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Research report

Ketogenic diet attenuates spatial and item memory impairment in pentylenetetrazol-kindled rats

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ABSTRACT

The ketogenic diet (KD) controls seizure and improves cognition in patients with drug refractory epilepsy. However, few experimental models have shown this neuroprotective effect on cognition. In this study, we investigated the cognitive protective effects of KD in pentylenetetrazol (PTZ)-kindled rats. We used two relatively low-stress behavioral assessment methods, the novel object recognition (NOR) task and the novel placement recognition (NPR) task, to reveal impairment in item and spatial memory, respectively. We used the Morris water maze (MWM) test for comparisons amongst memory assessment methods. The KD group had a slower body weight gain and shorter bregma-lambda length than the control normal diet (ND) group. KD did not increase anxiety or decrease motor activities in an open-field test. KD attenuated the decrease in exploration ratio both in NOR and NPR tasks in kindled rats. Compared to the kindled ND rats, kindled KD rats stayed longer in target quarter during the probe trial testing of MWM. However, there were no differences in memory acquisition based on the MWM test results. In conclusion, KD attenuated the spatial and item memory impairment in PTZ-induced seizures.

1. Introduction

Epilepsy is one of the most prevalent disabling neurological disorders. Approximately 20–50% of epileptic patients experience intellectual disability problems (Caller et al., 2015), which negatively impact their daily activities and quality of life (Kleen et al., 2012). Several factors have been implicated in the development of cognitive and behavioral impairment in epileptic patients, including the underlying etiology, age of onset, seizure semiology, seizure severity and frequency, adverse effects secondary to antiepileptic drugs (AEDs), and surgery (Elger et al., 2004; Gulati et al., 2014).

The ketogenic diet (KD), a high-fat, medium-protein, and low-carbohydrate diet, is used as an alternative to AEDs in subjects with drug refractory epilepsy. KD, which has antiepileptic, antiepileptogenic (Hansen et al., 2009; Hori et al., 1997; Jiang et al., 2012), and neuroprotective effects (Jiang et al., 2012; Linard et al., 2010), enhances alertness and attention and improves sleeping patterns and quality of life (Farasat et al., 2006; Hallböök et al., 2007; Kossoff et al., 2012; Pulsifer et al., 2001). It is unclear whether these cognitive improvements are due to direct effect of the KD, less seizure, the need for fewer sedating anticonvulsants, or a combination of these factors. To understand the protective effects of KD on cognition, several animal models have been developed.

However, there are limitations with animal models. First, while researchers have focused on the effects of KD on seizure activity in animals, e.g., KDs can increase induced-seizure threshold, delay seizure development, attenuate seizure risk, and/or decrease the seizure severity (Hansen et al., 2009; Hori et al., 1997; Jiang et al., 2012; Patel et al., 2010), less is known about its effects on cognition. Second, the effects of KD on cognition have not been consistent in animals. Studies have reported that amygdaloid-kindled epileptic rats on KD do not perform any differently from control rats in spatial learning or open-field tasks (Hori et al., 1997). Moreover, KD contributes to severe impairments in visual spatial memory and brain growth in spite of reducing the number of seizures in status epilepticus (SE) (Su et al., 2000; Zhao et al., 2004). However, several factors, including epileptic model, age, ketogenic ratio, methods of assessing cognition, may have contributed to these results. Furthermore, the evaluation of behavioral tasks, which is commonly used to assess the effect of KD on cognition, may be challenging. First, the vast majority of trials rely on the Morris water maze (MWM) test to assess cognitive function.

Abbreviations: KD, ketogenic diet; ND, normal control diet; PTZ, pentylenetetrazol; SE, status epilepticus; NOR, novel object recognition; NPR, novel placement recognition; MWM, morris water maze

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However, the use of aversive techniques (water, shocks, and food restriction) introduces a possible confound of stress (Pearson et al., 2014). Therefore, whether KD plays a role in cognition still requires further investigation. Second, a broad spectrum of cognitive defects including several forms of aphasia, apraxia, frontal lobe dysfunction, visuospatial disability, and selective memory deficits are associated with epilepsy (Gulati et al., 2014). The MWM test, which is particularly sensitive in the assessment of hippocampal lesions, is considered to be a behavioral test of visuo-spatial learning and memory that is not as sensitive to other aspects of memory.

The main purpose of this study was to investigate the effects of KD on learning and memory performance in pentylenetetrazol (PTZ)-kindled rats. We evaluated cognition in fully kindled rats, which overcame the interference of the epileptic seizure and the AED effect. To reduce possible confounding effects of task requirements on learning and psychological performance variables, we evaluated behavior by using two minimally stressful methods: the novel object recognition task (NOR) and the novel placement recognition task (NPR). Both of these methods require minimal learning. The NOR focuses on the measurement of item recognition memory, while the NPR focuses on visuo-spatial recognition memory testing. Additionally, we measured locomotion and anxiety-related behavior in an open-field before recognition task to determine whether they could account for differences in learning and memory tasks. Finally, we compared the NOR and NPR results to those obtained from the MWM test.

2. Results

2.1. Body weight, ketonemia, and kindling acquisition

Body weight increased in both groups, however, the KD group had a significantly lower growth gain than the ND group ($p < 0.001$, Fig. 1A). Additionally, the KD group had shorter bregma-lambda length than the ND group ($p < 0.05$, Fig. 1B), which revealed that KD decreased body weight gain and skeletal growth.

