosuppressive agent, cyclosporine A. It was further shown in this epilepsy model that both the KD and KB restore intrinsic deficits in hippocampal long-term potentiation, a cellular electrophysiological model of synaptic plasticity and a major mechanism underlying learning and memory [25]. This study revealed the first direct link between mPT and seizure threshold, and further supports the notion that KB are anti-seizure agents.

Inhibition of Histone Deacetylases

Histones are important proteins that regulate chromatin structure in eukaryotic cells and are heavily post-translationally modified. Acetylation of lysine residues on histones through

acetyltransferases enables unbound DNA to undergo transcription, whereas removal of acetyl groups by histone deacetylases (HDACs) results in tight binding of histones to DNA and transcriptional repression. HDAC inhibitors, while increasingly studied as anti-cancer and anti-inflammatory agents [53], may also play a role in ictogenesis and/or epileptogenesis [54]. Valproic acid (VPA), a broad-spectrum anti-seizure drug in clinical use, has been shown to inhibit both class I and II HDACs and is cytotoxic to many different cancer types [55]. HDAC inhibition may be a key anti-seizure mechanism of VPA action, as prior studies had failed to provide a compelling explanation for a variety of methodological reasons [56, 57].

Recently, BHB was shown to inhibit histone deacetylases (HDACs) both in vitro and in vivo, effects that were associated with increased resistance to oxidative stress [58]. Specifically, acetylation of histone H3 lysine 9 (H3K9) and histone H3 lysine 14 (H3K14) was increased by BHB, as was the transcription of genes regulated by FOXO3A (i.e., the antioxidant enzymes catalase and manganese superoxide dismutase). Additionally, in vivo administration of BHB via osmotic minipumps over 24 h resulted in decreased carbonylation and levels of lipid peroxides and 4-hydroxynonenal in the kidney. It should be noted that these effects were not reported in brain tissue or cells, but it is reasonable to speculate that BHB inhibition of HDACs and the subsequent transcriptional changes may mediate some of the brain antioxidant (and perhaps anti-seizure) effects known to occur with the KD.

HCA2 Receptors and the NRLP3 Inflammasome

Hydroxy-carboxylic acid receptor 2 (HCA2, GPR109A, also known as NIACR1 or the niacin receptor 1, and encoded by the *HCAR2* gene) is a G protein-coupled receptor found on adipocytes, neutrophils, tissue macrophages, and in the anterior cingulate cortex. BHB has been reported to activate HCA2 receptors on adipocytes, which prevents release of free fatty acids and induces vasodilatation. Rahman et al. [59] hypothesized that the neuroprotective effects of the KD might be mediated in part by BHB's actions on HCA2 receptors. They found that mice fed a KD or given BHB through subcutaneous minipumps showed smaller ischemic infarcts after distal middle cerebral artery occlusion compared to controls, and this effect was lost in *HCAR2*-null mice. Additionally, using chimeric mice and cell ablation techniques, these investigators showed that HCA2 receptors were present on bone marrow-derived monocytes and macrophages that infiltrated the brain, and that the neuroprotective effect of BHB required HCA2 receptor activation on these immune cells, and further, involved prostaglandin synthesis. This study was the first clear demonstration that BHB can exert a profound anti-inflammatory effect, something that has been clearly recognized to reduce seizure activity [60].

Along the same vein, BHB appears to target other important components of the immune system. The innate immune sensor NOD-like receptor protein 3 (NLRP3) inflammasome is a multiprotein complex responsible for caspase-1 activation and release of the pro-inflammatory cytokines IL-1β and IL-18 in macrophages. In response to excess glucose, ceramides and amyloids, the macrophage experiences a loss of cytoplasmic K^+ promoting ASC oligomerization, speck formation and the assembly of NLRP3. Intriguingly, Youm et al. [61] recently reported that BHB inhibits NLRP3 inflammasome assembly by preventing

K^+ efflux, but not through starvation-regulated mechanisms such AMP-activated protein kinase (AMPK), ROS, autophagy or glycolytic inhibition, all of which have previously been implicated in KD action. BHB inhibition of NRLP3 assembly was also independent of mitochondrial uncoupling protein-2 (UCP2), SIRT2 and HCA2 [61]. Other investigators later suggested that inhibition of the inflammasome by BHB may occur through suppression of oxidative stress in the endoplasmic reticulum [62].

As compelling as these studies appear, do either or both of these anti-inflammatory effects of BHB play a role in ictogenesis and/or epileptogenesis? None of these studies provide direct evidence in this regard, but the fact that the KD can induce epigenetic changes in the epileptic brain [63], and is highly effective against neuroinflammation-induced epilepsy such as FIRES (febrile infection-related epilepsy syndrome) support the notion that the anti-inflammatory effects of BHB may explain in part its anti-seizure properties [60, 64].

Summary

Not surprisingly, investigators have taken a simple reductionist approach to studying KD mechanisms and have asked whether the principal by-products of fatty acid oxidation (i.e., KB such as BHB, ACA and acetone) might exert direct effects on brain network excitability, beyond their well-established role as alternative fuels for bodily tissues under conditions of decreased glucose availability. With the emergence of the studies detailed above, it is becoming clearer that KB (and BHB, in particular) can exert profound and pleiotropic effects, which act in concert to block spontaneous recurrent seizures through novel mechanisms that yield neuroprotective activity in the epileptic brain. Clearly, the simple and intuitive notion that KB lie at the heart of KD action has been strengthened substantially nearly a century after its inception.

