Treadmill exercise in obese maternal rats during pregnancy improves spatial memory through activation of phosphatidylinositol 3-kinase pathway in the hippocampus of rat pups

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INTRODUCTION

Maternal nutrition is necessary for the growth of the fetus, and excessive intake of nutrients interferes with brain development in offspring. In the current study, the effect of treadmill running during pregnancy in obese maternal rats on spatial learning memory and spatial working memory in rat pups was investigated. Phosphorylation of phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), and extracellular signal-regulated kinase 1 and 2 (ERK1/2) was also identified in rat pups. Female rats were divided into the normal diet group and the high-fat diet group for 7 weeks, including pregnancy and lactation. Maternal treadmill running was performed for 4 weeks. The born rat pups were classified into a control group, a treadmill exercise group, a high-fat diet group, a high-fat diet and treadmill exercise group according to the status of maternal rats. Radial 8-arm maze task for spatial learning memory and Morris water maze task for spatial working memory were done. Western blot analysis was conducted to determine the expressions of PI3K, Akt, ERK1/2. In the current results, maternal treadmill running during pregnancy improved spatial learning memory and spatial working memory in rat pups born to obese maternal rats. This improving effect of memory was due to the enhanced phosphorylation of PI3K, Akt, and ERK1/2 by treadmill running.

Keywords: Maternal obesity, Pregnancy, Treadmill exercise, PI3K, Rat pups
phosphoinositide-3,4-diphosphate or phosphoinositide-3,4,5-triphosphate (Banadakoppa et al., 2014). Akt is a serine/threonine-specific protein kinase and regulates many downstream effectors such as nuclear factor-κB and Bcl-2 family proteins (Song et al., 2005). Akt plays critical role in glucose metabolism, apoptosis, cell proliferation, transcription, and cell migration (Numakawa et al., 2010). Activated Akt modulates activation or de-activation the substrate through kinase activity. Mitogen-activated protein kinase (MAPK) is one of the protein kinases and MAPK is specific to serine and threonine. MAPK regulates gene expression, proliferation, differentiation, mitosis, apoptosis, and cell survival (Pearson et al., 2001). ERK includes ERK1, ERK2, ERK5, and ERK7 etc. ERK 1 and 2 (ERK1/2) is called classical MAPK, and is stimulated by proliferation by epithelial growth factor or platelet-derived growth factor, or activated by cell differentiation factors such as nerve growth factor to promote cell proliferation or differentiation.

The purpose of current study was to evaluate the effect of treadmill running during pregnancy in obese maternal rats on spatial learning memory and spatial working memory in rat pups. Phosphorylation of PI3K, Akt, and ERK1/2 was also identified in rat pups to evaluate the mechanism of exercise.

MATERIALS AND METHODS

Animals

Four weeks old Sprague-Dawley male rats (n = 8) and female rats (n = 16) were used for this experiment. After mating with male rats for 48 hours, female rats were bred separately. Obesity was caused by feeding female rats a high-fat diet (60% fat). Female rats were divided into two groups: normal diet group (n = 8) and high-fat diet group (n = 8). The speed of maternal treadmill running was first 3 m/min for the 5 min, next 5 m/min for 5 min, and lastly 8 m/min for 20 min, once 30 min, 5 times a week, during 4 weeks. The born rat pups were provided 60 sec for the Morris water maze task, and if rat pups could not find the platform for 30 sec, and if rat pups could not find the platform within 60 sec, the hand guided them to the platform. The rat pups were water inhibited for 24 hours prior work measurement. Morris water maze task was performed 24 hr after the last training. When finding the platform, rat pups were left on the platform for 30 sec, and if rat pups could not find the platform within 60 sec, the hand guided them to the platform. All rat pups were trained for 3 consecutive days, 3 times a day prior to the Morris water maze task measurement. Morris water maze task was performed 24 hr after the last training. When finding the platform, rat pups were left on the platform for 30 sec, and if rat pups could not find the platform within 60 sec, the hand guided them to the platform. The rat pups were provided 60 sec for the Morris water maze task, and all data were automatically measured by Smart Video Tracking System (Smart ver. 2.5, Panlab, Barcelona, Spain).

