Neuropharmacology 99 (2015) 500–509

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Ketogenic diet prevents epileptogenesis and disease progression in adult mice and rats

Theresa A. Lusardi [a], Kiran K. Akula [a], Shayla Q. Coffman [a], David N. Ruskin [b], Susan A. Masino [b], Detlev Boison [a, *]

[a] RS Dow Neurobiology Laboratories, Legacy Research Institute, Portland, OR 97232, USA
[b] Department of Psychology and Neuroscience Program, Trinity College, Hartford, CT 06106, USA

ARTICLE INFO

Article history:
Received 3 April 2015
Received in revised form
8 July 2015
Accepted 3 August 2015
Available online 6 August 2015

Keywords:
TLE
Acquired epilepsy
Chemoconvulsant
Adenosine
Epigenetics
DNA methylation

ABSTRACT

Epilepsy is a highly prevalent seizure disorder which tends to progress in severity and become refractory to treatment. Yet no therapy is proven to halt disease progression or to prevent the development of epilepsy. Because a high fat low carbohydrate ketogenic diet (KD) augments adenosine signaling in the brain and because adenosine not only suppresses seizures but also affects epileptogenesis, we hypothesized that a ketogenic diet might prevent epileptogenesis through similar mechanisms. Here, we tested this hypothesis in two independent rodent models of epileptogenesis. Using a pentylenetetrazole kindling paradigm in mice, we first show that a KD, but not a conventional antiepileptic drug (valproic acid), suppressed kindling-epileptogenesis. Importantly, after treatment reversal, increased seizure thresholds were maintained in those animals kindled in the presence of a KD, but not in those kindled in the presence of valproic acid. Next, we tested whether a KD can halt disease progression in a clinically relevant model of progressive epilepsy. Epileptic rats that developed spontaneous recurrent seizures after a pilocarpine-induced status epilepticus were treated with a KD or control diet (CD). Whereas seizures progressed in severity and frequency in the CD-fed animals, KD-fed animals showed a prolonged reduction of seizures, which persisted after diet reversal. KD-treatment was associated with increased adenosine and decreased DNA methylation, the latter being maintained after diet discontinuation. Our findings demonstrate that a KD prevented disease progression in two mechanistically different models of epilepsy, and suggest an epigenetic mechanism underlying the therapeutic effects.

1. Introduction

Epilepsy affects about 50 million persons worldwide; it is estimated that up to 30–35% of all cases are pharmacoresistant. In particular, seizures originating from the temporal lobe tend to progress in severity and frequency and may become refractory to treatment. Epileptogenesis refers to a complex process, involving epigenetic changes, inflammatory mechanisms, glial activation, and reorganization of neuronal circuitry, during which a normal brain becomes epileptic (Pitkanen and Lukasiuk, 2011; Vezzani et al., 2011). Antiepileptogenic and disease-modifying therapies that halt disease progression are therefore urgently needed. A ketogenic diet (KD) is a high-fat, low-carbohydrate and restricted-protein diet that is often a last resort to treat epilepsy in children who are resistant to antiepileptic drugs (Kossoff and Rho, 2009). Beyond its anticonvulsant effects, clinical evidence suggests that a KD might also have antiepileptogenic effects. Follow-up studies of epileptic children treated with a KD suggest that the diet may afford long-lasting seizure protection even after its discontinuation (Caraballo et al., 2011). Whether this stems from an antiepileptogenic effect of the diet or simply reflects spontaneous remission of seizures cannot be decided. Unlike the clear anticonvulsant success of the KD, which has been related to metabolic changes induced by the diet (Bough, 2008; Yellen, 2008), the potential antiepileptogenic activity of a KD remains poorly documented and evaluated.

We recently provided direct evidence that changes in DNA methylation patterns are a key determinant of the progression of epilepsy and that treatment with anticonvulsants that boost adenosine may reverse DNA hypermethylation and break the cycle of increasing seizure severity. Accordingly, adenosine has been

* Corresponding author. Robert Stone Dow Neurobiology Laboratories, Legacy Research Institute, 1225 NE 2nd Ave, Portland, OR 97232, USA.
E-mail address: dboison@downeurobiology.org (D. Boison).

http://dx.doi.org/10.1016/j.neuropharm.2015.08.007
0028-3908/

identified as an endogenous agent of the brain with potent and long-lasting antiepileptogenic properties (Williams-Karnesky et al., 2013). Because a KD was shown to suppress seizures by augmenting adenosine signaling in the brain (Masino et al., 2011) and because adenosine has not only anticonvulsive, but also antiepileptogenic properties (Williams-Karnesky et al., 2013), we hypothesized that a KD might prevent epileptogenesis or halt disease progression via augmentation of adenosine signaling. We tested the anti-epileptogenic effect of a KD in two different rodent models of epileptogenesis and show consistent increases in seizure thresholds and decreases in spontaneous seizure activity, which were maintained even after reversal to a normal diet. Seizure suppression in KD-fed epileptic animals was in line with a rise in brain adenosine and a sustained decrease in DNA methylation status.

2. Materials & methods

2.1. Animals, drug, and diet treatment

All animal procedures were conducted in accordance with protocols approved by the Legacy Institutional Animal Care and Use Committee (IACUC) and the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals. All studies were performed in adult male CD-1 mice or Wistar rats (Charles River Laboratories, Wilmington, MA, USA). Pentylenetetrazole, pilocarpine, scopolamine, and valproic acid (all from Sigma Aldrich, St. Louis, MO, USA) were dissolved in 0.9% w/v saline to achieve the desired dosages via the intraperitoneal (i.p.) or subcutaneous (s.c.) routes. Ketogenic diet (KD: 8.6% protein w/w, 75.1% fat w/w, 3.2% carbohydrates w/w) for rodents was obtained from Bio-Serv (#F3666, Bio-Serv, Frenchtown, NJ, USA) and supplied ad libitum. Caloric composition of the KD is: 93.4% fat, 4.7% protein, and 1.8% carbohydrates. Standard chow (control diet, CD) with a caloric composition of 13.5% fat, 28.5% protein, and 58.0% carbohydrates was used in control animals or after diet reversal.

