Please cite this article in press as: Shafiee SM et al. Effects of maternal hypothyroidism during pregnancy on learning, memory and hippocampal BDNF in rat pups: Beneficial effects of exercise. Neuroscience (2016), http://dx.doi.org/10.1016/j.neuroscience.2016.04.048

Neuroscience xxx (2016) xxx–xxx

EFFECTS OF MATERNAL HYPOTHYROIDISM DURING PREGNANCY ON LEARNING, MEMORY AND HIPPOCAMPAL BDNF IN RAT PUPS: BENEFICIAL EFFECTS OF EXERCISE

SEYED MORTEZA SHAFIEE, ABBAS ALI VAFAEI [†] AND ALI RASHIDY-POUR [*]

Laboratory of Learning and Memory, Research Center and Department of Physiology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran

Abstract—Hypothyroidism during early development leads to numerous morphological, biochemical and functional changes in developing brain. In this study, we investigated the effects of voluntary and treadmill exercise on learning, memory and hippocampal BDNF levels in both hypothyroid male and female rat pups. To induce hypothyroidism in the mothers, 6-propyl-2-thiouracil (PTU) was added to their drinking water (100 mg/L) from their embryonic day 6 to their postnatal day (PND) 21. For 14 days, from PNDs 31 to 44, the rat pups were trained with one of the two different exercise protocols, namely the mild treadmill exercise and the voluntary wheel exercise. On PNDs 45–52, a water maze was used for testing their learning and memory ability. The rats were sacrificed one day later and their BDNF levels were then measured in the hippocampus. The findings of the present study indicate that hypothyroidism during the fetal period and the early postnatal period is associated with the impairment of spatial learning and memory and reduced hippocampal BDNF levels in both male and female rat offspring. Both the short-term treadmill exercise and the voluntary wheel exercise performed during the postnatal period reverse the behavioral and neurochemical deficits induced by developmental thyroid hormone insufficiency in both male and female rat offspring. The findings of this study thus demonstrate a marked reversibility of both behavioral and neurochemical disorders induced by developmental thyroid hormone insufficiency through the performance of exercise.

Key words: hypothyroidism, exercise, spatial memory, BDNF, rat.

INTRODUCTION

Thyroid hormones are essential throughout life for normal brain development and function (Aranda and Pascual, 2001; Williams, 2008; Giné et al., 2013). These hormones enhance neurogenesis and the maturation of new neurons in the brain during its development (Ambrogini et al., 2005). The expression of thyroid hormone receptors in the brain in early gestation suggests that thyroid hormones play a crucial role in the body during this period (Perez-Castillo et al., 1985). The lack of thyroid hormones during this period can have dramatic effects on the proper functioning of many regions of the brain, including the hippocampus (Bernal, 2007). The structural integrity of the hippocampus is crucial for some types of learning and memory processes, which, in turn, depend on the sufficient intake of thyroid hormone supplements during the stage of development (Madeira et al., 1991). Thyroid hormone deficiency during the critical period of brain development is therefore associated with morphological, electrophysiological and biochemical alterations in the hippocampus (Gilbert et al., 2007), resulting in severe cognitive and neurological disorders (Desouza et al., 2005). Prenatal hypothyroidism is reported to decrease neuronal survival, the density of the dendritic spines and the functioning of the synapses, thus leading to learning and memory impairments (Wang et al., 2010).

The results of recent animal experiments suggest that exercise increases learning and memory abilities within a variety of behavioral tasks (Cotman and Berchtold, 2002; Swain et al., 2012). Moreover, exercise up-regulates the expression of growth factors, including the nerve growth factor (NGF) and the brain-derived growth factor (BDNF) in the hippocampus, which comprise a key brain structure in the medial temporal lobe and are essential for activity-dependent learning and memory (Neeper et al., 1996; Lynch, 2004). The hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition (Vaynman et al., 2004). Exercise also increases neurogenesis in the hippocampus (Helfer et al., 2009) through BDNF and NGF-mediated mechanisms (Baek et al., 2012). In addition, exercise delays neuronal apoptosis in the dentate gyrus of the hippocampus (Kim et al., 2010b). The results of cross-sectional and longitudinal intervention studies on humans have shown that physical activity helps attenuate the memory and cognitive decline associated with normal aging and caused by neurodegenerative diseases such as Alzheimer's disease (Kramer et al., 2004; Bherer et al., 2013). In children, aerobic fitness is associated with a better neuroelectric responsiveness and a faster cognitive processing (Hillman et al., 2005). A meta-analysis confirmed the positive relationship

[*]Corresponding author. Tel: +98-23-33621622; fax: +98-23-33627316.
E-mail address: rashidy-pour@semums.ac.ir (A. Rashidy-Pour).
[†] Equal contribution with the first author.
Abbreviations: BDNF, brain derived growth factor; GD, gestation day; NGF, nerve growth factor; PND, postnatal day; PTU, 6-propyl-2-thiourycil; WM, water maze.

http://dx.doi.org/10.1016/j.neuroscience.2016.04.048
0306-4522/

between physical activity and cognitive and academic performance in school-aged children (Sibley and Etnier, 2003).