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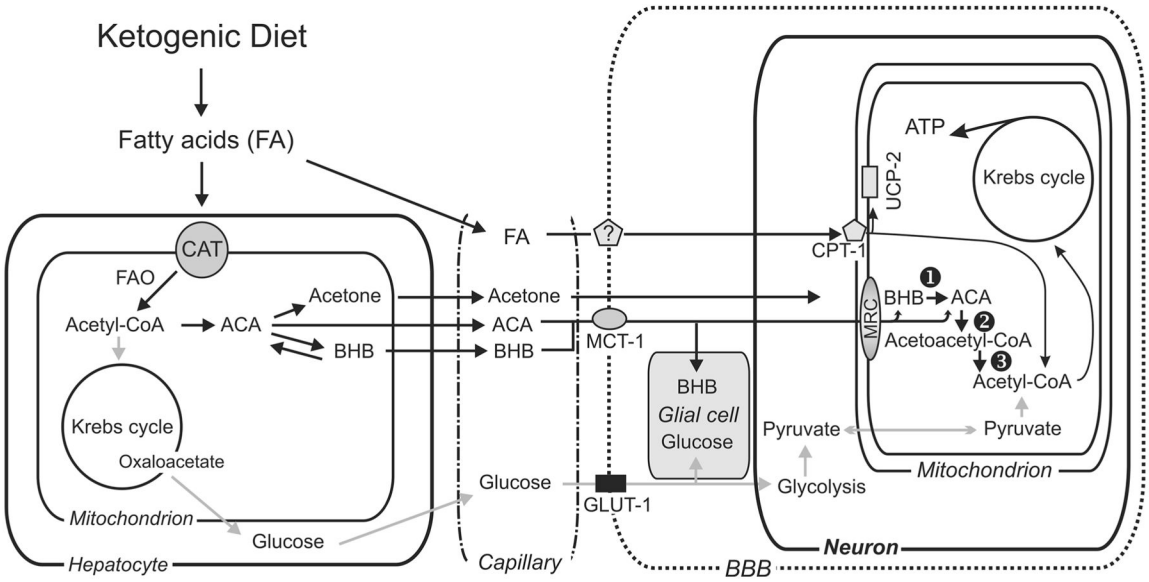

Fig. 1.

Metabolic pathways involved in KD treatment. *CAT* carnitine-acylcarnitine translocase, *GLUT-1* glucose transporter-1, *BBB* blood–brain barrier, *CPT-1* carnitine palmitoyl transferase, *numbered black circle* 1,3-hydroxybutyrate dehydrogenase, *numbered black circle 2* succinyl-CoA3-oxoacid CoA transferase, *numbered black circle 3* mitochondrial acetoacetyl-CoA thiolase, *MRC* mitochondrial respiratory complex. Reprinted with permission [15]

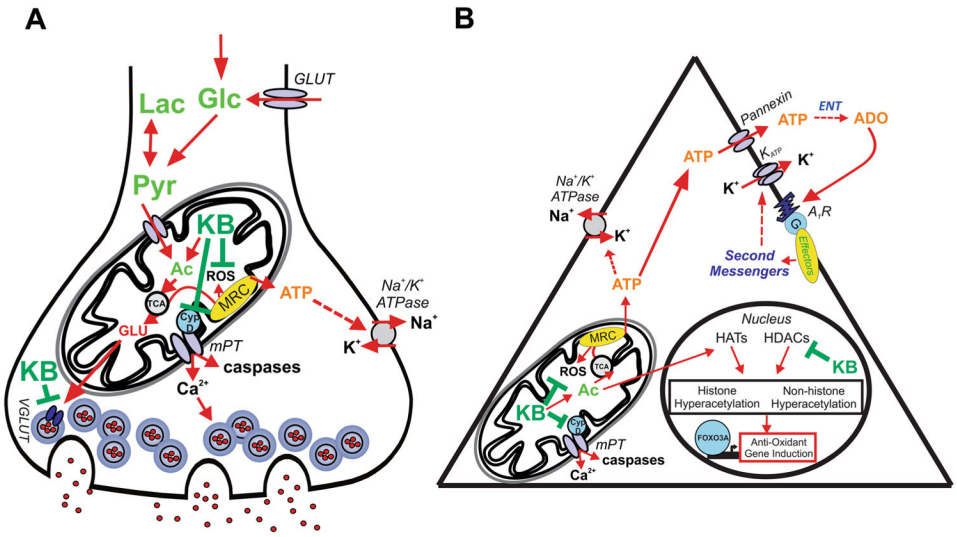

Fig. 2.

Illustration depicting potential mechanisms contributing to ketone body attenuation of synaptic hyperexcitability and neuroprotection. **a** Presynaptically, KB may inhibit vesicular glutamate transporters (VGLUT), thereby decreasing the amount of glutamate loaded in vesicles and reducing the size of glutamate quanta released during synaptic transmission. Concomitantly, KB may enhance ATP production, increasing the ability of the membrane to quickly repolarize after stimulation via the Na^+/K^+ ATPase, and possibly limiting the amount of neurotransmitter released. Lastly, KB may inhibit production of ROS and the mitochondrial permeability transition (mPT), protecting the cell against oxidative injury and preventing excessive release of calcium. **b** Postsynaptically, similar mitochondrial effects of KB confer neuroprotection and dampen cellular excitability. Furthermore, the increase of ATP may result in indirect opening of K_{ATP} channels by adenosine type 1 receptors (A_1R) via ATP release through pannexin hemichannels and subsequent conversion to adenosine (ADO) by extracellular ectonucleotidases (ENT). KB also promote histone and non-histone hyperacetylation by increasing acetyl-CoA, a substrate for histone acetyltransferases (HATs), and directly inhibiting histone deacetylases (HDACs)—with the end result of increasing endogenous anti-oxidants (among other actions)