

Peculiarities of Energy Metabolism of Cardiac Hystiocytes in White Rats under Chronic Stress Conditions

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ABSTRACT. Identification of the role of NO in cardiac hystiocytes of white rats in the energy generation process as well as investigation of the activity of a number of enzymes under conditions of biorhythm disturbance and isolation has been studied. Inhibition of enzyme activity under conditions of a 30-day chronic stress has been observed. © 2010 Bull. Georg. Natl. Acad. Sci.

Key words: cardiac hystiocytes, NO synthase, creatine kinase, chronic stress, succinate dehydrogenase.

In studying the vasodilation mechanism, it was found that acetylcholine was able, when interacting with the endothelial cell receptors, to initiate the formation of small molecules and to cause relaxation. These molecules have been named the endothelium-derived relaxing factor (EDRF) [1]. Subsequently EDRF was found to stimulate the formation of a second messenger – the cyclic guanosine monophosphate (cGMP) and was identified as nitric oxide (NO) [2]. Intracellular NO is generated as a result of NO synthase (NOS). The synthesized NO activates the cGMP synthesis, cGMP-dependent protein kinase, and Ca²⁺ATPase, leading to Ca²⁺ release from cardiac hystiocytes, their relaxation and vasodilation. At present, NO is deemed to be an endogenous vasodilator and is characterized by antihypertensive and anti-aggregatory properties [3]. Of special interest currently is the capacity of NO to affect the activity of many enzymes, especially of the respiratory chain components and glycolysis, which leads to programmed cell death – apoptosis [4].

The purpose of this study is to identify the role of NO in cardiac hystiocytes of white rats in the energy generation process as well as to investigate the activity

of a number of enzymes participating in the formation of ATP (adenosine triphosphate) under conditions of biorhythm disturbance and isolation.

Materials and Methods

Experiments were held on sexually mature male albino laboratory rats. The rats were kept in individual cages in the dark (dark-to-light ratio – 23.5/0.5 hrs) and were socially isolated. The animals were kept under the above-described conditions for 30 days. The control group consisted of animals kept in a common cage under natural conditions (10.00/14.00 hrs). Cell fractions were obtained through differential centrifugation [8].

Creatine kinase activity was determined by the quantity of inorganic phosphorus through spectrophotometry at $\lambda=400$ nm [9].

Succinate dehydrogenase activity in the mitochondria was determined by colorimetry by means of 3-(4,5-dimethylthiasol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and blue formasane. Intensity of blue coloring was measured by spectrophotometry at $\lambda=540$ nm [10]. The concentration of creatine in the samples was measured by means of diagnostic test systems (Dia Sys, Germany).

Intensity of nitric oxide synthesis was assessed by the product of NaNO_2 reaction, whereupon optic density was measured by spectrophotometry at $\lambda=540$ nm [11]. The results were processed by Student's test.

Results and Discussion

Chronic stress is known to cause variability of biochemical indices of cellular metabolism [5], although the data concerning the character of the quantitative content of NO during a stress are not simple [6, 7]. Therefore, the NO action mechanism and its quantitative variability are of interest to cardiologists.

In the first series of experiments, the dynamics of the quantitative variability of NO in the cytosolic and mitochondrial fractions of cardiac hystiocytes of white rats under conditions of a 30-day chronic stress were investigated. The obtained results are given in Fig. 1. As can be seen, after a 10-day stress a reduction of the NO content in the fractions is observable against the control. However, following a 20-day stress, NO reduction was noted only in the cytosol of cardiac hystiocytes, whereas in the mitochondria it increased. The NO content growth in the cytosol of cardiac hystiocytes of the 30-day-stress-subjected white rats accounted for, against the control, approximately 30%, and much higher for mitochondria. Given the character of NO action, a marked relaxation of the myocardium is supposed to occur as a result of the Ca^{2+} ATPase activity, as well as release of Ca^{2+} -ions from cardiac hystiocytes [8].

On the basis of literary data concerning the NO role in the activity regulation of glycolytic enzymes and Krebs cycle, the activity of succinate dehydrogenase enzyme against the background of the quantitative variability of NO during chronic stress was studied. The

obtained results are presented in Fig. 2. As is seen, enzymatic activity during the 10- and 20-day stresses is markedly reduced. Particular by notable reduction of the enzymatic activity is observable in the 20-day stress period. During this period, the activity of succinate dehydrogenase against the background of the quantitative variability of NO shows reduction by about 90%. The obtained results indicate a sharp reduction in the mitochondrial aerobic processes and regeneration of ATP molecules which, in turn, leads to the depletion of cardiac cells and formation of various pathologies.

The obtained data give ground to suppose a reduction in the activity of not only succinate dehydrogenase but also of creatine phosphokinase, which restores the baseline of ATP through transphosphorylation between creatine and phosphorous creatine. Five enzyme isoforms are known, of which two are mitochondrial and three cytosolic [9]. Based on it, the dynamics of the enzymatic activity variability in cardiac hystiocytes of white rats during the 30-day stress was studied. The obtained data are given in Fig. 3. These data are indicative of the enzymatic activity variability during the stress. In particular, following the 10-day stress, the enzymatic activity of the mitochondrial isoforms increases, to be followed by a reduction of the value that is characteristic of the control animals. The enzymatic activity of the cytosolic isoforms as a result of the 10-day stress also increases (approx. by 40%), to be followed by a reduction (approx. by 30%) in enzymatic activity. It should be mentioned that the activity of both isoforms, against succinate dehydrogenase, is characterized by lesser sensitivity to stress conditions. The activity of both isoforms increases on the 10th day of stress, then slowing down to approach the control value.

