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Research Report

Type 1 diabetes alters astrocytic properties related with neurotransmitter supply, causing abnormal neuronal activities

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ABSTRACT

Glutamine synthetase (GS), an astrocytic protein in the brain, mediates the process by which glutamate (Glu) is transformed into glutamine (Gln) during Glu and gamma-aminobutyric acid (GABA) *de novo* synthesis. There are many types of neural complications related with those neurotransmitters in type 1 diabetes (T1D) patients, but there is little information about the change GS. Therefore, we examined changes in GS activity and expression, as well as the amount of Glu, Gln, and GABA in the brain of a T1D animal model. Using primary culture we found that glucose fluctuation caused glial fibrillary acidic protein (GFAP) and GS changes but constant high glucose level didn't. In T1D mouse, GS expression increased in the prefrontal cortex (PFC) and hippocampus (HI), but decreased GS activity was only observed in the HI whereas GFAP expression decreased in both regions. Gln increased in both regions, but Glu and GABA were only increased in the HI of T1D animals where GS activity decreased with higher reactive oxygen/nitrogen species. Collectively, low GS activity may be closely related with high levels of Glu and GABA in the HI of T1D brain, and this would result in abnormal neurotransmissions.

Abbreviations: AMPK, AMP kinase; DCF, 2′,7′-dichlorodihydrofluorescein; DM, diabetes mellitus; GABA, gamma-aminobutyric acid; GFAP, glial fibrillary acidic protein; Gln, glutamine; Glu, glutamate; Glu/Gln cycle, glutamate/glutamine cycle; GS, glutamine synthetase; HI, hippocampus; IHC, immunohistochemistry; PFC, prefrontal cortex; RNS, reactive nitrogen species; ROS, reactive oxygen species; STZ, streptozotocin; T1D, type 1 diabetes; UPLC, ultra performance liquid chromatography

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1. Introduction

Astrocytes are the most abundant glia cell in the brain and play regulatory roles in neurogenesis, synaptogenesis, metabolic activity, blood-brain barrier maintenance, and cerebral blood flow (Parpura et al., 2012). Abnormal astrocytic functions are known to be involved in diverse brain disorders including seizure (Coulter and Eid, 2012), dementia (Rodriguez et al., 2009), and major depressive disorder (MDD) (Lee et al., 2013; Sanacora and Banasr, 2013) as well as in various inflammatory responses both in animal models and human patients (Barreto et al., 2011; Brosnan and Raine, 2013; Chiang et al., 2012; Lee et al., 2010). It has recently been suggested that uncontrolled glutamatergic neurotransmission might be a common underlying mechanism in these diseases (Eid et al., 2013; Sanacora et al., 2012; Walton and Dodd, 2007). From the viewpoint of interactions between astrocytes and neurons in the brain, the glutamatergic regulatory role of astrocytes may be the most important function.

The Glutamate/Glutamine (Glu/Gln) cycle is a major regulatory mechanism for fine-tuning Glu and Gln level in the brain. Glu is released from presynaptic neurons, and Glu left in the synaptic cleft is taken up by surrounding astrocytes, where it is converted to Gln by glutamine synthetase (GS). Newly synthesized Gln is transported back to the presynaptic neurons and reconverted into Glu. This process is critical for maintaining continuous glutamatergic signaling and tripartite glutamatergic synapses (Schousboe et al., 2013). Many studies have demonstrated altered GS expression in various pathological states. For example, GS expression and activity are decreased in Alzheimer's disease (Robinson, 2001), MDD (Miguel-Hidalgo et al., 2010), and epilepsy (Eid et al., 2013). Moreover, we previously reported that inhibition of GS activity resulted in depressive-like behaviors in mice (Lee et al., 2013).

