



Short communication

The ketogenic diet metabolite beta-hydroxybutyrate (β -HB) reduces incidence of seizure-like activity (SLA) in a K_{ATP} - and $GABA_B$ -dependent manner in a whole-animal *Drosophila melanogaster* model

Jinglu Li^{a,b}, Emma I. O'Leary^{a,c,1}, Geoffrey R. Tanner^{a,b,d,e,*}^a Department of Biological Sciences, Mount Holyoke College, South Hadley, MA 01075, United States^b Program in Neuroscience and Behavior, Mount Holyoke College, South Hadley, MA 01075, United States^c Program in Biochemistry, Mount Holyoke College, South Hadley, MA 01075, United States^d Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269, United States^e Connecticut Institute for the Brain and Cognitive Sciences, United States

ARTICLE INFO

Keywords:

Ketogenic diet

Ketone bodies

 K_{ATP} channel $GABA_B$ receptor

Seizure model

Bang sensitive

ABSTRACT

The high-fat, low-carbohydrate ketogenic diet (KD) is an effective clinical treatment for epilepsy in juveniles, especially for drug-resistant seizures. The KD results in elevated production of ketone bodies (KB's), such as beta-hydroxybutyrate (β -HB), which are thought to have anticonvulsant properties; however, their exact mechanism of action is unknown. *In vitro*, KB effects on reducing neuronal firing rates are mediated in part by K_{ATP} channel activity and $GABA_B$ signaling. In order to study metabolic and pharmacological effects in a whole-animal model, we used the *eas* “bang-sensitive” (BS) mutant strain of *Drosophila*, which exhibits seizure-like activity (SLA) upon mechanical stimulation. Direct application of the KB β -HB to food reduced BS SLA. Application either of tolbutamide, a K_{ATP} blocker, or of CGP-55845, a $GABA_B$ antagonist, concomitantly with β -HB, partially reversed these KB effects on SLA, verifying a role for K_{ATP} channels and $GABA_B$ signaling in mediating the anticonvulsant effects of KB's and validating this whole-animal model of KD effects on seizure.

1. Introduction

The ketogenic diet (KD) is a high-fat, low-carbohydrate dietary therapy featuring an elevated ratio (typically 4:1) of lipids to non-lipids (Wilder, 1921; Thiele, 2003). The KD is used effectively in the clinic to reduce drug-resistant seizures in cases of pediatric epilepsy (Thiele, 2003; Neal et al., 2008) and may be effective in treating adult cases of epilepsy as well (for example, see Lambrechts et al., 2012). The KD produces high levels of circulating ketone bodies (KB's), such as beta-hydroxybutyrate (β -HB) (Appleton and DeVivo, 1974; DeVivo et al., 1978), which may be used as fuel by the brain (Sokoloff, 1973) and are thought to produce the KD's anticonvulsant properties (Bough and Rho, 2007; McNally and Hartman, 2012). Recent research on “bang-sensitive” (BS) flies—*Drosophila* lines that exhibit seizure-like activity (SLA) following mechanical stimulation (Pavlidis et al., 1994; Fergestad et al., 2006)—has shown that flies with mutations or drug treatments affecting metabolism exhibit reduced SLA (Stone et al., 2013, 2014). It has also been demonstrated that K_{ATP} channels and $GABA_B$ signaling mediate

KB effects on neuronal activity *in vitro* (Ma et al., 2007; Tanner et al., 2011), and that K_{ATP} channel knockout aggravates SLA in an *in vivo* mouse seizure model (Giménez-Cassina et al., 2012). We sought to examine molecular targets of KB effects in a whole-animal model of seizure using *Drosophila* “bang-sensitive” (BS) seizure mutants as a model system.

