Neurochemistry International 108 (2017) 246–253

Contents lists available at ScienceDirect

Neurochemistry International

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

Proteomic approach to detect changes in hippocampal protein levels in an animal model of type 2 diabetes

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ARTICLE INFO

Article history:
Received 27 December 2016
Received in revised form
16 March 2017
Accepted 19 April 2017
Available online 21 April 2017

Keywords:
Apolipoprotein A-I preprotein
Myelin basic protein
Type 2 diabetes
Hippocampus

ABSTRACT

In our previous study, we demonstrated that type 2 diabetes affects blood-brain barrier integrity and ultrastructural morphology in Zucker diabetic fatty (ZDF) rats at 40 weeks of age. In the present study, we investigated the possible candidates for diabetes-related proteins in the hippocampus of ZDF rats and their control littermates (Zucker lean control, ZLC), by using two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). Approximately 2756 protein spots were detected by 2D-DIGE, and an increase or decrease of more than 1.4-fold was observed for 13 proteins in the hippocampal homogenates of ZDF rats relative to those of ZLC rats. Among these proteins, we found four proteins whose levels were significantly lower in the hippocampi of ZDF rats than in those of ZLC rats: glial fibrillary acidic protein (GFAP), apolipoprotein A-I preprotein (apoAI-P), myelin basic protein (MBP), and rCG39881, isoform CRA_a. Among these proteins, apoAI-P protein levels were decreased most prominently in ZDF rats than in ZLC rats, based on Western blot analysis. In addition, immunohistochemical and Western blot studies demonstrated that MBP, not GFAP, immunoreactivity and protein levels were significantly decreased in the hippocampus of ZDF rats compared to ZLC rats. In addition, ultrastructural analysis showed that ZDF rats showed myelin degeneration and disarrangement in the hippocampal tissue. These results suggest that chronic type 2 diabetes affects hippocampal function via reduction of MBP and apoAI-P levels as well as disarrangement of myelin.

1. Introduction

Diabetes is one of the most prevalent metabolic disorders worldwide, and obesity is a common driving factor for the development of type 2 diabetes mellitus (T2DM) (Ford et al., 1997; Kang et al., 2016; Mokdad et al., 2003; Resnick et al., 2000). T2DM is closely associated with insulin resistance and subsequent dysregulation of insulin receptor signaling (Accardi et al., 2012; Baker et al., 2011; Bergman, 2013). T2DM results in a series of secondary diseases, including cardiovascular disease, renal dysfunction, and memory impairment (Di Pino et al., 2016; Gold et al., 2007; Nosadini and Tonolo, 2011; Paredes et al., 2016; van Bussel et al., 2016).

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http://dx.doi.org/10.1016/j.neuint.2017.04.011
0197-0186/

In particular, insulin receptors are widely expressed in the central nervous system, with high concentrations in the olfactory bulb, hippocampus, cerebellar cortex, and some parts of the cerebral cortex (Zhao et al., 1999). In addition, patients with T2DM have atrophy of the hippocampus (den Heijer et al., 2003; Gold et al., 2007; Hayashi et al., 2011) and reduced functional connectivity between the hippocampus and other parts (i.e. frontal, temporal, and parietal) of the brain (van Bussel et al., 2016; Zhou et al., 2010). Diabetic patients show cognitive decrements (Awad et al., 2004; van den Berg et al., 2010) and an increased risk of developing dementia and Alzheimer's disease (Awad et al., 2004; Kloppenborg et al., 2008; Luchsinger, 2008; Spauwen et al., 2013). In the previous study, our colleagues demonstrated that high glucose leads to increased Aβ production and apoptosis of SK-N-MC (Lee et al., 2016).

Zucker diabetic fatty (ZDF) and Goto-Kakizaki (GK) rats are widely used as animal models for T2DM. The GK rat is a non-obese model for T2DM, while ZDF rat is an obese model of defective leptin receptors. ZDF rats develop marked hyperglycemia between weeks 7 and 12 of age (Peterson et al., 1990; Schmidt et al., 2003). However, these rats do not show any cognitive impairment at 26–28 weeks of age based on the Morris water maze tasks, or electrophysiological dysfunction based on long-term potentiation, compared to age-matched Zucker lean control (ZLC) rats (Bélanger et al., 2004). However, in our previous study, we demonstrated that the blood-brain barrier (BBB) integrity is reduced in the hippocampus of ZDF rats at 40 weeks of age (Yoo et al., 2016). There are few comprehensive reports on the changes of hippocampal proteins in the T2DM hippocampus (Abdul-Rahman et al., 2012; Ma et al., 2013; Yoo et al., 2015; Nam et al., 2016), although the reduced integrity of the BBB shows a possibility that hippocampal function may be affected at 40 weeks of age (Yoo et al., 2016).

