Nitric Oxide Level in the Rat Tissues Increases after 30-Day Hypokinesia: Studies by Electron Paramagnetic Resonance (EPR) Spectroscopy

Kh. L. Gainutdinov[1,2], R. I. Faisullina[2], V. V. Andrianov[1], R. I. Gilmutdinova[2], V. S. Iyudin[1], G. G. Jafarova[1,2], and F. G. Sitdikov[2]

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Studies by EPR spectroscopy showed that 30-day exposure of rats to augmenting hypokinesia led to a 3-fold increase in nitric oxide (NO) production in the heart and 2-fold in the liver. These results indicated that long-term hypokinesia stimulated NO synthesis.

Key Words: *nitric oxide; hypokinesia; heart; liver; electron paramagnetic resonance*

Nitric oxide (NO) is one of the most important regulators of physiological functions of the organism and of cell metabolism [4,11]. The role of NO in the functioning of the cardiovascular and nervous systems of vertebrates is highly significant. It regulates the vascular tone, AP, endothelial and vascular wall smooth muscle cell proliferation, is involved in the pathogenesis of atherosclerosis and hypertension, regulates myocardial contractility [7,9]. In the nervous system, NO participates in the development, maturation, and aging of the brain, in learning and memory processes; changes in NO production in nervous tissue can cause diseases [13]. Dysregulation of the cerebral blood-flow and blood supply to the heart and the relevant changes in NO production can lead to brain and heart ischemia with subsequent development of stroke and infarction [12,14]. In addition, NO is involved in the inflammation process in rheumatic, autoimmune, and viral diseases, modulates the formation of interstitial liquid and edema, plays the key role in suppression of bacterial and tumor cell activities, and can initiate the growth of tumor cells [9]. It reacts with numerous substances: thiols, proteins, sugars, metal ions, protein hemes, *etc.*, located in many tissues and organelles, which fact suggests the presence of NO and its complexes in various tissues [8,10]. The NO system plays an important role in adaptation to environmental changes and environmental conditions, for example, in exhausting swimming [2,6]. These data demonstrate the contribution of NO to regulation of physiological function in many tissues.

The problem of hypokinesia (HK) and/or hypodynamia now focused the attention of scientists. Hypokinesia (motor activity limitation) is one of the priority medicosocial problems, caused by the lifestyle, professional activity, long-term forced bed rest, *etc.* Hypokinesia results in reduction of muscle system work, which leads to changes in the functional and morphological characteristics of tissues, up to morbid conditions, depending on the duration and degree of HK [1,5].

We studied the time course of NO production in HK by analyzing NO content in various tissues of rats developing under conditions of limited motor activity.

MATERIALS AND METHODS

Experiments were carried out on little outbred albino rats. The animals were divided into 2 groups (10 per

[1]Kazan Physico-Technological Institute, Kazan Research Center, the Russian Academy of Sciences; [2]Kazan (Volga Region) Federal University, Russia. ***Address for correspondence:*** kh_gainutdinov@mail.ru. Kh. L. Gainutdinov

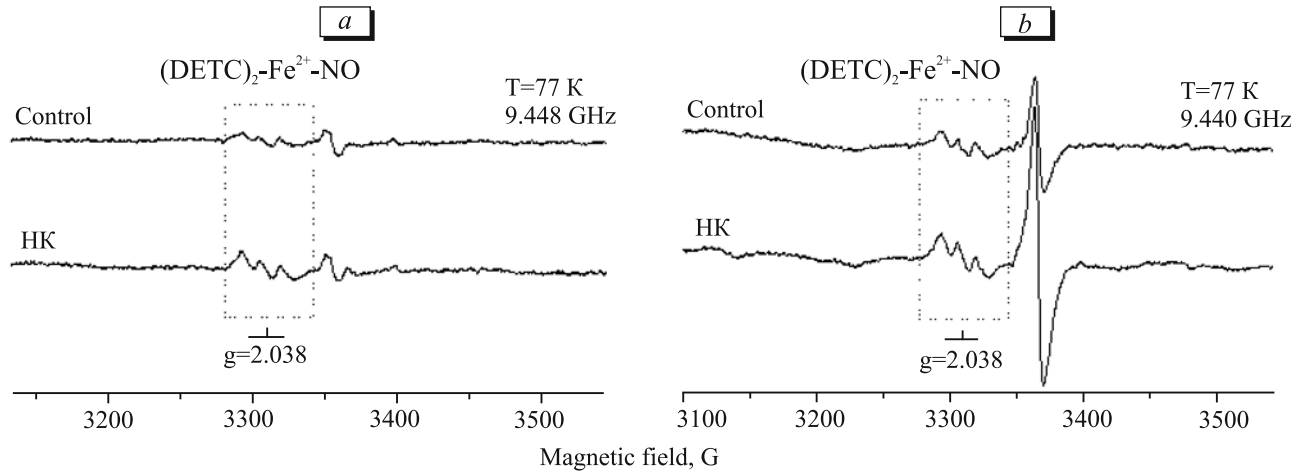

Fig. 1. EPR spectra of cardiac (*a*) and hepatic (*b*) tissues of control rats and rats exposed to 30-day HK. Dotted line: signal from $(DETC)_2$-Fe^{2+}-NO complex.

