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Prior regular exercise reverses the decreased effects of sleep deprivation on brain-derived neurotrophic factor levels in the hippocampus of ovariectomized female rats

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ABSTRACT

Previous studies indicated that brain-derived neurotrophic factor (BDNF) is the main candidate to mediate the beneficial effects of exercise on cognitive function in sleep deprived male rats. In addition, our previous findings demonstrate that female rats are more vulnerable to the deleterious effects of sleep deprivation on cognitive performance and synaptic plasticity.

Therefore, the current study was designed to investigate the effects of treadmill exercise and/or sleep deprivation (SD) on the levels of BDNF mRNA and protein in the hippocampus of female rats.

Intact and ovariectomized (OVX) female Wistar rats were used in the present experiment. The exercise protocol was four weeks treadmill running and sleep deprivation was accomplished using the multiple platform method. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and immunoblot analysis were used to evaluate the level of BDNF mRNA and protein in the rat hippocampus respectively.

Our results showed that protein and mRNA expression of BDNF was significantly ($p < 0.05$) decreased after 72 h SD in OVX rats in compared with other groups. Furthermore, sleep deprived OVX rats under exercise conditions had a significant ($p < 0.05$) up-regulation of the BDNF protein and mRNA in the hippocampus.

These findings suggest that regular exercise can exert a protective effect against hippocampus-related functions and impairments induced by sleep deprivation probably by inducing BDNF expression.

1. Introduction

Human and animal studies suggest that sleep has an important role in certain types of learning and memory and neuronal plasticity [1]. Accordingly, sleep deprivation causes memory deficit and decreases hippocampal level of BDNF [2,3]. In addition, BDNF is present in high concentration in the hippocampus and cerebral cortex and is very important for learning and memory [4].

Sleep disorders are almost frequent in modern society particularly in menopausal women. In menopausal women, sleep disturbances may be the most important indications for hormone therapy and certainly necessitate to be used as specific treatment in this group [5,6]. Additionally, despite the beneficial effects of estrogen on the brain functions, hormone replacement therapy increased adverse cardiovascular and oncological effects [7]; there is noticeable attention in developing healthier therapeutic approaches to alleviate sleep deprivation-associated impairments.

It has been indicated that exercise is one of the most potent non-pharmacological interference that can improve the cognitive functions around the postmenopausal period [8].

It has been demonstrated that exercise can alter some neurotransmitters and neurotrophin expression [4]. Altered expression of neurotrophic factors, for example BDNF is recognized to play a vital role in the hippocampus-related functions, synaptic plasticity [9,10] and psychiatric disorder [11]. Furthermore, it has been indicated that regular exercise can modulate the induction of mRNA and protein of BDNF within the hippocampus which may contribute to the maintenance of brain health and synaptic plasticity [4,12,13].

Several lines of evidence indicate that BDNF is a potential mediator of the central effects of estrogen. In particular, there are the extensive similarities between the functions of estrogen and BDNF in the CNS [14]. BDNF also provides both neurotrophic and neuroprotective support to different subpopulations of neurons, and is mostly associated

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with both learning and memory processes within the hippocampus [4,15,16]. It is possible that such properties may enhance specific learning and memory processes and help to reduce cognitive impairment associated sleep loss [17] and neurodegenerative disease [18,19].

Results from previous studies revealed that 24 h SD negatively affects cognitive function of rats by suppressing related signaling cascade such as BDNF in the hippocampus and such impairments can be prevented by regular exercise [17,20]. Interestingly, we have previously showed that female rats are more susceptible to the impairing effects of 72 h SD on spatial learning and memory in the Morris water maze task [21].

The aim of the current study was to examine the effects of regular exercise and/or SD on the level of BDNF protein and mRNA in the hippocampus of female rats.

2. Material and methods

2.1. Animals

Female Wistar rats (weighing 200–250 g) were used in the present study. Animals were caged in groups of four with access to food and water ad libitum. The rats were housed under a 12-h light–dark schedule (lights on: 07:00–19:00 h) and standard conditions of temperature (23 ± 1 °C). Two groups of intact and ovariectomized (OVX) rats were randomly chosen as the following subgroups: control (stayed in home cages), SD, exercised and exercised plus SD group. All procedures were performed in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee of Kerman Neuroscience Research Center (Ethics Code: KNRC-92-33).

2.2. Surgical procedures

All of the operations were carried out under general anesthesia using a mixture of ketamine (60 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). Both ovaries were removed by a small mid-abdominal incision under aseptic situations. After ovariectomy, all of the rats were kept in a controlled animal room for one month [22].

