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EFFECT OF DAILY INTENSE EXERCISE ON ASYMMETRY IN SYMPATHOADRENOMEDULLARY ACTIVITY**DELOVANJE SVAKODNEVNOG INTENZIVNOG VEŽBANJA NA ASIMETRIJU SIMPATO-ADRENOMEDULARNE AKTIVNOSTI***Ljubica Gavrilović, Slađana Dronjak i Vesna Stojiljković*

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Summary: In this study we tested changes in quantity of phenylethanolamine N-methyltransferase (PNMT), a "rate limiting" enzyme in the synthesis of adrenaline (A), as an indicator of sympatho-adrenomedullary activity in the left and right adrenal medulla of control animals and animals exposed to intensive physical activity. We utilised the model of chronic forced running (CFR: 12 weeks of treadmill running), which according to intensity and duration falls under intensive physical activity. The tested parameters were quantified by using the Western blot. We discovered that CFR considerably increases the level of PNMT proteins in the right adrenal medulla, which suggests that, in the conditions of daily intensive exercise, the sympatho-medullary link greatly influences the right adrenal medulla. The considerable increase of PNMT proteins in the right adrenal medulla confirms that the left hemisphere of the brain is much more sensitive to intensive physical activity.

Key words: adrenal medulla, asymmetry, adrenaline, PNMT, daily intense exercise

Sažetak: U ovoj studiji ispitivali smo promene količine feniletanolamin N-metiltransferaze (PNMT), "rate limiting" enzima u sintezi adrenalina (A), kao pokazatelja simpato-adrenomedularne aktivnosti u levoj i desnoj srži nadbubrežne žlezde, kod kontrolnih i životinja izlaganih intenzivnoj fizičkoj aktivnosti. Primenili smo model hroničnog forsiranog trčanja (CFR: 12 nedelja trčanja po pokretnoj traci), koji po intenzitetu i trajanju spada u intenzivnu fizičku aktivnost. Ispitivane parametre kvantifikovali smo korišćenjem Western blot analize. Pronašli smo da CFR značajno povećava nivo PNMT proteina u desnoj srži nadbubrežne žlezde, što sugeriše da u uslovima svakodnevnog intenzivnog vežbanja postoji veliki uticaj simpatomedularne veze sa desnom srži. Značajno povećanje nivoa PNMT proteina u desnoj srži nadbubrežne žlezde može da potvrdi da je leva hemisfera mozga mnogo osetljivija na intenzivnu fizičku aktivnost.

Ključne reči: srž nadbubrežne žlezde, asimetrija, adrenalin, PNMT, svakodnevno intenzivno vežbanje

1. INTRODUCTION

The literature data indicate that exercise improves physical and psychological quality of life. It is known that exercise training reduces the risk of developing diseases related to chronic stress. For example, in humans, regular exercise has a beneficial impact on depression [1]. Our previous data showed that exercise training acts as an important modulator of sympatho-adrenomedullary system [2]. Although a number of markers are frequently used to assess the involvement of the

sympatho-adrenal response (plasma and tissue noradrenaline-NA and adrenaline-A levels), it is important to examine more specific variables such as gene expression of key enzymes involved in catecholamine biosynthesis. Phenyl ethanolamine N-methyltransferase (PNMT) is considered as the "rate limiting" enzyme for the synthesis of A [3]. Our earlier studies showed that long-term treadmill running (12 weeks) of adult rat males produced an increased of PNMT protein level in the adrenal medulla [2] and increased concentrations of A in the plasma [4]. The literature data confirm that most internal

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organs are asymmetric in respect to the left and right sides [5]. In our previous study we found hippocampal asymmetry in expression of catecholamine synthesizing enzyme in socially isolated rats [6]. However, asymmetry in gene expression of A biosynthetic enzymes in right and left adrenal medulla in the conditions of daily intense exercise is still unknown. This prompted us to investigate whether daily intense exercise affects adrenal asymmetry regarding the expression of A synthesizing enzyme. In this study we examined how chronic forced running (CFR) affects protein levels of PNMT as an index for sympatho-adrenomedullary activity in right and left adrenal medulla.

2. MATERIALS AND METHODS

2.1. Animals

Wistar male rats (11-week-old) were under standard laboratory conditions with water and food ad libitum and kept three to four per cage [7]. The care was taken to minimize the pain and discomfort of the animals according to the recommendations of the Ethical Committee of the Vinča Institute of Nuclear Sciences, Belgrade, Serbia, which follows the guidelines of the registered "Serbian Society for the Use of Animals in Research and Education". Animals were divided into two groups: CONTROL group (n=10) was not exposed to any treatment and CFR group (n=10) consisted of animals exposed to chronic forced running for a period of 12 weeks. Chronic forced running is achieved by the rats' daily running on the treadmill for a period of 12 weeks. The treadmill running intensity is gradually increased from week to week, from the initial 10 minutes-10m/min up to 20 minutes-20m/min at 00 incline, [8,9,10]. Animals are being exposed to treadmill training 5 days a week for 12 weeks [8]. After 12 weeks, the animals were decapitated, the adrenals excised, right and left adrenal medulla dissected, frozen separately in liquid nitrogen and stored at -70 °C until analyzed [11].

