Available online at http://www.ijabbr.com

International journal of Advanced Biological and Biomedical Research

Volume 2, Issue 1, 2014: 170-175

Cerebellum and Reelin under chronic treadmill exercise conditions in male rats

Nasser Ahmadiasl[1], Farzam Sheikhzadeh Hesari[2]*, Elham Karimi Sales[3]

1. Professor of Physiology, Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

2. Assistant Professor of Physiology, Animal Sciences Group, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.

3. MSc in Animal Physiology, Animal Sciences Group, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.

ABSTRACT

Reelin is an extracellular matrix neuroprotein which plays important roles during development and maturation of cerebellum. In the postnatal cerebellum, Reelin is synthesized by cerebellar granule cells and secreted to extracellular matrix. This secreted protein modulates adult synaptic function, neurotransmitter release and regulates plasticity. Exercise has beneficial effects on central nervous system. This study investigated the effects of short and long-term training program on Reelin protein levels in the cerebellum. Forty male rats divided into four main groups; test 1 (15 days exercise-trained rats) and test 2 (60 days exercise-trained rats), and control 1 (rats were kept alive for 15 days) and control 2 (rats were kept alive for 60 days). At the end of the training period, Reelin levels in the cerebellum were measured by ELISA assay. Results showed that short and long-term regular exercise had no effect on Reelin protein levels in the cerebellum. Present study showed that regular exercise could not change Reelin protein concentration which mediated plasticity, dendritogenesis and synaptogenesis in the cerebellar neurons. Results of this study could correlate with unaffected plasticity, dendritogenesis and synaptogenesis in the cerebellum under exercise conditions as reported in previous studies.

Key words: Cerebellum, Exercise, Plasticity Reelin.

INTRODUCTION

Reelin is a 420-kDa glycoprotein structurally resembling extracellular matrix proteins. This glycoprotein and its signaling pathway play pivotal roles during development and maturation of laminated structures of the brain including the brain cortex, cerebellum and hippocampus (Rice and Curran 2001; Tissir and Goffinet 2003). In developing brain, Reelin is secreted by transient pioneer neurons (Cajal–Retzius cells) located in the marginal zone of the cerebral cortex and pioneer granule cells in the external granular layer of the cerebellum (Alcántara et al. 1998). In this period Reelin regulates migration of cortical plate neurons and newborn cerebellar neurons and is crucial for the proper cytoarchitecture of the laminated structure such as the cerebellum (D'Arcangelo et al. 1997; De Bergeyck et al. 1997). In the postnatal cerebellum, Reelin is synthesized by nearly all cerebellar granule cells and secreted to the extracellular matrix of cerebellar neurons. This secreted glycoprotein, at the axon terminals of the parallel fibers, adheres to integrin or very-low-density lipoproteins and apolipoprotein E2 receptors. Reelin pathway

Corresponding Author E-mail: drhesari@gmail.com

involves a cascade of intracytoplasmic events that initiated by binding of Reelin to its receptors (D'Arcangelo et al. 1999; Hiesberger et al. 1999). Reelin signaling pathway plays an important role in determination of Purkinje neuron positioning in the Purkinje cell layer (Giompres and Delis 2005; Maloku et al. 2010), modulation adult synaptic function, neurotransmitter release and regulation of plasticity (Beffert et al. 2005; Fatemi 2004; Hellwig et al. 2011; Niu et al. 2004). Also this pathway is involved in late steps of postnatal neuronal development such as stimulation of dendritic protein synthesis, dendritic differentiation, spine formation and promotion of axonal branching (Borrell et al. 1999; Chen et al. 2005; Del Río et al. 1997; Niu et al. 2004; Niu et al. 2008). Cerebellum is a large region in the brain that has an essential role in motor learning, motor control, physical coordination, motor planning, proper sequencing and timing of motor responses and balance. Also recent data suggest that distinct regions of the cerebellum have a significant role in cognition and learning (Bauer et al. 2009; Fine et al. 2002; Kandel et al. 2000). Exercise has beneficial effects on central nervous system such as maintaining and improving of neural function in the brain of human and animals (Molteni et al. 2002). Exercise improves neurogenesis and maturation of new born neurons and significantly increases dendritic complexity and angiogenesis (Kleim et al. 2002; Van Praag et al. 2005; Zhao et al. 2006). Both voluntary and forced exercise protocols have been used to explore the effect of exercise on brain function but the effect of forced exercise on neurogenesis is stronger than voluntary exercise (Burghardt et al. 2004; Leasure and Jones 2008). Exercise and Reelin have similar effects on structure and function of the brain. In this study the effects of treadmill running exercise on Reelin protein concentration in the cerebellum of adult rats was investigated.

MATERIALS AND METHODS

Animals

Forty male Wistar rats with 200±50 g weight were used in this study. All animals were housed in an environmentally controlled room with rat chow and water ad libitum.

Experimental training protocols

All male rats were familiarized with the treadmill to minimize novelty stress. Then, rats were randomly divided into exercised and non-exercised group and then each group were divided into two subgroups; test 1 (15 days exercise-trained rats –T1) and test 2 (60 days exercise-trained rats –T2), and control 1 (rats were kept alive for 15 days –C1) and control 2 (rats were kept alive for 60 days –C2). Rats of test groups ran for 1 min/day at 22 m/min and 0-degree inclination, 5 days/week.

