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Research article

Recovery of motor coordination after exercise is correlated to enhancement of brain-derived neurotrophic factor in lactational vanadium-exposed rats

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HIGHLIGHTS

- Lactational vanadium exposure impairs motor coordination in the late adolescent male rats.
- Exercise during childhood-adolescence prevents the impairment of motor coordination.
- We find a strong correlation between enhanced BDNF level and improvement of motor coordination.

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ABSTRACT

Lactational exposure to vanadium can reduce the locomotor activity in adult animals. In this study, we investigated whether lactational vanadium exposure impairs the motor coordination and whether exercise ameliorates this dysfunction. Sprague–Dawley dams were treated with or without vanadium during lactation. The weaned male offspring were trained to treadmill running for 5 weeks and then examined their motor coordination on a rotarod. The neuroprotective effect of exercise was evaluated by the brain-derived neurotrophic factor (BDNF) in plasma and cerebellum. The results demonstrated that vanadium-exposed rats exhibited impaired motor coordination and reduced plasma and cerebellar BDNF levels. Treadmill running during childhood-adolescence prevented the impaired motor coordination in the lactational vanadium-exposed rats. The beneficial effect of treadmill running on motor coordination in the vanadium-exposed rats was correlated to the normalization of plasma and cerebellar BDNF levels, as well as the increased TrkB phosphorylation in the cerebellum. The result suggests that exercise may prevent the impairment of motor coordination in the lactational vanadium-exposed rats.

1. Introduction

Exercise induces significant improvements in the performance of tasks requiring declarative and nondeclarative memories [1,2]. One of the most investigated exercise-induced mediators is the brain-derived neurotrophic factor (BDNF). BDNF is a member of the neurotrophin family of growth factors and plays a major role in neuronal survival, synaptic plasticity, and learning and memory [3]. The expression of BDNF and its tropomyosin-related kinase B (TrkB) receptor are induced in response to exercise, which is strongly correlated to the enhancement in cognition and motor skill learning [4,5]. Exercise-induced BDNF secretion promotes neuronal survival and facilitates the recovery after injury. Animal studies demonstrate that exercise-induced BDNF secretion is associated with an improvement in cognitive performance after traumatic brain injury and beta amyloid peptides infusion [6,7]. Meanwhile, exercise-induced BDNF secretion may account for recovery of motor control in the animal models of Parkinson's disease and Huntington's disease [8,9]. These studies suggest that BDNF plays a major role in the exercise-induced neuroprotection against brain injury.

Vanadium is considered as the most abundant metallic ingredient in petroleum. Burning of fossil fuels increases vanadium levels in the atmosphere [10]. In humans, exposure to vanadium results in neurobehavioral alteration, such as the reduced functions in emotion, cognition, and motor accuracy [11,12]. In adult rats, vanadium exposure causes neuropathological lesions in hippocampal neurons [13], as well as oxidative stress and demyelination in the cerebellum [14,15]. Because vanadium is present in breast milk and easily passes the blood brain barrier, it could cause developmental neurotoxicity in the exposed neonatal animals [16]. Lactational exposure of vanadium delays the development and impairs the

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locomotor activity in adult rats [17]. These data suggest that vanadium exposure at early life may exert adverse effects on the development and maturation of nervous system.

The present study aimed to determine the effects of exercise on the motor coordination in the lactational vanadium-exposed rats. We hypothesize that exercise could induce the release of BDNF and improve the motor coordination in the lactational vanadium-exposed rats. To test this hypothesis, weaned rats with or without lactational vanadium exposure were trained to exercise on the treadmill during the childhood-adolescence period. At late adolescence (8 weeks of age), motor coordination were behaviorally tested on a rotarod, and then the plasma and cerebellar levels of BDNF were measured by enzyme-linked immunosorbent assay (ELISA). The results can reveal whether lactational vanadium exposure alters the motor coordination and whether exercise ameliorates this dysfunction.

2. Materials and methods

2.1. Administration of DEHP to lactating rat dams

All procedures have been reviewed and approved by the Animal Care and Use Committee of Kaohsiung Medical University.

Pregnant female Sprague–Dawley rats ($n = 12$) had free access to Altromin 1320 rat pellets (Altromin, DE) and double distilled water. Because the same food used, we assumed that all animals were exposed to equivalent levels of food-borne trace elements. After delivery, male pups ($n = 36$) were maintained until weaning on postnatal day 21 (PND 21). The dams were administered intraperitoneally with $NaVO_3$ (Sigma–Aldrich, USA) or distilled water daily during lactation. The dose used was 3 mg/kg/day (1.25 mg V/kg/day). The dose and the administration were chosen based on the studies showing lactational $NaVO_3$ exposure impairs locomotor activity in adult rats [14,17].

