Psychiatry Research 250 (2017) 71–77

Contents lists available at ScienceDirect

Psychiatry Research

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

Physical exercise ameliorates mood disorder-like behavior on high fat diet-induced obesity in mice

Hye-Sang Park, Jae-Min Lee, Han-Sam Cho, Sang-Seo Park, Tae-Woon Kim*

Department of Physiology, College of Medicine, KyungHee University, Seoul, Republic of Korea

ARTICLE INFO

Keywords:
Depression
Anxiety
Dorsal raphe
Serotonin
Treadmill exercise
Obesity

ABSTRACT

Obesity is associated with mood disorders such as depression and anxiety. The aim of this study was to investigate whether treadmill exercise had any benefits on mood disorder by high fat diet (HFD) induced obesity. Mice were randomly divided into four groups: control, control and exercise, high fat diet (HFD), and HFD and exercise. Obesity was induced by a 20-week HFD (60%). In the exercise groups, exercise was performed 6 times a week for 12 weeks, with the exercise duration and intensity gradually increasing at 4-week intervals. Mice were tested in tail suspension and elevated plus maze tasks in order to verify the mood disorder like behavior such as depression and anxiety on obesity. In the present study, the number of 5-HT- and TPH-positive cells, and expression of 5-HT_{1A} and 5-HTT protein decreased in dorsal raphe, and depression and anxiety like behavior increased in HFD group compared with the CON group. In contrast, treadmill exercise ameliorated mood disorder like behavior by HFD induced obesity and enhanced expression of the serotonergic system in the dorsal raphe. We concluded that exercise increases the capacity of the serotonergic system in the dorsal raphe, which improves the mood disorders associated with HFD-induced obesity.

1. Introduction

Obesity can negatively affect an individual's quality of life and health. The prevalence of obesity in most countries is increasing steadily and is a large health burden (Wang et al., 2011). Obesity increases the risk of various chronic diseases, such as cardiovascular disease, high blood pressure, and diabetes (Nguyen et al., 2008). Obesity is also associated mood disorders with altered brain functions as well as changes in the peripheral organs. Wroolie et al. (2015) reported that cognitive performance in adult patients with mood disorders is affected by insulin resistance, steady-state plasma glucose levels, and body mass index.

Mood disorders associated with depression and anxiety symptoms are divided into many categories, including major depressive disorder (MDD) and bipolar disorder (BD). Many studies have demonstrated the association of obesity and mood disorders (Stunkard et al., 2003; Hung et al., 2015; Mansur et al., 2015). For example, several polymorphisms in fat mass- and obesity-associated genes are linked to an increase in body mass index (BMI) in people with MDD (Rivera et al., 2012), and people with MDD may show a predisposition to be overweight or obese (Farmer et al., 2008; Łojko et al., 2015; Nigatu et al., 2015). In addition, diseases such as mental disorders and obesity occur frequently in patients with BD (Regier et al., 1990; Harris and Barraclough 1997; Hsu et al., 2015). Furthermore, in chronic MDD and BD, there is a high risk of developing the metabolic changes of obesity (Díaz-Anzaldúa et al., 2015).

Mood is regulated by the central nervous system, with the neurotransmitter 5-hydroxytryptamine (5-HT), or serotonin, linked to the pathogenesis of depression (Cowen, 2008). Serotonin levels are regulated by tryptophan hydroxylase (TPH), which is the rate-limiting enzyme for serotonin biosynthesis and controls the release of synaptic 5-HT by the $5HT_{1A}$ receptor. The $5HT_{1A}$ receptor acts as an auto-receptor for 5-HT, and is one of several serotonin receptors localized to the dorsal raphe nucleus and the medial raphe nucleus of the brain. The raphe nucleus regulates serotonin release in various regions of the brain, as a key modulator of central serotonin secretion (Graeff, 1997). The sodium-dependent serotonin transporter (5-HTT) has been shown to play an important role in the pathogenesis of both mood disorders and eating disorders (Borkowska et al., 2015). The central serotonergic system originates in the dorsal raphe nucleus (DRN) in the midbrain, has widespread projections to emotion-related brain regions including the amygdala, hippocampus, basal ganglia, and cortex, and has been associated with antidepressant/anxiolytic properties (Vertes, 1991; Jacobs and Azmitia, 1992). In particular, the activity of serotonergic neurons in the dorsal raphe is known to be affected by various forms of metabolic, psychological, and physical stresses (Jocobs and Azmitia,

* Corresponding author.
E-mail address: twkim0806@naver.com (T.-W. Kim).

http://dx.doi.org/10.1016/j.psychres.2017.01.012
Received 29 July 2016; Received in revised form 5 December 2016; Accepted 3 January 2017
Available online 05 January 2017
0165-1781/

1992). The raphe nuclei contain the majority of the cell bodies of 5-HT neurons and are responsible for the production of 5-HT in the brain (Goda et al., 2015). Furthermore, depression decreases the activity of 5-HT and TPH and reduces the secretion of neurotransmitters and TPH levels in the raphe nucleus of the brainstem (Quan et al., 2010).

Many studies have suggested that physical exercise has a positive effect on brain function. Exercise ameliorates the neural response to stress in the brain and is protective against the progression of several neurological diseases, including Alzheimer's disease (Adlard et al., 2005), Parkinson's disease (Sung et al., 2012), and ischemic stroke (Willey et al., 2009). Studies have also provided strong evidence to suggest that exercise also improves anxiety and depression (Wipfli et al., 2011). However, little information is available regarding the effects of exercise on mood disorders such as depression and anxiety related to the behavioral effects associated with obesity. Further, the links between exercise, mood, and the central serotonin system are poorly understood. In the present study, we investigated the effects of treadmill exercise on mood disorders in relation to the serotonergic system in the dorsal raphe in an animal model of HFD-induced obesity.

