Biochimica et Biophysica Acta 1852 (2015) 1787–1795

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Mechanism for increased hepatic glycerol synthesis in the citrin/mitochondrial glycerol-3-phosphate dehydrogenase double-knockout mouse: Urine glycerol and glycerol 3-phosphate as potential diagnostic markers of human citrin deficiency

Mitsuaki Moriyama [a,1], Yuki Fujimoto [b,c,1], Shizuka Rikimaru [c], Miharu Ushikai [d,e], Eishi Kuroda [d], Kenji Kawabe [a], Katsura Takano [a], Akihiro Asakawa [d], Akio Inui [d], Kazuhiro Eto [f], Takashi Kadowaki [g], David S. Sinasac [h], Yoshiyuki Okano [i,j], Masahide Yazaki [k,l], Shu-ichi Ikeda [k,l], Chunhua Zhang [m], Yuan-Zong Song [n], Osamu Sakamoto [o], Shigeo Kure [o], Hiroshi Mitsubuchi [p], Fumio Endo [q], Masahisa Horiuchi [e], Yoichi Nakamura [a], Ken-ichi Yamamura [b], Takeyori Saheki [b,c,*]

[a] Laboratory of Integrative Physiology in Veterinary Sciences, Osaka Prefecture University, Izumisano, Japan
[b] Laboratory of Yamamura Project, Institute of Resource Development and Analysis, Kumamoto University, Kumamoto, Japan
[c] Institute for Health Sciences, Tokushima Bunri University, Tokushima, Japan
[d] Department of Psychosomatic Internal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
[e] Department of Hygiene and Health Promotion Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
[f] Department of Internal Medicine, Teikyo University, Tokyo, Japan
[g] Department of Diabetes and Metabolic Diseases, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
[h] Alberta Children's Hospital Research Institute, Department of Medical Genetics, Cumming School of Medicine, University of Calgary, Alberta, Canada
[i] Department of Genetics, Hyogo College of Medicine, Nishinomiya, Japan
[j] Okano Children's Clinic, Izumi, Japan
[k] Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine, Matsumoto, Japan
[l] Institute for Biomedical Sciences, Shinshu University, Matsumoto, Japan
[m] Department of Research Development, MILS International, Kanazawa, Japan
[n] Department of Pediatrics, The First Affiliated Hospital, Jinan University, Guangzhou, China
[o] Department of Pediatrics, Tohoku University Graduate School of Medicine, Sendai, Japan
[p] Department of Neonatology, Kumamoto University Hospital, Kumamoto, Japan
[q] Department of Pediatrics, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan

ARTICLE INFO

Article history:
Received 27 January 2015
Received in revised form 21 April 2015
Accepted 27 April 2015
Available online 5 May 2015

Keywords:
Citrin deficiency
Glycerol
Glycerol 3-phosphate
Mitochondrial aspartate-glutamate carrier
Redox state
Pyruvate

ABSTRACT

The mitochondrial aspartate-glutamate carrier isoform 2 (citrin) and mitochondrial glycerol-3-phosphate dehydrogenase (mGPD) double-knockout mouse has been a useful model of human citrin deficiency. One of the most prominent findings has been markedly increased hepatic glycerol 3-phosphate (G3P) following oral administration of a sucrose solution. We aimed to investigate whether this change is detectable outside of the liver, and to explore the mechanism underlying the increased hepatic G3P in these mice. We measured G3P and its metabolite glycerol in plasma and urine of the mice under various conditions. Glycerol synthesis from fructose was also studied using the liver perfusion system. The citrin/mGPD double-knockout mice showed increased urine G3P and glycerol under normal, fed conditions. We also found increased plasma glycerol under fasted conditions, while oral administration of different carbohydrates or ethanol led to substantially increased plasma glycerol. Fructose infusion to the perfused liver of the double-knockout mice augmented hepatic glycerol synthesis, and was accompanied by a concomitant increase in the lactate/pyruvate (L/P) ratio. Co-infusion of either pyruvate or phenazine methosulfate, a cytosolic oxidant, with fructose corrected the high L/P ratio, leading to reduced glycerol synthesis. Overall, these findings suggest that hepatic glycerol synthesis is cytosolic NADH/NAD^+ ratio-

