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Short report

Exercise induces age-dependent changes on epigenetic parameters in rat hippocampus: A preliminary study

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ABSTRACT

Regular exercise improves learning and memory, including during aging process. Interestingly, the imbalance of epigenetic mechanisms has been linked to age-related cognitive deficits. However, studies about epigenetic alterations after exercise during the aging process are rare. In this preliminary study we investigated the effect of aging and exercise on DNA methyltransferases (DNMT1 and DNMT3b) and H3-K9 methylation levels in hippocampus from 3 and 20-months aged Wistar rats. The animals were submitted to two exercise protocols: single session or chronic treadmill protocol. DNMT1 and H3-K9 methylation levels were decreased in hippocampus from aged rats. The single exercise session decreased both DNMT3b and DNMT1 levels in young adult rats, without any effect in the aged group. Both exercise protocols reduced H3-K9 methylation levels in young adult rats, while the single session reversed the changes on H3-K9 methylation levels induced by aging. Together, these results suggest that an imbalance on DNMTs and H3-K9 methylation levels might be linked to the brain aging process and that the outcome to exercise seems to vary through lifespan.

1. Introduction

Recently, epigenetic mechanisms have been linked to normal aging-related changes in the brain, as well as neuropsychiatric and neurodegenerative diseases (Saha and Pahan, 2006). Epigenetic typically involves modifications in the micro and macrostructure of chromatin, DNA and nuclear proteins, particularly histones, which can modulate the transcriptional machinery and allow long lasting modifications in the genome. DNA and histone methylation, in addition to histone acetylation, are the most extensively studied post-translational modifications, which can influence gene transcription (Kouzarides, 2007).

The histones can be methylated on either lysine (K) or arginine (R)-residues by histone methyltransferases. Site-specific methylation of amino acid residues can condensate or relax the chromatin structure, such as mono-methylation of histone H3 at K9 (H3-K9) is associated to transcriptional activation, whereas transcriptionally silent regions contain di- and tri-methylation of H3-K9 (Gupta et al., 2010). The impact of aging process and exercise on H3-K9 methylation is poorly exploited. While, DNA methylation is catalyzed by a group of enzymes called DNA methyltransferases (DNMTs), DNMT1, DNMT2, DNMT3a, and DNMT3b that transfer the methyl group from the donor S-adenosylmethionine (SAM) to 5′ position of the cytosine pyramidal ring. This process usually represses the gene transcription. DNMT1 is primarily involved in maintenance of DNA methylation after replication, while DNMT3a and DNMT3b are particularly important for *de novo* methylation (Reik et al., 1999). It has been described a genome-wide tendency to DNA hypomethylation in multiple vertebrate organs during aging process (Richardson, 2002; Wilson et al., 1987). In addition, the age-related global hypomethylation is related to DNMT1 deficits in senescent human fibroblasts (Lopatina et al., 2002). However, studies reporting DNMT content in the brain during aging process are lacking.

Interestingly, epigenetic mechanisms have been linked to the age-related cognitive decline, since histone deacetylase (HDAC) inhibitors have been shown to improve memory in aged rodents (Levenson and Sweatt, 2005; Reolon et al., 2011). Accordantly, some evidences demonstrated that exercise ameliorates aging-related cognitive function

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in rodents (Pietrelli et al., 2012; Radak et al., 2001), in addition, recent findings have demonstrated that the exercise was able to modulate the histone acetylation status, enhancing transcription of genes related to brain function (Elsner et al., 2011; Gomez-Pinilla et al., 2011).

Considering that exercise restores the age-related memory deficits and epigenetic mechanisms which may be related to protective effects of exercise, it is crucial to assess the modulation of exercise on epigenetic parameters in the normal aging process. Therefore, the aim of this investigation was to study the effect of aging and two treadmill exercise protocols, single session of treadmill or chronic treadmill protocol on methylation parameters, specifically, DNA methyltransferases 1 and 3b (DNMT1and DNMT3b) and histone H3 lysine 9 (H3-K9) methylation levels.

2. Material and methods

2.1. Animals and training

Male Wistar rats of different ages, 3 and 20-months-old were used. The animals 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" (No. 80-23, revised 1996) was followed in all experiments. The Local Ethics Committee (CEUA/UFRGS) approved all handling and experimental conditions (nr. 21449).

Rats were randomly divided into sedentary (SED) or exercised group (EXE). The exercise training consisted of running sessions in an adapted motorized rodent treadmill (INBRAMED TK 01, Porto Alegre, Brazil) at 60% of the animals' maximal oxygen uptake (Brooks and White, 1978). Peak oxygen uptake (VO_2) was measured indirectly in all animals before training (Arida et al., 1999; Brooks and White, 1978; Elsner et al., 2011). Two exercise protocols were employed: a single session of treadmill exercise (20 min) and chronic treadmill protocol (20 min running session each day for 2 weeks). SED was handled exactly as the experimental animals and was left on the treadmill for 5 min without any stimulus to run. All the procedures took place between 14:00 and 17:00 h.

