Cell Mol Neurobiol (2014) 34:1097–1101
DOI 10.1007/s10571-014-0096-z

SHORT COMMUNICATION

Treadmill Exercise Alters Histone Acetyltransferases and Histone Deacetylases Activities in Frontal Cortices from Wistar Rats

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Received: 23 June 2014 / Accepted: 2 August 2014 / Published online: 24 August 2014

Abstract Studies have pointed out the relationship between neuroprotective exercise effects and epigenetic mechanisms on the hippocampus. Considering the role of frontal cortex on brain functions, we investigated the impact of different exercise protocols on enzymatic system involved with histone acetylation status, histone acetyltransferases (HATs), and histone desacetylases (HDACs) in frontal cortices from Wistar rats. Male Wistar rats aged 3 months were submitted to a single session or a daily running protocol during 2 weeks. The single session enhanced HAT activity, while the moderate daily exercise protocol reduced the HDAC activity. Our results indicate that frontal cortex is susceptible to epigenetic modulation following exercise and that both exercise protocols seem to induce a histone hyperacetylation condition in this brain area.

Keywords Forced exercise · Treadmill · Wistar rats · Frontal cortices · Histone acetyltransferases · Histone deacetylases

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Introduction

Several evidences have pointed out a central role for epigenetic mechanisms, such as DNA methylation, histone modifications, and non-coding RNA expression on gene expression (Kouzarides 2007). Moreover, epigenetic marks have been modulated by environmental factors like exercise, diet, maternal care, and xenobiotic exposure (Szyf et al. 2008; Stankiewicz et al. 2013; Ntanasis-Stathopoulos et al. 2013; Pareja-Galeano et al. 2014). Interestingly, although epigenetic modulation was firstly linked to permanent changes through life of the organism, recent works have indicated that these markers can be fast (minutes) and transiently (less than 24 h) modified (Miller and Sweatt 2008).

Histone acetylation is widely associated with enhanced transcriptional activity (Kimura et al. 2005; Choi and Howe 2009), whereas deacetylation is usually linked with transcriptional repression (Kouzarides 2007; Kimura et al. 2005; Choi and Howe 2009). Histone acetyltransferases (HATs) and histone desacetylases (HDACs), respectively, add and remove the acetyl groups of lysine residues from amino-terminal tails of histones (Strahl and Allis 2000; Wade 2001; McKinsey et al. 2001). It is important to note that the balance between HAT and HDAC activities has been associated with

acetylation and cellular homeostasis through its role on transcriptional activation (Saha and Pahan 2006).

Interestingly, the histone hyperacetylation status observed following exercise seems to be related to improved brain functions, although these findings have been focused only in hippocampus (Elsner et al. 2011; Gomez-Pinilla et al. 2011; Collins et al. 2009; Lovatel et al. 2013).

Gomez-Pinilla and colleagues (2011) showed that exercise induces hipocampal acetylation of histone H3 in the BDNF promoter IV in rats. In addition, a single session of exercise (20 min) in treadmill increased HAT activity and decreased HDAC activity, immediately and 1 h after the exercise in young adult rat hippocampus, without any effect of daily protocol or at long-term (Elsner et al. 2011). It was also demonstrated that a moderate daily protocol (20 min/day during 2 weeks) improved transitorily global histone 4 (H4) acetylation in hippocampi from 20-months-old rats, which was positively correlated with the inhibitory avoidance aversive memory performance (Lovatel et al. 2013). Taken together, these findings support the idea that exercise might induce histone acetylation in hippocampus (Elsner et al. 2011), showing also the time window of exercise effects.

Even though several evidences have demonstrated the effect of exercise on different brain areas such as cerebral cortex (Magistretti and Pellerin 1996; Vissing et al. 1996; Huang et al. 2012), there are no studies, to our knowledge, reporting the impact of exercise on epigenetic modulation in this brain area. It is remarkable to note that the frontal cortex has a pivotal role in high-order cognitive functions such as decision-making, attention, and working memory (Liston et al. 2006).