2. Materials and methods

2.1. Animals and housing conditions

All animal experimental procedures conformed to the regulations stipulated by the National Institutes of Health (NIH) and the guidelines of the Korean Academy of Medical Science. This study was approved by the Kyung Hee university Institutional Animal Care and Use Committee (Seoul, Korea) (KHUASP (SE)-14-018). The mice were housed under controlled temperature (20 ± 2 °C) and lighting (07:00 to 19:00 h) conditions with food and water available ad libitum. Male 4 weeks old C57BL/6 mice were randomly divided into 4 groups (n=20 per group): control (CON), control and exercise (CON+EX), high fat diet (HFD), and high fat diet and Exercise (HFD+EX). Obesity was induced by a 20-week HFD (60%). For histological assessment, 10 animals and for western blot, 10 animals were used.

2.2. Exercise protocol

Table 1.

2.3. Behavior test

In order to evaluate level of depression- and anxiety-like behaviors in mice, depression was determined in the tail suspension task, and anxiety level was determined in an elevated plus maze task.

2.3.1. Tail suspension test

Mice were suspended individually by their tails from a wooden shelf 50 cm above the surface of a table in the test room. The tip of the tail was fixed using adhesive tape. Each subject was suspended by the tail for 6 min. During the test session, the cumulative immobility time was analyzed using a Smart version 2.5 video tracking system (Panlab, Barcelona, Spain).

Table 1
Treadmill exercise started after 20 weeks of the intake of high fat.

Exercise period (weeks)	Warm-up (m/min)	Exercise (m/min)	Cool-down (m/min)	Time (min)
0–2	5	10	5	30
3–4		10		40
5–6		13		30
7–8		13		40
9–10		16		40
11–12		16		50

2.3.2. Elevated plus maze test

The elevated plus maze apparatus consisted of two open arms (45×10 cm), crossed at right two opposed arms of the same size. The junction area of central measured platform (10×10 cm), set up 65 cm above the floor. Mice were placed on the central platform facing a closed arm and were allowed to explore the maze freely for 7 min. Entry into an arm was defined as entry of all four paws into the arm. Time and entries in the open arms were measured.

2.4. Tissue preparation

The animals were sacrificed immediately after determination of behavior test. To prepare the brain slices, the animals were fully anesthetized with Diethyl Ether after which the mice were transcardially perfused with 50 mM phosphate-buffered saline (PBS) and then fixed with freshly prepared solution of 4% paraformaldehyde in 100 mM phosphate buffer (PB, pH 7.4). The brains were then removed, post-fixed in the same fixative overnight, and transferred into a 30% sucrose solution for cryoprotection. Coronal sections with thicknesses of 40 μm were made using a freezing microtome (Leica, Nussloch, Germany).

2.5. Immunohistochemistry for TPH and 5-HT

To visualize TPH and 5-HT expression, immunohistochemistry for TPH and 5-HT in the dorsal raphe nuclei was performed. The dorsal raphe nucleus spanning from Bregma −7.20 mm to −8.00 mm were obtained from each brain. The sections were incubated in PBS for 10 min, and then washed three times in the same buffer. The sections were then incubated in 1% hydrogen peroxide (H_2O_2) for 30 min. The sections were selected from each brain and incubated overnight with rabbit anti-TPH antibody (1:1000; Oncogene research Product, Cambridge, UK) and rabbit anti-5-HT antibody (1:500; abcam, Cambridge, UK) and then with biotinylated rabbit secondary antibody (1:200; Vector Laboratories) for another 1 h. The secondary antibody was amplified with the Vector Elite ABC kit® (1:100; Vector Laboratories). Antibody-biotin-avidin-peroxidase complexes were visualized using 0.03% DAB, and the sections were mounted onto gelatin-coated slides. The slides were air-dried overnight at room temperature, and the coverslips were mounted using Permount®.

2.6. Western blot for 5-HT_{1A} and 5-HTT

The dorsal raphe nuclei was collected, then immediately frozen at −70 °C. Protein from dorsal raphe nuclei were extracted. The tissues were homogenized with lysis buffer containing 50 mM Tris–HCl (pH 8.0), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM $MgCl_2 \cdot 6H_2O$, 1 mM EGTA, 1 mM PMSF, 1 mM Na_2VO_4, and 100 mM NaF, then ultra- centrifuged at 50,000 rpm for 1 h. Protein content was measured using a Bio-Rad colorimetric protein assay kit (Bio-Rad, Hercules, CA, USA). Protein (30 μg) was separated on SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane. Anti-ß actin (1:1000; santa cruze), anti-5-HT_{1A} and 5-HTT (1:1000; abcam) were used as the primary antibodies. Horseradish peroxidase-conjugated anti-mouse antibody for β-actin, anti-rabbit antibody for 5-HT_{1A} and 5-HTT were used as the secondary antibody. Experiments were performed in normal laboratory conditions and at room temperature, except for the transferred membranes. Transferred membranes were performed at 4 °C with the cold pack and pre-chilled buffer. Band detection was performed using the enhanced chemiluminescence (ECL) detection kit (GE healthcare, Amersham™). To compare the relative expression of proteins, the detected bands were calculated densitometrically using Molecular Analyst™, version 1.4.1 (Bio-Rad).