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Effects of NO Synthase Blocker L-NAME on Functional State of the Neuromotor System during Traumatic Disease of the Spinal Cord

G. G. Yafarova[1,2,3], V. V. Andrianov[1,3], R. Kh. Yagudin[2], I. I. Shaikhytdinov[2], and Kh. L. Gainutdinov[1,3]

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Functional state of the neuromotor system after administration of a nonspecific NO synthase blocker L-NAME was studied on the model of experimental contusion of the spinal cord. Electron paramagnetic resonance measurements of NO production in the damaged segment of the spinal cord were performed for estimation of the dynamics of intensity of NO production during traumatic disease of the spinal cord and selection of optimal period for L-NAME administration. The status of the neuromotor system was evaluated by stimulation electromyography. Treatment with L-NAME during the acute period of traumatic injury to the spinal cord sharply reduced the intensity of evoked motor responses and more pronounced increase in excitability of peripheral motor structures. The results suggest that NO system is a factor of regulation of the stress-induced and adaptive responses of the body at the early stage of spinal cord injury.

Key Words: *nitrogen oxide; L-NAME blocker; electron paramagnetic resonance; spinal cord; electroneuromyography*

Nitrogen oxide (NO) is an important transmitter of inter- and intracellular interactions in the body. Studies of the properties and biological role of this molecule showed that NO is a gaseous messenger that plays a role of a universal modulator of diverse body functions, including regulation of respiration, immune status, cardiovascular homeostasis, activity of macrophages, gene expression, plasticity of the nervous tissue, memory, and neurotransmitter release [7-9,13]. The role of NO under conditions of nervous system injury is still debated [10]. Along with vasodilatory, neurotransmitter, and stress-limiting properties, NO takes a part in the development of oxidative stress, glutamate-calcium cascade, and inflammation [9,14]. Spinal injury is associated with Ca^{2+} accumulation in cells, energy deficiency, changes of active ion transport, glutamate excitotoxicity, and oxidative stress. According to previous research [2], activation of inducible NO synthase in the spinal cord is observed on day 3 after trauma. Under these conditions, the reaction between NO and superoxide leads to generation of a strong oxidant peroxynitrite (ONOO—) that oxidizes lipids, proteins, and DNA and induce neuronal death [12,13]. On the other hand, moderate increase in NO concentration (to 0.5 μM) improves cell survival or produces a cytoprotective effect [4]. Thus, NO can produce either damaging or protective effects depending on the conditions. The effects of NO can also depend on its concentration in cells, presence of oxygen, metabolites of oxidative stress and antioxidants, which can alter its amount, signal function, and physiological activity. NO system is of specific interest during spinal cord injury, as it may significantly contribute to adaptive metabolic and structural changes in this

[1]Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University; [2]Republican Clinical Hospital, Ministry of Health of Tatarstan Republic; [3]Kazan Physical-Technical Institute, Kazan Research Center of the Russian Academy of Sciences, Kazan, Tatarstan Republic, Russia. ***Address for correspondence:*** kh_gainutdinov@mail.ru. Kh. L. Gainutdinov

pathology. However, quantitative estimation of NO production in body tissues during traumatic disease of the spinal cord has not been performed yet. This analysis can be conducted by using electron paramagnetic resonance (EPR) spectroscopy, a direct method for estimation of concentration of paramagnetic particles based on the reaction between the radical and spine trap [15]. Experimental measurements of NO production and neurophysiological estimation of the effects of its modulation in spinal cord injury seem to be a promising approach for detailed study of the pathogenesis of this disorder.

Here we studied the dynamics of NO production by EPR-spectroscopy and effects of NO production blocker L-NAME on the functional state of neuromotor system after experimental spinal cord trauma.

