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RADIOSPECTROMETRIC METHODS IN SCIENCES OF LIFE

Changes in Nitric Oxide and Copper Content in Rat Liver and Hippocampus after Brain Ischemia Modeling

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Abstract—Results of analysis of nitric oxide and copper content in rat liver and hippocampus after brain ischemia modeling are provided. The studies are carried out using the electron paramagnetic resonance spectroscopy method with spin traps. It was shown that, the day after brain ischemia modeling, nitric oxide content in hippocampus decreases on average by 50% and a tendency toward its decrease was observed in liver tissues. Two days after brain ischemia modeling, nitric oxide content in the brain recovered and a significant increase by 46% against control indices was observed in the liver. On the second day of the postischemic period, the copper content, which is associated with superoxide dismutase content, increased on average by 2.5-fold in the liver. No significant changes in copper content was found in the hippocampus.

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INTRODUCTION

The function of tissues in an organism depends on a number of factors. One such factor is the necessity for a sufficient amount of oxygen, which is delivered with the bloodstream, for oxidation processes to take place. Oxygen insufficiency results in pathological processes in the organism, which are preceded by hypoxia [1, 2]. Hypoxia is a pathological process occurring at an insufficient oxygen supply of organism tissues or impaired oxygen utilization in the course of biooxidation—a condition of oxygen starvation both of the whole organism and individual organs and tissues [2, 3]. This is an important component of pathogenesis of many diseases [4–6]. Hypoxia is a universal pathological condition in the most diverse human diseases: cardiovascular and respiratory failures, myocardial ischemia, impaired brain and peripheral circulation, etc. [2, 5, 7]. When there is an insufficient oxygen supply to an organism, and to the brain in particular, brain ischemia occurs, which may end in ischemic stroke [3, 8].

During hypoxia, the functioning of neuromediatory systems is impaired, including that of the nitric oxide (NO) system [9]. NO is known as one of the most important signal molecules, regulating the physiological function of the organism and cell metabolism. It is widespread in the nervous system [10, 11]. The participation of NO in the development of different pathological conditions in the organism is attracting great interest [10, 12–14]. At present, the development of brain ischemia and the ensuing occurrence of stroke are associated with the impairment of brain circulation, as well as the impairment of its regulation by the NO system [14–16]. NO performs its physiological functions, either binding with iron ions (Fe) included in a gem, or via S-nitrosylation of proteins, and also participates in a whole number of biochemical reactions [10, 17, 18]. It is found that NO plays both protective and destructive roles in pathological processes, which is caused by many factors [19].

Therefore, two diametrically opposite actions of NO can be considered: a stimulating positive one and a toxic damaging one [19]. It may be that this is related to the amount of nitric oxide in tissues. The issue appears of using a modern method of detection and quantitative determination of NO content in tissues of living organisms in the norm and experimentally modeled pathologies.

Electron paramagnetic resonance (EPR) is one of the most effective methods of detection and quantitative determination of NO in biological tissues [17, 20, 21]. The effectiveness of the method is determined by a technique, developed by Vanin et al. [22], which is based on the reaction of a radical (in this case, NO) with a spin trap. An adduct with a typical EPR spectrum is formed as a result of the reaction. The authors used a complex of Fe^{2+} with diethyl dithiocarbamate (DETC) to capture NO and form stable triple

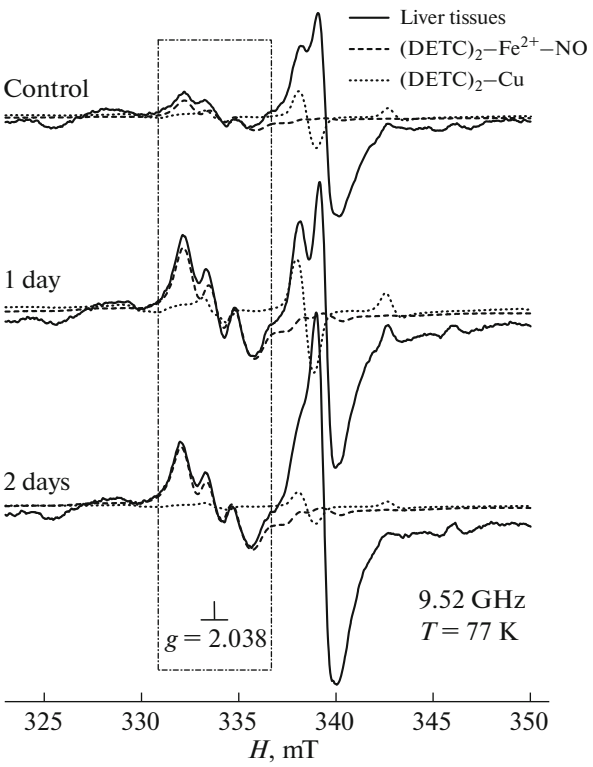

Fig. 1. EPR spectra of liver tissue of intact rat ("Control") and of a rat on the next day ("1 day") and after 2 days ("2 days") after modeling brain ischemia. The rats were administered with components of spin trap to form the $(DETC)_2$–Fe^{2+}–NO complex. Abscissa shows the magnetic field.

