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Research Article

Nitrogen Oxide Production Dynamics upon Recovery after Hyperkinesia

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ABSTRACT:
Hypokinesia (restricted physical activity) is one of the most pressing medical and social problems caused by lifestyle, occupational activity, prolonged bed rest, and a range of diseases. Prolonged hypokinesia may cause changes in the contractile function of the cardiac muscle, and poor oxygen supply to the tissues. Objective of the study was to investigate the role of NO in the consequences resulting from the recovery after hypokinesia by analyzing the NO-containing paramagnetic complexes in various tissues of rats growing under restricted physical activity. We applied electron paramagnetic resonance to analyze the production of nitric oxide (NO) in the hepatic and heart ventricle tissues of rats kept under hypokinesia for 30 days, and during their subsequent recovery after 1 and 2 weeks. We have discovered that the hypokinetic regime leads to an increase in NO production in the tissues by two times. We have found that during the subsequent recovery from hypokinesia there is further increase in NO production in the heart ventricles, while in the hepatic tissues NO production remains unchanged. We have shown that during the subsequent recovery from hypokinesia there is further increase in Cu content (a possible indicator of superoxide dismutase activity) in both the heart ventricles and the hepatic tissues.

Keywords: nitric oxide, heart, hypokinesia, immobilization stress, electron paramagnetic resonance.

1. INTRODUCTION.

Nitric oxide (NO) – a gaseous chemical messenger that is a free radical, regarded now as a new signaling molecule playing the role of a universal regulator of many physiological processes in the body [1; 2]. The role of NO in the animal vital activities is especially significant in the functioning of the cardiovascular [3; 4; 5] and nervous systems [6; 7]. It has been found that NO impairs the progress of myocardial infarction, and this impairment lies in the reduced heart rate, reduced blood pressure, stroke volume and cardiac output [8; 9; 10]. There is also an opposite point of view, according to which an excess of NO is a compensatory factor that helps to maintain tissue perfusion and provides antiarrhythmic effect during reperfusion [5; 11; 12]. The described literature data indicate two opposite effects of NO: a stimulating, positive, as well as toxic, and damaging effect that can lead to cell death [13; 14]. There are clear contradictions in the assessment of the effects of the NO donors and the NOS blockers, when it is considered that these impacts or stimuli provide unambiguous effect [15; 16], although the exact quantification of NO in the tissues has not been conducted under these conditions.

Hypokinesia (restricted physical activity) is one of the most pressing medical and social problems caused by lifestyle, occupational activity, prolonged bed rest, and a range of diseases, etc. [17; 18; 19]. Limitation of muscle activity is one of the most important components of the symptoms of hypokinetic syndrome [20;

21; 22]. Prolonged hypokinesia causes significant changes in the contractile function of the cardiac muscle. All these phenomena will inevitably lead to a serious deterioration of tissue oxygen supply, i.e. hypoxia [20; 23].

Previously, we have conducted EPR spectroscopic studies of the dynamics of NO production in cardiac and hepatic tissues during hypokinesia of various duration, which found a significant increase in NO content at 30- and 60-day hypokinesia [19; 24; 25]. Therefore, the objective of the study was to investigate the role of NO in the consequences resulting from the recovery after hypokinesia by analyzing the NO-containing paramagnetic complexes in various tissues of rats growing under restricted physical activity.

2. METHODS

The studies were conducted in white laboratory outbred rats divided into 2 groups: 1 – control group kept under standard vivarium conditions, at unrestricted motion activity (K, n=7); II – experimental group kept at restricted physical activity (HK, n=19). The experimental group was divided into 3 sub-groups: 1) animals, kept under hypokinetic conditions for 30 days [19; 24; 25], 2) animals, kept for 1 week under recovering conditions after 30-day hypokinesia, and 3) animals, kept for 2 weeks under recovering conditions after 30-day hypokinesia.

Restriction of motor activity of growing rats was achieved by placing them in the pen-case cages. Hypokinesia started from age of 20 days: the first two days hypokinesia duration was 1 hour, and further increased by 2 hours every 2 days. By day 25 of hypokinesia, the time of stay in the cages reached 23 hours, and thereafter remained constant until the end of the experiment. At 22-23-hour hypokinesia, animals were released from the cages for 1-2 hours. We recorded the EPR spectra in rats after 30-day hypokinesia, and the intact animals of corresponding age (50-day-old) were tested as control. We also recorded the EPR spectra in animals, kept for 1 and 2 weeks under recovering conditions after 30-day hypokinesia.

