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Full-length Article

The signaling mechanisms of hippocampal endoplasmic reticulum stress affecting neuronal plasticity-related protein levels in high fat diet-induced obese rats and the regulation of aerobic exercise

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ABSTRACT

High fat diet (HFD)-induced obesity has been shown to reduce the levels of neuronal plasticity-related proteins, specifically brain-derived neurotrophic factor (BDNF) and synaptophysin (SYN), in the hippocampus. However, the underlying mechanisms are not fully clear. Endoplasmic reticulum stress (ERS) has been reported to play a key role in regulating gene expression and protein production by affecting stress signaling pathways and ER functions of protein folding and post-translational modification in peripheral tissues of obese rodent models. Additionally, HFD that is associated with hyperglycemia could induce hippocampal ERS, thus impairing insulin signaling and cognitive health in HFD mice. One goal of this study was to determine whether hyperglycemia and hyperlipidemia could cause hippocampal ERS in HFD-induced obese SD rats, and explore the potential mechanisms of ERS regulating hippocampal BDNF and SYN proteins production. Additionally, although regular aerobic exercise could reduce central inflammation and elevate hippocampal BDNF and SYN levels in obese rats, the regulated mechanisms are poorly understood. Nrf2-HO-1 pathways play roles in anti-ERS, anti-inflammation and anti-apoptosis in peripheral tissues. Therefore, the other goal of this study was to determine whether aerobic exercise could activate Nrf2-HO-1 in hippocampus to alleviate obesity-induced hippocampal ERS, which would lead to increased BDNF and SYN levels.

Male SD rats were fed on HFD for 8 weeks to establish the obese model. Then, 8 weeks of aerobic exercise treadmill intervention was arranged for the obese rats. Results showed that HFD-induced obesity caused hyperglycemia and hyperlipidemia, and significantly promoted hippocampal glucose transporter 3 (GLUT3) and fatty acid transport protein 1 (FATP1) protein expression. These results were associated with the activation of hippocampal ERS and ERS-mediated apoptosis. At the same time, we found that excessive hippocampal ERS not only significantly decreased proBDNF—the precursor of mature BDNF, but also attenuated p38/ERK-CREB signaling pathways and activated NLRP3-IL-1β pathways in obese rats. These results were associated with reduced BDNF and SYN protein production. However, these adverse changes were obviously reversed by aerobic exercise intervention through activating the Nrf2-HO-1 pathways.

These results suggest that dietary obesity could induce hippocampal ERS in male SD rats, and excessive hippocampal ERS plays a critical role in decreasing the levels of BDNF and SYN. Moreover, aerobic exercise could activate hippocampal Nrf2 and HO-1 to relieve ERS and heighten BDNF and SYN production in obese rats.

1. Introduction

Emerging evidence indicates that high-fat diet (HFD)-induced obesity has adverse effects on hippocampal structure and function, and could cause hippocampal-dependent learning and memory decline. Brain-derived neurotrophic factor (BDNF) and synaptophysin (SYN), which are important neuronal plasticity-related proteins, play important roles in neurogenesis and synaptic plasticity. Hippocampal BDNF and SYN levels could be significantly decreased by HFD-induced obesity (Hao et al., 2016; Langdon et al., 2011). However, the underlying mechanisms are not fully understood.

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The endoplasmic reticulum (ER) is an important site of secreted and membrane proteins folding, post-translational modification and maturation. Obesity that is associated with hyperglycemia and hyperlipidemia could increase the ER workload and cause the accumulation of unfolded or misfolded proteins to induce ER stress (ERS) (Mandl et al., 2009). Moderate ERS serves as a protective mechanism through activating unfolded protein response (UPR), but prolonged or excessive ERS can induce apoptosis (Tabas and Ron, 2011). Some animal studies have shown that HFD-induced obesity could cause hypothalamic ERS, which is linked to leptin/insulin resistance and diminished cognitive function (Liang et al., 2015; Won et al., 2009). The most recent study shows that HFD associated with hyperglycemia could cause hippocampal ERS to impair insulin signaling in mice (Sims-Robinson et al., 2016). However, it's unknown that whether obesity could induce hippocampal ERS in obese SD rats. It has been found that glucose transporter 3 (GLUT3) and fatty acid transport protein 1 (FATP1), which are mainly expressed in the brain, can transport glucose and long-chain fatty acids from blood across the blood-brain barrier (BBB) into the brain (Salkovic-Petrisic et al., 2014; Wang et al., 2014). Several studies have suggested that hyperglycemia reaching the brain can promote GLUT3 expression and damage the brain (Mergenthaler et al., 2013), which probably induces ERS by perturbing intracellular glucose metabolic homeostasis. Moreover, increased long-chain fatty acids could affect FATP1 expression and exert toxic effects on cellular functions (Deguil et al., 2011), which possibly causes ERS. However, the effect of HFD-induced obesity on hippocampal GLUT3 and FATP1 expression in SD rats is unknown, and whether hyperglycemia and hyperlipidemia combining with GLUT3 and FATP1 expression could cause hippocampal ERS is also unknown.