In the present study, therefore, we determined T2DM-related proteins using two-dimensional fluorescence difference gel electrophoresis (2D-DIGE), following matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) in the hippocampus.

2. Materials and methods

2.1. Experimental animals

Male and female heterozygous (*Lepr*$^{fa/+}$) Zucker diabetic fatty (ZDF) rats were purchased from Genetic Models (Indianapolis, ME, U.S.A.) and mated. They were housed under standard conditions with adequate temperature (22 °C) and humidity (60%) control, a 12-hr light/12-hr dark cycle, and free access to food and water. The handling and care of the animals conformed to the guidelines established to comply with current international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985, revised 1996) and were approved by the Institutional Animal Care and Use Committee (IACUC) of Seoul National University (Approval number: SNU-120312-10). All the experiments were conducted with an effort to minimize the number of animals used and suffering caused by the procedures employed in the present study.

2.2. Protein preparation for 2D-DIGE

The 2D-DIGE procedure was performed under conditions of darkness, as described by Na et al. (2009). ZLC (n = 9) and ZDF (n = 9) rats at 40 weeks of age were anesthetized with 2 g/kg urethane (Sigma-Aldrich, St. Louis, MO, USA), following which hippocampal tissues were isolated from the brain. The overall procedures are summarized in Fig. 1. Hippocampi were suspended in the sample buffer, which consisted of 30 mM Tris, 7 M urea, 2 M thiourea, 65 mM dithiothreitol (DTT), and 4% (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate [CHAPS]) with 40 μL protease inhibitor (pH 8.5). Suspensions were sonicated 5 times for 10 s and centrifuged at 45,000 rpm for 45 min. Proteins in the supernatants were quantified using the 2D Quant kit (GE Healthcare, Uppsala, Sweden).

2.3. Labeling with CyDye™ and separation of proteins by 2-D DIGE

For CyDye labeling, 1 μL of Cy5 working solution (400 pmol) and 1 μL of Cy3 working solution (400 pmol) were added to two samples of hippocampal homogenates from ZLC and ZDF rats (50 μg). Samples labeled using each CyDye™ DIGE fluor (Cy2, Cy3, and Cy5) and 2 × buffer (2 M thiourea, 7 M urea, 2% Pharmalyte®, 130 mM DTT, 4% CHAPS, and a trace of bromophenol blue) were used for 2-D DIGE analysis. Mixed samples were loaded into the anode cup of a Multiphor II IEF system (GE Healthcare Bio-Sciences). First-dimension isoelectric focusing started from 100 V for 3 h and gradually increased to a final voltage of 8,000 V. A total of 80,000 V/h was provided by setting the maximum current to 75 μA. To make a preparative picking gel for identification of protein spots, 2 mg of preparative hippocampal protein samples were mixed with rehydration buffer (6 M urea, 2 M thiourea, 4% CHAPS, 0.4% DTT, 2% v/v IPG buffer pH 3 to 10, pH 6 to 11, and pH 4 to 7) to a final volume of 450 μL.

For the DIGE experiment, 8%–16% linear gradient sodium dodecyl sulfate (SDS) polyacrylamide gels were made using low fluorescent glass plates, and a general glass plate was applied for (200 mm × 250 mm × 1.0 mm) preparative gels. The equilibrated immobilized pH gradient (IPG) strips after the first-dimension electrophoresis were placed onto SDS polyacrylamide gels. The gels were placed into the 24 cm Ettan DALT *twelve* system (GE Healthcare Bio-Sciences), then run at 2.5 W per gel for 30 min and at 12 W per gel for 7 min.

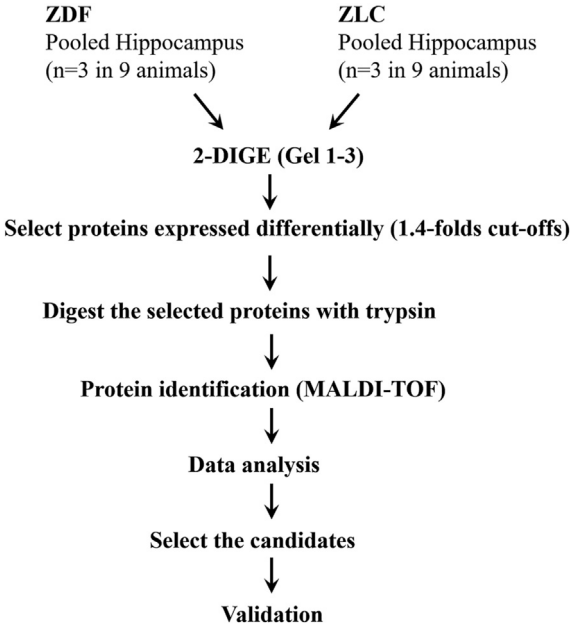

Fig. 1. Schematic drawing of the overall workflow in the proteomics study.