Schizophrenia Research xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Schizophrenia Research

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

Effects of a ketogenic diet on auditory gating in DBA/2 mice: A proof-of-concept study

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ARTICLE INFO

Article history:
Received 18 May 2015
Received in revised form 18 September 2015
Accepted 21 September 2015
Available online xxxx

Keywords:
Gating
Ketogenic diet
Hippocampus
P50
Schizophrenia

ABSTRACT

Although the ketogenic diet has shown promise in a pilot study and case report in schizophrenia, its effects in animal models of hypothesized disease mechanisms are unknown. This study examined effects of treatment with the ketogenic diet on hippocampal P20/N40 gating in DBA/2 mice, a translational endophenotype that mirrors inhibitory deficits in P50 sensory gating in schizophrenia patients. As expected, the diet increased blood ketone levels. Animals with the highest ketone levels showed the lowest P20/N40 gating ratios. These preliminary results suggest that the ketogenic diet may effectively target sensory gating deficits and is a promising area for additional research in schizophrenia.

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1. Introduction

Hippocampal inhibitory dysfunction and its associated neurophysiological abnormalities are a topic of great interest in schizophrenia, due in large part to their utility as potential targets for therapeutic intervention. Neuroimaging studies in human patients frequently have observed increased activity in the hippocampus in patients with schizophrenia, relative to comparison subjects (reviewed by Heckers and Konradi, 2015; Tamminga et al., 2012, 2010; Tregellas, 2014). The magnitude of this abnormality may predict positive (Ebmeier et al., 1993; Friston et al., 1992; Gur et al., 1995; Kawasaki et al., 1996; Liddle et al., 1992; Molina et al., 2005; Schobel et al., 2013, 2009), negative (Schobel et al., 2009; Tregellas et al., 2014), and cognitive (Tregellas et al., 2014) symptoms. It follows that treatments that can reduce hippocampal activity may confer therapeutic benefit in schizophrenia.

One strategy for targeting this phenotype is to attempt to repurpose interventions for other brain disorders associated with neuronal hyperactivity, such as epilepsy (Smucny et al., 2015a, 2015b). One relatively low-risk intervention is the ketogenic diet, which has been approved for treatment-resistant epilepsy (reviewed by Klein et al., 2014). The diet typically consists of a high ratio (for example, 4:1) of fats to carbohydrates plus proteins. The ketogenic diet and related diets have shown potential efficacy in a limited number of case studies and small pilot studies in schizophrenia (Kraft and Westman, 2009; Pacheco et al., 1965). Its effects on schizophrenia patients and in animal models of disease mechanisms are, however, largely unknown.

As a first step towards preclinical evaluation of the ketogenic diet, the goal of this study was to administer the diet in the DBA/2 mouse to examine its effects on a hippocampal hyperactivity-associated physiological abnormality that mirrors the schizophrenia endophenotype (Singer et al., 2009). At present, perhaps the most well-established translational, clinically predictive physiological assay to assess hippocampal inhibitory circuitry in rodent models of schizophrenia is hippocampal auditory P20/N40 gating (reviewed by Smucny et al., 2015a). This assay, conducted using depth electrodes implanted into the mouse hippocampus, was developed to mirror P50 gating deficits in schizophrenia. P50 gating deficits refer to the phenomenon in which patients are unable to filter early (50 ms post-stimulus) neuronal response to the second of a pair of identical, repeated auditory click stimuli (Adler et al., 1982; Freedman et al., 1983; Javitt and Freedman, 2015; Miwa et al., 2011). P50 gating dysfunction is one of the most frequently studied and consistently replicated electrophysiological endophenotypes in the illness, with an established genetic basis (polymorphisms on the α7 nicotinic receptor gene potentially contributing to reduced receptor expression) (Olincy and Freedman, 2012; Sinkus et al., 2015) leading to α7 nicotinic receptor-targeted investigational therapies (Olincy et al., 2006; Winterer et al., 2013; Zhang et al., 2012). Neuroimaging and depth recording studies in humans and rodents have localized P50 gating generators to the hippocampus, among other brain areas (Bak et al., 2014; Bickford-Wimer et al., 1990; Grunwald et al.,

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http://dx.doi.org/10.1016/j.schres.2015.09.022
0920-9964/Published by Elsevier B.V.