NO content in the rat organs was determined by technique developed at the Institute of Chemical Physics, Russian Academy of Sciences, by professor A.F. Vanin and his colleagues [26; 27], which uses spin trapping method. As previously [19], we applied complex of Fe^{2+} with diethyldithiocarbamate (DETC). The animals were anesthetized with 25% urethane solution intraperitoneally, 1200 mg per kbw. Spin trap components are separately administered into the animal: DETC-Na was intraperitoneally administered at a dose of 500mg/ kg in 2.5 ml of water, and the solution mixture: ferrous sulfate ($FeSO_4 \cdot 7\ H_2O$, Sigma, USA) at a dose of 37.5 mg/kg and citrate sodium (chemically pure) at a dose of 187.5 mg/kg, prepared immediately prior to administration, were administered subcutaneously at three points - into the right and left thigh and the withers. The mixture of ferrous sulphate and sodium citrate forms iron citrate. DETC-Na and iron citrate are distributed through the body, and their interaction forms a water-insoluble complex DETC-Fe^{2+}, which is capable of reacting with NO with formation of a stable radical $(DETC)_2$-Fe^{2+}-NO. This complex can be detected by electron paramagnetic resonance (EPR). Furthermore, the spin trap reacts with Cu and forms a $Cu(DETC)_2$ complex, which can also be detected by EPR spectroscopy. 40 minutes after drug administration the rats were decapitated. The cardiac and hepatic tissues were removed, the left ventricle was separated from the heart for further measurements, the removed tissues were quickly dried and frozen in special capillaries with liquid nitrogen for measurements. The weight of the samples was 100 mg. The EPR spectra of the prepared samples were registered with a spectrometer ER 200 SRC, Bruker, in the X-range (9.50 GHz) with the magnetic field modulation of 100 kHz, modulation amplitude of 2Gs, microwave power of 30 mW, the time constant of 200 ms and a temperature of 77 K in the Finger Dewar, Bruker. Modulation amplitude, amplification and RF power in all experiments were chosen subject to the lack of over modulation and saturation of the EPR signal, and remained the same for all measurements.

The EPR spectra amplitude was always

normalized to the weight of the sample and the EPR signal amplitude of the reference sample (details of EPR signal measurement method were described previously) [7]. NO amount was estimated based on the intensity of characteristic EPR signal, relating to $(DETC)_2$-Fe^{2+}-NO. The assessment was conducted by the employee of KPTI KSC RAS Iiudin V.S. To measure the number of spins in the sample the signals of the test and the reference samples were compared. The coal was used as a reference (Ncoal = 33.663*1018 spins/cm^3). For these measurements we used the resonator ER 4105 DR. The average of the measured value and standard error of mean M ± SEM were obtained upon statistical processing. The significance of differences of average values was tested using Student's t-test and Mann-Whitney U-test. Differences were considered significant at $p<0.05$.

3. RESULTS

We applied EPR method to study the left ventricle and hepatic tissues of rats kept under hypokinetic conditions for 30 days, as well as animals kept for 1 and 2 weeks under recovering conditions after 30-day hypokinesia, and the 50-day-old control rats, which corresponds to the age of the rats after 30-day hypokinesia. In all measured EPR spectra we recorded a characteristic triplet signal from the complex based on the spin trap $(DETC)_2$-Fe^{2+}-NO [26], which integrated intensity is directly proportional to the amount of NO in the sample.

