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Research report

Exercise modifies amphetamine relapse: Behavioral and oxidative markers in rats

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HIGHLIGHTS

- Rats developed preference and relapse to amphetamine (AMPH).
- AMPH increased intensified anxiety-like symptoms related to abstinence.
- AMPH increased oxidative damages and modified enzymes activity in hippocampus.
- Exercise reduced AMPH-relapse and anxiety-like symptoms after re-exposure to drug.
- Exercise increased enzymes activity and prevented oxidative damages in hippocampus.

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ABSTRACT

Exercise has been reported to attenuate rewarding symptoms related to addictive drugs mainly by affecting the brain neuroplasticity and neurotransmission. In this study, we investigated the influence of physical exercise on the behavioral and enzymatic status related to drug relapse in rats. Animals were primarily treated with amphetamine (AMPH; 4.0 mg/kg, i.p.) or vehicle (C; NaCl 0.9% solution) in the conditioned place preference (CPP) paradigm for 14 days. Half of each experimental group was then submitted to swimming sessions (60 min/day, 5 days/week) for 5 weeks. Animals were re-exposed to AMPH- or vehicle-CPP paradigm for another 3 days, in order to observe drug relapse and anxiety-like symptoms, which were observed 24 h after AMPH reconditioning in CPP, and elevated plus maze (EPM), respectively, and brain biochemical evaluations were carried out subsequently. While AMPH was related to place preference and anxiety, indicating drug addiction and abstinence symptoms, respectively, physical activity was able to prevent relapse symptoms after AMPH reconditioning, as observed through consecutive decreased CPP and anxiety-like symptoms. In addition, AMPH exposure increased reactive species (RS) generation and protein carbonyl (PC) levels together with decreased activity of catalase- and Na^+K^+-ATPase in hippocampus. On the other hand, while all AMPH-induced effects were prevented by physical activity, there was a negative correlation between PC levels ($r = 0.65$; $p < 0.003$) and CAT activity, and a positive correlation between RS generation and PC levels ($r = 0.54$; $r = 0.52$, $p < 0.05$) with AMPH–CPP after exercise. These results indicate that exercise has a clear beneficial influence on the prevention of psychostimulant drug relapse.

1. Introduction

Physical exercise has been associated with different health benefits, including increased self-esteem and well-being and reduced incidence of anxiety and depression [1]. Together, these factors are able to retard the progress of degenerative diseases, thus ameliorating brain plasticity and the cognitive process [2]. Recently, studies of our group have shown the beneficial influence of moderate exercise on movement disorders [3], anxiety-like symptoms and memory acquisition [4], besides modulating the dopamine transporter (DAT) activity in rat striatum [5]. So far, different studies have shown beneficial influences of exercise on the prevention and reversal of addiction [6], but few studies into relapse and abstinence symptoms of psychostimulants like AMPH have been performed.

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Drug dependence is a serious problem of public health for which treatment options are scarce, making physical exercise an economical and promising tool. Psychostimulant drugs have been widely used around the world and their effects include increased attention, motivation and well-being [7]. In this context, amphetamine-like (AMPH) drugs are psychostimulant agents clinically used to treat narcolepsy, morbid obesity [8], and attention deficit hyperactivity disorder (ADHD) [9]. On the other hand, AMPH-like drugs, represented by methamphetamine and methyllene-dioxy-methamphetamine (MDMA or "ecstasy") are illicit, whose recreational use has increased alarmingly, causing serious concerns due their association with positive reinforcement, tolerance and dependence [10].

Biochemically, AMPH is able to increase the neurotransmission in the central nervous system (CNS), facilitating processes of monoamine release and re-uptake, leading to increased extracellular levels of dopamine (DA), serotonin (5-HT) and nor epinephrine (NE) [11]. High doses or continued administration of AMPH is related to psychosis-like symptoms such as disordered thinking, anxiety and restlessness, besides stereotyped behavior [12]. On the other hand, the withdrawal of this substance is frequently related to loss of interest or pleasure in common life rewarding stimuli [13]. Experimentally, discontinuation of AMPH administration after its chronic use is related to anxiety-like and depressive behaviors. Even after long periods of abstinence, psychostimulant drugs are associated with high relapse rates upon re-exposure to drug [14], reinforcing its high addictive power. Indeed, relapses to psychostimulant drugs have been related to different factors, including drug priming, environmental cues and stress, which can be reflected on specific brain areas, including hippocampus [15], amygdala, orbit- and pre-frontal cortex, nucleus accumbens and pallidum. All these brain areas are essentially innervated by dopaminergic projections, whose changes involve adaptive conditions that occur in addiction [16]. Of particular importance, the hippocampus is primarily stimulated by monoamines such as dopamine (DA), whose release may be significantly increased by AMPH. In fact, this drug is able to partially inhibit monoamine oxidase (MAO) activity, contributing to accumulation of DA and other monoamines in the synaptic cleft. This excess of monoamines has been described as pivotal in drug rewarding symptoms, which are related to pleasure and euphoria, whereupon dopamine (DA) plays a critical role [17].