ELISA assay

At the end of the training period, animals of all groups were weighed, anesthetized with intraperitoneal injection of ketamine and xylazine at doses of 100 mg and 5 mg per kilogram of body weight, respectively, and killed by rapid decapitation (Ayoub 2009). Ultimately brains were rapidly removed; the cerebellum was dissected, washed with ice-cold saline and quickly frozen in liquid nitrogen. All samples were stored at -80^{o}C freezer. The tissues were homogenized in 5-10 mL of PBS with a glass homogenizer. ELISA assay was performed according to manual of rat Reelin ELISA kit (E92775Ra) which was purchased from Life Science Inc Company.

Statistical analysis

Results are presented as means ± SE. One-way analysis of variance, followed by Tukey's post hoc test was used to analyze the differences between groups. Statistical significance was set at $p<0.05$.

Results

Results demonstrated that treatment with short-term regular moderate exercise (15 days) could not significantly affect Reelin protein concentration in the cerebellum of treadmill running group as compared to control group which was kept alive for 15 days (fig 1). Also results of this study showed that long-term exercise had no significant effect on Reelin protein concentration in the cerebellum of test group which were exercised on treadmill for 60 days as compared to control group which was kept alive for 60 days (fig 1). Also changes in Reelin protein levels in the cerebellum of both test groups when compared to each other were not significant (fig 1).

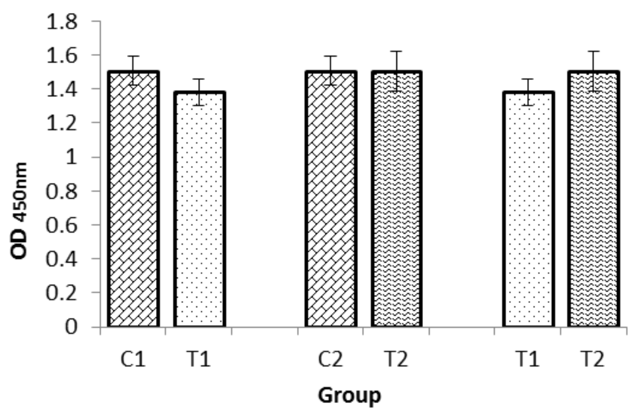

Fig 1. Effect of exercise on Reelin concentration in the cerebellum (Means of optical density ± SE). Comparisons between four groups showed no significant difference in runner compared with control rats in Reelin concentration in the cerebellum, ($p<0.05$). C1: Control group that kept alive for 15 days, C2: Control group that kept alive for 60 days, T1: Test group that treated with 15 days of exercise, T2: Test group that treated with 60 days of exercise.

Discussion

This study investigated various durations of regular exercise on Reelin protein levels of cerebellum. In embryonic and early postnatal period, cerebellum synthesize high levels of Reelin by young granule cells in the external granular layer but during postnatal development, the pattern of Reelin expression changes (Alcántara et al. 1998). In the adult period, Reelin expression continues by granule cells in the internal granule layer. This different expression pattern reflects the different functions exerted by Reelin in the embryonic and postnatal brain. In the embryonic and early postnatal period Reelin has important role in correct neural migration and the formation of cellular layers during development of cerebellum (Huang and D'Arcangelo 2008). In adults, Reelin promotes plasticity, dendritogenesis and synaptogenesis in cerebellar neurons (Beffert et al. 2005; Fatemi 2004; Niu et al. 2008) and also mediated neurotransmitter release (Hellwig et al. 2011Hellwig et al. 2011). In this study treadmill exercise protocols have been used to explore the effect of regular moderate exercise on Reelin protein levels in the cerebellum of adult male rats. Before only very few studies about the link of Reelin and treadmill running exercise in the brain of adult rats was performed (Hesari and Sales 2013). Reelin plays important roles in the synaptogenesis, dendritic branching and dendritic spine formation during adult period (Alcántara et al. 1998; Borrell et al. 1999; Niu et al. 2008; Weeber et al. 2002). This study showed that long and short-term regular exercise could not significantly affect this protein levels in the cerebellum of adult rats. Previous studies indicated that although motor skill learning can alter the number of synapses per Purkinje neuron (Anderson et al. 1996), physical exercise is not generally affect cerebellar plasticity and the number of dendritic spines and synaptic inputs on Purkinje neurons in the cerebellum. Previously documented that exercise only could affect and increase angiogenesis in the cerebellum (Black et al. 1990; Kleim et al. 2007). So, these previous studies are in agreement with results of the present study. Kennerd et al investigated the effect of treadmill running exercise with moderate speed on cerebellum and showed runners which treated with moderate speed were not significantly different from sedentary controls in rotorod performance and improvement of cerebellum function (Kennard and Woodruff-Pak 2012). So study of Kennard et al is in agreement with results of present study about inability of this type of regular exercise on plasticity of cerebellum. Present study suggested that molecular mechanism of unaffected structure and activity of cerebellum under regular exercise conditions, is unchanged Reelin protein levels in this area.

Conclusion

This study investigated diverse durations of regular exercise on Reelin protein levels in the cerebellum of healthy adult rats and showed that regular physical activity had no significant effect on Reelin protein concentration which mediated plasticity, dendritogenesis and synaptogenesis in the cerebellar neurons.

Acknowledgements

This work was supported by Neurosciences Research Center, Tabriz University of Medical Sciences and natural sciences faculty, University of Tabriz, Tabriz, Iran.

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