2.2. Preparation of samples

In order to verify the acute and delayed effects of exercise, rats were decapitated 1 h and 18 h after a single session or after the last training session of chronic treadmill exercise. The whole hippocampi were quickly dissected out and immediately snap-frozen in liquid nitrogen, and stored at −80 °C. In the day of the assay, the whole hippocampi were homogenized in 1:3 volumes of specific ice-cold lysis buffer.

2.3. Determination of DNMT1, DNMT3b and methylation of histone H3 lysine 9 (H3-K9) levels

Specific ELISA Assay Kits (Colorimetric Detection, catalog #P-3011, #P-3013, #P-3018, respectively, *EpiQuik*®) were used. All the procedures were done according to the manufacturer's instructions. The Pierce BCA Protein Assay kit ® was used to determine the protein concentration.

2.4. Statistical analysis

All results were expressed as the percent of control (mean± S.E.M.). The results were analyzed by Three-Way Analysis of Variance (ANOVA) with age, exercise and time points after the exercise as factors followed by post hoc Tukey's multiple range test when appropriate. The influence of age on epigenetic markers was evaluated by Student's *t*-test. In all tests, $p<0.05$ was considered.

3. Results

3.1. Aging process reduces epigenetic markers in rat hippocampus

Firstly, we evaluated the effect of the aging process on histone methylation levels. 20-months-old rats hippocampi displayed lower histone H3-K9 methylation levels (about 50%) compared to the young adult group (Fig. 1; $p=0.0002$). It was also observed that DNMT1 levels were significantly diminished (about 25%) in the aged group (Fig. 1; $p=0.009$). The level of DNMT3b enzyme was not modified by age.

3.2. Exercise decreased DNMT3b and DNMT1 levels in hippocampi from young adult rats

The DNMT3b levels after single session of exercise are illustrated in Fig. 2A. Three-way ANOVA revealed significant effect of the exercise factor ($F_{(1,39)}=5.845$; $p=0.021$), without any effect of age. When measured 1 h after session ended, young adult exercised rats exhibited lower levels of DNMT3b (about 30%) when compared to its sedentary group ($p=0.042$), while no delayed (18 h) effects of exercise were observed. The DNMT3b levels were not modified by the chronic exercise regimen in all groups (data not shown). Three-way ANOVA showed the effects of both factors, single session of exercise ($F_{(1,39)}=29.505$; $p<0.001$) and age ($F_{(1,39)}=10.073$; $p=0.003$), on DNMT1 levels (Fig. 2B). In addition, there was a significant interaction between age and exercise factors ($F_{(1,39)}=7.302$; $p=0.011$). This exercise protocol diminished acutely DNMT1 levels in hippocampi from 3 months-aged rats (approximately 45%; $p<0.001$), without any change on this parameter 18 h after exercise. There was no significant effect of chronic exercise protocol on DNMT1 levels in both young adult and aged groups (data not shown).

3.3. Exercise affects differently hippocampal H3-K9 methylation levels in young adult and aged rats

Fig. 3 shows H3-K9 methylation levels in both 3 and 20-months-aged rat hippocampus at different time-points. Three-way ANOVA indicated effect of age ($F_{(1,39)}=11.818$; $p=0.002$) and a significant interaction between age and exercise factors (Fig. 3A; $F_{(1,39)}=42.165$; $p<0.0001$). This exercise protocol diminished acutely and delayed H3-K9 methylation levels in young adult hippocampi (about 50%, $p=0.006$ and 60%, $p<0.0001$, respectively). Differently, hippocampi from 20-months-old rats have higher H3-K9 methylation levels 1 h and 18 h after single exercise session (respectively, about 30 and 100%; $p=0.024$ and $p<0.001$).

Additionally, it was observed the effect of both factors, chronic exercise (Fig. 3B; $F_{(1,39)}=7.431$; $p=0.011$), and age ($F_{(1,39)}=10.709$, $p=0.003$), in addition to an interaction between the factors

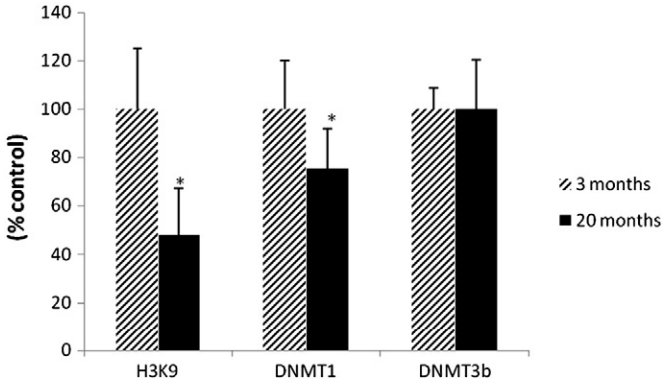

Fig. 1. Effects of aging process on methylation parameters in rat hippocampus. Results are expressed as percentage of young adult group and columns represent mean±S.D. (n=9–12). Student *t*-test, * = different as compared to control group.