Blood glucose and serum BHB levels were measured in ND and KD groups (Fig. 1C and D). After week 1, KD induced persistent hypoglycemia and ketonemia. Blood glucose levels were lower in KD than in ND ($p < 0.001$), while serum BHB levels were higher in KD than in ND rats ($p < 0.001$). KD delayed the progression of seizure stages during PTZ-induced kindling ($p < 0.05$, Fig. 1E).

2.2. Open field task

The number of central square entries in the KD-kindled group was higher than in the non-kindled KD group ($p < 0.01$, Fig. 2A), but similar to ND-kindled group. There were no significant differences in the time spent in the center of the arena between KD and ND whether kindled or not (Fig. 2B). Both kindled groups displayed larger total distance travelled in the open-field, compared to the non-kindled groups ($p < 0.05$ in KD and $p < 0.01$ in ND, Fig. 2C). Additionally, the speed within the arena of both the kindled group was higher than that of the non-kindled group ($p < 0.01$ in ND and $p < 0.05$ in KD, Fig. 2D). However, no significant difference in the distance or motor speed was obtained between KD and ND groups, whether kindled or not.

2.3. NOR and NPR tasks

The non-kindled ND rats naturally spent significantly more time exploring the novel object or place ($p < 0.01$ in NOR task and $p < 0.001$ in NPR task, Figs. 3A and C), but this trait disappeared when kindled (Fig. 3B and D). Therefore, ND-kindled rats exhibited reduced exploration ratios both in the NOR task ($p < 0.05$, Fig. 3E) and NPR task ($p < 0.01$, Fig. 3F), which suggested the impaired exploratory ability in the ND-kindled group.

KD rats spent relatively more time with the novel object and

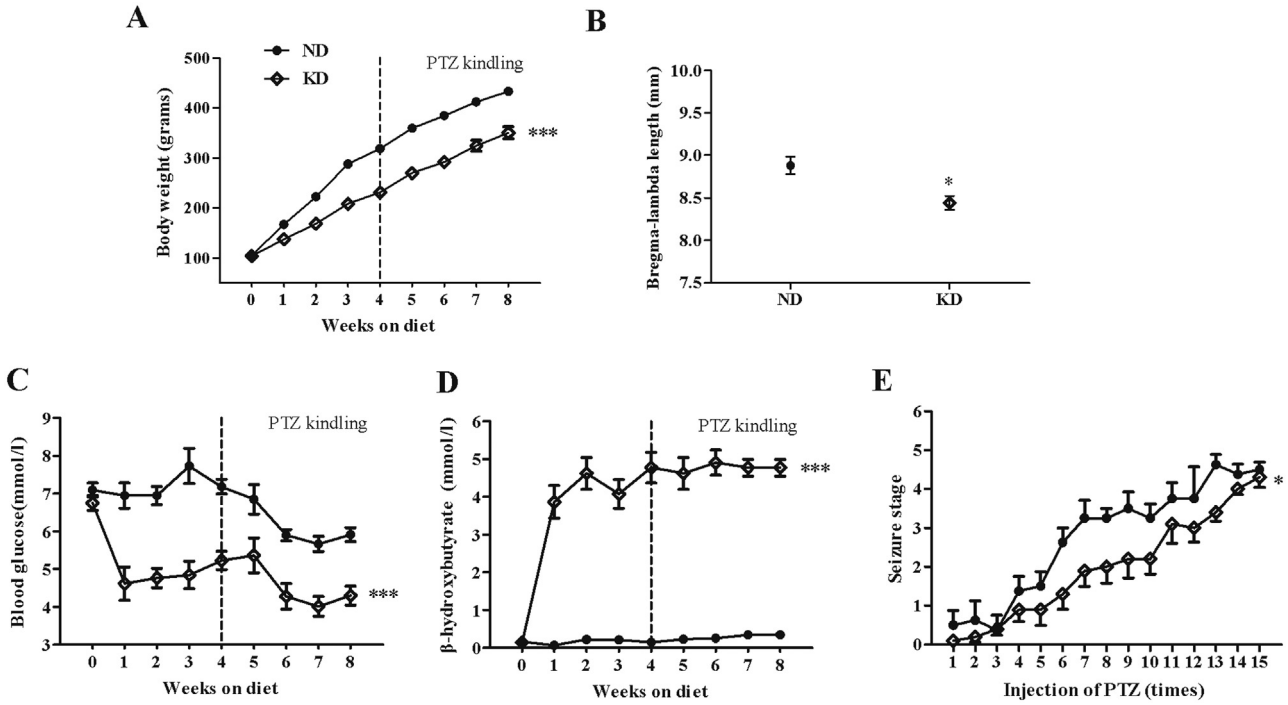

Fig. 1. (A) Body weight. (B) Bregma-lambda length. (C) Blood glucose levels. (D) Serum beta-hydroxybutyrate (BHB) levels and (E) Seizure stage during PTZ-kindling acquisition. n = 6 for each group in A, B, C and D. For E, n = 10 in kindled KD group, n = 8 in kindled ND group. Two-way repeated measures analysis of variance (ANOVA) was used for comparison between KD and ND over different time points in A,C,D and E. Independent sample *t*-test was used for B. *$p < 0.05$ and ***$p < 0.001$ compared with ND group.