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Treadmill exercise induces age-related changes in aversive memory, neuroinflammatory and epigenetic processes in the rat hippocampus

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ABSTRACT

It has been described that exercise can modulate both inflammatory response and epigenetic modifications, although the effect of exercise on these parameters during the normal brain aging process yet remains poorly understood. Here, we investigated the effect of aging and treadmill exercise on inflammatory and epigenetic parameters specifically pro and anti-inflammatory cytokines levels, activation of NF-kB and histone H4 acetylation levels in hippocampus from Wistar rats. Additionally, we evaluated aversive memory through inhibitory avoidance task. Rats of 3 and 20 months of age were assigned to non-exercised (sedentary) and exercised (running daily for 20 min for 2 weeks) groups. The effect of daily forced exercise in the treadmill was assessed. The levels of inflammatory and epigenetic parameters were determined 1 h, 18 h, 3 days or 7 days after the last training session of exercise. It was observed an age-related decline on aversive memory, as well as aged rats showed increased hippocampal levels of inflammatory markers, such as TNFα, IL1-β and NF-kB and decreased IL-4 levels, an anti-inflammatory cytokine. Moreover, lower levels of global histone H4 acetylation were also observed in hippocampi from aged rats. Interestingly, there was a significant correlation between the biochemical markers and the inhibitory avoidance test performance. The forced exercise protocol ameliorated aging-related memory decline, decreased pro-inflammatory markers and increased histone H4 acetylation levels in hippocampi 20-months-old rats, while increased acutely IL-4 levels in hippocampi from young adult rats. Together, these results suggest that an imbalance of inflammatory markers might be involved to the aging-related aversive memory impairment. Additionally, our exercise protocol may reverse aging-related memory decline through improving cytokine profile.

1. Introduction

Several studies have pointed out that both normal aging process and neurodegenerative diseases are associated with chronic neuroinflammatory response (de Magalhaes, Curado, & Church, 2009; Lynch, 2010; Salminen et al., 2008). It has been suggested that alterations on pro-inflammatory cytokines levels, such as interleukin-1β (IL-1β) and tumor-necrosis factor alpha (TNF-α), and anti-inflammatory cytokines, such as interleukin-4 (IL-4) and interleukin-10 (IL-10), may contribute to aged-related decline of brain functions (Buchanan, Sparkman, Chen, & Johnson, 2008; Griffin et al., 2006; Lynch, 2010; Nolan et al., 2005; O'Donnell et al., 2000).

Interestingly, a great body of evidences has shown that cytokines can alter memory formation and synaptic plasticity, where the both the over-expression and absence of IL-1β and TNF-a may directly influence the long-term potentiation (LTP) maintenance, an animal model for learning and memory. However, the involvement the inflammatory cytokines on hippocampal-dependent learning and memory behavioral tasks; i.e., spatial memory, object recognition and contextual fear conditioning has been poorly studied. There are no studies, to our knowledge, reporting the association of inflammatory cytokines with aversive memory formation in the aging process.

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The neuroinflammation is a dynamic process subject to aging process and several factors, including physical activity, seem to modulate inflammatory signaling (Wannamethee et al., 2002). Treadmill exercise can decrease TNF-α and IL-1β levels in different brain regions from healthy young rodents (Ang, Wong, Moochhala, & Ng, 2004; Chennaoui, Drogou, & Gomez-Merino, 2008; Funk et al., 2011), however Ding et al. (2005) showed controversial results. We recently demonstrated that the treadmill exercise alters some neuroinflammatory parameters, such as cyclooxygenase-2 (COX-2), prostaglandin E2 and E-prostanoid receptors levels in young adult rats (Lovatel et al., 2012). Besides, the aversive memory enhancing effects by exercise seems be related to higher COX-2 (Lovatel et al., 2012).

In addition, the aging process has been associated with increased activation of the nuclear factor-kappaB (NF-kB), an inducible transcription factor complex that regulates the expression of inflammatory molecules (Baeuerle & Baichwal, 1997; Manning & Anderson, 1994). NF-kB activation is commonly short-lived, but this pattern may be different during the aging process (Yu & Chung, 2006). Interestingly, the NF-κB-signaling pathway may be regulated by histone acetylation, an important epigenetic mechanism that can control the expression of specific genes with opposite directions depending on the cell type (Chen, Fischle, Verdin, & Greene, 2001; Jenuwein & Allis, 2001; Viatour et al., 2003; Yamamoto, Verma, Prajapati, Kwak, & Gaynor, 2003). There are evidences indicating that histone acetylation status is implicated with the inflammatory responses. However, these findings were mostly based in hematopoietic cell, monocytes and macrophages (Bode et al., 2007; Rahman, Marwick, & Kirkham, 2004; Roger et al., 2011). However, studies reporting the relationship between NF-kB or histone acetylation status and neuroinflammation cytokines during aging process are lack.