2. Material and methods

2.1. *Drosophila* strains

Canton-S (wild-type strain) and *tko*, *sesB*, and *eas*^{alaE13} BS mutants were obtained from the Bloomington *Drosophila* Stock Center (BDSC, Bloomington, IN). These BS strains of flies feature mutations in genes closely tied to metabolism: *eas* is mutant for ethanolamine kinase (Pavlidis et al., 1994), which phosphorylates membrane lipid precursors using ATP (Lykidis et al., 2001); *tko* is mutant for the mitochondrial ribosomal protein S12 (Royden et al., 1987); and *sesB* is mutant for the

* Corresponding author at: Department of Physiology and Neurobiology, University of Connecticut, 75 North Eagleville Road, Unit U-3156, Storrs, CT 06269, United States.

E-mail address: geoffrey.tanner@uconn.edu (G.R. Tanner).

¹ Present address: Laboratory of Protein Conformation and Dynamics, Biochemistry and Biophysics Center, National Heart, Lung, and Blood Institute, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892, United States.

mitochondrial adenine nucleotide translocator (Zhang et al., 1999). These mutants' deficits affect ATP production and handling. Seizure susceptibility of these mutants has been previously described (Fergestad et al., 2006). For generation of experimental animals, virgin female flies of a given BS mutant strain were paired with male flies of the same strain. Mating pairs were distributed one pair per food vial. All experimental flies were raised and maintained at room temperature.

2.2. Seizure behavior recording and analysis

To induce SLA, individual flies in an enclosed square recording arena (1.5 cm × 1.5 cm, designed and built in-house) were shaken for 10 seconds on a Vortex Genie2 (Scientific Industry; Bohemia, NY) set to its highest intensity (10). After vortexing, arenas were placed under an Olympus SZ40 zoom stereo microscope (Micro-Tech Optical Inc.; Bloomfield, CT) connected to a Pixelink USB microscope camera (Pixelink; Ottawa, ON). Videos of fly behavior were recorded using Camtasia Studio software (TechSmith Corporation; Okemos, MI) at a framerate of 10 fps and a size of 640 × 480 pixels. Each recording was 3 minutes long, including 10 seconds vortexing time and 5 seconds transfer time. Recorded videos were analyzed off-line using either manual scoring (visualized in Windows Media Player, Microsoft; Redmond, WA) or automated scoring with Ethovision software (Noldus Information Technology, Leesburg, VA). Behaviors were analyzed for the entire recording period between 5 and 165 seconds after vortexing.

For automated scoring, Ethovision reported SLA episodes that resulted in rapid motor shifts occurring faster than the video framerate as the detection parameter “subject not found”. Preliminary assessments of the model corroborated this detection setting using manual scoring in conjunction with video tracking; we therefore used the percentage of time of “subject not found” as a proxy for SLA incidence.

For manual scoring, four types of SLA could be reliably identified and classified across all genotypes and treatments. “Jump”: movement of more than one body length away from the original location. “Flip”: either body rotation or body spinning about the antero-posterior axis. “Disappearance” (interpreted as high-frequency tremors): a video frame with no detectable fly image while the fly was still at the same position before and after image loss. “Mix”: presence of more than one SLA type in a given behavioral epoch. Because of their connection with apparent hyperactivity in flies, these SLA classifications may correspond to tonic-clonic or clonic episodes in human patients, although further work is needed to understand the exact relationship between fly SLA and human seizures.

The total duration of each individual manually-scored SLA was counted for each individual fly. From these numbers, the mean durations of each SLA type were calculated.

2.3. Diets and pharmacology

Diets were based on the standard Bloomington Formulation (“control diet”; Genesee Scientific, San Diego, CA) with the anti-fungal agents Tegosept (15 g/L food; Apex BioResearch Products, Whitmore Lake, MI) and propionic acid (4.9 mL/L food). The proportion of nutrients in the control diet was approximately 48 carbohydrates:7.5 protein:1 fat. KB-supplemented diets contained 2 mM sodium R,S-beta-hydroxybutyrate (β -HB), a concentration consistent with concentrations used in previous *in vitro* studies (e.g. Ma et al., 2007; Tanner et al., 2011). Drug treatments included either 200 μ M tolbutamide or 2 μ M CGP-55845. All chemicals were obtained from Sigma Chemical (St. Louis, MO) unless otherwise noted.

