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Review article

Do ketone bodies mediate the anti-seizure effects of the ketogenic diet?

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ABSTRACT

Although the mechanisms underlying the anti-seizure effects of the high-fat ketogenic diet (KD) remain unclear, a long-standing question has been whether ketone bodies (i.e., β-hydroxybutyrate, acetoacetate and acetone), either alone or in combination, contribute mechanistically. The traditional belief has been that while ketone bodies reflect enhanced fatty acid oxidation and a general shift toward intermediary metabolism, they are not likely to be the key mediators of the KD's clinical effects, as blood levels of β-hydroxybutyrate do not correlate consistently with improved seizure control. Against this unresolved backdrop, new data support ketone bodies as having anti-seizure actions. Specifically, β-hydroxybutyrate has been shown to interact with multiple novel molecular targets such as histone deacetylases, hydroxycarboxylic acid receptors on immune cells, and the NLRP3 inflammasome. Clearly, as a diet-based therapy is expected to render a broad array of biochemical, molecular, and cellular changes, no single mechanism can explain how the KD works. Specific metabolic substrates or enzymes are only a few of many important factors influenced by the KD that can collectively influence brain hyperexcitability and hypersynchrony. This review summarizes recent novel experimental findings supporting the anti-seizure and neuroprotective properties of ketone bodies.

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1. Introduction

The ketogenic diet (KD) is a high-fat, low-carbohydrate and adequate-protein formulation that has been used for nearly a

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century to treat medically intractable epilepsy. Although the mechanisms underlying the KD's clinical effects remain unclear (Rho and Stafstrom, 2012; Rogawski et al., 2016), it remains controversial whether any or all of the major ketone bodies (e.g., beta-hydroxybutyrate, acetoacetate and acetone) produced by the liver are directly responsible for the KD's anti-seizure profile. One reason for this persistent uncertainty is the clinical observation that blood ketone (i.e., β-hydroxybutyrate) levels do not correlate well with seizure control (Kossoff et al., 2009; Kossoff and Rho, 2009; but see Gilbert et al., 2000; van Delft et al., 2010), although local ketone levels at the neuronal or synaptic level may be a more accurate reflection of ketone effects on excitability (Stafstrom, 2004). Further, another diet that has been used successfully to treat patients with medically intractable epilepsy, the low glycemic index treatment (LGIT), does not induce systemic ketosis (Muzykewicz et al., 2009). While definitive evidence in this regard is not yet forthcoming, recent clinical data indicate that ketone bodies (specifically, β-hydroxybutyrate) may yet be relevant to an anti-seizure effect (Buchhalter et al., 2017). In contrast, experimental data are more compelling, with recent studies highlighting pleiotropic actions of β-hydroxybutyrate and novel molecular targets (Puchalska and Crawford, 2017). While some of these mechanistic observations have yet to be firmly and causally linked to an attenuation of seizure activity, the scientific rationale for both ketone-induced anti-seizure and neuroprotective effects has grown substantially over the past few years (Gano et al., 2014; Puchalska and Crawford, 2017). This review summarizes the evidence for ketone bodies as important contributors to ketogenic diet effects in the clinical setting (see Table 1, Fig. 1).

2. Evidence for the anti-seizure activity of ketone bodies in *in vivo* seizure models

Since the introduction of the KD and the hypothesis that ketone bodies are responsible for its therapeutic effects, there has been a relative paucity of *in vivo* studies demonstrating the therapeutic efficacy of ketones against seizures. However, since the turn of the 21st century, evidence has been quickly accumulating, supporting the notion that ketone bodies can indeed contribute to seizure control. Initial screening for anti-seizure activity usually begins with determination of dose-dependent protection of an acutely administered compound against an induced seizure in normal (i.e., non-epileptic) animals. In the case of ketone bodies, the first documented testing began with Keith (1933, 1935) who found that acetone and acetoacetate, but not β-hydroxybutyrate, protected rabbits against seizures induced by thujone, a constituent of wormwood oil and a known antagonist of γ-aminobutyric acid, type A ($GABA_A$) receptors (Höld et al., 2000).