It is important to note that histone acetylation is controlled by histone acetyltransferases (HAT) and histone deacetylases (HDAC) enzymes (Choi & Howe, 2009; Kouzarides, 2007). It has been widely recognized that histone acetylation is associated with enhanced transcriptional activity whereas deacetylation is typically associated with transcriptional repression (Kimura, Matsubara, & Horikoshi, 2005; Kouzarides, 2007). Interestingly, several groups have demonstrated that memory formation is associated with increased levels of histone acetylation (Barrett & Wood, 2008; Mikaelsson & Miller, 2011). Evidences have indicated that histone deacetylase inhibitors (HDACi) are able to improve memory performance. Studies demonstrated that administration of HDACi improved memory in object recognition (Reolon et al., 2011), conditioned context (Vecsey et al., 2007), LTP (Levenson et al., 2004) and water maze (Peleg et al., 2010; Ricobaraza, Cuadrado-Tejedor, & Garcia-Osta, 2011; Ricobaraza et al., 2009). We previously showed that a single session of treadmill exercise increased HAT activity, in addition decreased HDAC activity in hippocampi from young adult rats, suggesting that this exercise protocol can induce histone acetylation (Elsner et al., 2011). Accordantly, several studies have demonstrated that exercise improves performance in different memory and learning tasks in aged and young rodents (Berchtold, Castello, & Cotmana, 2010; Radak et al., 2006; van Praag, Shubert, Zhao, & Gage, 2005). Despite these findings, to our knowledge there are no studies reporting the impact of exercise on global histone acetylation in rat brain during normal aging process.

Our working hypothesis was that memory behavioral tasks performance is correlated to neuroinflammation and epigenetic markers, as well forced exercise would be able to reverse the aging effects. Thus, the aim of this study was to investigate the effects of treadmill exercise protocol (20 min/day during 2 weeks) on TNFα, IL-1β, IL-4, NF-kB and histone H4 acetylation levels in hippocampi from 3 and 20-months-old Wistar rats. Moreover, we also investigated the time course of the exercise effects, specifically, 1 h, 18 h, 3 days and 7 days after the last session of treadmill exercise.

2. Methods

2.1. Animals

Male Wistar rats of different ages, 3 and 20-months-old were used. The animals were provided by Centro de Reprodução de Animais de Laboratório (CREAL) at Universidade Federal do Rio Grande do Sul (UFRGS) and were maintained under standard conditions (12-h light/dark, 22 ± 2 °C) with food and water *ad libitum*. The NIH "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 80-23, revised 1996) was followed in all experiments. The Local Ethics Committee (CEUA de Ética em Pesquisa – UFRGS) approved all handling and experimental conditions (nr. 21449).

2.2. Training

Rats were randomly divided into sedentary (SED) or exercised (EXE) groups. EXE were submitted to exercise protocol consisted on 20 min running session each day for 2 weeks (Fig. 1). SED was handled exactly as the experimental animals and was left on the treadmill for 5 min without any stimulus to run. The exercise training consisted of running sessions in an adapted motorized rodent treadmill (INBRAMED TK 01, Porto Alegre, Brazil), with individual Plexiglas lanes, at 60% of the animals maximal oxygen uptake (Brooks & White, 1978). Peak oxygen uptake (VO_2) was measured indirectly in all animals before training. Each rat ran on a treadmill at a low initial speed with the speed being increased by 5 m/min every 3 min until the point of exhaustion (i.e., failure of the rat to continue running). The time to fatigue (in min) and workload (in m/min) were taken as indexes of exercise capacity, which was in turn taken as VO_2 max (Arida, Scorza, dos Santos, Peres, & Cavalheiro, 1999; Brooks & White, 1978; Cechetti et al., 2007, 2008; Elsner et al., 2011; Scopel et al., 2006). Rats of 3-months-old were adapted to the treadmill by running, in the first few sessions, at 6.7 m/min for the first 2 min, 10 m/min for the next 4 min, 15 m/min for 8 min, 10 m/min for 4 min and 6.7 m/min for the last 2 min. Thereafter, animals ran at 6.7 m/min for the first 4 min, 15 m/min for 12 min and 6.7 m/min for the last 4 min. Rats of 20-months-old were adapted to the treadmill by running, in the first few sessions, at 4.2 m/min for the first 2 min, 6.3 m/min for the next 4 min, 9.5 m/min for 8 min, 6.3 m/min for 4 min and 4.2 m/min for the last 2 min. Thereafter, animals ran at 4.2 m/min for the first 4 min, 9.5 m/min for 12 min and 4.2 m/min for the last 4 min. Animals that initially refused to run were encouraged by gently tapping their backs. Neither electric shock nor physical prodding was used in this study and all the procedures took place between 14:00 and 17:00 h.

2.3. Inhibitory avoidance test

The inhibitory avoidance apparatus was consisted on 50 × 25 × 25 cm acrylic box (Albarsch, Porto Alegre, Brazil) whose floor consists of parallel caliber stainless steel bars (1 mm diameter) spaced 1 cm apart. A 7 cm wide, 2.5 cm high platform was placed on the floor of the box against the left wall. We used the single-trial step-down inhibitory avoidance conditioning as an established model of fear-motivated memory. At step-down inhibitory avoidance training, animals learn to associate a location in the training apparatus (a grid floor) with an aversive stimulus (footshock). The general procedures for inhibitory avoidance behavioral