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Effect of NO Synthase Blockade on NO Production in Rat Heart under Conditions of Hypokinesia

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Electron paramagnetic (EPR) spectroscopy study showed that 90-day hypokinesia in rats is accompanied by an increase in NO production in the heart. A nonselective NO synthase inhibitor L-NAME decreased the content of NO in the heart atria and ventricles of hypokinetic rats by 67-70%. A selective inhibitor of inducible NO synthase, aminoguanidine, also decreased the level of NO in the heart atria and ventricles of hypokinetic rats by 60-65%. Our results indicate that the increase in NO production during hypokinesia is associated with activation of NO synthases.

Key Words: *NO; heart; rat; hypokinesia; electron paramagnetic resonance*

Cardiac activity is regulated by the sympathetic–parasympathetic interactions [3]. NO plays an important role in these regulatory influences. NO formation in the body is realized via the enzymatic and nonenzymatic pathways. Enzymatic synthesis of NO in the cells involves a protein family of NO synthases (NOS), which are usually classified into the constitutive (cNOS) and inducible types (iNOS) [8,13]. cNOS-synthesized NO provides adequate blood supply, affects activity of neurons, and regulates cell metabolism [9,10,14]. Immunogenic and proinflammatory stimulation is followed by the expression of genes for iNOS synthesis [11,13]. The nonenzymatic pathway suggests reduction of NO^{2-} or NO^{3-} to NO (nitric reductase mechanism) [7].

Changes in physiological functions and mechanisms of their realization during hypokinesia are an urgent problem. It is related to an increase in the life span and significant reduction of physical activity, particularly in children and subjects not engaged in physical labor.

Here we studied the effect of NOS inhibitors on NO content in tissues of hypokinetic rats.

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MATERIALS AND METHODS

Experiments were performed on outbred albino rats. The animals were divided into 3 groups. Group 1 rats (control) were kept under standard vivarium conditions (n=30, heart; n=20, liver). Group 2 rats were subjected to 90-day hypokinesia (n=28, heart; n=20, liver). Group 3 rats were injected with the following NOS inhibitors: L-NAME (n=15, heart; n=15, liver) and aminoguanidine (n=15, heart; n=15, liver). Hypokinesia in animals was modeled starting from day 21 of life. The period of hypokinesia over the first 2 days was 1 h. In the follow-up period, the duration of hypokinesia increased by 2 h at 2-day intervals. By day 25, the animals were maintained in box cages for 23 h. This time remained unchanged until day 90. Under conditions of 22-23-h hypokinesia, we let the animals out of box cages for 1-2 h [1].

NO content in the heart ventricles, atria, and liver was measured by the spin trap method. The experiment and method were previously described in details [2]. Components of a spin trap were administered 40 min before decapitation. Electron paramagnetic resonance (EPR) spectra of test samples were recorded on an EMX/plus X-band EPR spectrometer (Bruker) equipped with an ER 4131VT temperature-controlling device at 140 K under the following conditions: modu-

lation frequency 100 kHz; modulation amplitude 5 G; SHF radiation power 2 mW; time constant 81 msec; and SHF radiation frequency 9.444 Hz.

The animals were intraperitoneally narcotized with 25% urethane in a dose of 1200 mg/kg. Nonselective NOS inhibitor L-NAME in a dose of 10 mg/kg was injected intraperitoneally 60 min before decapitation. Selective iNOS inhibitor aminoguanidine (10 mg/kg) was injected intraperitoneally 60 min before decapitation. These agents were dissolved in physiological saline. The doses of inhibitors were selected from published data [12,15].

The results were analyzed statistically. The significance of differences was evaluated by Student's *t* test and Mann–Whitney *U* test.

RESULTS

EPR spectra for the heart tissue of rats are shown in Figure 1. The amount of a NO-containing paramagnetic complex $(DETC)_2$-Fe^{2+}-NO in the heart and liver tissues of rats after 90-day hypokinesia was 104.8±6.6 and 225.0±9.4 rel. units, respectively (Fig. 2). The content of this complex in the heart and liver of L-NAME-receiving rats was 33.2±2.6 and 39.2±5.5 rel. units, respectively. Therefore, a nonselective NOS inhibitor L-NAME decreased the content of NO in the heart and liver tissues of rats by 68.3 and 82.6%, respectively. This antagonist not only abolished activation of NO synthesis during hypokinesia, but also decreased the content of NO above the control level. These data indicate that the increase in NO production during hypokinesia is associated with activation of the enzymatic pathway of NO synthesis. The intensity of NO formation by the nitrite reductase mechanism is reduced under conditions of hypokinesia.

