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Treadmill running frequency on anxiety and hippocampal adenosine receptors density in adult and middle-aged rats

Marcelo S. Costa *, Ana Paula Ardais, Gabriela T. Fioreze, Sabrina Mioranzza, Paulo Henrique S. Botton, Luis Valmor Portela, Diogo O. Souza, Lisiane O. Porciúncula

Laboratory of Studies on the Purinergic System, Graduation Program in Biological Sciences/Biochemistry, Federal University of Rio Grande do Sul, Health and Basic Sciences Institute, Department of Biochemistry, Porto Alegre/RS 90035-003, Brazil

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ABSTRACT

Physical exercise protocols have varied widely across studies raising the question of whether there is an optimal intensity, duration and frequency that would produce maximal benefits in attenuating symptoms related to anxiety disorders. Although physical exercise causes modifications in neurotransmission systems, the involvement of neuromodulators such as adenosine has not been investigated after chronic exercise training. Anxiety-related behavior was assessed in the elevated plus-maze in adult and middle-aged rats submitted to 8 weeks of treadmill running 1, 3 or 7 days/week. The speed of running was weekly adjusted to maintain moderate intensity. The hippocampal adenosine A_1 and A_{2A} receptors densities were also assessed. Treadmill running protocol was efficient in increasing physical exercise capacity in adult and middle-aged rats. All frequencies of treadmill running equally decreased the time spent in the open arms in adult animals. Middle-aged treadmill control rats presented lower time spent in the open arms than adult treadmill control rats. However, treadmill running one day/week reversed this age effect. Adenosine A_1 receptor was not changed between groups, but treadmill running counteracted the age-related increase in adenosine A_{2A} receptors. Although treadmill running, independent from frequency, triggered anxiety in adult rats and treadmill running one day/week reversed the age-related anxiety, no consistent relationship was found with hippocampal adenosine receptors densities. Thus, our data suggest that as a complementary therapy in the management of mental disturbances, the frequency and intensity of physical exercise should be taken into account according to age. Besides, this is the first study reporting the modulation of adenosine receptors after chronic physical exercise, which could be important to prevent neurological disorders associated to increase in adenosine A_{2A} receptors.

1. Introduction

Over the past years, a wide range of studies have shown that increased physical activity or regular physical exercise promote positive effects on brain functions (Cotman and Berchtold, 2002; Dishman et al., 2006; Gillum and Obisesan, 2010; Kramer et al., 1999; Rolland et al., 2010). Otherwise, the sedentary lifestyle has been correlated with a higher risk for development of obesity, cardiovascular diseases, type 2 diabetes, osteoporosis, cancer and depression (Roberts and Barnard, 2005).

The prevalence of mental health disorders among the elderly is often unrecognized, but it has been estimated that one in four older adults presents symptoms of depression, anxiety disorders or other psychiatric disorders. Mental health disorders are frequently comorbid in older adults, occurring with a number of common chronic diseases (Fiske et al., 2009; Flint, 2005). In this scenario, the prevalence of anxiety disorders is estimated to be as high as 25% (Craske et al., 2009; Hettema et al., 2001).

Adenosine is a neuromodulator in the central nervous system (CNS), which via mainly adenosine A_1 and A_{2A} metabotropic receptors control synaptic transmission of many neurotransmitters, such as glutamate and dopamine. Adenosine A_1 receptors are expressed throughout the brain while adenosine A_{2A} is more restricted to the basal ganglia (Cunha, 2001). The physical exercise influences the central dopaminergic, noradrenergic and serotonergic systems (Meeusen and De Meirleir, 1995). In a previous study, the participation of adenosine receptors in physical activity was reported, in which caffeine increased treadmill running time and spontaneous locomotor activity on the open field task, whereas the adenosine A_1/A_{2A} receptor agonist, 5′-N-ethylcarboxamidoadenosine (NECA) decreased it (Davis et al., 2003). However, the effects of chronic physical exercise on this important neuromodulator were not investigated yet.

Pharmacological approaches and genetic deletion of adenosine A_1 and A_{2A} receptors revealed that both receptors are implicated in the

Abbreviation: VO_{2max}, maximum oxygen uptake.

* Corresponding author at: Federal University of Rio Grande do Sul, Department of Biochemistry, Rua Ramiro Barcelos, 2600, anexo, Porto Alegre/RS 90035-003, Brazil. Tel.: +55 51 3308 5557; fax: +55 51 3308 5540.
E-mail address: marsilcos@yahoo.com.br (M.S. Costa).

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etiology of anxiety (for review see Cunha et al., 2008). The knockout mice for adenosine A_1 as well as for adenosine A_{2A} receptor display an exacerbation of anxiety-related behavior (Giménez-Llort et al., 2002; Johansson et al., 2001; Lang et al., 2003; Ledent et al., 1997). In addition, anxiogenic effects of caffeine, a recognized nonselective adenosine A_1 and A_{2A} receptor antagonist, are demonstrated in a wide range of rodent studies (File et al., 1988; Florio et al., 1998; Jain et al., 1995).

According to epidemiological studies, the physical activity can attenuate anxiety-related symptoms (Bhui and Fletcher, 2000; Dunn et al., 2001; Goodwin, 2003; Lampinen et al., 2000; Motl et al., 2004), being applied as a non-pharmacological intervention in patients, mainly in older adults (Kligman and Pepin, 1992). However, studies evaluating effects of exercise on anxiety-related behavior in rodents are more controversial, ranging from a reduction on anxiety (Greenwood et al., 2003), no effect (Pietropaolo et al., 2008), to even more anxiety after voluntary exercise (Burghardt et al., 2004; Leasure and Jones, 2008; Van Hoomissen et al., 2004).