Therefore, our aim was to investigate the acute effects, immediately and 1 h after two treadmill exercise protocols, single session or moderate daily treadmill protocol, specifically HAT and HDAC enzyme activities, in frontal cortices from young adult Wistar rats.

Materials and Methods

Animals

Male Wistar rats aged 3 months were used. The animals were maintained under standard conditions (12 h light/dark, 22 ± 2 °C) with food and water ad libitum and housed five per cage. 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 (Comitê de Ética em Pesquisa-UFRGS) approved all handling and experimental conditions (number. 13001).

Training

Rats were randomly divided into sedentary (SED) or exercised (EXE) groups. SED was handled exactly as the experimental animals, and left on the treadmill for 5 min without any stimulus to run. The exercise training consisted of running sessions on an adapted motorized rodent treadmill (INBRAMED TK 01, Porto Alegre, Brazil), with individual Plexiglas lanes, at 60 % of the animals' maximal oxygen uptake (Brooks and White 1978). Peak oxygen uptake ($V'O_2$) was measured indirectly in all animals before training. Each rat ran on a treadmill at a low initial speed, which was increased by 5 m/min every 3 min until the point of exhaustion (i.e. the rat stopped 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 $V'O_2$ max (Brooks & White 1978; Cechetti et al. 2007, 2008; Elsner et al. 2011; Lovatel et al. 2013). The animals which initially refused to run were encouraged by gently tapping their backs. Neither electric shock nor physical prodding was used in this study. These procedures took place between 2:00 and 5:00 p.m.

The exercise consisted of running sessions at 60 % of $V'O_2$ max (Brooks and White 1978). Rats were subjected to a single session of treadmill running or daily running for 2 weeks. In the single session of treadmill exercise, animals were subjected to a single 20-min session 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. The moderate daily treadmill protocol consisted of one 20 min running session each day for 2 weeks. In the first two sessions, rats were adapted to the treadmill by running 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.

Preparation of Samples

Rats were decapitated immediately and 1 h after the single session or after the last training session of daily protocol. The frontal cortices were quickly dissected out and immediately snap-frozen in liquid nitrogen, stored at −80 °C until the determination of HDAC and HAT enzyme activity. On the day of the assay, frontal cortex was homogenized in three volumes of ice-cold lysis buffer pH 7.4 containing (in mM): 250 sucrose; 20 Tris–HCl; 1 EDTA; 1 EGTA; 10 KCl; 1 DTT; 0.1 PMSF; 0.0001 okadaic acid). The lysates were centrifugated (16,000*g* for 5 min) at 4 °C in a microcentrifuge tube, and the supernatant was removed for analysis. The protein concentration was measured by the Coomassie Blue method using bovine serum albumin as standard (Bradford 1976).

