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RESEARCH ARTICLE

Age-dependent peculiarities modulation of activity of aldehyde scavenger enzymes in mitochondria of rat thigh muscle during stress

Vadim V. Davydov (✉)[1], Evgenya R. Grabovetskaya[2], Amjad Hamdallah[3]

[1] *Chair of Biochemistry State Academican I.P.Pavlov Medical University, Ryazan, 390026, Russia*
[2] *Chair of Medical Chemistry Kharkov National Medical University, Kharkov, 61022, Ukraine*
[3] *Chair of Biochemistry V.N. Karazin Kharkov National University, Kharkov, 61077, Ukraine*

Abstract **The purpose of this study was a comparative investigation of activity of aldehyde scavenger enzymes in mitochondrial fraction of a thigh muscle in intact and immobilized rats of different ages. It has been shown that 12-month-old (adult) rats have high basal levels of aldehyde dehydrogenase, aldehyde reductase and glutathione transferase activity in mitochondrial fraction of thigh muscle. Aldehyde dehydrogenase activity increases during immobilization stress in adult rats. This change promote to enhance the effectiveness of utilization of carbonyl products of free radical oxidation in mitochondria of skeletal muscle of 12-month-old rats during stress. Immobilization of old and pubertal rats is accompanied by metabolic preconditions leading to accumulation of endogenous aldehydes in mitochondria, and, as a result, to the injury of muscular fibers and intensification of sarcopenia manifestations.**

Keywords aldehydes, mitochondria, muscle, immobilization stress, aging

Introduction

Structure and properties of skeletal muscles change during ontogenesis. Late ontogenesis is characterized by development of sarcopenia – condition accompanied by loss of skeletal muscle mass and strength of muscle contraction (Bautmans et al., 2009; Naricini and Maffulli, 2010). Sarcopenia is one of the global problems of old people, which leads to decreased quality of life and acceleration of death (Rossi et al., 2008; Naricini and Maffulli, 2010). Notwithstanding widespread sarcopenia during aging, mechanisms of its pathogenesis are still not clear. Oxidative stress (Chen et al., 2008; Hindle et al., 2010) and mitochondrial dysfunction are considered to be significant for sarcopenia development (Kang et al., 2013; Marzetti et al., 2013).

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Correspondence: Vadim V. Davydov
E-mail: vaddavydov@mail.ru

Damage of a cell in oxidative stress is due to accumulation of aldehyde products of free radical oxidation (Uchida, 2000). All mentioned facts allow assuming that resistance to sarcopenia development is largely dependent on the condition of the mitochondrial system of endogenous aldehydes catabolism. This system includes aldehyde dehydrogenases (ALDH), aldehyde reductases (ALR) and glutathione transferases (GT) (Davydov et al., 2004; Davydov et al., 2012). However, they are still not investigated in skeletal muscle. Peculiarities of modulation activity of these enzymes under stimulated formation of free radical oxidation carbonyl products, i.e. during immobilization stress (Meerson, 1984; Davydov and Shvets, 2003), which, in turn, increase the development of sarcopenia in aging, are not studied yet (Chen et al., 2008). At the same time, we have previously shown that long-term immobilization of animals is accompanied by age-specific changes in the manifestation of oxidative stress in skeletal muscles (Davydov et al., 2014). Taking this into consideration, the aim of the present study was the evaluation of activity of endogenous aldehydes scavenger enzymes in mitochondrial fraction from thigh muscle of intact and immobilized rats of different ages.

Materials and methods

Male Wistar rats of three different age groups (50 animals) were used in the present study: 1 – 1.5-month-old (pubertal); 2 – 12-month-old (adult); 3 – 24-month-old (old) rats. Each age group was divided into two subgroups: 1 – intact animals and 2 – those affected by immobilization stress. Immobilization stress was modeled by tying of animals to a stationary plank for 5 h per day for 2 days. The effectiveness of stress was controlled by the level of epinephrine in the blood (Atrac and Madnusson, 1978).