Diabetes is a group of metabolic diseases characterized by hyperglycemia due to insufficient production of insulin by pancreatic β-cells (Type 1 diabetes, T1D) or the failure of cells to respond to insulin (type 2 diabetes, T2D). It was recently reported that patients with diabetes showed increased incidences of CNS complications including seizure (Verrotti et al., 2012), cognitive impairment (Gispen and Biessels, 2000; Lyoo et al., 2009), and MDD (Ajilore et al., 2007; Lyoo et al., 2009).

It was also reported that the decreased expression of glial fibrillary acidic protein (GFAP) in the brain (Coleman et al., 2004, 2010) and the increased Glu levels in the retina of streptozotocin (STZ)-induced T1D animal models (Lieth et al., 1998). Moreover, Glu/Gln/γ-aminobutyric acid (GABA) levels were determined to be elevated in the prefrontal cortex (PFC) of T1D patients (Lyoo et al., 2009). These reports implicate that the Glu regulatory functions and astrocyte structure might be altered by diabetes, and these changes could be involved in diabetes-induced CNS pathogeneses. However, the etiological mechanisms remain unclear. Therefore, we investigated changes in GS and its metabolites in the brain of a T1D mouse model and assessed the influence of glucose concentration on GS expression and activity in primary astrocyte cultures.

2. Results

2.1. STZ increased blood glucose levels

A T1D mouse model was induced by administering STZ, a compound that selectively destroys pancreatic β-cells that is used extensively in diabetes research. After STZ injection, body weights and blood glucose levels were measured every week for eight weeks (Fig. 1). STZ-treated groups showed significantly elevated blood glucose levels compared to controls (CTL: 265±23.8 mg/dl; STZ: >500 mg/dl) at two weeks and remained elevated until eight weeks (Fig. 1A). We defined diabetes as blood glucose >400 mg/dl. If the blood glucose level was >500 mg/dl, it was considered as 500 mg/dl. Diabetes also resulted in a significant decrease in body weight (Fig. 1B).

2.2. GFAP expression decreased in T1D mouse brain

In the present study, we examined GFAP expression in four brain regions and found that it was decreased in the PFC and hippocampus (HI) of the T1D group compared to control group (Fig. 2A and B), which is consistent with previous reports (Coleman et al., 2004, 2010).

2.3. GS activity and expression showed region-specific changes in T1D mouse brain

We measured GS activity and expression level in the PFC, HI, amygdala (Am), and hypothalamus (HY) of T1D mouse brain. While GS activity was increased in the PFC (Fig. 3A) of T1D mice

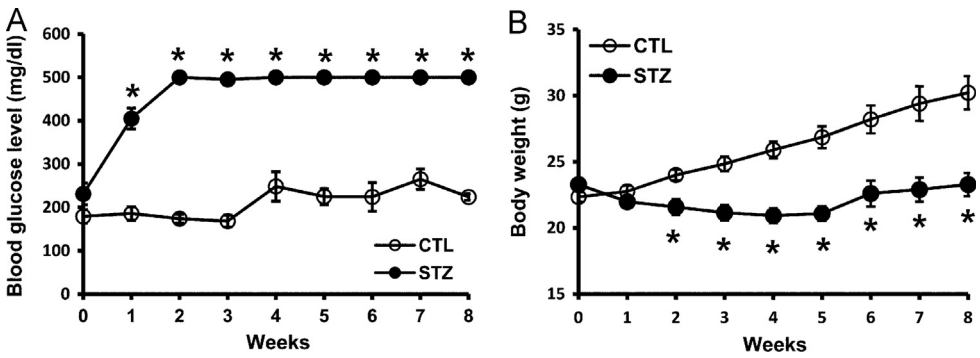

Fig. 1 – The changes in blood glucose levels (A) and body weights (B) of STZ and CTL groups over 8 weeks. Note the higher blood glucose levels and lower body weights of STZ mice compared with CTL, indicating successful DM induction. Data are presented as mean±SEM. Statistical significance is as follows: *$p<0.05$ from CTL. ($n=5$/group).