group), control and experimental. Controls were kept under standard vivarium conditions, 3-4 per cage. Experimental animals were subjected to 30-day HK. Exposure to HK started from the age of 21 days: 1 h during the first 2 days, after which the HK exposure was prolonged by 2 h every third day. By day 25 of HK, the animals spent 23 h in penal cages every day and the duration of exposure remained unchanged until the end of the experiment [1].

The levels of NO were measured in heart and liver tissues. The difficulty of NO measurements consisted in its high activity and short lifespan, manifesting by its low concentration. The samples for measurements of EPR spectra were prepared for measurements by the spin trap method, detecting NO in low concentrations [15]. The experiment and methods were described previously [3]. A sample weighed 100 mg. The EPR spectra of the samples were recorded on a EPR spectrometer (X-range), Bruker EMX/plus, with an ER 4112HV thermal attachment at 77°K.

The results were statistically processed, the means and standard errors in the means were calculated ($M\pm SEM$). The significance of differences in the mean NO levels in different tissues of control and experimental rats was verified by the Student t and Mann–Whitney U tests.

RESULTS

Figure 1 presents the EPR spectra of heart and liver tissues of control and experimental animals. A characteristic triplet signal from the $(DETC)_2$-Fe^{2+}-NO complex is clearly seen, its integral intensity directly proportional to NO content in the sample.

Exposure of rats to HK for 30 days led to a 3-fold increase of NO content in the heart and 2-fold increase in liver tissues (Fig. 2). Excessive formation of NO can decrease significantly the smooth muscle cell tone, deteriorate the endothelial function, and directly suppress myocardial contractility, which was observed in septic and hemorrhagic shock and acute myocardial infarction; a significant reduction of cardiac output, blood stroke volume, and hepatic microcirculation were recorded after injection of drugs blocking NO synthase activity [14]. It was found that NO augmented the course of myocardial infarction, this deterioration consisting in reduction of heart rate, AP, stroke and minute blood volumes [7]. According to an opposite opinion, NO excess served as a compensatory factor, maintaining tissue perfusion and exhibiting antiarrhythmic effect in reperfusion [6,9]. Excessive NO production in the cell can also cause DNA injuries and support inflammation in endotoxemia, septic shock, inflammatory diseases of the lungs [4,8]. The toxic effect of NO manifested primarily by inhibition of the mitochondrial enzymes, this leading to decrease of production of ATP and of

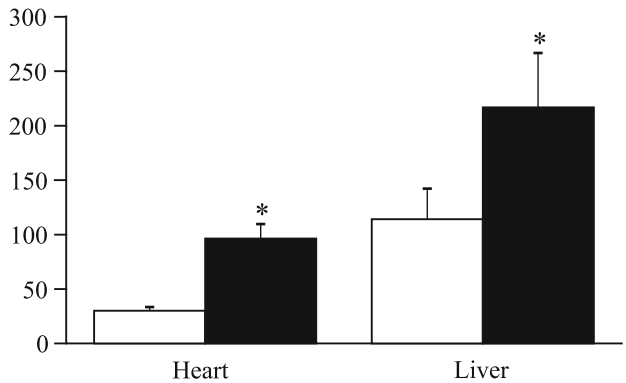

Fig. 2. Changes in NO production in cardiac and hepatic tissues of control rats (light bars) and rats subjected to 30-day HK (dark bars). Ordinate: integral intensity of signal from $(DETC)_2$-Fe^{2+}-NO complex. *$p<0.05$ in comparison with the control.

enzymes involved in DNA replication. Moreover, NO and peroxynitrite can directly damage DNA, which triggers defense mechanisms (*e.g.* activation of polysynthetase) and leads to further decrease in ATP content and cell death [8].

The important role of NO in many processes, including heart work, and insufficient information about the volume of NO synthesis and its functions in a growing organism exposed to HK necessitate studies in this direction. Our results indicate that HK is associated with a significant increase of NO levels in the organs studied in our experiment. A previous study on rats subjected to intense training (hyperkinesia) has shown a reduction of NO production in various tissues [2]. Hence, more intense production of NO in HK in our experiment suggests a close relationship between NO level and the motor activity. According to published data, HK causes significant changes in the cardiovascular system, in the viscera, in the blood flow and oxygen supply system, and it is therefore logical to suggest that some of these changes are caused by stationary increase of NO production in the key for the organism tissues.

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