Rats were decapitated under light ether anesthesia, then thigh muscle was removed and immediately placed in chilled 0.9% sodium chloride solution. Pieces of muscle tissue were thoroughly minced with scissors after washing from blood. Mince was mixed at a ratio of 1: 3 (weight : volume) with saline medium containing 0.05 M Tris, 0.005 M magnesium sulfate, and 0.001 M EDTA (pH 7.4) and then homogenized for 3 min in a glass Potter-Elvehejm homogenizer with PTFE pestle. The homogenate was filtered through 4 layers of gauze and centrifuged at 1000 *g* for 10 min. The resulting supernatant was transferred to clean tubes and centrifuged at 10000 *g* for 20 min. The sediment was washed twice in homogenization medium at 10000 *g* for 20 min and used as a mitochondrial fraction. All fractionation procedures of muscle homogenate were performed at 4 – 5°C.

Activity of ALDH (NAD) (Pirozhkov and Panchenko, 1988), ALR (NADH) (Srivastava et al., 2001) and GT (Mannervik and Guthenberg, 1981) in the mitochondrial fraction of thigh muscle was determined. Protein content in samples was determined by the Lowry method.

Statistical calculations were done by means of Excel and SPSS Statistics 17.0 software using Wilcoxon-Mann-Witney nonparametric test. Differences between the data were considered significant at $p < 0.05$.

Results and discussion

Table 1 shows changes of ALDH activity in the mitochondria from thigh muscle during ontogenesis. It 8 times increases in 12-month-old rats as compared to 1.5-month-old animals, and 3.6 times decreases in old rats as compared to adult ones. At the same time activity of ALDH in old animals becomes 123% higher than in pubertal rats. Similarly, activity of ALR in the thigh muscle mitochondrial fraction of 12-month-old animals is twice higher than that in 1.5-month-old rats. In 24-month-old animals it is the same as in 1.5-month-old rats, but its value is 2.5 times lower than in adult ones. Activity of GT in animals of all studied age groups is at the same level.

Long-term immobilization of 1.5-month and 12-month-old rats induces elevation of ALDH activity in mitochondrial fraction from thigh muscle by 223% and 101% respectively, compared to their initial level. ALR activity in the mitochondrial fraction of rat thigh muscle in 1.5-month immobilized rats decreases by 41% as compared to its initial level. At the same time, there is no change in activity of this enzyme in 12-month-old and 24-month-old animals under immobilization stress. GT activity in the mitochondrial fraction from rat thigh muscle in all investigated age groups after immobilization remains at the initial level.

Table 1 Activity of aldehyde scavenger enzymes (nmol/(mg protein ·min)) in the mitochondrial fraction of the thigh muscle rats of different age groups during immobilization stress (mean±SEM)

Age (months)	Experimental group of rats	ALDH	ALR	GT
1.5	intact	1.3±0.2	1.7±0.5	1.3±0.1
	immobilized	4.2±0.7 *	1.0±0.1 *	1.5±0.2
12	intact	** 10.4±1.2	** 3.5±0.8	1.5±0.1
	immobilized	20.9±3.1*	4.7±1.0	1.1±0.1
24	intact	** 2.9±0.4***	1.4±0.3 ***	1.3±0.3
	immobilized	3.2±0.2	1.5±0.4	1.2±0.2

* The data are positively distinguished from intact 1.5-month-old rats ($p < 0.05$).
*** The data are positively distinguished from intact 12-month-old rats ($p < 0.05$).
** The data are positively distinguished from intact rats of same age group ($p < 0.05$).
The table represents the average data from 6 investigations.

The resulting changes are accompanied by alteration of the ratio investigated enzymes activity under stress. This can lead to the change of carbonyl metabolic products utilization pathways in the muscle cells. Figure 1 shows that long-term immobilization causes elevation of index value ALDH/GT in 1.5- and 12-month-old rats of more than 160% and 130%

