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Anticonvulsant profile of a balanced ketogenic diet in acute mouse seizure models

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Summary Anticonvulsant effects of the ketogenic diet (KD) have been reported in the mouse, although previous studies did not control for intake of vitamins, minerals and antioxidants. The aim of this study was to examine the effects of balanced ketogenic and control diets in acute mouse seizure models. The behavior in four mouse seizure models, plasma D-β-hydroxybutyrate (D-BHB) and glucose levels were determined after feeding control diet, 4:1 and 6:1 KDs with matched vitamins, minerals and antioxidants. Feeding 4:1 and 6:1 KDs *ad lib* to 3-week-old (adolescent) mice resulted in 1.2—2.2 mM D-BHB in plasma, but did not consistently change glucose levels. The 6:1 KD reproducibly elevated the CC50 (current that initiates seizures in 50% mice tested) in the 6-Hz model after 14 days of feeding to adolescent CD1 mice. Higher plasma D-BHB levels correlated with anticonvulsant effects. Despite ketosis, no consistent anticonvulsant effects of KDs were found in the fluorothyl or pentylenetetrazole CD1 mouse models. The 4:1 KD was neither anticonvulsant nor neuroprotective in hippocampus in the C3H mouse kainate model. Taken together, the KD's anticonvulsant effect was limited to the 6-Hz model, required chronic feeding with 6:1 fat content, and was independent from lowering plasma glucose.

Introduction

The ketogenic diet (KD) is a high fat, low protein, low carbohydrate diet, and is anticonvulsant in many drug-resistant epileptic children (Vining et al., 1998; Vining, 1999; Sinha and Kossoff, 2005; Freeman et al., 2006, 2007; Hartman and Vining, 2007). Its anticonvulsant mechanism of action is unknown and is therefore being researched in animal models. Different KDs for mice and rats have been developed that were shown to be anticonvulsant in certain seizure paradigms (Appleton and DeVivo, 1974; Hori et al., 1997; Bough and Eagles, 1999; Bough et al., 1999b; Muller-Schwarze et al., 1999; Rho et al., 1999; Su et al., 2000; Todorova et al., 2000; Hartman et al., 2007). The KD was also neuroprotective after kainate-induced status epilepticus (SE, Noh et al., 2003), after brain injury (Prins et al., 2005) and in an amyotrophic lateral sclerosis model (Zhao et al., 2006). To our knowledge, the intake of vitamins and minerals in the control and KDs were not precisely matched in studies using mice. The aim of this study was to examine

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the effects of balanced ketogenic and control diets in acute mouse seizure models. We (1) developed 4:1 and 6:1 ketogenic and control diets that are matched in their amounts of vitamins, minerals and antioxidants relative to their caloric content and (2) assessed the anticonvulsant and neuroprotective effects of these KDs in four acute mouse seizure models, including the 6-Hz, fluorothyl, kainate, and pentylenetetrazole models. There has been concern that different body weights of ketogenic vs. control diet-fed rodents may influence seizure behavior in chemical seizure models (e.g. Nylen et al., 2005), because the pharmacokinetics of convulsant drugs may be influenced by body fat composition. Therefore, we included the 6-Hz model of limbic seizures, in which seizure thresholds appear to be independent from body weight (Hartman et al., 2008).

Materials and methods

Diets and mice

Many rodent studies are based on the F3666 diet (Bio-Serv; e.g. Bough and Eagles, 1999; Bough et al., 2002, 2006; Nylen et al., 2005; Hartman et al., 2008). The F3666 diet often has been reported to be a 6.3:1 KD (6.3 parts fat relative to one part protein plus carbohydrates, w/w), but according to the datasheet it contains 8.6 times more fat than protein and carbohydrates together. Here, 4:1 and 6:1 ketogenic and control diets were developed based on a 4:1 KD previously used in mice (TD.96355, Rho et al., 1999; Noh et al., 2003). The amounts of vitamins, minerals and antioxidants were adjusted according to the newest nutritional standards and were equal among all diets relative to their caloric densities (Table 1). 4:1 (TD.06233) and 6:1 (TD.07797) ketogenic and control diets (TD.06316, all Harlan-Teklad) were fed *ad lib* for 1—3 weeks to 3-week-old CD1 (Charles River) and C3Heb/Fe male mice (Jackson Laboratories). For the pentylenetetrazole seizure tests, we restricted caloric intake of 4:1 and 6:1 KD to about 80% of the daily caloric intake in adult male CD1 mice, mimicking the experimental conditions used for rats (e.g. Bough et al., 2006). In the restricted feeding experiments, mice were given 1.4—1.6 g of 4:1 or 6:1 KD per day and mouse body weights were monitored at least every other day. All vitamins and minerals were increased by 25% in the restricted KDs to avoid dietary deficiencies.