Tissue preparation

After Morris water maze task, Zoletil 50 (10 mg/kg, Vibac Laboratories, Carros, France) was injected intraperitoneally to anesthesia, and 50 mM phosphate-buffered saline was injected through heart, and then fixed with 4% paraformaldehyde in 100 mM phosphate buffer (pH 7.4). Brains were excised and the brains were fixed overnight using the same fixative and transferred to a 30% sucrose solution to prevent freezing. A frozen microtome (Leica, Nussloch, Germany) was used to make 40-μm-thick sagittal sections.

Western blot analysis

As mentioned below, western blotting for PI3K, Akt, and
ERK1/2 was done (Park et al., 2019). Hippocampal tissues were lysed in a lysis buffer comprising 1 mM phenylmethylsulfonyl fluoride, 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% sodium dodecyl sulfate, 0.5% deoxycholic acid, 1% Nonidet P40, 100-mg/mL leupeptin. Rabbit anti-GAPDH antibody (1:1,000; Cell Signaling Technology, Beverly, MA, USA), mouse anti-total PI3K (t-PI3K) antibody (1:1,000; Santa Cruz Biotechnology, CA, USA), rabbit anti-phosphorylated-PI3K antibody (p-PI3K) (1:1,000; Santa Cruz Biotechnology), rabbit anti-total-Akt (t-Akt) antibody (1:1,000; Santa Cruz Biotechnology), rabbit anti-phosphorylated-Akt (p-Akt) antibody (1:1,000; Santa Cruz Biotechnology), rabbit anti-phosphorylated-ERK1/2 (p-ERK1/2) antibody (1:1,000; Cell Signaling Technology) were used as the primary antibody. Horseradish peroxidase-conjugated secondary antibodies were treated after washing. Image-Pro Plus computer-assisted image analysis system (Media Cyberbetics Inc., Silver Spring, MD, USA) was used for the quantification of the bands.

Data analysis
Data was analyzed by IBM SPSS Statistics ver. 21.0 (IBM Co., Armonk, NY, USA), and one-way analysis of variance followed Duncan post hoc test compared among the groups. The results were expressed as the mean±standard error of the mean with \( P < 0.05 \) considered as statistically significant.

RESULTS

Spatial learning ability
For the effect of treadmill running during pregnancy of obese maternal rats on the spatial learning ability of rat pups, the radial 8-arm maze task was conducted (Fig. 1). In the high-fat diet group, the time taken to find all the water in 8 mazes was increased compared to the control group \( (P < 0.05) \). Treadmill running reduced the time taken to find all the water in the high-fat diet group \( (P < 0.05) \). In the high-fat diet group, the number of correct choices that found water in succession was reduced compared to the control group \( (P < 0.05) \). Treadmill running increased the number of correct choices to water continuously in the high-fat diet group \( (P < 0.05) \). In the high-fat diet group, the number of errors entering the maze was increased compared to the control group \( (P < 0.05) \). Treadmill running reduced the number of errors in the high-fat diet group \( (P < 0.05) \).

Spatial working memory
For the effect of treadmill running during pregnancy of obese maternal rats on the spatial working memory of rat pups, the Morris water maze task was conducted (Fig. 2). In the high-fat diet group, the time spent around probe quadrant was decreased compared to the control group \( (P < 0.05) \). Treadmill running increased the time spent around probe quadrant in the high-fat diet group \( (P < 0.05) \).

PI3K expression
For the effect of treadmill running during pregnancy of obese maternal rats on the PI3K expression in the hippocampus of rat pups, PI3K expression was determined (Fig. 3). The ratio of p-PI3K/t-PI3K was decreased in the high-fat diet group compared to the control group \( (P < 0.05) \). Treadmill running increased the ratio of p-PI3K/t-PI3K in the high-fat diet group \( (P < 0.05) \).