2003; Williams et al., 2011). The neuronal basis for gating deficits is hypothesized to be due to the reduced ability of hippocampal interneurons to generate persistent inhibition to an auditory stimulus. The area is therefore less able to reduce its response to repeated stimuli, contributing to a sensory "flooding" of irrelevant information as first reported in case studies in patients in the 1960s (McGhie and Chapman, 1961; Venables, 1964). P50 gating may therefore be a translational, physiological measure of hippocampal inhibitory dysfunction in schizophrenia contributing to hyperactivity. Relative to other mouse strains, the DBA/2 mouse shows a similar deficit in hippocampal auditory gating of the mouse analog of the P50, the P20/N40 wave (Stevens et al., 1996). The aim of this study, therefore, was to determine if the ketogenic diet can improve hippocampal auditory P20/N40 gating in the DBA/2 mouse, establishing proof-of-concept that the ketogenic diet could be repurposed to target an electrophysiological endophenotype associated with inhibitory dysfunction in schizophrenia.

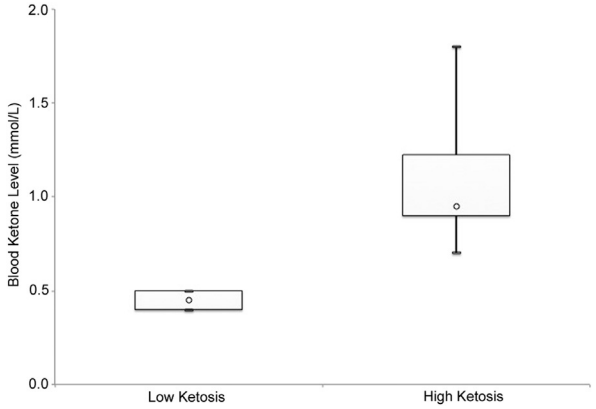

Fig. 1. Box plot demonstrating distribution of blood ketone levels for mice in the low ketosis group ($n = 6$) and mice in the high ketosis group ($n = 6$).

2. Materials and methods

2.1. Animals

DBA/2 male mice (Harlan SP, Indianapolis, ID) were group housed on aspen chip bedding with nestlets, and food and water continuously available (except for an overnight fasting period prior to electrophysiological recording). Lights cycled at 12 h intervals (on at 0600). The Institutional Animal Care and Use Committee of the University of Colorado Anschutz Medical Campus approved the experimental protocols.

2.2. Ketogenic diet

Upon arrival, mice were fed with a standard diet (60% carbohydrate, Harlan Teklad) for at least 2 weeks. Mice were then transitioned to the TD 96355 ketogenic diet (0.6% carbohydrate, Harlan Teklad), an extremely low-carbohydrate diet similar to diets used previously to cause ketosis in mice (Brownlow et al., 2013). Mice were maintained on the diet for 3 weeks, at which time they were studied, because blood ketone measurements indicated that ketosis had been achieved. Animals underwent an overnight fast prior to electrophysiological recordings.

2.3. Blood ketone measurement

Blood (collected by a tail snip) ketone levels were measured using a Nova Max Plus ketone meter (Nova Biomedical Corp., Bedford, MA). Ketones were measured immediately before animals began the ketogenic diet, weekly, and immediately before recordings. Prior to beginning the ketogenic diet, all animals showed blood ketone levels of 0.1 mmol/L or below. After completion of the diet, 6 animals showed ketone levels of 0.5 mmol/L or below ("low-ketosis" group), and 6 animals showed ketone levels greater than 0.5 mmol/L ("high ketosis" group) (Fig. 1).