Abbreviations: αKG, α-ketoglutarate; AGC, aspartate-glutamate carrier; Ala, alanine; AOA, aminooxyacetate; Arg, arginine; ASA, argininosuccinate; Asp, aspartate; Cit, citrulline; CTLN2, adult-onset type II citrullinemia; Cr, creatinine; DHAP, dihydroxyacetone phosphate; Gln, glutamine; Glu, glutamate; G3P, glycerol 3-phosphate; KO, knockout; Lac, lactate; L/P ratio, lactate-to-pyruvate; MCT, medium-chain triglyceride; mGPD, mitochondrial glycerol-3-phosphate dehydrogenase; Na-Pyr, sodium pyruvate; NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency; PMS, phenazine methosulfate; Pyr, pyruvate; wt, wild-type

* Corresponding author at: Laboratory of Yamamura Project, Institute of Resource Development and Analysis, Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811 Japan. Tel.: +81 996 373 6818; fax: +81 996 373 6639.

[1] Both equally contributed.

http://dx.doi.org/10.1016/j.bbadis.2015.04.023
0925-4439/© 2015 Elsevier B.V. All rights reserved.

dependent and reveal a likely regulatory mechanism for hepatic glycerol synthesis following a high carbohydrate load in citrin-deficient patients. Therefore, urine G3P and glycerol may represent potential diagnostic markers for human citrin deficiency.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Human citrin (encoded by *SLC25A13*) is a liver-type mitochondrial aspartate-glutamate carrier (AGC isoform 2; AGC2) [1,2], and was initially found to be deficient in the autosomal recessive disease, adult-onset type II citrullinemia (CTLN2) [3] — a disorder characterized by late-onset and recurring symptoms of abnormal behavior and sudden disturbances of consciousness due to hyperammonemia. *SLC25A13* mutations have also now been identified in cholestatic infants with transient multiple aminoacidemias, galactosemia and hypoproteinemia with growth failure (designated neonatal intrahepatic cholestasis caused by citrin deficiency; NICCD) [4–6]. Further examination of known patients outside of the neonatal period has also found persistent failure to thrive and dyslipidemia [7]. In total, the age-dependent clinical spectrum that characterizes the collective disease entity called citrin deficiency (a.k.a., AGC2 deficiency) has now been established [8–10].

In spite of the clear relationship between *SLC25A13* mutations and the various age-specific presentations, the first mouse model of citrin deficiency, the *Slc25a13* knockout (KO) mouse, showed virtually no physiological consequences despite measurable *in vitro* biochemical abnormalities [11]. Considering the two classical pathways for transporting cytosolic NADH reducing equivalents into the mitochondria, the malate-aspartate (Asp) and glycerophosphate shuttles (both demonstrated to be highly active in rodent liver), we have created an improved citrin-deficient mouse model that exhibits a variety of CTLN2 and NICCD phenotypes. By crossing the citrin-KO mouse with the *Gpd-2* (encoding mitochondrial glycerol-3-phosphate dehydrogenase; mGPD)-KO mouse [12], the citrin/mGPD double-KO mice exhibit symptoms of growth retardation, hypoglycemia, and hyperammonemia under fed conditions that are exaggerated by oral administration of a sucrose solution [12]. The double-KO mice also show loss of body weight when fed a high carbohydrate diet (AIN-93 M) due to reduced intake, while they recover their body weight when they are switched to a higher protein diet, or one enriched with amino acids, sodium pyruvate (Na-Pyr) or medium-chain triglycerides (MCT) [13]. A metabolomic analysis of the citrin/mGPD double-KO mice further revealed a variety of changes in hepatic metabolites following oral administration of sucrose: marked elevations of glycerol 3-phosphate (G3P) and citrulline (Cit), inhibition of lysine metabolism resulting in increased lysine, and decreased TCA cycle intermediates along with glutamate (Glu) and glutamine (Gln) [14]. Furthermore, these metabolite changes could be ameliorated by the simultaneous administration of Na-Pyr, amino acids including alanine (Ala) and Glu, or MCT [13].

