Hormones and Behavior 63 (2013) 158–165

Contents lists available at SciVerse ScienceDirect

Hormones and Behavior

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

Voluntary exercise does not ameliorate spatial learning and memory deficits induced by chronic administration of nandrolone decanoate in rats

Fatemeh Tanehkar [a], Ali Rashidy-Pour [a,*], Abbas Ali Vafaei [a], Hamid Reza Sameni [b], Saeed Haghighi [b], Hossien Miladi-Gorji [a], Fereshteh Motamedi [c], Maziar Mohammad Akhavan [d], Kowsar Bavarsad [a]

[a] Laboratory of Learning and Memory, Research Center and Department of Physiology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran
[b] Research Center of Physiology and Department of Anatomical Sciences, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran
[c] Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[d] Laboratory of Protein and Enzyme, Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 8 June 2012
Revised 30 September 2012
Accepted 2 October 2012
Available online 12 October 2012

Keywords:
Anabolic androgenic steroids
Nandrolone decanoate
Morris water maze
Learning
Memory
Voluntary exercise
BDNF
Rat

ABSTRACT

Chronic exposure to the anabolic androgenic steroids (AAS) nandrolone decanoate (ND) in supra-physiological doses is associated with learning and memory impairments. Given the well-known beneficial effects of voluntary exercise on cognitive functions, we examined whether voluntary exercise would improve the cognitive deficits induced by chronic administration of ND. We also investigated the effects of ND and voluntary exercise on hippocampal BDNF levels. The rats were randomly distributed into 4 experimental groups: the vehicle-sedentary group, the ND-sedentary group, the vehicle-exercise group, and the ND-exercise group. The vehicle-exercise and the ND-exercise groups were allowed to freely exercise in a running wheel for 15 days. The vehicle-sedentary and the ND-sedentary groups were kept sedentary for the same period. Vehicle or ND injections were started 14 days prior to the voluntary exercise and continued throughout the 15 days of voluntary exercise. After the 15-day period, the rats were trained and tested on a water maze spatial task using four trials per day for 5 consecutive days followed by a probe trial two days later. Exercise significantly improved performance during both the training and retention of the water maze task, and enhanced hippocampal BDNF. ND impaired spatial learning and memory, and this effect was not rescued by exercise. ND also potentiated the exercise-induced increase in hippocampal BDNF levels. These results seem to indicate that voluntary exercise is unable to improve the disruption of cognitive functions by chronic ND. Moreover, increased levels of BDNF may play a role in ND-induced impairments in learning and memory. The harmful effects of ND and other AAS on learning and memory should be taken into account when athletes decide to use AAS for performance or body image improvement.

Introduction

Anabolic steroids, which are technically known as anabolic androgenic steroids (AAS) are a large class of synthetic androgens that mimic the effects of the male sex hormones testosterone and dihydrotestosterone. They increase protein synthesis within cells, which results in the buildup of cellular tissue (anabolism), especially in muscles (Bhasin et al., 1996; Bhasin et al., 2003; Forbes, 1985). Although AAS were originally developed for therapeutic purposes (Basaria et al., 2001), they are now commonly used for illegal self-administration at supra-physiological doses to improve performance or body image (Kanayama et al., 2008; Trenton and Currier, 2005). Studies have shown that high doses of AAS can cause serious adverse effects, such as skeletal muscle injuries including an increased rate of muscle strains/ruptures, harmful changes in cholesterol levels, acne, high blood pressure, liver damage (mainly with oral steroids) and dangerous changes in the structure of the left ventricle of the heart (van Amsterdam et al., 2010).

Recent studies have suggested that the misuse of AAS in supraphysiological doses also affects several CNS-related behaviors, such as aggression, anxiety, depression and cognitive functions (Su et al., 1993; Trenton and Currier, 2005). Long-term administration of the AAS nandrolone decanoate (ND) leads to behavioral and neurochemical changes in the central nervous system in rodents (Clark and Henderson, 2003; Henderson et al., 2006; Kindlundh et al., 2004; Kurling et al., 2005; Penatti et al., 2009; Rossbach et al., 2007; Thiblin et al., 1999) which may underlie some of the behavioral changes that are observed in human AAS abusers. Two recent studies have shown that the chronic administration of ND to male rats impairs social and spatial memories via central androgen receptors

* Corresponding author at: Laboratory of Learning and Memory, Research Center and Department of Physiology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran. P.O. Box: 35145-331. Fax: +98 231 3354186.
E-mail address: rashidy-pour@sem-ums.ac.ir (A. Rashidy-Pour).

0018-506X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yhbeh.2012.10.003

and dynorphinergic actions in the hippocampus (Kouvelas et al., 2008; Magnusson et al., 2009).

Ample evidence from human and nonhuman animal studies has shown that exercise can improve cognitive functions in a variety of physiological and pathophysiological conditions (Anderson et al., 2000; Ang and Gomez-Pinilla, 2007; Ang et al., 2006; Baruch et al., 2004; Bekinschtein et al., 2011; Luo et al., 2007; Vaynman et al., 2004). Recent studies in animal models have investigated the biological mechanisms that underlie the beneficial effects of exercise. It is now clear that several neurotropic factors and neurotransmitters participate in exercise-induced cognitive benefits. Among various neurotropic factors, hippocampal BDNF plays a crucial role in learning and memory. Exercise enhances the level of hippocampal BDNF in mice or rats, and this effect is controlled by neuronal activity, neurotransmitters and interactions with peripheral factors, such as estrogen, corticosterone and possibly IGF-1 (Cotman and Berchtold, 2002). Inhibition of hippocampal BDNF has been shown to blunt the exercise-induced enhancement of learning and memory, which suggests a critical role for BDNF in mediating exercise effects on learning and memory (Vaynman et al., 2004).

