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IS THE INTERACTION BETWEEN FATTY ACIDS AND TRYPTOPHAN RESPONSIBLE FOR THE EFFICACY OF A KETOGENIC DIET IN EPILEPSY? THE NEW HYPOTHESES OF ACTION

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Abstract—The effects of a ketogenic diet in controlling seizure activity have been proven in many studies, although its mechanism of action remains elusive in many regards. We hypothesize that the ketogenic diet may exert its antiepileptic effects by influencing tryptophan (TRP) metabolism. The aim of this study was to investigate the influence of octanoic and decanoic fatty acids (FAs), the main components in the MCT diet (medium-chain triglyceride diet, a subtype of the ketogenic diet), on the metabolism of TRP, the activity of the kynurenic pathway and the concentrations of monoamines and amino acids, including branched-chain amino acids (BCAA) and aromatic amino acids (AAA). The acute effects of FA on the sedation index and hippocampal electrical after-discharge threshold were also assessed. We observed that intragastric administration of FA increased the brain levels of TRP and the central and peripheral concentrations of kynurenic acid (KYNA), as well as caused significant changes in the brain and plasma concentrations of BCAA and AAA. We found that the administration of FA clearly increased the seizure threshold and induced sedation. Furthermore, we have demonstrated that blocking TRP passage into the brain abolished these effects of FA but had no similar effect on the formation of ketone bodies. Given that FAs are major components of a ketogenic diet, it is suggested that the anticonvulsant effects of a ketogenic diet may be at least partly dependent on changes in TRP metabolism. We also propose a more general hypothesis concerning the intracellular mechanism of the ketogenic diet. © 2015 Published by Elsevier Ltd. on behalf of IBRO.

Key words: seizure activity, ketogenic diet, tryptophan, kynurenic acid, octanoic and decanoic fatty acids, NAD^+/NADH ratio.

INTRODUCTION

The medium-chain triglyceride (MCT) ketogenic diet appears to be one of the most effective therapeutic approaches to drug-resistant epilepsy in the pediatric population. Its efficacy in controlling seizure activity has been well documented in several retrospective, prospective, and randomized clinical studies (Levy et al., 2012).

There are many hypotheses regarding the mechanisms of the anticonvulsant action of the MCT diet. The crucial mechanism appears to be the increased production of ketone bodies (acetone, acetoacetic acid, and β-hydroxybutyric acid (BHM)) in the liver, despite the fact that in animal models of epilepsy, ketosis correlates poorly with seizure control (Likhodii et al., 2000; Thavendiranathan et al., 2000; Rho and Stafstrom, 2012). Other explanations include the possible stabilization of glucose metabolism and the influence on amino acids and neurotransmitter systems that are involved in the regulation of neuronal excitability (Hartman et al., 2007). Other studies have also evaluated the potential neuroprotective effects of ketone bodies (Hartman et al., 2007; Streijger et al., 2013).

The MCT diet largely comprises two unbranched fatty acids (FAs): octanoic (C8) and decanoic (C10) acids, with a predominance of the C8 FA (Haidukewych et al., 1982). The MCT diet causes C8 and C10 to accumulate in blood plasma. However, the role of these substances in seizure control has not been clearly established (Sills et al., 1986). The main constituent of the MCT diet, C8, was found to possess some acute anticonvulsant properties (Wlaź et al., 2012; Liu and Wang, 2013).

Previously, we demonstrated that valproic acid (VPA), the short-chain FA commonly used in the treatment of epilepsy and bipolar disorders and in the prevention of

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Abbreviations: 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, serotonin; AAA, aromatic amino acids; AAT, aspartate aminotransferase; ADT, large neutral amino acid mixture; ALA, alanine; ASP, aspartate; BBB, blood–brain barrier; BCAA, branched-chain amino acids; BHM, β-hydroxybutyric acid; C10, decanoic acid; C8, octanoic acid; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; FAs, fatty acids; GLTM, glutamine; GLU, glutamate; GLY, glycine; HVA, homovanillic acid; ILEU, L-isoleucine; KYN, kynurenine; KYNA, kynurenic acid; LAC, lactate; LDH, lactate dehydrogenase; LEU, L-leucine; LNAA, large neutral amino acid; LYS, L-lysine; MAS, malate-aspartate shuttle; MCT, medium-chain triglyceride; MCTr, monocarboxylate transporter; METH, L-methionine; NA, noradrenaline; NAD, nicotinamide adenine dinucleotide; OXA, oxaloacetate; PC, pyruvate carboxylase; PEPCK-M, mitochondrial phosphoenolpyruvate carboxykinase; PHE, L-phenylalanine; PYR, pyruvate; TAU, taurine; TDO, tryptophan 2,3-dioxygenase; THR, L-threonine; TRP, tryptophan; VAL, L-valine; VPA, valproic acid.