2.2. Western blot analysis

Adrenal medulla were homogenized in 0.05 M sodium phosphate buffer (pH 6.65). Antibodies used for quantification of specific proteins were as follows: for PNMT the polyclonal anti-PNMT primary antibody, rabbit (dilution 1:1000, Protos Biotech Corporation, USA) and for β -actin the rabbit polyclonal anti- β -actin (ab8227, dilution 1:5000, Abcam, USA). After washing, the membranes were incubated in the secondary anti-rabbit (dilution 1:5000,

Amersham ECLTM Western Blotting Analysis System, UK) antibody conjugated to horseradish peroxidase. Secondary antibody were then visualized by the Western blotting enhanced chemiluminescent detection system (ECL, Amersham Biosciences, UK). Membranes were exposed to ECL film (Amersham Biosciences, UK). Densitometry of protein bands on ECL film (Amersham Biosciences, UK) was performed by Image J analysis PC software. The result was expressed in arbitrary units normalized in relation to β actin, which is in accordance with protocol of Gavrilović et al. [12].

2.3. Data analysis

The data are presented as means \pm S.E.M. Differences of PNMT levels between control and CFR animals in the right and left adrenal medulla were analyzed by t-test. Statistical significance was accepted at $p < 0.05$. Correlations of PNMT levels in the right and left adrenal medulla and A levels in the plasma were analyzed by the Pearson test, using the Sigma Plot v10.0 (with SigmaStat integration).

3. RESULTS

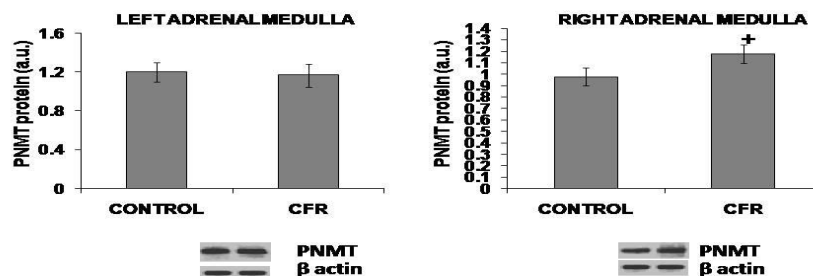
The animals exposed to CFR showed an increase of PNMT protein by 20% ($p < 0.05$, t-test, Figure 1) in the right adrenal medulla, compared with the control animals. However, we did not find changes in protein levels of PNMT in the left adrenal medulla in animals exposed to CFR. The significant positive correlation was found between the levels of PNMT in the right adrenal medulla and plasma concentration of A (Pearson $R = 0.569$; $p < 0.05$) of animals exposed to CFR.

4. DISCUSSION

In the present study we have found that the CFR treatment has increased PNMT protein levels in the right adrenal medulla. In addition, very important result in this study is a significant positive correlation between the levels of PNMT protein in the right adrenal medulla and A in the plasma of the animals exposed to CFR. Our results indicate that CFR treatment may lead to continuous accumulation of PNMT protein in the right adrenal medulla as an adaptation on applied intense physical activity. This adaptive response is necessary to maintain the A biosynthetic capacity in the adrenal medulla during periods of sustained A secretion. Our results confirm that during daily intense exercise the increased synthesis of PNMT protein only in the right adrenal medulla affects the sustained increase of A secretion.

Figure 1. Effects of chronic forced running (CFR) on phenylethanolamine N-methyltransferase (PNMT) protein levels in the left and right adrenal medulla

Slika 1. Efekti hroničnog prinudnog trčanja (CFR) na nivo proteina feniletanolamin-N-metiltransferaze (PNMT) u levoj i desnoj adrenalnoj meduli.



The values are means \pm S.E.M. of 10 rats. Statistical significance: $+p < 0.05$ animals exposed to chronic forced running vs. control animals (t-test). The result was expressed in arbitrary units normalized in relation to β actin.

The asymmetric increase in the protein levels of PNMT in the adrenal medulla suggests a higher impact of sympathoadrenomedullary input in the right than in the left medulla in the condition of daily intense exercise. Toth et al. [13] reported that the regulation of adrenomedullary function such as catecholamine release is under complex and multifactorial control, including both hormonal and neural regulatory processes. A significant increase in protein levels of PNMT in right adrenal medulla may confirm that the left brain hemisphere is more susceptible to intense exercise. Based on our results, it may be concluded that sympathoadrenomedullary

activity is asymmetric in the conditions of daily intense exercise. The results presented here confirm that the CFR in rats is forced exercise and it shows adaptations that are indicative of chronic stress.

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Conflict of interest

The authors report no conflict of interest.

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