MATERIALS AND METHODS

Experiments were performed on outbred male and female rats weighting 200±20 g and aging <1 year (n=41). Standard open spinal cord injury at Th_{IX} level was modeled by the method of A. Allen according to bioethical standards. Diethyldithiocarbamate (DETC) and Fe^{2+} served as a spine trap that bind NO yielding $(DETC)_2$-Fe^{2+}-NO complex characterized by easily discriminated spectrum with hyperfine triplet structure [3]. EPR-measurements of NO production were performed in the damaged segment of the spinal cord (days 1 and 3 after trauma), and in a similar spinal cord area in intact animals (n=21). Spectra of prepared samples were recorded on an X-ray ER 200E SRC EPR spectrometer and EPR EMX/plus spectrometers (Bruker). L-NAME (methyl ether of N-nitro-L-arginine), a non-specific blocker of NO synthases, was injected intraperitoneally in a dose of 20 mg/kg (n=20) on day 2 after the trauma. The status of the neuromotor system in rats in intact and experimental animals was estimated by stimulation electromyography before surgery and on day 6 after the experimental contusion both in controls and rats receiving L-NAME. M-responses of the gastrocnemius muscle to electric stimulation of the left and right sciatic nerves were recorded using needle electrodes. Stimulating needle electrodes were inserted at the sites corresponding to projections of the sciatic nerve near the hip joint; stimulus intensity varied from 0.35 to 60 V, duration was 0.5 msec. The following parameters of M-responses were analyzed: threshold, latent period (LP, time between stimulation onset and the first change of the wave), and amplitude of maximal responses (Amax, intensity of response from maximal positive peak to maximal negative peak to supramaximal stimulation of the nerve). Stimulation and registration of induced responses was performed using an electromyograph (Medickor).

The results were analyzed using Biostat software; Student's t test and Wilcoxon's test were applied.

RESULTS

EPR spectra of the spinal cord of intact rat and rat after spinal cord trauma are presented in Figure 1. Two components were distinguished: signal from $(DETC)_2$-Fe^{2+}-NO complex with g-factor equal to 2.038 and a

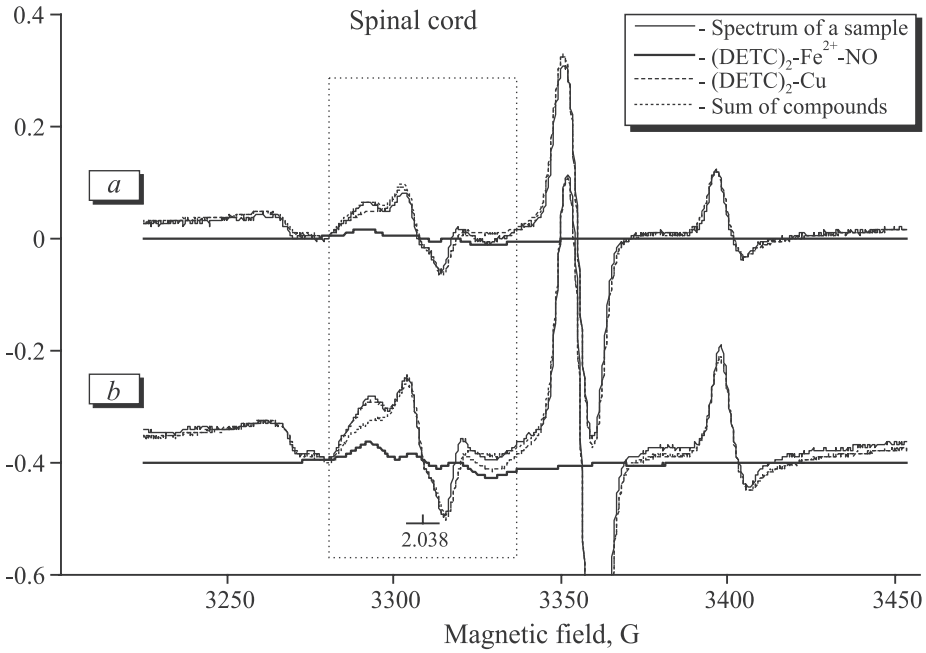

Fig. 1. EPR spectrum of intact rat spinal cord (*a*) and on day 3 after spinal cord trauma (*b*). The signal from spine trap bind to NO is isolated.