We maintained *eas* flies on three different dietary paradigms. One group of flies was raised on the control diet and another was raised on the KB diet, both groups from hatching through day 3 post-eclosion from the pupal casing as adults. A third group was raised from hatching on the control diet, then switched to the KB diet from eclosion until day

3 post-eclosion. For drug conditions, flies were raised on the indicated diet, plus drug, from hatching through day 3 post-eclosion. We found that *eas* flies' pre-eclosion development took about 7 days on average, resulting in a total of 10 days on each diet.

We measured SLA in all three groups on day 3 post-eclosion.

2.4. Statistics

Statistical analysis was performed using Microsoft Excel (Microsoft; Redmond, WA) and SPSS (IBM; Armonk, NY) software. Univariate analysis of variance (ANOVA), Mann-Whitney *U* tests and Kruskal-Wallis tests were used to compare SLA between different diets and genotypes.

3. Results and discussion

3.1. The ketone body β -hydroxybutyrate (β -HB) reduces SLA in the *eas* BS mutant *Drosophila* strain

In addition to Canton-S flies as a control strain, we tested for sensitivity to mechanical induction of SLA in several BS strains of flies whose mutations are tied to metabolism: *eas*, *tks*, and *sesB* (see Material and Methods). We found that the *eas* strain had both the highest incidence of automatically-scored mechanically-induced SLA and the highest rate of reproduction (data not shown). We therefore chose this strain to investigate whether KB's may exert an anticonvulsant effect in *Drosophila*.

As compared with control-diet-fed counterparts, *eas* flies raised for their entire lives on β -HB-supplemented food, or switched to β -HB-supplemented food at eclosion, exhibited a reduction in SLA on day 3 post-eclosion, following a trend of reduced SLA with increased time dieted on β -HB-supplemented food (see Fig. 1.). For flies raised on β -HB-supplemented food for their entire lives, this reduction was striking and significant both by automated and manual scoring measures. These results validate SLA analysis in *Drosophila* BS mutants as a model for the KD.

3.2. The anticonvulsant effects of the ketone body β -HB on SLA are partially mediated by both K_{ATP} channels and GABA_B signaling

There exists an established link between K_{ATP} channel activity, as well as GABA_B signaling, and KB effects in brain slice models (Ma et al., 2007; Tanner et al., 2011), and for K_{ATP} channels in ameliorating SLA in *in vivo* mouse models (Giménez-Cassina et al., 2012). We tested for similar such effects in this *Drosophila* model by comparing the average percentage of time in SLA between the standard control diet and a KB (β -HB) diet containing 200 μ M tolbutamide, a K_{ATP} channel blocker (“ β -HB + tolbut”). Incidence of *eas* flies' SLA on the β -HB + tolbut diet was significantly greater ($p < 0.01$) than that of flies on the β -HB-only diet, suggesting that the observed effects of the KB β -HB on SLA are at least in part mediated by K_{ATP} channels (Fig. 2).

We also used a tolbutamide-only diet as a control for KB-independent effects of the drug and found that incidence of SLA on the tolbutamide-only diet (mean $0.100 \pm 0.025\%$; $n = 20$) was significantly lower ($p < 0.001$) than that of the control diet (mean $0.800 \pm 0.266\%$; $n = 20$). This result is consistent with earlier work showing that blockade of K_{ATP} channels by tolbutamide ameliorates SLA in *Drosophila* (Stone et al., 2013) and may result from increased metabolic stability subsequent to increased hemolymph glucose availability via upregulation of adipokinetic hormone (Kim and Rulifson, 2004).

Finally, we applied the GABA_B antagonist CGP-55845 on top of the KB β -HB, and found that SLA on the β -HB + CGP diet was significantly greater ($p < 0.01$) than that of flies on the β -HB-only diet in our model, implicating GABA_B signaling in the KB effect on SLA mitigation.

Download English Version:

<https://daneshyari.com/en/article/5628591>

Download Persian Version:

<https://daneshyari.com/article/5628591>

[Daneshyari.com](https://daneshyari.com)