Nearly seventy years passed before interest in ketone bodies was revived. Acetone and acetoacetate, but not β-hydroxybutyrate, protected against sound-induced seizures in the Frings audiogenic seizure-susceptible mouse model (Rho et al., 2002). Shortly thereafter, acetone was found to increase the seizure threshold of rats in multiple models of seizure induction, including the maximal electroshock test which models tonic-clonic seizures, the pentylenetetrazol test which models typical absence seizures, the amygdala kindling test which models complex partial (more recently termed focal with impaired awareness) seizures with bilateral spread, and the AY-9944 test which models chronic atypical absence seizures (Likhodii and Burnham, 2002; Likhodii et al., 2003). Collectively, these data suggest that acetone has a broad-spectrum anti-seizure profile similar to the clinical experience with the KD. Effects of acetone in the pentylenetetrazol test and electroshock test have been confirmed, and extended to protection against tonic seizures in the 4-aminopyridine (4-AP) test and reduced seizure severity during lithium-pilocarpine status epilepticus (Gasior et al., 2007; Inoue et al., 2009; Hasebe et al., 2010). Similar to acetone, acetoacetate reduced seizures induced by intrahippocampal 4-AP infusion in rats (Juge et al., 2010). Furthermore, acetoacetate and its analog 2-phenylbutyrate decreased hippocampal seizure activity in the intrahippocampal kainate model of chronic epilepsy (Kadowaki et al., 2017).

Recently, ketone esters have been investigated as a potential "pro-drug" capable of sustained elevation of ketone bodies (D'Agostino et al., 2013). The *R,S*-1,3-butanediol acetoacetate diester (BD-AcAc2) resulted in elevated blood acetone, acetoacetate and β-hydroxybutyrate levels in rats and increased the latency to hyperbaric oxygen-induced seizures. In contrast, 1,3-butanediol raised only blood β-hydroxybutyrate levels and failed to affect seizure latencies. Single or repeated dosing of BD-AcAc2 has been further demonstrated to increase the threshold of pentylenetetrazole-induced seizures in rats (Viggiano et al., 2015, 2016) and audiogenic- and kainate-induced seizures in a mouse model of Angelman syndrome (Ciarlone et al., 2016).

Based on the aforementioned studies, it would appear that β-hydroxybutyrate does not contribute to the anti-seizure efficacy of the ketogenic diet. However, three recent studies indicate that β-hydroxybutyrate may yet play a role. In an effort to determine if the higher endogenous β-hydroxybutyrate levels in suckling neonates confers seizure protection, Minlebaev and Khazipov (2011) performed a series of depth electrode experiments on postnatal day 5–9 non-anesthetized rat pups. Inhibiting ketogenesis had no effect on seizures provoked by a single flurothyl exposure. However, seizures during a second exposure were exacerbated. This effect was reversed with administration of exogenous β-hydroxybutyrate, suggesting a reduction of hyperexcitability and a direct role of β-hydroxybutyrate in raising the seizure threshold in neonates (Minlebaev and Khazipov, 2011). The second study investigated the effects of β-hydroxybutyrate in the betamethasone-NMDA model of infantile spasms (Yum et al., 2015). A single administration of β-hydroxybutyrate failed to affect the spasms, but repeated injections over three days increased the latency and decreased the number of spasms. Furthermore, this anti-seizure effect was enhanced with repeated bouts of NMDA-triggered spasms (Yum et al., 2015). The third study involved chronic infusion of β-hydroxybutyrate via osmotic minipumps over a two-week period in a genetic model of epilepsy and reported that β-hydroxybutyrate reduced spontaneous recurrent seizures similar to a ketogenic diet (Kim et al., 2015a,b). Collectively, these studies suggest that the anti-seizure effects of β-hydroxybutyrate *in vivo* may have been missed in previous experiments due to limited, single dosing and/or the use of acute seizure models rather than models replicating aspects of spontaneous recurrent seizures.

In pediatric and adult patients or animals clinically used KDs generally raise plasma levels of ketone bodies to between 1 and 10 mM (Gilbert et al., 2000; van Delft et al., 2010; Simeone et al., 2016; 2017). Importantly, all of the *in vivo* studies described above used doses that would raise plasma ketones to within this range (e.g., an intraperitoneal injection of 10 mmol/kg β-hydroxybutyrate raises β-hydroxybutyrate to 5–7 mM, injection of 8 mmol/kg acetone raises acetone to 4–6 mM and intragastric administration of the pro-drug BD-AcAc2 raises acetone to 1 mM, β-hydroxybutyrate to 4 mM and acetoacetate to 4 mM; Eiger et al., 1980; Likhodii and Burnham, 2002; Likhodii et al., 2003; D'Agostino et al., 2013; Yum et al., 2015). Moreover, those studies performing dose response experiments calculated anti-seizure ED_{50}'s that would result in this mM range.

Before delving into the *in vitro* evidence of ketone-mediated