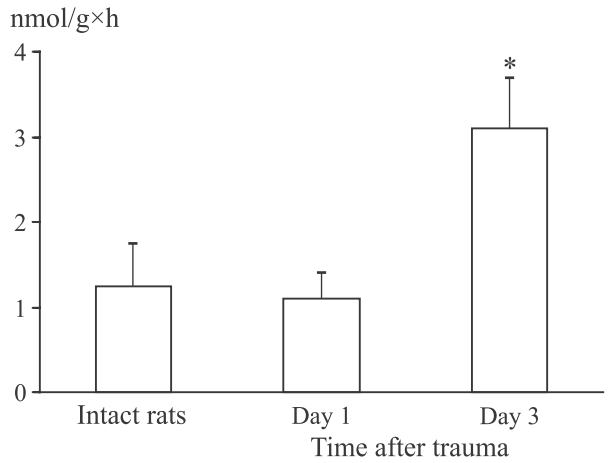

Fig. 2. Changes in NO production in the spinal cord after spinal cord trauma. Here and in Fig. 3: *$p<0.05$ in comparison with intact animals.

signal from $(DETC)_2$-Cu complex. NO concentration was estimated using an integral area of spectrum of $(DETC)_2$-Fe^{2+}-NO complex normalized by the reference sample with known amount of paramagnetic particles. NO production in the spinal cord tissue of intact rats was ~1.3 nmol/g×h. Slight reduction in NO concentration was observed 24 h after spinal trauma, but 3 days after the trauma, the intensity of NO production in tissues of the spinal cord was by 3 times higher than in intact animals ($p<0.05$; Fig. 2).

Electromyography showed that LP of M-responses characterizing conduction velocity in motor fibers remained practically unchanged in both groups. Amax of motor responses of the gastrocnemius muscle on day 6 after spinal trauma in the group not treated with L-NAME did not significantly differ from the corresponding parameter in intact animals. In animals treated with L-NAME, Amax of motor responses was significantly lower ($p<0.05$) than prior to surgery and this parameter in non-treated animals (Fig. 3, *a*). Significant reduction in the amplitude of M-responses during blockage of NO synthases attests to possible protective role of NO in this pathology. It is known that the decrease in the amplitude of M-response can be determined by degenerative changes in both neuronal apparatus of the spinal cord and muscles. NO can protect neurons form toxic effects of glutamate by increasing of cGMP synthesis or blocking NMDA receptors via binding of nitrosonium ion (NO^+) to their regulatory center [10]. Neuronal NO synthase was also found in skeletal muscles and activity of this enzyme decreased during long-term hypokinesia [11]. Recent animal experiments revealed interaction between NO amount in the skeletal muscle (*m. soleus*), activation of neuronal NO synthase, and synthesis of cytoskeletal and contractile proteins; the involvement of neuronal NO synthase of rat skeletal muscle in the regulation of the synthesis of myosin type I heavy chains was demonstrated [5,11]. Thus, NO can modulate muscle status upon changes in motor activity. Intensification of destroying of cytoskeletal proteins during single eccentric load and reduction of animal working capacity was observed after the administration of the neuronal NO synthase blocker. Injection of NO precursor L-arginine 2 days before physical activity prevented protein degradation and enhanced their working capacity [5].

A decrease in the threshold of M-responses of the gastrocnemius muscle to stimulation of the tibial nerve was observed in rats after trauma, and this may reflect an increase in the excitation of peripheral motor structures. This reduction was more pronounced in the group of animals receiving NO synthases blocker L-NAME (Fig. 3, *b*). The decrease in the threshold of induced responses during this pathology is related to the development of denervation hypersensitivity. More pronounced decrease in the threshold in animals can be explained by the fact that NO play a role of retro-