BDNF and SYN gene transcription are directly upregulated by cAMP response element-binding protein (CREB) (Pinnock et al., 2010), which is activated by phosphorylation at Ser^{133} by various stress signaling molecules including p38 mitogen-activated protein kinase (p38 MAPK) and extracellular signal-related kinase (ERK), two MAPK isoforms that are important for long-term memory (LTM) formation (Liu et al., 2014). There is evidence that HFD-induced obesity suppresses the activation of ERK-CREB pathways, thus leading to decreased BDNF expression in mice brains (Chen et al., 2007; Liu et al., 2015). Moreover, BDNF could upregulate the SYN protein expression and plays a key role in synaptic plasticity in the hippocampus (Santos et al., 2010). Conversely, evidence suggests that obesity is also associated with heightened levels of pro-inflammation cytokines in the brain, and increased pro-inflammation cytokine Interleukin-1β (IL-1β) expression could cause hippocampal inflammation (Boitard et al., 2014; Sobesky et al., 2014), which is directly linked to the decreased protein expression of BDNF and SYN and synaptic plasticity (Tong et al., 2012; Barrientos et al., 2004). The secretion of caspase-1-dependent maturation of IL-1β is mediated by NLRP3 (NLR family, pyrin domain containing 3) inflammasome, which is known as a central regulator of the immune response in inflammation and a potential sensor of ERS (Kanneganti and Dixit, 2012). Recent studies have suggested that glutamate neurotoxicity induced hippocampal ERS could activate NLRP3 and increase IL-1β secretion (Li et al., 2015). However, little is known about the effects of obesity-induced hippocampal ERS on p38/ERK and NLRP3 activation in obese SD rats. We made a hypothesis that excessive ERS will reduce hippocampal BDNF and SYN protein expression in obese rats most likely via negatively regulating the p38/ERK-CREB pathways and positively regulating the NLRP3-IL-1β pathways. In addition to the above stress signaling mechanisms, ER also could regulate the biosynthesis of mature BDNF. BDNF is synthesized as pro-isoforms (proBDNF) that is post-translationally cleaved to mature BDNF, and this conversion is accomplished in the ER (Barker, 2009). However, it's unknown that whether excessive ERS will perturb the process of proBDNF cleavage to reduce the levels of mature BDNF.

The beneficial effects of aerobic exercise on ameliorating obesity associated with glucose and lipid metabolic abnormities, central inflammation and enhancing plasticity-related protein expression have been reviewed elsewhere (Gomes da Silva et al., 2013). However, the mechanisms that are involved are poorly understood, especially whether and how aerobic exercise alleviates obesity-induced hippocampal ERS. It has been found that heme oxygenase-1 (HO-1) has many roles, including anti-ERS, anti-inflammatory and anti-apoptotic roles, and its gene transcriptional activation is mediated by nuclear factor-erythroid 2-related factor 2 (Nrf2) (Son et al., 2013). However, it's not clear whether aerobic exercise could enhance Nrf2 and HO-1 protein expression to protect the hippocampus against obesity-induced ERS impairment.

Therefore, the purpose of this study was to investigate whether HFD-induced obesity that is associated with glucose and lipid metabolic disorders could cause hippocampal ERS, and how ERS regulates hippocampal BDNF and SYN levels. In addition, we examined the roles of Nrf2-HO-1 pathways, which might be activated by aerobic exercise, in affecting the hippocampal ERS and the BDNF and SYN production.

2. Materials and methods

2.1. Animals

A total of 150 male Sprague–Dawley (SD) rats were purchased from Shanghai Lab Animal Center (Certificate SCXK 2013-0016) at the age of 7 weeks (220 ± 10 g), and they were housed in the SPF animal research center of Shanghai University of Sport (SYXK 2014-0002), which has specific environment conditions such as a constant temperature of (22 ± 2) °C, a relative humidity of (50 ± 10)% and a 12-hour light-dark cycle from 07:00 to 19:00. The rats had free access to water and diet. All of the studies were performed in accordance with the Science Research Ethics Committee of Shanghai University of Sport (No. 2015013). The experimental protocols were approved by the Animal Care and Use Committee at the Shanghai University of Sport. All efforts were made to minimize the number of animals involved and the potential for suffering.

2.2. High fat diet-induced obesity and aerobic exercise intervention

The animals were randomly divided into two groups with similar body weight: one group was assigned to the high-fat diet (HFD, 40% kcal from fat, n = 110), and the other group was assigned to the normal chow diet (NCD, 12.5% kcal from fat, n = 40). The normal chow feed formula was based on AIN and AOAC purified diet for maintenance of the adult rats. The rats were housed five rats per cage, and they started consuming the high-fat or normal diet (SLAC Laboratory Animal CO. Ltd, Shanghai, China). Every cage included a wooden chew toy. The body weight (BW) was monitored once a week throughout the experiment. At the end of the 8th week, the BW value of the NCD group was expressed as the mean and standard deviation (SD). The animals in the HFD group that had a BW greater than the NCD group mean BW + 1.4 SD were designated as diet-induced obesity (DIO) rats, according to the method that others have previously used (Yu et al., 2014). Then, rats from NCD group and DIO were randomly selected and based on above body weight inclusion criteria were placed into one of four groups (n = 12/each group) according to their diet and exercise status: normal diet control sedentary group (CS), normal diet with aerobic exercise group (CE), obesity sedentary group (OS), obesity with