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ABSTRACTS


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Application of EPR spectroscopy to study changes in the content of nitric monoxide and copper in the rats frontal lobes after modeling a combined injury of brain and spinal cord

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Nitric oxide (NO) is known as one of the most important signaling molecules regulating the physiological functions of the organism and the metabolism of cells [1]. Studies of the role of NO in the transmission of signals in the nervous system began shortly after its discovery [2]. It was found that the production of endogenous NO is observed in almost all tested groups of animals, as well as in plants, diatoms, slime molds and bacteria [3]. By activating soluble heme-containing guanylate cyclase and ADP-ribosyltransferase, NO participates in the regulation of intracellular concentration of Ca^{2+} ions, is involved in pH regulation against the background of cerebral ischemia [4]. The two main mechanisms stabilizing the unpaired electron $^{\bullet}NO$ are its reaction with other free radicals and interactions with d-orbitals of transition metals [5]. Since NO is a chemically highly reactive free radical capable of acting both as an oxidizer and as a reducing agent [1], there is an assumption about its diverse effects in biological tissues. Therefore, NO is an example of the classic two-faced Janus.

There are many methods of measuring NO production in biological systems. Precise measurement of both the steady concentration of NO and the speed of NO generation in biological systems is a difficult task due to the low activity of NO synthases and its short half-life. In last years electronic paramagnetic resonance (EPR) proved to be one of the most efficient methods for the detection and quantification of nitric oxide in biological tissues [2, 5]. Therefore, we conducted a study of the dynamics of NO production in injured and non-injured areas (frontal lobes) of the brain when modeling combined brain and spinal cord injury in rats.

We used EPR spectroscopy to study the dynamics of NO in the brain and heart of rats after modeling a number of pathological processes. The intensity of NO production by EPR spectroscopy was measured using the spin trap technique [1, 6], which is based on the reaction of a radical (in this case NO) with the spin trap. The complex of Fe^{2+} with diethyldithiocarbamate (DETC) was used to capture NO and to form a stable ternary complex $(DETC)_2$-Fe^{2+}-NO in the animal tissues. Those complexes are characterized by an easily recognizable EPR spectrum with *g*-factor $g = 2.035–2.040$ and a triplet hyperfine structure [6, 7]. The spectra of the complex $(DETC)_2$-Fe^{2+}-NO were measured on Brucker X spectrometers (9.50 GHz) EMX/plus with a temperature module ER 4112HV with a magnetic field modulation of 100 kHz and a modulation

amplitude of 2 G ,with a microwave power of 30 mW, a time constant of 200 ms and a temperature of 77 K in a finger Dewar of the Bruker company.

By the methods of EPR spectroscopy our team has evaluated effect of brain stroke on the intensity of NO production in the tissues of the brain of rats in vivo. A simulation of brain injury and then spinal cord injury was performed. Seven days after the operation, tissues were extracted from the injured and non-injured areas of the brain and measurements were made. The measurements of NO were carried out by EPR spectroscopy. The results of the analysis demonstrate a significant decrease in NO production 7 days after the simulation of trauma in the injured brain region by an average of 6 times (P = 0.029, Mann-Whitney) and also a significant decrease in NO production in the non-injured (contralateral) brain region by an average of 3 times. There is a difference in NO production in the injured and contralateral regions of the brain (P = 0.050, t-test).

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