2.2. Treadmill running

The weaned male pups were divided into four groups ($n = 9$ in each group): control (C), vanadium exposure (V), exercised control (Cex), and exercised vanadium exposure (Vex). On PND 22, exercised rats were allowed to run on a motor-driven treadmill without footshock (Model Exer 3/6, Columbus Instruments, USA), starting at a very low speed and gradually reaching 8 m/min for 30 min each day for 5 days to become familiar with treadmill running. Then the animals were exercised for 30 min/day, 5 days/wk for 4 weeks. The running speed started at 12 m/min, increased 3 m/min every week, and reached to 21 m/min at the end of the training period. In contrast, animals in the sedentary groups were placed on the treadmill without running for 10 min each day for 5 weeks. This treatment for sedentary and exercised animals has been successfully used in the previous study [18].

2.3. Measurement of extensor thrust response

The extensor thrust response [19] was measured at PND 59. A Plexiglas plate (6 cm × 3 cm × 0.5 cm) was attached to the force gauge. The tester supported the rat in a vertical position and the plate was placed against the hindlimb foot pads of both feet, and the legs were pressed inward to an approximate midpoint between a fully extended and fully flexed position. The apparatus was then held stationary until a resistance value (g of force) was obtained. Three values were obtained in succession, and the average value was used as the score of extensor thrust response. Then the score of thrust was subdivided to the body weight to obtain the ratio of thrust-to-body weight.

2.4. Rotarod test

All animals underwent a rotarod test at PND 60. A motor-driven plastic roller (6 cm in diameter and 10 cm in length) was elevated 30 cm. Animals were initially trained to maintain themselves on the rotating rod at 5 rotations per minute (RPM) for 2 min (habituation phase). After a period of 24 h, animals were evaluated for their ability to stay on the rotating rod for three successive trials, starting at 10 RPM and increasing to 20, 30, and 40 RPM in the next sessions with a lapse of 20 sec between each session. The latency to fall for each trial was recorded, and the times of three consecutive trials were averaged. The coordination index was determined by: [(latency at 10 RPM) × 1.0] + [(latency at 20 RPM) × 2.0] + [(latency at 30 RPM) × 3.0] + [(latency at 40 RPM) × 4.0].

2.5. Immunohistochemistry

After the rotarod test, animals were returned to their home cage for 24 h. Then the animals were sacrificed by inhalation of CO_2 and the blood was collected from the atria. Blood samples were centrifuged at 1500 × *g* rpm for 30 min and the supernatants were collected for ELISA assay. Animals were perfused transcardially with phosphate buffered saline (PBS) for 3 min and their brains were removed and immersed in ice-cold PBS. The right cerebellar hemispheres were stored at −80 °C for Western blotting and ELISA assay. Another half of cerebellar hemispheres were soaked in 4% paraformaldehyde for 24 h. Frozen saggital sections of 20 μm were processed for immunohistochemical detection of calbindin using mouse anti-calbindin antibody (1:200, Synaptic System, USA), and then incubated for 1 h with Alexa Fluor 488 Goat anti-mouse IgG antibody (1:1000, Life Technologies, USA). Images were captured using a Zeiss microscope. We sampled 2 sagittal slices (20 μm thickness) from cerebellar vermis, and the total number of calbindin-positive cells was counted using Image-Pro Plus software (Media Cybernetics, USA). The number of Purkinje cells in each slice was subdivided to the length of Purkinje layer along the sagittal plane of cerebellum. The averaged data was presented by cells/mm.

2.6. ELISA assay

Plasma and cerebellar BDNF levels were measured using a BDNF E_{max} Immunoassay kit optimized for small sample volumes (Promega, USA), according to the manufacturer's instructions. For cerebellar BDNF level, the isolated cerebellum was immediately put on ice and homogenized in lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, and complete protease inhibitor tablets). Homogenate was centrifuged at 13,000 g for 20 min at 4 °C. Protein in the supernatant was quantified using a Bradford assay. Equal amounts of protein from each sample were used to ELISA assay. The detection limit of this assay is 7.8 pg/mL. Each sample was measured in duplicate and the inter-assay variation was <11.3% and <13.5 for plasma and cerebellar samples, respectively. The average BDNF level from the same rat was calculated for comparison.

2.7. Western blotting

Equal amounts of protein from each sample were mixed with LDS sample buffer (Invitrogen, USA). Samples were separated using pre-cast 10% Bis–Tris gel (Invitrogen, USA) in MOPS running buffer (Invitrogen, USA) and transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, MA, USA) in NuPage transfer buffer (Invitrogen, USA). Following incubation with the primary antibodies for 24 h at 4 °C: rabbit anti-phospho TrkB antibody (Tyr706) (1:100, Santa Cruz, CA, USA), mouse anti-calbindin