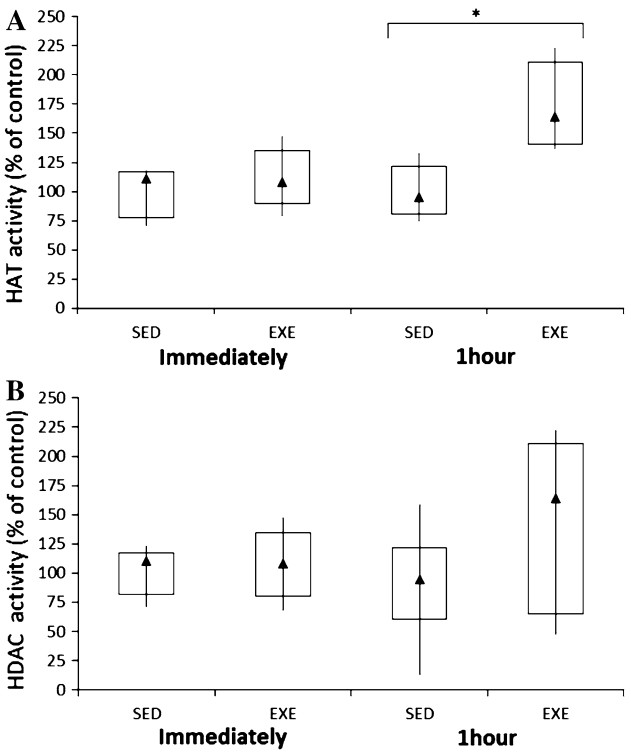

Fig. 1 Effects of the single exercise session on HAT (**a**) and HDAC (**b**) activities in frontal cortices from Wistar rats. Results are expressed as percentage of the SED group (immediately) ($n = 4$–5). Kruskal–Wallis followed by Dunńs test, *values significantly different from its respective SED control group ($p = 0.031$)

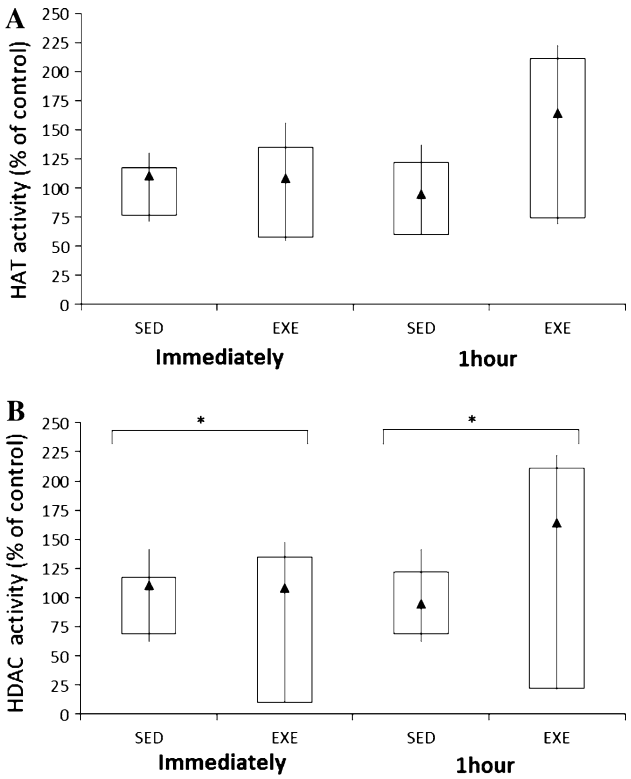

Fig. 2 Effects of the moderate daily treadmill protocol on HAT (**a**) and HDAC (**b**) activities in frontal cortices from Wistar rats. Results are expressed as percentage of the SED group (immediately) ($n = 4$–5). Kruskal–Wallis followed by Dunńs test, *values significantly different from its respective SED control group ($p = 0.003$)

Determination of Global HDAC Enzyme Activity

The global HDAC enzyme activity was determined using an HDAC Assay Kit (Fluorometric Detection catalog # 17-372, Upstate Biotechnology, Temecula, CA, USA) according to the manufacturer's instructions. Briefly, the samples were incubated with the substrate at 30 °C for 45 min before addition of the activator solution. The mixtures were incubated at room temperature for additional 10 min and HDAC enzyme activity was measured on a microplate reader (excitation: 360 nm, emission: 450 nm).

Determination of HAT Enzyme Activity

The HAT enzyme activity was determined using an ELISA HAT Assay Kit (Colorimetric Detection catalog # K332-100, Biovision, Milpitasa CA, USA) according to the manufacturer's instructions. The mix, containing 5 µl of sample, HAT buffer, substrate and NAD generating enzyme, was incubated for 1 h 30 min. The HAT enzyme activity was evaluated in a microplate reader at 440 nm.

Statistical Analysis

The results were expressed as percentage of control, where the SED (immediately) group was taken as 100 %. Results were expressed as medians (25th/75th of percentiles). Kolmogorov–Smirnov was used as a normality test, and statistical significance between group comparisons was determined by Kruskal–Wallis, followed by Dunn test. Analysis was performed using the Statistical Package for the Social Sciences (SPSS) software on a PC-compatible computer. In all tests, $p < 0.05$ was considered to indicate statistical significance.