2.2. Pentylenetetrazole (PTZ) kindling

Kindling is a process whereby repeated stimulation of the brain initiates permanent alterations in neural circuitry, resulting in increased convulsive response to the same stimulus. Kindling paradigms are therefore widely used to assess the efficacy of potential antiepileptogenic treatments (Loscher, 2002). We used a chemical kindling paradigm, based on the repeated administration of sub-convulsant doses of PTZ (El Yacoubi et al., 2008). Prior to the induction of kindling, 4 month old mice were randomly assigned to one of three groups: CD, KD-I, or KD-II. Mice were fed either a KD or CD for 8 weeks, and KD treatment was maintained throughout the 29 day kindling period. During chemical kindling, sub-convulsive doses of PTZ were injected i.p. every other day (including weekends) adhering to the following schedule: 25 mg/kg from study day 1–15 and 30 mg/kg from day 17–29. After each PTZ injection, mice were monitored 30 min for incidence and severity of seizures using a modified Racine scale (0 = no response, 1 = mouth and facial jerks, 2 = nodding or myoclonic body jerks, 3 = forelimb clonus, 4 = rearing, falling down, hindlimb and forelimb clonus). For diet reversal, half of the animals on the KD received a glucose injection (30% w/v; 2 g/kg) on study day 29 after the last kindled seizure, and those mice were then reverted to CD feeding for the remainder of the experiment. Five days later, all animals were subjected to a single 30 mg/kg, i.p. dose of PTZ to assess the maintenance of previous seizure thresholds. Identical protocols for kindling and seizure threshold studies were followed when kindling a different set of animals in the presence or absence of the antiepileptic drug valproic acid (VPA, 200 mg/kg, i.p.). In those studies VPA was injected 30 min prior to each PTZ injection.

2.3. Pilocarpine model of epileptogenesis

Temporal lobe epilepsy (TLE) is characterized by repeated spontaneous seizures that are initiated in the hippocampal formation. A period of prolonged status epilepticus (SE) initiates epileptogenic processes leading to spontaneous convulsive seizures and histologic changes associated with TLE (Dudek et al., 2006). SE was induced in adult male Wistar rats (250–275 g) with pilocarpine (280 mg/kg, i.p.) (Klitgaard et al., 1998). Scopolamine (1 mg/kg, s.c.) was injected 30 min before pilocarpine to reduce peripheral cholinergic effects. Animals that experienced no SE during 60 min after the first pilocarpine administration were treated a second time with half the initial dose. Animals that did not develop SE after the second dose were excluded from further experimentation. SE was terminated 2 h after its onset with diazepam (5 mg/kg, s.c.) to reduce post-ictal mortality. Only rats that exhibited at least 2 h of convulsive Racine stage-4 seizures were used. Control rats were treated with saline (0.9% w/v NaCl, i.p.) instead of pilocarpine, but likewise received scopolamine and diazepam. Starting 3 weeks after the initial SE, all animals were intermittently (3–4 week bins) video monitored (24 h/day, 7 days/week) to quantify the number of convulsive stage 4–5 seizures. All rats were video monitored at the same time, and all captured videos were included in the analysis. A custom “software robot” was utilized to create sequential 8 h video files. To account for occasional video loss due to technical difficulties, seizures per week calculations were normalized to the number of hours analyzed. Diet treatment was initiated after the conclusion of week 6 after the SE. At this time point all animals had a seizure rate of at least 3 seizures per week. All animals were monitored for up to 23 additional weeks by intermittent video monitoring and retrospective scoring and analyses performed by investigators blinded to the experimental conditions. Behavioral seizures were confirmed by EEG analysis in selected animals, with bilateral recording screw electrodes implanted at stereotaxic coordinates: AP, −4.5 mm; ML, ±4.0 mm. Electrical brain activity was amplified (Grass Technologies) and digitized (PowerLab; AD Instruments). EEG seizure activity was defined as high-amplitude rhythmic discharges that clearly represented a new pattern of tracing that lasted at least 5 s.

2.4. Quantification of β-hydroxybutyrate

Blood levels of β -hydroxybutyrate (BHB) were measured by Precision Xtra ketone test strips (Abbott Laboratories). Blood was collected from the lateral tail vein on study day 18. Increased blood concentration of BHB was taken as evidence of ketonemia in animals fed with the KD.

2.5. Adenosine quantification

After decapitation, the brain was removed and hippocampal tissue dissected out, frozen in liquid nitrogen, and stored at −80 °C. Adenosine analyses were performed on a high performance liquid chromatography (HPLC) system coupled to a multiple wavelength detector (Agilent 1100 series). Samples were eluted on a C18 column with a particle size of 5 μm and a flow rate of 0.8 mL/min using a mobile phase containing water, acetonitrile and methanol (88:7:5, %v/v). The retention time of adenosine was around 6 min at a detection wavelength of 258 nm. All peak areas were within the linear range of the standard curves. Adenosine values were extrapolated from the linear regression curve calculated on the basis of standard solutions. Extracellular adenosine levels are presented as the mean of four samples.