Conditioned place preference (CPP) is an animal model commonly used to study the reinforcement and relapse of addictive drugs such as AMPH [18]. In this model, animals are previously conditioned with an abuse drug in the non-preferred compartment of the apparatus. After some days of conditioning, this stimulus is used to estimate the preference and relapse for the drug, which may be related to reinforcement symptoms and dependence reinstatement [19]. For this, CPP paradigm was used to quantify the amphetamine-preference and relapse, while the elevated plus maze (EPM) was used to evaluate fear- and anxiety-like symptoms, which have been related to drug abstinence [18]. Thus, the present study was developed to evaluate the influence of chronic and moderate physical exercise on withdrawal and relapse symptoms after AMPH conditioning.

2. Methods

2.1. Animals and experimental procedure

Twenty adult male Wistar rats weighing 350 g ± 50 g, from the breeding facility of Universidade Federal de Santa Maria (UFSM), RS, Brazil, were kept in Plexiglas cages with free access to food and water in a room with controlled temperature (23 ± 1 °C) and on a 12 h light/dark cycle with lights on at 7:00 a.m., following international norms of animal care and maintenance.

2.2. Drugs and solutions

D,L-Amphetamine (Merck, Germany) 4 mg/mL, was used according to Kuhn et al. [18].

2.3. Conditioned place preference (CPP) paradigm

CPP is an animal model used to evaluate hedonic reinforcement effects of drug. It uses a three-compartment box separated by manual guillotine doors: two compartments of equal size (45 cm × 45 cm × 50 cm) with equivalent intensity of light but different visual clues: one with a white floor and striped walls, and the other with a striped floor and smooth white walls. These two compartments converge to a third smaller compartment. The apparatus was cleaned with alcohol 20% using wet sponge and paper towel before the introduction of each animal. On day 1, rats were kept for 15 min in each compartment for habituation; on day 2 we performed the pre-test, which consists in letting the animal freely choose one of the compartments for 15 min. On the next 14 days, animals were conditioned with D,L-amphetamine (4,0 mg/kg, i.p.) for 25 min in the non-preferred compartment, and with vehicle in the preferred compartment, with an interval of 4 h between each administration. After this conditioning phase, on the testing day, rats were placed in the common compartment with free access to both compartments. Time spent in the drug-paired environment was interpreted as preference, whereas time spent away from that environment was interpreted as aversion [18]. After 5 weeks of training (described below), the animals were submitted to more three days of AMPH reconditioning in CPP, which was followed of an additional test of drug preference as described above. Relapse symptoms were quantified by the higher time spent in the drug-paired environment in this second drug exposure (adapted from Cruz et al. [19])

2.4. Exercise protocol

After 14 days of drug conditioning, the exercise group were subjected to a swimming protocol in a plastic container (diameter 180 cm × depth 45 cm) under continuous supervision, with water temperature set to 29 ± 1 °C, for 1 h per day (two 30 min sessions with a 10 min rest interval), five times per week for 5 weeks (adapted from Teixeira et al. [5]). During the interval, the animals were kept on a platform that allowed them to rest without leaving the water (adapted from Teixeira et al. [5]).

2.5. Elevated plus maze (EPM)

To evaluate the influence of exercise on anxiety-like symptoms, animals were observed in the EPM, which is based on the innate fear rodents have for open and elevated spaces [20]. The apparatus consists in a platform elevated 50 cm from the floor. Forty-centimeter (40 cm) high walls enclose two opposite arms (50 cm × 10 cm) whereas the other two arms have no walls. All of the arms have a central intersection (10 cm × 10 cm). At the beginning of the test, the rat was placed in central intersection facing the open arm. Time spent in the closed arms, head dipping frequency in the open arms and entries number in both closed and open arms (number of total entries) were quantified for 5 min in the EPM.

2.6. Biochemical measurements

Twenty-four hours after behavioral evaluations, animals were anesthetized with sodium thiopental, 50 mg/kg, i.p.) and