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migraine headaches, may exert its antiepileptic effect by influencing tryptophan (TRP) metabolism (Maciejak et al., 2013). We have also demonstrated that VPA causes an increase in kynurenic acid (KYNA) levels in the brain, most likely due to TRP displacement from its binding site on plasma albumin. In the present study, we expected to achieve the same effect with the administration of different medium-chain FAs, which are components of the MCT diet that also possess strong affinity for plasma albumin and may influence TRP metabolism.

The aim of the present study was to determine whether two unbranched FAs, C8 and C10, administered together or separately, possess anticonvulsant activity. To this end, the acute electrophysiological and functional effects of FA on hippocampal electrical after-discharges and on the sedation index were assessed. Moreover, in order to investigate the biochemical basis of FA action, the concentrations of monoamines, branched-chain amino acids (BCAA), aromatic amino acids (AAA) and other amino acids, as well as changes in the concentrations of TRP, kynurenine (KYN) and KYNA elements of the KYN pathway, in the brain and plasma followed by the FA administration, were assessed.

EXPERIMENTAL PROCEDURES

Animals

Male Wistar rats, weighing 250 ± 50 g at the beginning of the experiment, were used in the study. The animals were housed under standard laboratory conditions (temperature 21 ± 2 °C, 12 h light/dark cycle, light on at 7:00 AM). The rats were given free access to food and water. All experiments were performed between 9:00 AM and 3:00 PM. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study was conducted in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC). The study protocol was approved by the Committee for Animal Care and Use at the Medical University in Warsaw (permission No. 13/2014). All care was taken to minimize suffering during the experimental procedure as well as at the time of sacrifice.

Chemicals

Octanoic acid (C8), decanoic acid (C10), L-phenylalanine (PHE), L-leucine (LEU), L-isoleucine (ILEU), L-methionine (METH), L-valine (VAL), L-tyrosine (TYR), L-threonine (THR) and L-lysine (LYS) were used in the study. All compounds were purchased from Sigma–Aldrich, Poland. All compounds were suspended in a 0.5% solution of methylcellulose and administered through intra-gastric gavage.

Experimental design (Table 1)

Experiment 1: The time changes in concentrations of TRP, KYN and KYNA in the hippocampus and plasma after p.o. FA administration. The changes in the hippocampal and plasma concentration of TRP, KYN and KYNA were determined 15 min, 1.5 h, or 6 h after FA administration. The FA group received 5 ml of the mixture of C8 (81%) and C10 (19%): [4.05 ml (61 mmol/kg) of C8 + 0.95 ml (12.36 mmol/kg) of C10] (Haidukewych et al., 1982). The control group received 5 ml of a 0.5% methylcellulose solution. The substances were administered by intra-gastric gavage.

Experiment 2: The dose-dependent changes in concentrations of TRP, KYN and KYNA in the hippocampus and plasma after p.o. FA administration. The changes in the hippocampal concentration of KYNA, KYN and TRP were determined 1.5 h (the time point, in which the most pronounced biochemical changes were observed (see Section Experiment 1)) after FA administration of 1 ml, 3 ml or 5 ml of the mixture of FA. The control group received 5 ml of a 0.5% methylcellulose solution. The substances were administered by intra-gastric gavage.

To carry out these experiments, the following procedures were used:

Tissue preparation. One and half hours after FA administration, the rats were sacrificed by decapitation, and the brains were removed. The hippocampi were isolated using a scalpel and a magnifying glass. Trunk blood was collected in a tube containing heparin.

The hippocampus sample was weighed, placed in a cool, dry polypropylene vial, and homogenized in 20 volumes of ice-cold 2% perchloric acid with a sonic vibra-cell (30 s at 4 °C). The homogenates were then centrifuged at 26,880×*g* for 8 min at 4 °C. Next, the supernatants were collected and filtered through a 0.45-μm filter (Millipore), and immediately frozen and stored at −78 °C until they were assayed.

The blood samples were centrifuged at 2600×*g* for 10 min at 4 °C. The samples were deproteinized with 50 μl of 2.4 M perchloric acid for every 0.5 ml of plasma, after centrifuged at 26,880×*g* for 8 min at 4 °C, the supernatant was transferred to an Eppendorf tube and filtered through a 0.45-μm filter (Millipore). The samples were stored at −78 °C until use (Herve et al., 1996).

Determination of KYN, KYNA and TRP concentration in the hippocampus and plasma (Experiment No. 2, 4, 7)

KYN, KYNA and TRP concentrations in the brain were measured according to the modified methods of Wu et al. (1992) and Herve et al. (1996) as described in detail in our previous study (Maciejak et al., 2014). High-performance liquid chromatography (HPLC) with fluorescence was performed to detect KYN, KYNA and TRP. The HPLC system consisted of the following components: a pump (Shimadzu, LC-10AD VP) and a fluorescence detector (Shimadzu, RF-10 XL). KYNA and TRP were separated on a Phenomenex Luna C18 (150 mm × 3 mm) column with a Phenomenex KJO-4286 precolumn set at a flow rate of 0.4 ml/min operating at room temperature. Chromatogram registration and analysis were performed using ChromaX 2004 software. The concentrations of KYN, KYNA and TRP were

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