The next series was performed to evaluate the type of NOS, which serves as a source for the increase in NO production during hypokinesia. The study was conducted with a selective iNOS inhibitor aminoguanidine. A selective iNOS inhibitor aminoguanidine decreased the content of NO in the heart atria and ventricles of hypokinetic rats by 64.5 and 57.3%, respectively. NO content in the liver tissue decreased by 71.2% (Fig. 3). These data indicate that activation of NO synthesis during hypokinesia is mainly related to iNOS activity.

Hypokinesia is accompanied by suppression of myocardial function, decrease in the energy potential of the heart, reduction of the minute volume, and dysfunction of venous and arterial vessels. These changes are followed by hypoxia, which results in the increased synthesis of NO, NO^{3-}, and NO^{2-} [4,5]. We cannot exclude the effect of restraint stress on the body. It was hypothesized that the stress response

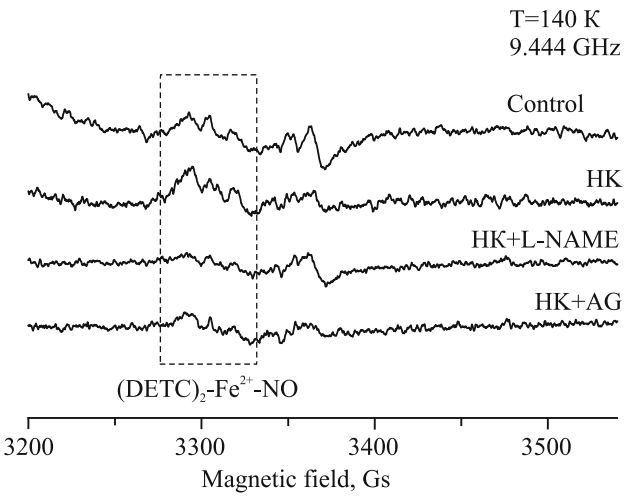

Fig. 1. EPR spectra for rat heart tissue. Dotted area: fragment of the signal from $(DETC)_2$-Fe^{2+}-NO. Here and in Figs. 2 and 3: HK, hypokinesia; AG, aminoguanidine.

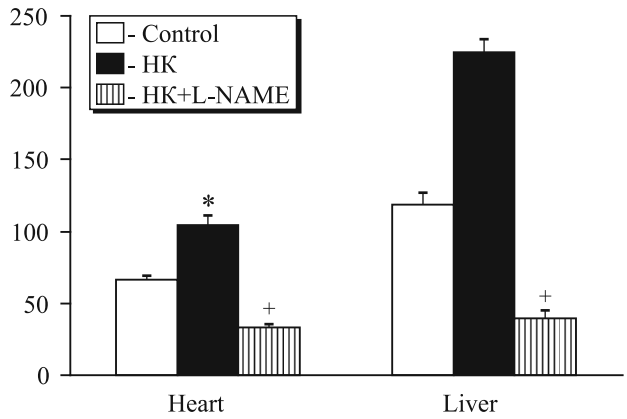

Fig. 2. Nonselective blockade of NO production in the heart and liver tissues of rats with L-NAME. Here and in Fig. 3: ordinate, integral intensity of the signal from $(DETC)_2$-Fe^{2+}-NO (rel. units). $p<0.05$ in comparison with *control, +HK.

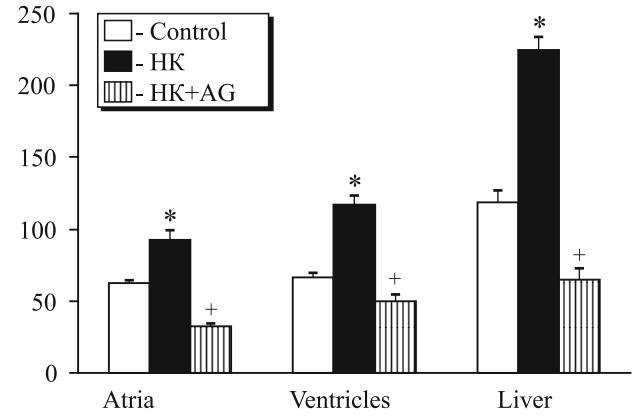

Fig. 3. Blockade of iNOS-catalyzed NO production in tissues of the liver, heart atria, and ventricles of rats with aminoguanidine.

and long-term adaptation are followed by (or result from) the decrease or increase in NO production [6]. Previous studies revealed that 30-day hypokinesia is

followed by an increase in the synthesis and secretion of proinflammatory (IL-1, IL-6, and TNF) and anti-inflammatory (IL-4, IL-10, and IL-12) cytokines [4]. IL-1, TNF-α, and IL-6 activate iNOS. We revealed a significant increase in the production of NO, which can be realized via this mechanism.

Studying the role of the NO system during stress and adaptation is of considerable interest. Various shock states in the body are accompanied by overproduction of NO, while stress-related dysfunction results in the decrease of NO generation.

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