The purpose of the present study was to examine whether we could find evidence of the hepatic changes observed in the citrin/mGPD double-KO mice in plasma or urine, especially the marked increase in G3P, that would serve as either diagnosis markers, or potential therapeutic outcome measures, of citrin deficiency. The double-KO mice do in fact show increased urine G3P and glycerol under fed conditions, and increased plasma glycerol under fasted conditions as well as after oral administration of various carbohydrates. We further explored the effect of a carbohydrate load on hepatic glycerol synthesis in the double-KO mice using the liver perfusion system to test whether elevated urine and plasma glycerol are derived from liver metabolism. The presence of fructose in the perfusate of the double-KO mice led to increased hepatic glycerol production that was coupled to the elevated lactate/pyruvate (L/P) ratio, revealing that hepatic glycerol synthesis is dependent on the cytosolic NADH/NAD^+ ratio. Finally, our examinations of urine from citrin-deficient patients revealed, for the first time, a subset of them have increased urine glycerol and G3P levels. Overall, our findings implicate urine G3P and glycerol as potential markers of citrin deficiency.

2. Materials and methods

2.1. Materials

Fructose was purchased from Merck Co. (Darmstadt, Germany). Glucose, ethanol (99.5%), L-Ala, Na-Pyr and phenazine methosulfate (PMS) were obtained from Wako Pure Chemical Co. (Osaka, Japan). Aminooxyacetate (AOA) was obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA). Difco Bacto Tryptone was supplied from Voigt Global Distribution Inc. (Lawrence, USA). Enzymes for metabolite determination were obtained from Roche Diagnostics (Indianapolis, IN, USA). Other reagents were of analytical grade.

2.2. Animals

All wild-type (wt), citrin-KO, mGPD-KO and citrin/mGPD double-KO mice used were congenic on the C57BL/6 J background. Mice used for the experiments were generated using the breeding scheme described previously by Saheki et al. [14]. Briefly, mGPD-KO and double-KO mice were obtained by mating heterozygous citrin-KO/homozygous mGPD-KO ($citrin^{+/-}$/$mGPD^{-/-}$) mice, while wt and citrin-KO mice were generated by mating heterozygous citrin-KO ($citrin^{+/-}$/$mGPD^{+/+}$) mice. Genotyping was performed with DNA extracted from ear punches using procedures specific for each of the targeted mutations in the citrin-KO [11] and mGPD-KO [15] mice, respectively. All mice were maintained at a constant temperature (23 ± 1 °C) on a 12-hour light/dark cycle (light on, 8:00 to 20:00) with free access to water and CE2 chow (25% protein, 4.6% fat, and 50% carbohydrate providing 343 kcal/100 g; CLEA Japan, Tokyo, Japan). The experimental protocols met the institution's and the National Research Council's standards for the care and use of laboratory animals in research and were approved by the Ethical Committees for Animal Experimentation at Kumamoto University (B25-147) and Osaka Prefecture University (22-2).

2.3. Sample preparation

Mice used for the experiments were analyzed between 80 and 160 days of age. Urine was collected overnight (between 18:00 and +9:00) from singly-housed mice in metabolic cages (Natsume Seisakusho Co., Ltd., Tokyo, Japan) with CE-2 chow under fed conditions, or 24 h without chow under fasted conditions. Plasma was separated from blood taken from the heart of mice under sodium pentobarbital (50 mg/kg body weight) anesthetic between 10:00 and 11:00, 1 h after administration of a solution by gastric tube. Solutions administered were sucrose, glucose, fructose, ethanol, or sucrose containing tryptone, Na-Pyr, Ala, or MCT. In separate experiments, following cervical dislocation, livers were quickly removed, freeze-clamped between aluminum tongs, and stored at −70 °C for analysis of G3P.

2.4. Contents of test solutions

Solutions administered were 20 ml/kg of 25% sucrose (5 g/kg), 25% glucose (5 g/kg), 25% fructose (5 g/kg), 9% (vol/vol) ethanol (1.4 g/kg), or 25% sucrose containing 25% tryptone (5 g/kg), 1 M Na-Pyr (20 mmol/kg), 1 M Ala (20 mmol/kg), or 5% MCT (contained 78.9%

Download English Version:

https://daneshyari.com/en/article/8259685

Download Persian Version:

https://daneshyari.com/article/8259685

Daneshyari.com