Results

The effects of the single session of treadmill exercise in frontal cortices are illustrated in Fig. 1. Kruskal–Wallis indicated a significant effect of exercise ($KW = 8.831$; $p = 0.031$) in HAT activity (Fig. 1a). This exercise protocol increased HAT activity 1 h after the last training session when compared to the SED group, while HDAC activity was unchanged (Fig. 1b).

The moderate daily treadmill protocol altered HDAC activity ($KW = 13.83$; Fig. 2b), specifically, reducing this parameter immediately (about 85 %; $p = 0.003$) and 1 h (about 70 %; $p = 0.003$) after the last session when compared to the SED group. This exercise protocol did not affect HAT activity (Fig. 2a).

Discussion

In the present study, the exercise altered HDAC and HAT activity in frontal cortices from young adult Wistar rats, demonstrating that this brain area is vulnerable to exercise-induced epigenetic modulation. Therefore, these data support the hypothesis that epigenetic modulation in frontal cortex may be involved, at least in part, on neuroprotective activity and memory enhancing effects of exercise, since this brain area is involved in memory performance and attention (Liston et al. 2006).

The results showed that the single session protocol increased HAT activity, while daily exercise protocol reduced HDAC activity, supporting the idea that both exercise protocols may induce histone hyperacetylation in frontal cortices. This finding may be related to previous studies reporting that expression of essential genes related to brain function, such as NMDAR1, VEGF, Flk-1 B and BDNF, is increased (Lou et al. 2008; Takamatsu et al. 2010; Hopkins et al. 2011, Gomez-Pinilla et al. 2011).

It has been suggested that an adequate stimulus-dependent regulation of histone acetylation and deacetylation is critical to memory formation (Stilling and Fischer 2011). Feng and colleagues (2007) showed that HAT and HDAC modulate, respectively, activation and inhibition of memory storage-related gene expression. In addition, it has been demonstrated that HDAC inhibitors increase histone acetylation, consequently, potentiating memory and synaptic plasticity and ameliorating cognitive deficits (Korzus et al. 2004; Levenson et al. 2005; Rando and Chang 2012).

It is remarkable to note that HAT activity seems to be diminished in neurodegenerative disorders (Rouaux et al. 2003; Jiang et al. 2003), while HDAC activity is increased (Saha and Pahan 2006). Therefore, HDAC inhibitors appear to be efficient in several neurological conditions, maintaining a dynamic regulation of neuronal plasticity. HDAC inhibitors have showed to reverse neuronal apoptosis induced by oxidative stress, which is associated with several neurodegenerative diseases such as Alzheimer's Parkinson's, Huntington's, stroke and multiple sclerosis (Ryu et al. 2003). Taken together, we can speculate that the modulation of exercise on histone acetylation can be related to these neuroprotective effects.

It is possible to speculate that there is a structure-dependent effect of exercise on epigenetic parameters, specifically in acetylation status, since previous findings demonstrated that hippocampus from daily EXE rats did not have any changes in these enzymes activities. Furthermore, the single session protocol was able to decrease HDAC activity immediately and 1 h after single session of treadmill exercise in young adult rat hippocampus (Elsner et al. 2011). Moreover, it was demonstrated that the gene expression profiles were dissimilar in hippocampus and cerebellum in response to exercise in rodents (Abel and Rissman 2013).

Conclusion

The present findings bring new insights into the exercise effects on epigenetic regulation in frontal cortices from Wistar rats. In summary, we demonstrated that both exercise protocols in treadmill, single session, and moderate daily exercise are able to modulate HATs or HDACs activities, what can be related to acetylation status in frontal cortices. Additional works will be required to clarify the mechanism behind this phenomenon.

Acknowledgments This work was supported by the Brazilian funding agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq (Dr. I.R. Siqueira; V.R. Elsner; C. Spindler); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES (K. Bertoldi; G.A. Lovatel; F. Moysés); Programa Institucional de Bolsas de Iniciação Científica–PIBIC CNPq-UFRGS (L.R. Cechinel; C.Basso).