Akt expression
For the effect of treadmill running during pregnancy of obese maternal rats on the PI3K expression in the hippocampus of rat pups, PI3K expression was determined (Fig. 3). The ratio of p-PI3K/t-PI3K was decreased in the high-fat diet group compared to the control group \( (P < 0.05) \). Treadmill running increased the ratio of p-PI3K/t-PI3K in the high-fat diet group \( (P < 0.05) \).
maternal rats on the Akt expression in the hippocampus of rat pups, Akt expression was determined (Fig. 4). The ratio of p-Akt/t-Akt was decreased in the high-fat diet group compared to the control group \((P < 0.05)\). Treadmill running increased the ratio p-Akt/t-Akt in the high-fat diet group \((P < 0.05)\).

**ERK1/2 expression**

For the effect of treadmill running during pregnancy of obese maternal rats on the ERK1/2 expression in the hippocampus of rat pups, ERK1/2 expression was determined (Fig. 5). The ratio
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Treadmill exercise increased the ratio p-ERK1/2/t-ERK1/2 in the high-fat diet group ($P < 0.05$).

**DISCUSSION**

Obesity causes systemic inflammatory conditions and is also associated with cognitive impairment (Bilbo and Tsang, 2010). Maternal swimming during pregnancy increased short-term memory in the hippocampus of rat pups (Lee et al., 2006). It is well known that exercise of the maternal rats increased memory in offspring (Akhavan et al., 2008; Kim et al., 2007). In the current results, spatial learning memory and spatial working memory were decreased in rat pups born to maternal rats with a high-fat diet compared to control maternal rats. Treadmill running of the maternal rats significantly improved spatial learning memory and spatial working memory in rat pups born to maternal rats with a high-fat diet.

Protein phosphorylation by the tyrosine kinase B receptor (TrkB) induces the activation of PI3K and Akt, regulating the survival of neurons and inducing the activity of ERK, thereby activating the transcription factor cyclic AMP-response element binding (Kaplan and Miller, 2000). PI3K and ERK activation is mediated by protein binding to phosphorylated TrkB receptor (Numakawa et al., 2010). The MAPK signaling system is a factor that regulates embryogenesis, cell differentiation, proliferation, and death, and ERK1/2 is an important kinase that determines cell fate in the MAPK signaling system. ERK is essential for cell survival by activating PI3K and Akt signaling pathways and promoting cell differentiation and growth (Santos et al., 2010). ERK is essential for cell survival by activating PI3K and Akt signaling pathways to promote cell differentiation and growth (Santos et al., 2010). In the current results, phosphorylation of PI3K, Akt, and ERK1/2 in rat pups born to maternal rats with a high-fat diet was decreased compared to the control group. This means maternal rats with high-fat diet caused dephosphorylation of PI3K, Akt, and ERK1/2 in rat pups.

In a previous study, maternal treadmill running during pregnancy improved short-term memory in rat pups born to obese maternal rats (Ji et al., 2020). The improving effect of short-term memory was achieved by enhancing neurogenesis through increased expression of BDNF and TrkB by treadmill running (Ji et al., 2020). In the current results, phosphorylation of PI3K, Akt, and ERK1/2, which are involved in neuronal birth and survival in the hippocampus, was significantly enhanced in rat pups by treadmill running of maternal rats with high-fat diet group. This indicates treadmill running in maternal rats with high-fat diet enhanced phosphorylation of PI3K, Akt, and ERK1/2 in rat pups.

In the current study, maternal treadmill exercise during pregnancy improved spatial learning memory and spatial working memory in rat pups born to obese maternal rats. This increasing effect was due to the enhanced phosphorylation of PI3K, Akt, and ERK1/2 in rat pups by treadmill running.

**CONFLICT OF INTEREST**

No potential conflict of interest relevant to this article was reported.

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