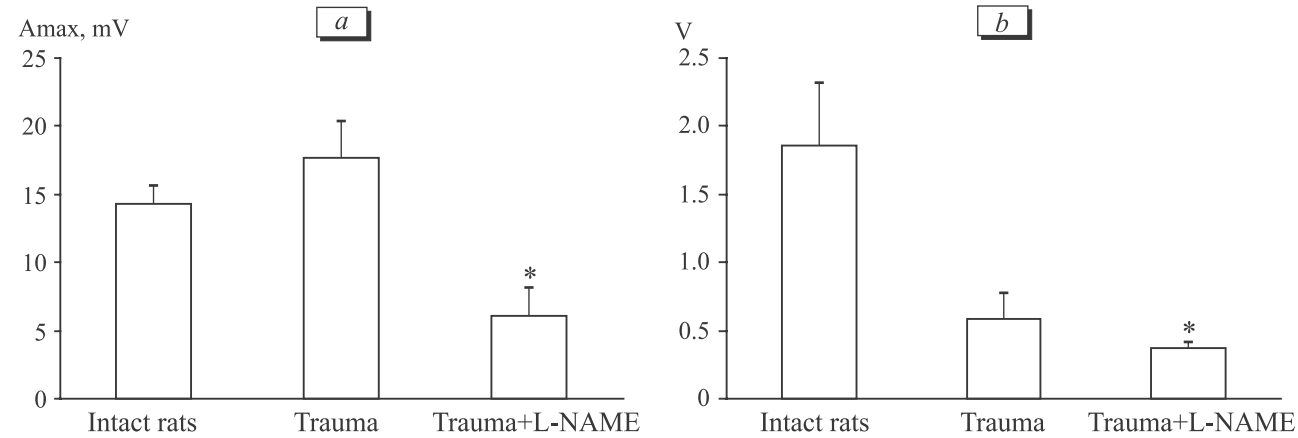

Fig. 3. Effects of L-NAME on the maximal amplitude (*a*) and threshold (*b*) of motor responses of the gastrocnemius muscle in 6 days after the spinal cord trauma.

grade messenger in the neuromuscular synapses. It is known that NO modulates the neurotransmitter release in the peripheral nervous system: endogenous tonic synthesis of NO is supposed to appear in the area of neuromuscular synapses followed by retrograde diffusion of NO to the nerve terminal and inhibition of acetylcholine release [8,9]. Blockage of NO synthesis reduces acetylcholine level, which can determine more pronounced decrease in motor responses in animals receiving L-NAME.

We have previously demonstrated an increase of NO production in the heart in 3 days after spinal cord trauma [1]. NO synthesis in the liver during this period also significantly surpassed the control values. The results of EPR analysis suggest that the increase in NO amount in the early post-trauma period is typical not only of the damaged nervous tissue, but also in other vital organs. This generalized response suggests activation of the NO-dependent stress-limiting system during this period. It was previously shown that long-term hypokinesia in animals is associated with changes in the dynamics of NO production [3] and NO-dependent mechanisms of restraint stress were revealed [6]. Now, it was hypothesized that NO contributes to the regulation of stress response by limiting its excessive activation and damaging effects [6]. According to this hypothesis, NOergic stress-limiting system work in parallel with the well-known stress-limiting systems (GABAergic, peptidergic, antioxidant, *etc*.). Taking into account high functional strain of the neuromotor and metabolic systems during spinal cord traumas, it can be hypothesized that regulation of intracellular and systemic interactions under these conditions is also related to modulation of the intensity of NO production in the body.

Therefore, an increase in NO production was observed in 72 h after spinal cord trauma. Blockage of NO synthesis is followed by a decrease in the intensity of induced motor responses and more pronounced increase in excitation of peripheral motor structures. Obtained data reflect that an increase in NO production during this pathology is an adaptive response, which promotes the integrity of peripheral motor structures after trauma of the spinal cord. Taking into account not only local, but also generalized enhancement of NO production on day 3 after the trauma, it may be concluded that NO serves as a regulators of stress-response of the body at the early stages of traumatic disorder of the spinal cord.

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