2.4. Hippocampal recordings of sensory inhibition

Mice were fasted overnight prior to testing for sensory inhibition. *In vivo* hippocampal recordings were performed as described previously (Smucny et al., 2014). Mice (22–30 g) were anesthetized with chloral hydrate (400 mg/kg, ip) and pyrazole (400 mg/kg, ip) to retard the metabolism of the chloral hydrate. The mouse was placed into a mouse adaptor for a stereotaxic instrument and maintained at 37 °C with a heating pad. The scalp was incised and retracted. A burr hole was opened over the dorsal hippocampus (1.8 mm posterior to bregma, 2.7 mm lateral to midline (Paxinos and Franklin, 2004)) for the recording electrode and a second hole opened over the contralateral cortex rostral to bregma for the reference electrode. A Teflon-coated, stainless steel wire recording electrode (127 μM diameter) was lowered to the pyramidal cell layer of hippocampal area CA3 (−1.5–1.8 mm below dura). Final recording position was determined by the presence of complex spiking patterns typical of pyramidal cells (Miller and Freedman, 1995). An identical electrode was placed on the anterior cortex to act as reference. Miniature earphones attached to hollow ear bars, placed at the externalization of the aural canal, delivered the computer-generated auditory stimuli. EEG responses to paired click stimuli (3000 Hz, 10 ms, 70 dB SPL, presented 0.5 s apart, with 9 s between pairs) were amplified 1000 times with bandpass filtering at 1–500 Hz. Data were collected and analyzed using SciWorks data acquisition and analysis program (DataWave, Loveland CO). The responses to 16 pairs of stimuli were collected and averaged at 5-min intervals. The maximum negativity between 20 and 60 ms after the stimulus (N40) was selected and measured relative to the preceding positivity (P20). This composite component has been shown to be less variable than either component (P20 or N40) alone (Hashimoto et al., 2005). Three parameters were measured per record: conditioning amplitude—the magnitude of the response to the first stimulus (S1), test amplitude—the magnitude of the response to the second stimulus (S2), and TC ratio—the ratio of the test amplitude/conditioning amplitude, which is a measure of the level of inhibition (Stevens et al., 1996). A TC ratio of 0.5 or less is evidence of normal sensory inhibition (Freedman et al., 1983).

Consistent with previous studies from our laboratory examining chronic effects of investigational compounds on P20/N40 gating (e.g., Stevens et al., 2008), the 3 most similar records per mouse were selected and conditioning amplitude, test amplitude and TC ratio averaged to obtain characteristic parameters for each mouse. Ketone levels were correlated against TC ratio for each mouse ($n = 12$). Data were compared between high and low ketosis groups for analysis of the 3 parameters ($n = 6$ per group).

3. Results and discussion

Representative S1 and S2 waveforms for a mouse in the low-ketosis group and a mouse in the high-ketosis group are shown in Fig. 2. Overall, ANOVA for TC ratio revealed a significant difference with the high ketosis group having a significantly lower TC ratio [$F(1,10) = 45.516$, $p < 0.001$] (Fig. 3). ANOVA for conditioning amplitude showed no significant difference between the two groups [$F(1,10) = 1.468$, $p = 0.253$] while that for test amplitude was significantly reduced for the high ketosis group [$F(1,10) = 7.463$, $p = 0.021$] (Fig. 3). A Pearson's correlation between ketone level and TC ratio showed a negative correlation such that the higher the ketone level the lower the TC ratio (improved sensory inhibition) ($r = -0.679$, $p = 0.008$) (Fig. 4).

To our knowledge, this study is the first to show that the ketogenic diet may improve hippocampal auditory P20/N40 gating in the DBA/2

Please cite this article as: Tregellas, J.R., et al., Effects of a ketogenic diet on auditory gating in DBA/2 mice: A proof-of-concept study, Schizophr. Res. (2015), http://dx.doi.org/10.1016/j.schres.2015.09.022