BDNF and its high-affinity receptor, namely TrkB, are widely expressed in the mammalian brain (Lewin and Barde, 1996) and play a crucial role in the development, maintenance and functioning of the CNS (Huang and Reichardt, 2003). In adolescence, BDNF affects almost all the aspects of development, such as the differentiation of neural stem cells into neurons and stimulating the growth and improving the survival of newly-generated cells and synaptogenesis (Tapia-Arancibia et al., 2004). In addition, neuronal activity regulates BDNF transcription, transport of BDNF mRNA and protein into dendrites and the activity-dependent secretion of BDNF, which, in turn, modulate synaptic plasticity, synaptogenesis and memory formation (Bekinschtein et al., 2008).

Developmental thyroid hormone insufficiency reduces cognitive functions and neural development in both humans and experimental animals (Chakraborty et al., 2012). In rats, maternal thyroidectomy significantly reduces BDNF expression in the brain of developing rat pups (Liu et al., 2010). Prenatal exposure to PTU also leads to reduced hippocampal BDNF in neonatal rats (Chakraborty et al., 2012). BDNF is known to be directly regulated by thyroid hormones and play essential roles during the critical period of fetal brain development (Wang et al., 2006). These findings suggest that reduced hippocampal BDNF during early development may contribute to the adverse functional and structural events in the CNS induced by lack of thyroid hormones during pregnancy.

A review of literature suggests that studies have not yet examined how physical activity helps reverse the behavioral and biochemical alternations induced by developmental thyroid hormone insufficiency. Therefore, we investigated whether both voluntary and mild treadmill exercise would restore cognitive deficits, and reduced hippocampal BDNF in both male and female hypothyroid rat pups. We used both voluntary and forced exercise because different kinds of physical activity induce different effects on neuronal adaptations in different brain regions, and cognitive functions (Liu et al., 2009; Lin et al., 2012).

EXPERIMENTAL PROCEDURES

Experimental animals

Female Wistar rats were obtained from the breeding colony of the Semnan University of Medical Sciences Semnan, Iran and housed in cages for a 12 h light/dark cycle at 22–24 °C, where they had *ad libitum* access to food and water. All experimental procedures were conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

Experimental design

Female rats were mated with normal males. Day of conception was confirmed by observation of vaginal plug and was designated as gestation day 0 (GD0). The rats were housed in individual cages until delivery. The day of birth was designated as postnatal day 0 (PND 0). On GD6, the pregnant rats were divided into two groups: the control group mothers and the hypothyroid group mothers. The dams (n = 30) in the hypothyroid group were administered the anti-thyroid agent PTU (Sigma Co. USA) solved in drinking water at a concentration of 100 ppm beginning on GD6 and continuing throughout lactation until PND 21. The dams in the control group (n = 30) received only tap water during this period. The PTU dose used was chosen based on the findings of previous studies on the subject (Zahedi Asl et al., 2009; de Picoli Souza and Nunes, 2014; Mallela et al., 2014). Two pups from each dam were sacrificed by decapitation on PND 21, that is, when the PTU treatment was terminated. Trunk blood was collected for the thyroid test. The rats' blood serum was separated through the centrifugation of the clotted samples and was then stored at −80 °C for later analyses through the Elisa method and for measuring serum TSH and total T4. On PNDs 45–52, spatial learning and memory were tested after the rats' BDNF, TSH and T4 were measured (see below).

Wheel running exercise protocol

The exercise rats were given 24-h access to a cage equipped with a running wheel (diameter = 34.5 cm, width = 9.5 cm). Each wheel's revolutions were counted and recorded every day at 6 am. The sedentary rats were confined to similar cages with no access to a wheel. After the 14-day period of the exercise, the running wheels were removed from the cages and the rats were trained and tested with the water maze (WM) task.

Treadmill exercise protocol

The rat pups in the treadmill exercise groups were forced to run on a motorized treadmill for 30 min once a day, starting on PND 31 for 14 days, by gentle tail touch without electrical shock. The exercise intensity consisted of running at a speed of 2 m/min for the first 5 min, 5 m/min for the next 5 min, and 8 m/min for the last 20 min with a 0° inclination. The rat pups in the control group and in the hypothyroid group were left on the treadmill without running for the same period as that in the exercise groups (Kim et al., 2010a).

Testing of spatial learning and memory in the WM

The WM was a black circular pool (140 cm in diameter and 60 cm high) that was filled with 22 °C water to a depth of 25 cm. The WM protocol was a stringent protocol of four trials per day for five consecutive days. During each trial, each rat was placed into the water at one of the four cardinal points of the compass (N, E, S, and W), which varied from trial to trial in a quasi-random order. The rat had to swim until it climbed onto the escape platform. Animals that failed to find the platform within the allocated time were gently guided to the

Please cite this article in press as: Shafiee SM et al. Effects of maternal hypothyroidism during pregnancy on learning, memory and hippocampal BDNF in rat pups: Beneficial effects of exercise. Neuroscience (2016), http://dx.doi.org/10.1016/j.neuroscience.2016.04.048