$(DETC)_2$–Fe^{2+}–NO complex in different animal tissues. These complexes are characterized by an easily recognizable EPR spectrum with a *g* factor value of 2.035–2.040 and a triplet hyperfine structure [17, 21, 23]. The method has a sensitivity of 0.04–0.4 nM, allows one to perform direct measurements, and is highly sensitive owing to the use of spin traps [24].

Earlier, our group performed an in vivo estimation of the effect of ischemic stroke on NO production in brain, heart, and liver tissues in rats via the EPR spectroscopy method [25]. To model ischemic stroke, animals were subjected to 5-min hypobaric hypoxia (by conventional 4500-m ascent above sea level); in this case, the whole organism is subjected to ischemia. The goal of this work was to study, via the EPR spectroscopy method, the intensity of NO production and copper content (as an index of superoxide dismutase) in liver tissues, as well as in the hippocampus of rats, in modeling brain ischemia via carotid ligation [26, 27], which allows only the brain to be subjected to an ischemic effect.

1. METHODS

Modeling of ischemic stroke was carried out according to the accepted protocol of the Ethics Committee of the Institute of Physiology of the National Academy of Science of Belarus, Minsk. Brain ischemia was modelled by ligation of common carotids at the bifurcation level in male Wistar rats ($n = 10$) under ketamine–xylazine–acepromazine narcosis [26, 27]. The second group consisted of intact animals ($n = 10$) that had not been subject to surgical manipulations in the brain area. Thirty minutes before the extraction of the studied tissues, the animals were administered with components of a spin trap for NO. Liver tissues, as well as the hippocampus, were extracted under ketamine–xylazine–acepromazine narcosis, and the samples were immediately frozen in liquid nitrogen.

During the preparation of samples for measuring EPR spectra, we based what we did on the spin trap technique suggested by Prof. A.F. Vanin et al. [17, 28]. We used the complex of Fe^{2+} with diethyl dithiocarbamate, $(DETC)_2$–Fe^{2+} as a spin trap. The spin trap complex with NO ($(DETC)_2$ –Fe^{2+}–NO) is characterized by an easily recognizable EPR spectrum with a *g* factor value of 2.038 and triplet hyperfine structure [14, 17, 21]. As was done earlier [20], DETC–Na was administered intraperitoneally in a dose of 500 mg/kg in 2.5 mL of water. The mixture of iron sulfate solutions ($FeSO_4 \cdot 7H_2O$, Sigma, United States) in a dose of 37.5 mg/kg and sodium citrate in a dose of 187.5 mg/kg (in a volume of 1 mL water per 300 g animal weight) prepared immediately before the administration was administered subcutaneously into three areas: the right and left femur and the rostral section of the interscapular region. Iron citrate is formed in the mixture of iron sulfate and sodium citrate. DETC–Na and iron citrate are distributed over the organism and, upon interaction, form a water-insoluble complex (DETC)–Fe^{2+}, which can interact with NO, forming a stable $(DETC)_2$–Fe^{2+}–NO radical, which can be detected via the EPR spectroscopy method. The spin trap components (DETC–Na, $FeSO_4$, sodium citrate) were administered to the animals 30 min before the extraction of studied tissues. Tissue samples (of the liver and hippocampus) were immediately frozen in liquid nitrogen and were in frozen state transferred from Minsk to Kazan in plastic containers with dry ice. The complex of a spin trap with NO ($(DETC)_2$–Fe^{2+}–NO) is well preserved in this condition, and the signal from the complex does not change for more than a month. The mass of samples were about 100 mg.

The spin trap also interacts with Cu, forming $Cu(DETC)_2$ complex, which also can be detected via the EPR spectroscopy method [29]. The measurements of the spectra of $(DETC)_2$–Fe^{2+}–NO) and Cu $(DETC)_2$ complexes were performed on X range (9.50 GHz) Bruker EMX/plus spectrometers with ER 4112HV temperature control systems and ER 200 SRC