Treadmill running is widely used by humans (Winter et al., 2007) because all variables implicated in physical training programs necessary to achieve great results can be controlled. Indeed, the frequency of the physical exercise is often controlled for maximizing the training and for avoiding negative effects of overtraining (ACSM, 1998). Nonetheless, effects of physical exercise on the anxiety-related behavior usually do not take into account the frequency of sessions and the intensity throughout training, which in turn can contribute for inconclusive results.

In this study, the effects of frequency of physical exercise were evaluated on anxiety-related behavior in adult and middle-aged rats for better understanding its effects on mental health. Considering the involvement of the adenosine receptors in anxiety disorders and its important role as a neuromodulator in the CNS, the density of adenosine A_1 and A_{2A} receptors was assessed in the hippocampus from adult and middle-aged rats trained on the treadmill running 1, 3 or 7 days/week during 8 weeks at moderate intensity. The hippocampus is a brain structure important in processes involving learning and memory, but its participation on anxiety has also been evidenced in other studies (Barkus et al., 2010; Oler et al., 2010; Sapolski et al., 1990; Xiang et al., 2011).

This schedule of exercise training (1, 3 and 7 days per week, 20 min per session at moderate intensity, for 8 weeks) was chosen to mimic exercise conditions in humans (ACSM, 1998; 2009). Thus, in this study we analyzed the effects of low and high exercise frequencies. Moreover, 8 weeks of training is considered sufficiently chronic to promote health-related adaptations.

2. Materials and methods

2.1. Animals

Adult (3 month-old) and middle-aged (14–16 month-old) male Wistar rats were used. The animals were housed into plastic cages under a light/dark cycle (lights on at 7:00 a.m.), with tap water and food ad libitum and kept at 23 °C. All procedures were carried out according to NIH Guide for Care and Use of Laboratory Animals, and Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care. This work was approved by the ethical committee of Federal University of Rio Grande do Sul. All animals were killed by decapitation after behavioral analysis.

2.2. Treadmill exercise protocol

The treadmill exercise protocol was started from 8 to 10 a.m. Prior to the exercise training, animals were habituated in the treadmill apparatus for rat (INBRAMED TK 01, Porto Alegre, Brazil) during one week, in order to minimize novelty-induced stress. They were randomly assigned to eight experimental groups according to age (adult and middle-aged rats): treadmill controls (0); treadmill running 1 day/week (1); treadmill running 3 days/week (3), and treadmill running 7 days/week (7).

At the first day of the second week, an incremental test in the treadmill was performed to indirectly determine the maximal oxygen uptake (VO_2max) as recommended by Brooks and White (1978). This test was to determine the running speed during the training period. Briefly, animals were placed in the treadmill to run during 25 min at a low initial speed. The speed was increased by 5 m/min each 3 min up to exhaustion of the animal. Exhaustion was achieved when animals could not respond to the increase of speed. The time to fatigue (in minutes) and workload (expressed by velocity in m/min) were taken as indexes of capacity for exercise, and as a measure of VO_2max. This measure was used to control the exercise intensity during the physical training program. The incremental test was repeated in the seventh week of treadmill running training to assess the efficacy of this protocol. The intensity of the physical training protocol was adapted for each animal and it never surpassed 60–75% of the respective maximum oxygen uptake. The exercise training protocol consisted of treadmill running during 8 weeks. Each training session lasted 20 min because great improvements in physical fitness were achieved at this time (ACSM, 1998). The velocity of treadmill was weekly increased for all treadmill groups. The treadmill controls (0) were always habituated to the experimental room and handled as treadmill runners but they were not submitted to the forced running protocol (adapted from Scopel et al., 2006). The treadmill controls were placed in the treadmill off 3 days/week during 20 min. Then, animals returned to their home cages. All groups were handled every day to avoid differences in handling in the elevated plus-maze. Importantly, in the frequency of 3 days/week the animals were not submitted to the treadmill running for 3 consecutive days. There was an interval of one day between exercise days. Three days/week for control groups was chosen because this was the intermediate frequency among groups.

The velocity of running at the beginning of training was 10 m/min and 7 m/min for adult rats and middle-aged rats, respectively. This velocity was weekly increased, and at the end of training reached 20 m/min and 12 m/min for adult and middle-aged rats, respectively. This procedure was essential to keep the intensity constant in 60–75% of the maximum oxygen uptake along the 8 weeks of treadmill running training.

2.3. Elevated plus-maze

The analysis in the elevated plus-maze took place during the early light portion of the light/dark cycle. All groups were exposed to the maze on Tuesday, 48 h after the last Sunday running session for all frequencies of exercise training. This time period between the last treadmill running session and analysis in the elevated plus-maze was chosen to avoid acute exercise-related stress (Contarteze et al., 2008), which could influence the effects of treadmill running training for 8 weeks. The apparatus was made of wood covered with a layer of black formica and had four elevated arms (52 cm from the floor) 50 cm long and 10 cm wide. The arms were arranged in a cross-like disposition, with two opposite arms being enclosed (by 40 cm high walls) and two being open, having at their intersection a central platform (10×13.5 cm) that gave access to any of the four arms. The open arms were surrounded by a raised ledge (1 mm thick and 5 mm high). The platform was under dim light (70 lx). Each rat was placed in the central platform facing an open arm and the following observations were manually recorded during 5 min by two observers blind to the experimental groups. Each animal was tested for 5 min. Four variables were measured: (1) time spent in the open arms; (2) number of entries into the open arms; (4) number of entries into the closed arms; and (4) number of rearing. The rat was considered to have entered or spent time in an arm only when all four paws were in the respective arm.