2.3. Treadmill exercise

Rats ran as forced exercise for five day per week (from Saturday to Wednesday at 0° inclination) for four weeks during the light cycle, between 9:00 and 14:30 (they received a mild shock, 0.25 mA, when they stopped running). The rats were allowed to habituate to treadmill environment for 30 min during 2 successive days before the beginning of the exercise protocol to minimize nonspecific stress responses. The exercise protocol used in the current experiment was mild-moderate in that it gradually increased in velocity and time across the 4 week period. The exercise procedure included the following stages: 30 min for the first two weeks (at 10 m/min speed), 45 min for the third week and 60 min for the fourth week (both at 15 m/min speed). Every 15 min during each session, the animals had a five minute rest period [17].

2.4. Induction of sleep deprivation

Columns-in-water method (multiple platforms method) was used to induce SD. This apparatus (90 cm × 50 cm × 50 cm) contained 10 columns (10 cm high, 7 cm diameter located 2 cm above the water level) which were designed in two rows and spaced 10 cm apart (edge to edge). The apparatus permits rats to move from one platform to another. The animal was placed on top of a small platform. As a result, SD was achieved when the animal began rapid eye movement sleep (REM), losing muscle tone causing the rats to contact the water and awaken.

During the study, the rats had free access to clean water bottles and food pellet baskets were always hanging from the top of the chamber. In the present research, SD was induced for 72 h as previously explained [21]. The cage mates (4 rats) were put together in a chamber to retain social stability and were kept under standard conditions [12:12-h light–dark cycle at a controlled temperature (23 ± 1 °C)] in the sleep deprivation period.

Sleep deprivation was accomplished for 24 h after performing the last exercise session in the exercise/SD groups.

2.5. Molecular experiments

For molecular experiments, animals were anesthetized with atmosphere CO_2 in desiccators jar with low pressure flow of CO_2 [23]. After decapitation, both whole hippocampi were rapidly separated on an ice-cold surface and frozen in liquid nitrogen. The dissected hippocampi from each rat were randomly distributed for further western blot and RT-PCR assays and stored at −80 until homogenization.

2.5.1. BDNF immunoblot analysis

Sample preparation for immunoblot analysis was performed as previously reported [24]. The dissected hippocampal tissues were homogenized in ice-cold buffer containing 10 mM Tris–HCl (pH 7.4), 1 mM EDTA, 0.1% SDS, 0.1% Sodium deoxycholate, 1% NP-40 with protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 2.5 μg/ml of leupeptin, 10 μg/ml of aprotinin) and 1 mM sodium orthovanadate, using a tissue homogenizer (Silent Crusher S Homogenizer, Germany) at a medium speed for 5 s, repeated 3 to 5 times. The homogenate was centrifuged at 14,000 rpm for 15 min at 4 °C. Protein concentrations were determined using the Bradford protein assay (Bio-Rad Laboratories, Muenchen, Germany). An equal amount of protein (40 μg) was resolved electrophoretically on 12.5% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membranes (Hybond ECL, GE Healthcare Bio-Science Corp., NJ, USA). During electrophoresis, the molecular weight was monitored with a pre-stained protein ladder (Fermentas, Life Science). Membranes were blocked with 5% nonfat powdered milk in Tris-buffered saline-Tween 20 (TBS-T) (0.1% Tween 20 in 150 mM Tris–HCl, pH 7.5) for 1.5 h at room temperature and thereafter, the membranes were incubated overnight with a primary rabbit polyclonal antibody for BDNF (1:1000, sc-20981; Santa Cruz Biotechnology, Santa Cruz, USA,) at 4 °C. After washing in TBS-T buffer (three times for 5 min, at room temperature) the membranes were incubated for 2 h at room temperature with an anti-rabbit IgG secondary antibody conjugated with horseradish peroxidase (1:15,000; GE Healthcare Bio-Sciences). Both primary and secondary antibodies were diluted in blocking buffer. The antibody-antigen complexes were visualized using the ECL system (Amersham Biosciences) and then exposed to Lumi-Film chemiluminescent detection film (Roch, Germany). Lab Work analyzing software (UVP, UK) was used to analyze the intensity of the expression. To control for loading, the membranes were stripped and reassayed using an antibody for β-actin (Cell Signaling Technology Inc., Beverly, MA, USA; 1:1000).

2.5.2. Semi-quantitative PCR

Total hippocampal RNA was isolated using a modification of the guanidine isothiocyanate-phenol-chloroform method using RNX+ reagent [25]. The isolated RNA was eluted with 20 μl of RNase-free water. All RNA was quantitated by spectrophotometer (Eppendorf AG, Hamburg, Germany) and optical density (OD) 260/280 nm ratios were determined. If A260/280 of extracted RNA had a value of ~2.0, it was considered as pure RNA samples and recruited in the next experimental steps. In addition, the extracted RNA was electrophoresed (1.5% agarose gel) and stained with ethidium bromide. After visual assessment of the 28 s and 18 s of rRNA band, we considered intact or good RNA. The same concentration of extracted RNA was used to make cDNA. Briefly, the single-strand cDNA was synthesized from