Conflict of interest The authors declare no conflicts of interest.

Limitations The current study has an important limitation. It was the small sample size, what can difficult extrapolation of the results. Further studies with larger sample sizes could be done to better understand these results.

References

Abel JL, Rissman EF (2013) Running-induced epigenetic and gene expression changes in the adolescent brain. Int J Dev Neurosci 31(6):382–390

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:218–254

Brooks GA, White TP (1978) Determination of metabolic and heart rate responses of rats to treadmill exercise. J App Physiol 45:1009–1015

Cechetti F, Rhod A, Simao F, Santin K, Salbego C, Netto CA et al (2007) Effect of treadmill exercise on cell damage in rat hippocampal slices submitted to oxygen and glucose deprivation. Brain Res 1157:121–125

Cechetti F, Fochesatto C, Scopel D, Nardin P, Goncalves CA, Netto CA et al (2008) Effect of a neuroprotective exercise protocol on oxidative state and BDNF levels in the rat hippocampus. Brain Res 1188:182–188

Choi JK, Howe LJ (2009) Histone acetylation: truth of consequences? Biochem Cell Biol 87:139–150

Collins A, Hill LE, Chandramohan Y, Whitcomb D, Droste SK, Reul JMHM (2009) Exercise improves cognitive responses to psychological stress through enhancement of epigenetic mechanisms and gene expression in the dentate gyrus. PLoS One 4:e4330

Elsner VR, Lovatel GA, Bertoldi K, Vanzella C, Santos FM, Spindler C, de Almeida EF, Nardin P, Siqueira IR (2011) Effect of different exercise protocols on histone acetyltransferases and histone deacetylases activities in rat hippocampus. Neuroscience 192:580–587

Feng J, Fouse S, Fan G (2007) Epigenetic regulation of neural gene expression and neuronal function. Pediatr Res 61(5 Pt 2):58R–63R

Gomez-Pinilla F, Zhuang Y, Feng J, Ying Z, Fan G (2011) Exercise impacts brain derived neurotrophic factor plasticity by engaging mechanisms of epigenetic regulation. Eur J Neurosci 33:383–390

Guan JS, Haggarty SJ, Giacometti E, Dannenberg JH, Joseph N, Gao J (2009) HDAC2 negatively regulates memory formation and synaptic plasticity. Nature 459:55–60

Hopkins ME, Nitecki R, Bucci DJ (2011) Physical exercise during adolescence versus adulthood: differential effects on object recognition memory and brain-derived neurotrophic fator. Neuroscience 194:84–94

Huang TY, Lin LS, Cho KC, Chen SJ, Kuo YM, Yu L, Wu FS, Chuang JI, Chen HI, Jen CJ (2012) Chronic treadmill exercise in rats delicately alters the Purkinje cell structure to improve motor performance and toxin resistance in the cerebellum. J Appl Physiol 1136:889–895

Jiang H, Nucifora FC Jr, Ross CA, DeFranco DB (2003) Cell death triggered by polyglutamine-expanded huntingtin in a neuronal cell line is associated with degradation of CREB-binding protein. Hum Mol Genet 12:1–12

Kimura A, Matsubara K, Horikoshi M (2005) A decade of histone acetylation: marking eukaryotic chromosomes with specific codes. J Biochem 138:647–662

Korzus E, Rosenfeld MG, Mayford M (2004) CBP histone acetyltransferase activity is a critical component of memory consolidation. Neuron 42:961–972

Kouzarides T (2007) Chromatin modifications and their function. Cell 4:693–705

Levenson JM, Sweatt J (2005) Epigenetic mechanisms in memory formation. Nat Rev Neurosci 6:108–118

Liston C, Miller MM, Goldwater DS, Radley JJ, Rocher AB, Hof PR, Morrison JH, McEwen BS (2006) Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. J Neurosci 26(30):7870–7874