Given the well-known beneficial effects of physical exercise on learning and memory, voluntary exercise may ameliorate learning and memory deficits induced by chronic ND. To address this issue, the present study was designed to examine the influence of exercise on the learning and memory impairing effects of chronic ND in rats. We also investigated effects of chronic ND and exercise on hippocampal levels of BDNF, a key molecule that links voluntary exercise with improvements in cognitive function (Vaynman et al., 2004).

Materials and methods

Animals and experimental groups

Adult male Wistar rats (210 ± 10 g) were individually housed in cages (50 × 26 × 25 cm) and kept on a 12-h light/dark cycle (6 am lights on—6 pm lights off) at 22–24 °C with food and water available *ad libitum*. All experiments were conducted between 8:30 and 12:00 h. The experimental protocol was approved by the research committee of Semnan University of Medical Sciences (Semnan, Iran). All of the experimental procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. In addition, care was taken to minimize the number of animals that were used in each experiment.

Experimental groups

The rats were randomly distributed into 4 experimental groups, and each group contained 8 rats: the vehicle-sedentary group (VEH/SED), the ND-sedentary group (ND/SED), the vehicle-exercise group (VEH/EXC), and the ND-exercise group (ND/EXC). The rats in both of the sedentary groups were not submitted to any type of physical activity. The ND-treated rats received a subcutaneous (s.c.) injection of ND (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) every third day (15 mg/kg, which is a supra-physiological dose), and the vehicle-treated groups received an s.c. injection of the vehicle (propylene glycol). The ND dosage and the treatment schedule were selected based on previous reports (Kouvelas et al., 2008; Magnusson et al., 2009). Vehicle or ND injections were started 14 days prior to the voluntary exercise and continued throughout the 15 days of voluntary exercise (10 injections over 29 days) (Fig. 1). The rats were weighed four times during the study.

Exercise paradigm

Each of the exercising rats was given access to a cage that was equipped with a running wheel (the diameter = 34.5 cm, and the width = 9.5 cm) that was freely rotated against a resistance of 100 g. Each wheel was equipped with a magnetic switch that was connected to a separate counter (located outside the animal house) that monitored the number of revolutions per hour. The number of revolutions for each wheel was recorded every day at 6 am. The sedentary rats were confined to similar cages without access to a wheel. After a 15-day period of exercise, the running wheels were removed from the cages, and the rats were trained and tested on the water maze task.

BDNF measurements

The BDNF protein levels were assessed using Rat BDNF ELISA kits (Boster Biological Technology Co., Wuhan, China) according to the manufacturer's recommendations. The hippocampal extracts were prepared in lysis buffer, and the homogenates were centrifuged to remove insoluble materials (12,500 × *g* for 20 min at 4 °C). The total protein concentration was determined according to the Micro BCA procedure (Pierce, Rockford, IL, USA). For the ELISA, 96 well flat-bottomed Immulon-2 plates were incubated overnight at 4 °C with carbonate coating buffer containing an anti-BDNF monoclonal antibody. The plates were blocked for 1 h with the block and the sample (B&S) buffer prior to incubation of the samples and the BDNF standards with shaking for 2 h at room temperature. A standard curve was established using serial dilutions of known amounts of BDNF that ranged from 0 to 500 pg/ml (diluted in B&S buffer). The plates were washed 3 times with TBS (20 mM Tris HCl, 150 mM NaCl, 0.05% v/v Tween 20) prior to a 1 h incubation (at room temperature) with a biotinylated anti-rat BDNF antibody. After the antibody incubation, the plates were washed three times with TBS and incubated for 1 h (at room temperature) with avidin–biotin–peroxidase complex (ABC). After the incubation, an ABC working solution was added to each well, incubated at room temperature for 30 min, and washed 5 times with TBS. Then, TMB color developing agent was added to each well and incubated for 30 min at room temperature. After the samples turned blue, the reaction was stopped by the addition of TMP stop solution, and the absorbance was measured at 450 nm using an automated ELISA plate reader. The sensitivity of the assay was <2 pg/ml.

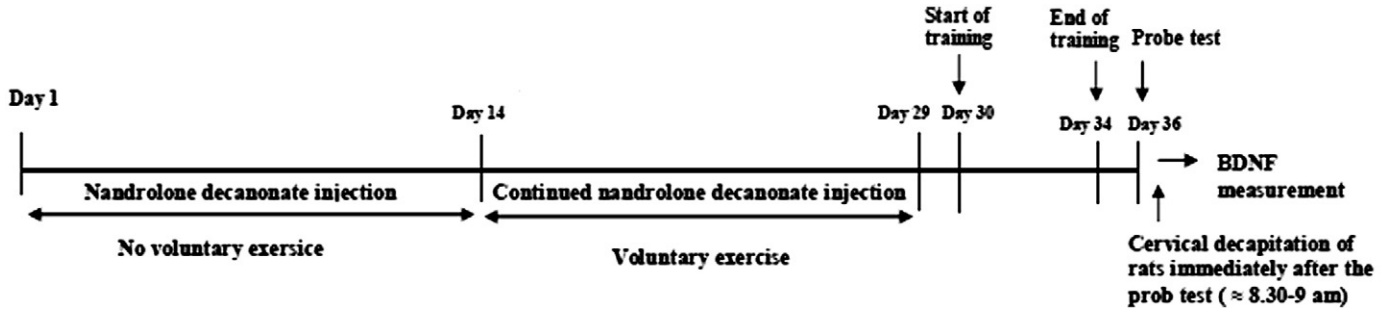

Fig. 1. Timeline of treatment, voluntary exercise and behavioral testing (see Materials and methods for details).