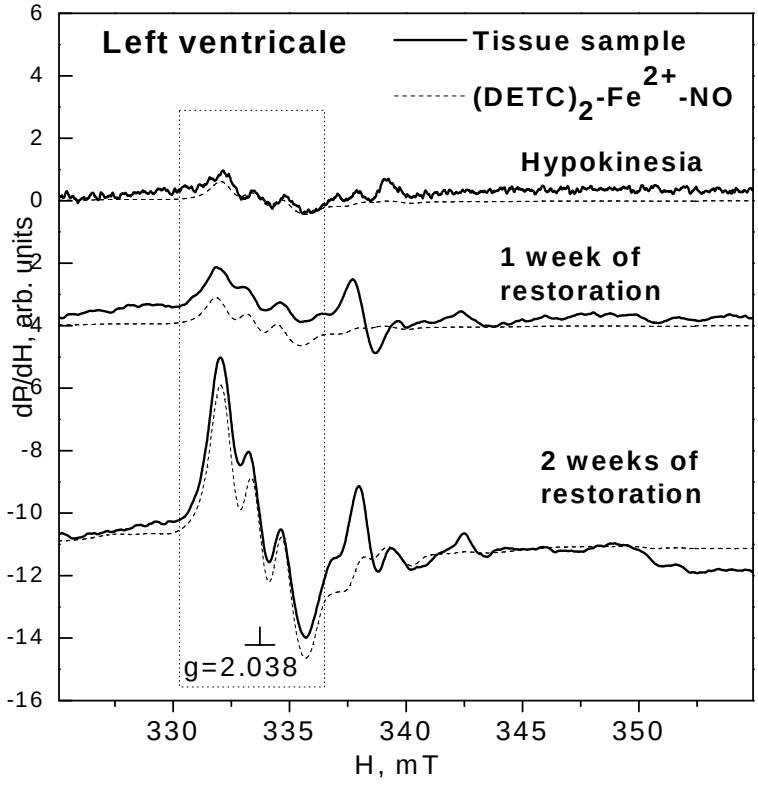

Fig. (1). The EPR spectra of left ventricular tissue of rats after 30-day hypokinesia and subsequent recovery after 1 and 2 weeks. A part of signal from $(DETC)_2$-Fe^{2+}-NO is shown in frame. The X-axis – the value of the magnetic field.

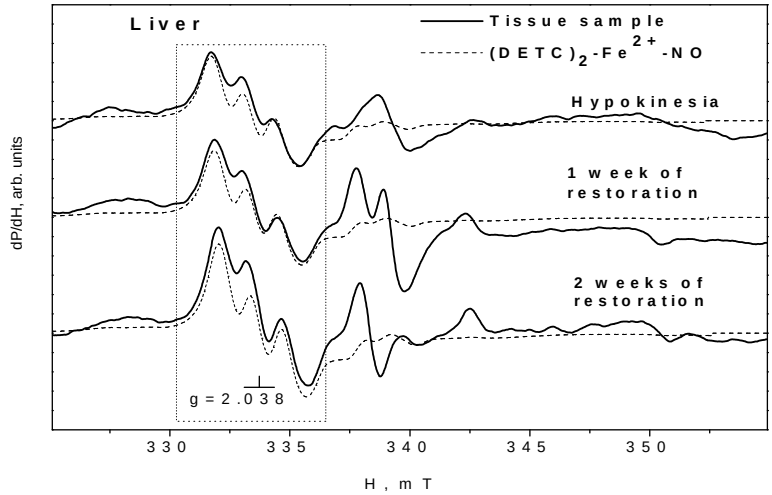

Fig. (2). The EPR spectra of hepatic tissue of rats after 30-day hypokinesia and subsequent recovery after 1 and 2 weeks. A part of signal from $(DETC)_2$-Fe^{2+}-NO is shown in frame. The X-axis – the value of the magnetic field.

Figures 1 and 2 show the samples of the EPR spectra of the left ventricle and hepatic tissues of rats kept under hypokinetic conditions for 30 days, as well as animals kept for 1 and 2 weeks under recovering conditions after 30-day hypokinesia.

Measurements of the integrated intensity of the EPR spectra of spin trap $(DETC)_2$-Fe^{2+}-NO show that NO production in the heart ventricular tissues averages 240 nmol/(g*h), in the hepatic tissue – 400 nmol/(g*h) for control rats (NO production per time unit, normalized to the weight of the tissue sample).

It was found that 30-day hypokinesia leads to an increase in the NO content by 2 times in both the left ventricular and hepatic tissues of rats (Figure 3).

When recovering after 30-day hypokinesia, no further changes occur in the NO production in the hepatic tissue as compared with the group of 30-day hypokinesia both after 1 and 2 weeks, however, there is a continuing increase in the NO production in the left ventricular tissues by about 2 and 3 times, respectively (Figure 3).

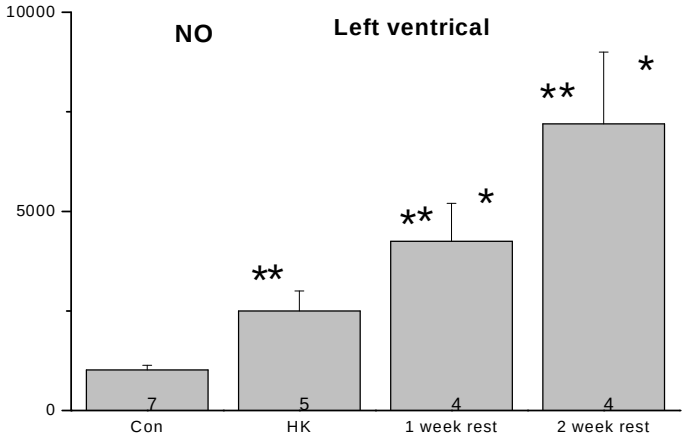

Fig. (3). Changes in the amount of NO-containing paramagnetic complex $(DETC)_2$-Fe^{2+}-NO) in the left ventricular and hepatic tissues of rats after 30-day hypokinesia and subsequent recovery after 1 and 2 weeks. The Y-axis – an integrated intensity of the EPR spectrum, rel.un. Note: * – significance as compared with the hypokinetic group: $p<0.05$, ** – reliability as compared with the control group: $p<0.05$.