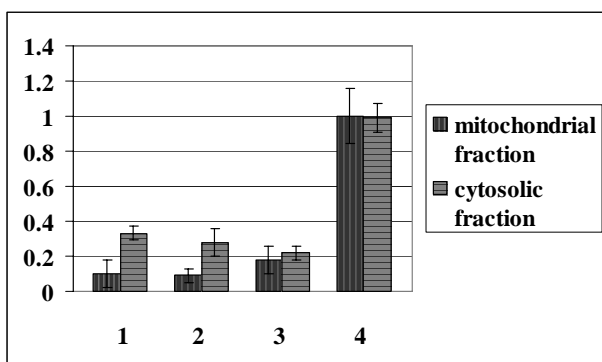


Fig. 1. Dynamics of the quantitative variability of NO in the cytosolic and mitochondrial fractions of cardiac hystiocytes of white rats under conditions of a chronic stress

X-axis – number of stress day (1 - control; 2 - 10-day stress; 3 - 20-day stress; 4 - 30-day stress)

Y-axis – NO concentration ($\mu\text{mol/ml}$)

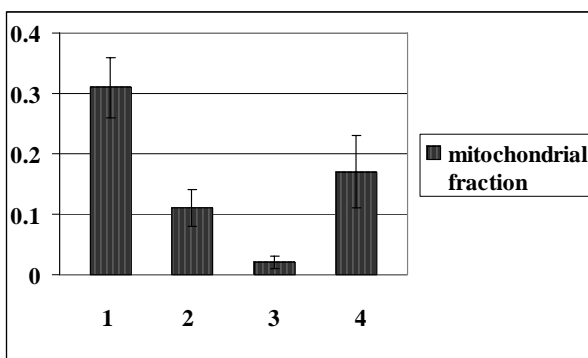


Fig. 2. Dynamics of the activity of succinate dehydrogenase enzyme in the mitochondrial fractions of cardiac hystiocytes of white rats under conditions of a chronic stress

X-axis – number of stress day (1 - control; 2 - 10-day stress; 3 - 20-day stress; 4 - 30-day stress)

Y-axis – Enzymatic Activity ($\mu\text{kat/L}$)

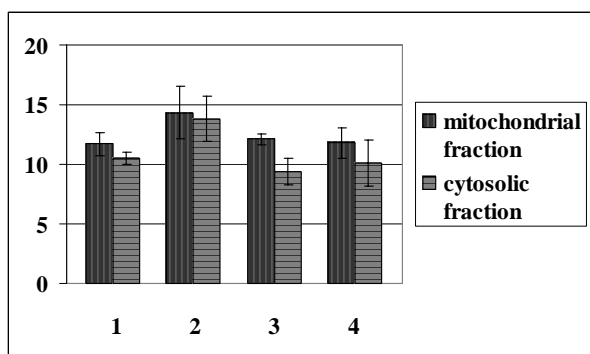


Fig. 3. Dynamics of the activity of creatine phosphokinase in the cardiac hystiocytes of white rats under conditions of chronic stress
 X-axis – number of stress day (1 - control; 2 - 10-day stress; 3 - 20-day stress; 4 - 30-day stress)
 Y-axis – Enzymatic Activity ($\mu\text{mol P}_i/\text{mg protein}^{-1} \text{min}^{-1}$).

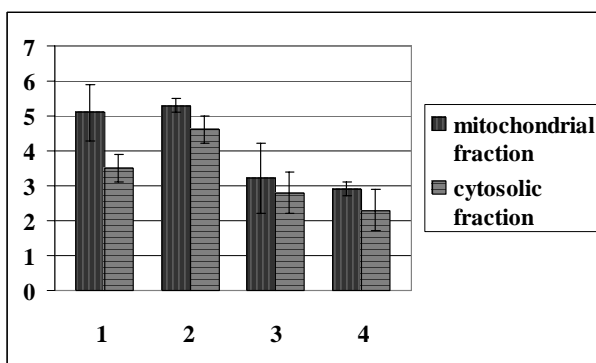


Fig. 4. Dynamics of the quantitative content of creatine in cardiac hystiocytes during chronic stress.
 X-axis – number of stress day (1 - control; 2 - 10-day stress; 3 - 20-day stress; 4 - 30-day stress)
 Y-axis – creatine concentration ($\mu\text{mol/ml}$)

A different picture is observable in studying creatine – a phosphokinase substrate. The content of creatine in cardiac hystiocytes during chronic stress was found to sharply vary. Fig. 4 presents data indicating quantitative variability of creatine. As it appears, the creatine content in both fractions increases after the 10-day stress, to be followed by a sharp reduction phase. For example, after the 20-day stress the creatine content in mitochondria makes up about 60% of the control value and about 25% in cytosol.

Based on the above-mentioned data, it can be presumed that a long-term chronic stress affects the processes in cardiac hystiocytes protecting the organism from cardiovascular disorders and supplying these cells with necessary energy.

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ბიოქიმია

თეთრი ვირთაგვების კარდიოპისტოციტებში ენერგეტიკული ცვლის თავისებურებანი ქრონიკული სტრესის პირობებში

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სტატიაში შესწავლილია თეთრი ვირთაგვების კარდიომიოციტებში NO-ს გავლენა ენერგეტიკული ცვლის პროცესში მონაწილე სხვადასხვა ფერმენტის აქტივობაზე ქრონიკული იზოლაციური სტრესის პირობებში. დადგენილია ფერმენტთა საგრძნობი ინჰიბირება 30-დღიანი სტრესის მოქმედების შედეგად.

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