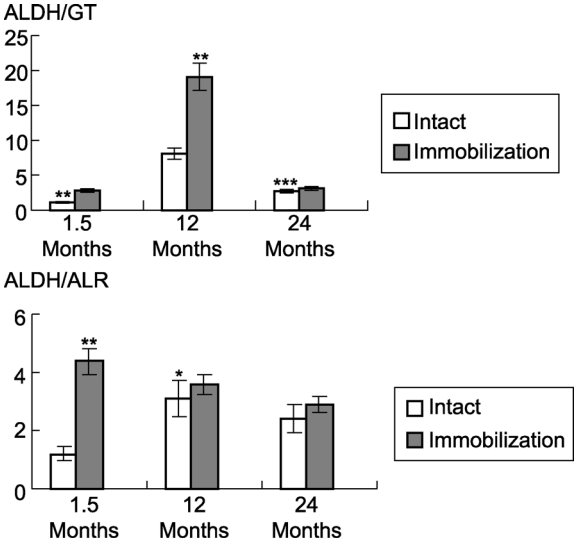

Figure 1 The ratio activities of aldehyde scavenger enzymes in mitochondria of the thigh muscle rats of different age groups during immobilization stress (mean±SEM). The figure represents the average data from 6 investigations.* $p < 0.05$, the data are positively distinguished from intact 1.5-months-old rats; ** $p < 0.05$, the data are positively distinguished from intact rats of same age group; *** $p < 0.05$, the data are positively distinguished from intact 12-months-old rats

respectively as compared to the initial level. Simultaneously, the index ALDH/ALR in 1.5-month old animals increases of more than 250% as compared with intact rats of this age.

A comprehensive assessment of the obtained results allows concluding that adaptive changes develop along with high basal aldehyde dehydrogenase and aldehyde reductase activity in mitochondria from thigh muscle of adult mature animals during immobilization. These changes are aimed to intensify utilization of endogenous aldehydes in redox reactions. Elevation of the rate of endogenous aldehydes oxidation in aldehyde dehydrogenase reaction becomes particularly important. Because of this, there is no accumulation of free radical oxidation products of proteins (protein carbonyls, Schiff bases and TBA-reactive substances) in the muscle tissue from immobilized rats of this age group, i.e., there are no manifestations of oxidative stress (Davydov et al., 2014). Perhaps, this leads to decrease of Schiff bases level in the mitochondrial fraction in adult animals.

Stable concentration of free radical oxidation products in mitochondria of old rats during immobilization stress is maintained without changes in the activity of aldehydes scavenger enzymes. Therefore, lack of oxidative stress manifestations in the mitochondria after long-term immobilization can be caused by restriction of prooxidant effects on the body due to suppression affinity of adrenergic receptors in tissues to epinephrine during aging (Michalíková et al., 1990) or antioxidant system increase in the late ontogenesis (Lopez-Torres and Perez-Campo, 1992).

Aldehyde dehydrogenase activity increases in the mitochondrial fraction from the thigh muscle in 1.5-month-old rats after long-term immobilization similar to that in the adult animals. However, in 1.5-month-old rats this change is accompanied by lowering of aldehyde reductase activity. We can assume that these facts cause a sharp decrease of reductive pathway of endogenous aldehydes catabolism and, as a consequence, development of tense state of the utilization of free radical oxidation carbonyl products intensively generated by stress. This is manifested by accumulation products of endogenous aldehydes transformation – protein carbonyls and Schiff bases in the mitochondria of the thigh muscle (Davydov et al., 2014). All these facts indicate limitation efficiency utilization of free radical oxidation carbonyl products in mitochondria of muscle tissue in case of stimulation their generation under stress. This is followed by increasing of skeletal muscle sensitivity to the stress damage during adolescence.

This age-related phenomenon is not unique for skeletal muscle, it is also found in myocardium (Grabovetskaya and Davydov, 2009), which means that it is generalized in immobilization stress. It can be formed due to age-related alterations of isozyme composition of mitochondrial aldehyde dehydrogenases and aldehyde reductases in muscle tissue. They are, in turn, may be due to peculiarities of the endocrine regulation of the synthesis isozymes of aldehyde dehydrogenases and aldehyde reductases during puberty. Our future researches will be devoted to these aspects.

Compliances with ethics guidelines

Davydov V V, Grabovetskaya E R and Amjad Hamdallah declare that they have no conflict or interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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