Seizure models

Different ages of mice were used according to the requirements of the seizure models studied. For the 6-Hz, fluorothyl and kainate models, we initiated the KD after weaning to obtain maximal ketosis (e.g. Nylen et al., 2005). For the pentylenetetrazole model, adult mice were chosen over adolescent mice because of the extreme difficulty of tail vein infusion in small animals. We used CD1 mice for the 6-Hz, fluorothyl and pentylenetetrazole models, because these mice are outbred. Inbred C3Heb/FeJ mice were used in the kainate model, because we were also interested in the neuroprotective effect of the KD. Previous experience in outbred mice in our hands showed high variability of the extent of hippocampal damage after status epilepticus (Borges et al., 2003).

6-Hz CD1 mouse model

The 6-Hz seizure model was performed as described (Brown et al., 1953; Kaminski et al., 2004; Hartman et al., 2008). The critical current intensity needed to induce seizures in 50% of mice tested (CC50) was determined after 7—21 days of feeding 4:1, 6:1 ketogenic and control diets *ad lib* to 3-week-old CD1 mice. A topical anesthetic (0.5% tetracaine hydrochloride ophthalmic solution) was applied to the corneas 10—15 min before stimulation. After wetting the electrodes with 0.9% NaCl immediately before testing, 0.2 ms duration pulses at 6-Hz with different current intensities were applied to the corneas for 3 s by a constant-current device (ECT Unit 57800, Ugo Basile). Mice were manually held during stimulation and then released for behavioral observation. Seizures were characterized by a stunned or fixed posture, rearing, forelimb clonus, or twitching. Mice were considered to be protected from seizures if they did not show seizure behavior and resumed normal exploratory behavior within 10 s (Brown et al., 1953). Electrical currents of varying intensities were applied to different mice to determine the CC50 using the ''up-and-down'' method (Kimball et al., 1957). This method requires fixed log steps between different current intensities, but the stimulation device cannot be programmed to deliver these exact current intensities. Therefore, we chose to use fixed 2 mA intervals, which when converted to log intervals have a coefficient of variation of 10%. For the CC50 calculation we used the average log interval of those current steps that determined the CC50.

Fluorothyl CD1 mouse model

After 7—21 days feeding of 4:1, 6:1 ketogenic and control diets *ad lib* to 3-week-old CD1 mice, we assessed the latencies to fluorothyl-induced clonic and tonic seizures with the observers being blinded to the diet groups. Mice were placed into a clear plastic observation chamber (15 cm × 20 cm × 28 cm). Fluorothyl (bis(2,2,2-trifluoroethyl)ether, Aldrich) was dripped at a flow rate of 20 μl/min onto a suspended filter paper near the top of the chamber from where it evaporated. The latencies to the first myoclonic seizure and tonic extension were timed.

Pentylenetetrazole CD1 mouse model

The seizure thresholds for myoclonic and tonic seizures induced by pentylenetetrazole (i.v.) were compared after 19 days of feeding restricted amounts of 4:1 or 6:1 KD vs. *ad lib* control diet to adult CD1 mice. Ten milligram per milliliters pentylenetetrazole dissolved in saline was infused into the tail vein at 150 μl/min. The latencies to the first myoclonic seizure and tonic extension were determined and converted to seizure thresholds expressed as mg/kg body weight using the following formula: time to seizure (min) × 1.5 mg PTZ/min/weight (kg). The pentylenetetrazole concentration and flow rate were chosen based on previous studies (Löscher and Lehmann, 1996; Stöhr et al., 2007) and because they provide seizure latencies that could well be distinguished by the experimenters, e.g. the first seizure was elicited after 19—51 s. The maximum infusion volume was 13 ml/kg body weight, which is similar to Löscher and Lehmann (1996).

Kainate C3Heb/FeJ mouse model

3-Week-old C3Heb/FeJ mice were placed on 4:1 ketogenic or control diet. After 16 days mice were injected with 25—35 mg/kg kainate (i.p.) dissolved at 2 mg/ml in phosphate-buffered saline (PBS, pH 7.4) and behavioral seizures were observed. Mice on control diet all received 35 mg/kg kainate (i.p.), while we lowered the kainate dose in some KD-fed mice to ensure survival. Control mice were injected with PBS only. SE onset was defined as the onset of whole clonic body seizures that became continuous. SE severity was scored by the most severe seizure activity observed, with whole body clonic seizures scored as (1), rearing and falling or loss of balance scored as (2), and tonic—clonic seizures or jumping scored as (3). After SE, the diets were continued for 3 more days until mice were anesthetized with 100 mg/kg pentobarbital (i.p.) and perfused with 4% paraformaldehyde through the heart. The brain was extracted and post-fixed for 1—2 days in 4% paraformaldehyde. All animal experiments were approved by TTUHSC and every effort was made to minimize animal suffering.