The content of $Cu(DETC)_2$ 1 week after recovery from 30-day hypokinesia in the left ventricular tissues decreases slightly, but after 2 weeks increases again by 2.5 times (Figure 3). The content of $Cu(DETC)_2$ in the hepatic tissues increases 1 week after recovery from 30-day hypokinesia by 7 times, and after 2 weeks increases by 9 times (Figure 3).

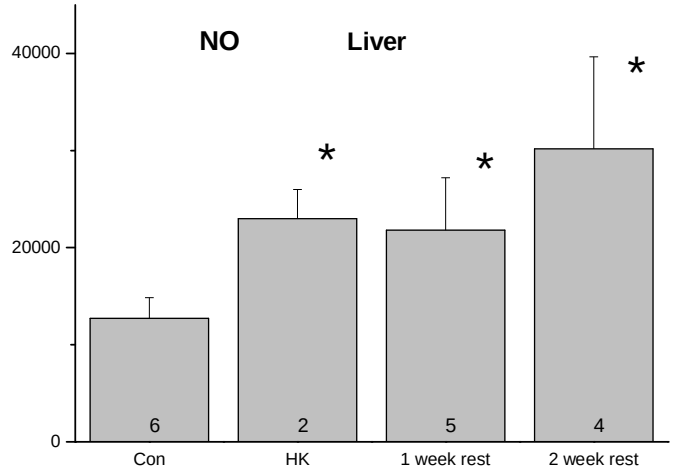

Fig. (4). Changes in the amount of $Cu(DETC)_2$ paramagnetic complex in the left ventricular and hepatic tissues of rats after 30-day hypokinesia and subsequent recovery after 1 and 2 weeks. The Y-axis – an integrated intensity of the EPR spectrum, rel.un. Note: * – significance as compared with the hypokinetic group: $p<0.05$.

4. SUMMARY

The hypokinetic regime leads to an increase in NO production in the tissues by two times. During the subsequent recovery from hypokinesia there is further increase in NO production in the heart ventricles, while in the hepatic tissues NO production remains unchanged. During the subsequent recovery from hypokinesia there is further increase in Cu content (a possible indicator of superoxide dismutase activity) in both the heart ventricles and the hepatic tissues.

5. CONCLUSION

Numerous experimental evidences suggest that hypokinesia is a stressor agent for warm-blooded animals and humans. The emergency stress phase of experimental hypokinesia continues from day one to five. It is characterized by a sharp increase in production of catecholamines and glucocorticoids, and by the prevalence of catabolic processes [20; 22]. Our experiments do not exclude the effect of immobilization stress on the body. The mediators of hematological stress syndrome can have either direct stress-limiting effects through the interaction with glucocorticoid receptors and adrenergic receptors, or indirect - by enhancing the NO production [11; 12]. It is known that hypokinesia is accompanied by the development of hypoxia, which, in turn, causes an increased synthesis of nitric oxide and NO^{3-} and NO^{2-} ions [12; 23]. Based on the electron-acceptor nature of NO^{2-} ions, we can assume that the cells can shift to nitrate (nitrite) respiration under the oxygen deficiency [5]. A significant increase in NO production, which we obtained in our experiments, can also indicate this mechanism. We have found data about increasing amount of NO at chronic immobilization. This response of the animal systems, probably, indicates the long-term immobilization stress, which is accompanied by an increased activity of NO-synthesizing system [11; 19; 25]. Also, we had previously shown a virtually complete suppression of NO generation in rats swimming with a heavy load, and NO overproduction at acute hypoxia and heat shock [11, 28]. Since the highest level of NO formation in atrial and ventricular tissues as well as in liver tissues was observed previously after 30-day hypokinesia [24], we chose the analysis of NO production

after recovery of the animals from this hypokinesia as the main task of this study. We obtained a result different from the possible assumptions - it turned out that NO production starts to increase in the heart and does not change in liver tissues. This result indicates that the recovery from hypokinesia is also a significant immobilization stress for animals. We have also shown that there is a simultaneous increase in $Cu(DETC)_2$ amount in the examined tissues, which is an indicator of the enzyme superoxide dismutase activity. This process is an expression of antioxidant properties and can neutralize the effects of the increased NO production. Thus, there are two opposite processes, and a cumulative effect on the body will depend on what process is dominant. The obtained data extend the understanding of the role of NO and NO-synthases in the activity of the internal organs of rats kept under stressful conditions at early postnatal ontogenesis. Hypokinesia does not exclude an immobilization stress – a reaction of the organism, and the shifts in NO content may be considered as the indicators of the stress resistance of animals and humans, and this may be one of the objective methods of stress resistance evaluation.

CONFLICT OF INTERESTS

The author declares that the provided information has no conflicts of interest.

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