Lou SJ, Liu JY, Chang H, Chen PJ (2008) Hippocampal neurogenesis and gene expression depend on exercise intensity in juvenile rats. Brain Res 1210:48–55

Lovatel G, Elsner V, Bertoldi K, Vanzella C, Moysés F, Vizuete A, Spindler C, Cechinel L, Netto CA, Muotri A, Siqueira IR (2013) Treadmill exercise induces age-related changes on aversive memory, neuroinflammatory and epigenetic parameters in rat hippocampus. Neurobiol Learn Mem 101:94–102

Magistretti PJ, Pellerin L (1996) Cellular bases of brain energy metabolism and their relevance to functional brain imaging: evidence for a prominent role of astrocytes. Cereb Cortex 1:50–61

McKinsey TA, Zhang CL, Olson EN (2001) Control of muscle developmentby dueling HATs and HDACs. Curr Opin Genet Dev. 5:497–504

McQuown SC, Barrett RM, Matheos DP, Post RJ, Rogge GA, Alenghat T (2011) HDAC3 is a critical negative regulator of long-term memory formation. J Neurosci 31(2):764–774

Miller CA, Sweatt JD (2008) Covalent modification of DNA regulates memory formation. Neuron 59:1051

Ntanasis-Stathopoulos J, Tzanninis JG, Philippou A, Koutsilieris M (2013) Epigenetic regulation on gene expression induced by physical exercise. J Musculoskelet Neuronal Interact 13:133–146

Pareja-Galeano H, Sanchis-Gomar F, Garcia-Gimenez JL (2014) Physical exercise and epigenetic modulation: elucidating intricate mechanisms. Sports Med 44:429–436

Radák Z, Goto S, Tahara S, Kaneco T, Kumagai S, Berkes I, Ogonovszky H (2005) The effects of moderate-, strenuous- and over-training on oxidative stress markers, DNA repair, and memory, in rat brain. Neurochem Int 46:635–640

Rando TA, Chang HY (2012) Aging, rejuvenation, and epigenetic reprogramming: resetting the aging clock. Cell 148:46–57

Rouaux C, Jokic N, Mbebi C, Boutillier S, Loeffler JP, Boutillier AL (2003) Critical loss of CBP/p300 histone acetylase activity by caspase-6 during neurodegeneration. EMBO J 22:6537–6549

Ryu H, Lee J, Olofsson BA, Mwidau A, Dedeoglu A, Escudero M, Flemington E, Azizkhan-Clifford J, Ferrante RJ, Ratan RR (2003) Histone deacetylase inhibitors prevent oxidative neuronal death independent of expanded polyglutamine repeats via an Sp1-dependent pathway. Proc Natl Acad Sci U S A 100:4281–4286

Saha RN, Pahan K (2006) HATs and HDACs in neurodegeneration: a tale of disconcerted acetylation homeostasis. Cell Death Differ 13:539–550

Stankiewicz AM, Swiergiel AH, Lisowski P (2013) Epigenetics of stress adaptations in the brain. Brain Res Bull 98:76–92

Stilling RM, Fischer A (2011) The role of histone acetylation in age-associated memory impairment and alzheimer's disease. Neurobiol Learn Mem 96:19–26

Strahl BD, Allis CD (2000) The language of covalent histone modifications. Nature 403:41–45

Szyf M, McGowan P, Meaney MJ (2008) The social environment and the epigenome. Environ Mol Mutagen 49:46–60

Takamatsu Y, Ishida A, Hamakawa M, Tamakoshi k, Jung C, Ishida K (2010) Treadmill running improves motor function and alters dendritic morphology in the striatum after collagenase-induced intracerebral hemorrhage in rats. Brain Res 1355:165–173

Vissing J, Andersen M, Diemer NH (1996) Exercise-induced changes in local cerebral glucose utilization in the rat. J Cereb Blood Flow Metab 4:729–736

Wade PA (2001) Methyl CpG-binding proteins and transcriptional repression. Bioessays 23:1131–1137