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Epilepsy Research 68 (2006) 207–211

Epilepsy Research

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A ketogenic diet and knockout of the norepinephrine transporter both reduce seizure severity in mice

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Received 26 August 2005; received in revised form 30 September 2005; accepted 8 November 2005
Available online 13 December 2005

Abstract

Ketogenic diets (KD) have been known to be effective against epilepsy for more than 80 years, yet the mechanism(s) responsible for this action remain unknown. Norepinephrine (NE) has been shown to have anti-ictal effects against a wide variety of pro-convulsants and in animal models of epilepsy. Loss of noradrenergic activity is also associated with loss of the seizure protection seen following consumption of ketogenic diets. By contrast, knockout of the NE transporter (NET) gene, which elevates synaptic levels of norepinephrine, decreases seizure severity in mice fed normal diets. The purpose of this study was to compare the severity of maximal electroshock seizures in mice lacking the NET (NET KO) with that of wild type (WT) mice fed either a normal or a KD. In general, NET KO mice and mice fed a KD had a similar reduction in seizure severity, and the anticonvulsant effects of the genetic deletion of NET and the ketogenic diet were additive. These observations suggest that, while the noradrenergic system is required for the anti-seizure effects of the KD, additional mechanisms are involved.

Keywords: Ketogenic diet; Calorie restriction; Diet; Maximal electroshock; Seizure; NET knockout

1. Introduction

Among the oldest and most generally effective treatments of epilepsy (Vining et al., 1998) is the use of high-fat, low-carbohydrate (ketogenic) diets (KD), originally described by Wilder (1921). Despite a long history of clinical use and numerous experimental studies (see, e.g., Stafstrom and Rho, 2004), the mechanisms underlying its effectiveness remain unknown. The ability of the KD to ameliorate flurothyl-induced seizures is lost in mice lacking norepinephrine (NE) due to knockout of the gene for dopamine β-hydroxylase (Dbh−/− mice), which encodes the enzyme that catalyzes the conversion of dopamine to NE (Szot et al., 2001). A general anti-epileptic role for NE has been shown by lesioning studies, pharmacological experiments and mutant rats and mice (for review see Weinshenker and Szot, 2002). Conversely,

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doi:10.1016/j.eplepsyres.2005.11.002

increasing NE signaling is typically anticonvulsant (Weinshenker and Szot, 2002). For example, the severity of maximal electroshock (MES) seizures is decreased in mice lacking the NE transporter (NET KO), which have elevated extracellular NE levels (Xu et al., 2000; Ahern et al., 2005). The KD modestly enhances the basal release of NE into the synapse and more than doubles the levels of NE in the extracellular space, provided the NE transporter (NET) is blocked (see Szot, 2004). Curiously, rats and mice fed KDs differ in their responses to MES. Rats fed a KD and subjected to MES show *more severe* seizures (Bough et al., 2000) while mice fed the same diet show less severe seizures (Millichap et al., 1964). The purpose of the present study was to compare seizure severity in wild type (WT) mice fed a KD with that seen in NET KO mice fed a chow diet and to test the hypothesis that knockout of the NET will have no additional effect upon seizure severity in mice fed the KD. We found that the reduction in seizure severity in WT mice fed the KD was equal to that seen in NET KO mice and that seizure severity in NET KO mice was further reduced if they were fed the KD, indicating that its action must involve more than just effects mediated by the NET.

2. Methods

2.1. Animals

A total of 31 adult male and female mice of the C57BL6/J strain between 3 and 7 months of age were used in this study, 14 of them norepinephrine transporter knockouts (NET KO) and 17 Wild Type controls (WT). Mice were age-matched between genotypes. These mice were derived from breeding pairs obtained from Mark Caron (Duke University). All had been exposed to a single MES seizure approximately 3 weeks prior to MES testing in the present set of experiments and were a subset of those contributing to Fig. 3 of Ahern et al. (2005). Animals were housed in groups of from 3 to 7 in colony cages on Alpha chips bedding and kept in quarantine for the entire duration of their stay at Georgetown University. All were treated in accordance with a Georgetown University Animal Care and Use protocol (#99-062). Animals were maintained at a temperature of 22 °C on a 12-h light:12-h dark cycle with lights on at 06.00 h.

2.2. Diets

All mice were fed Purina rodent chow (5001) ad libitum throughout the first study (Ahern et al., 2005) and all were then switched to a high-fat (ketogenic) diet (BioServe F3666), also fed ad libitum. All animals were provided water at all times.

2.3. Seizures

Maximum electroshock was applied via ear-clip electrodes from a constant-current device (Ugo Basile ECT Unit 7801, Varese, Italy) at a frequency of 299 pulses/s for 100 ms at 20 mA. The electrodes made contact via felt pads, which were wetted with 3 M NaCl for each animal. Seizure severity was judged by timing the period from initiation of shock until the hind limbs passed through a 90° angle with the body (flexion) and extension was taken as the time from that point until the hind limbs reached maximum extension, returned toward the normal posture, and the mouse palpably relaxed. This endpoint (relaxation) was chosen because it was more consistent than the attainment of a specific position by the relaxing hind limbs. The duration of each event was determined by an individual blinded with respect to the identity of the animal being tested. Mice were not weighed but there was no evident difference either between genotypes or for the two diets.

2.4. Statistics

Comparisons between WT and NET KO animals for extension, flexion and extension/flexion (E/F) ratio were made by Mann–Whitney "*U*" test and a *P*-value of <0.05 was considered significant, unless otherwise indicated.

3. Results

Both the KD and the lack of NET reduced the duration of hindlimb extension compared to WT mice fed the normal rodent chow diet (Fig. 1, compare group 1 to groups 2 and 3). Interestingly, the effects of the KD and of NET knockout were approximately equal, as the duration of hindlimb extension for wildtype mice fed the KD was similar to that of NET KO mice fed the