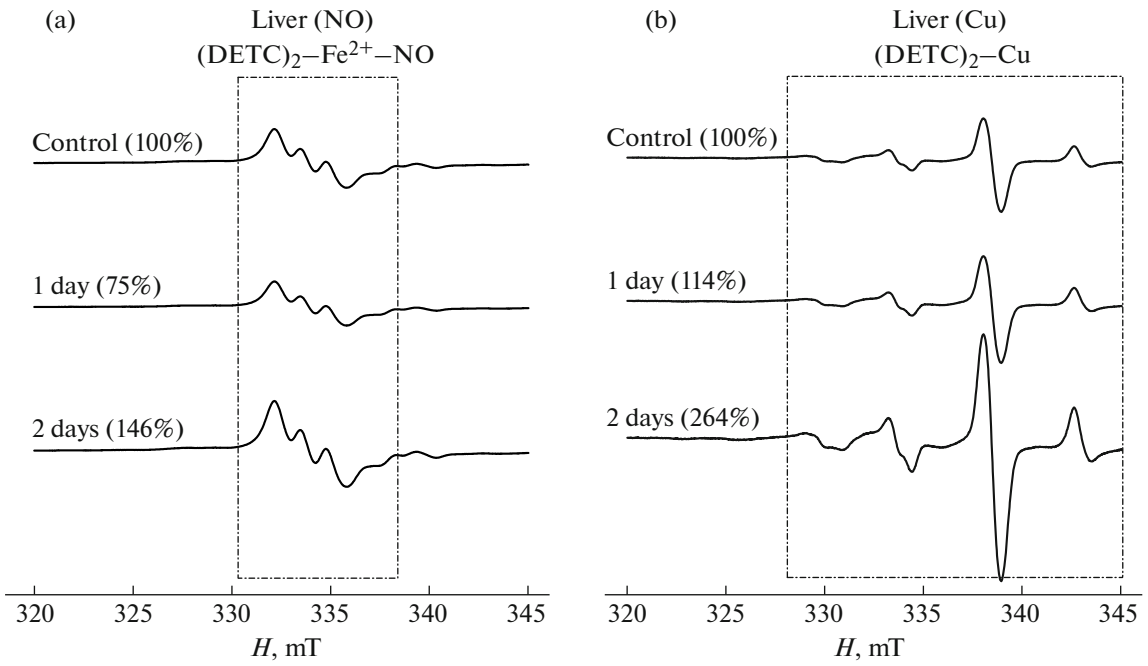

Fig. 2. Middle (medium) signals of complexes (a) $(DETC)_2–Fe^{2+}–NO$ and (b) $(DETC)_2–Cu$ picked out from EPR spectra of liver tissues of an intact rat ("Control") and of a rat on the next day ("1 day") and in 2 days ("2 days") after modeling brain ischemia. The dash-dot lines show areas of complexes (a) $(DETC)_2–Fe^{2+}–NO$ and (b) $(DETC)_2–Cu$ in an observed signal. Average levels of signals of these complexes are shown in percentage. Abscissa shows the magnetic field.

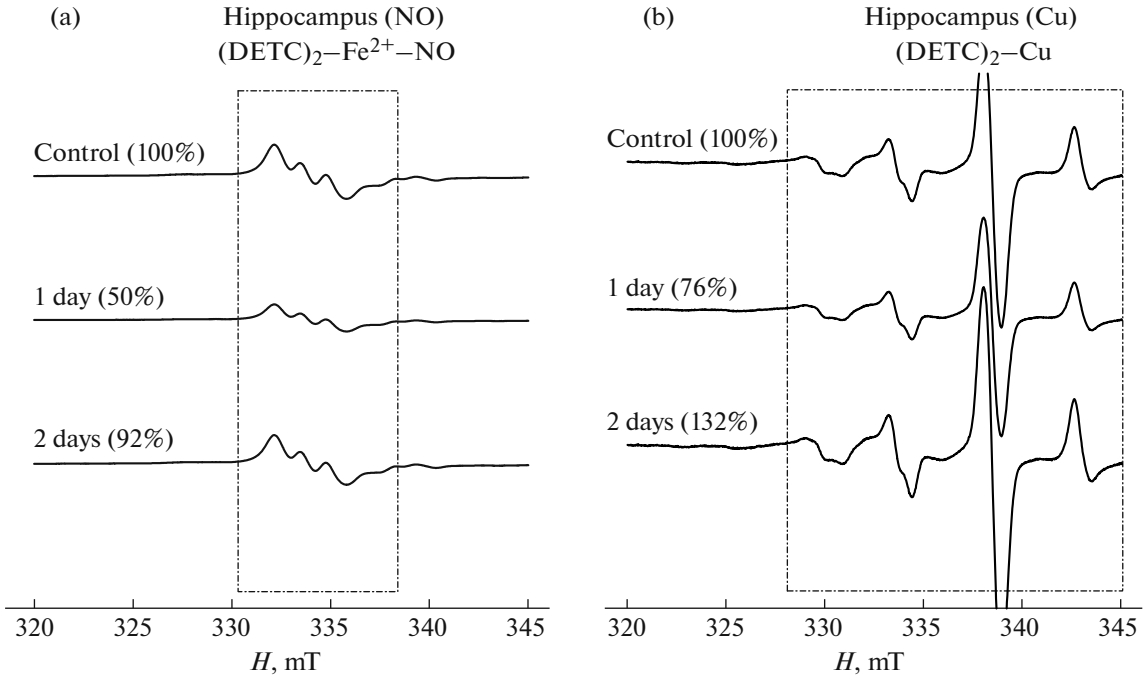

Fig. 3. Average signals of $(DETC)_2–Fe^{2+}–NO$ (a) and $(DETC)_2–Cu$ (b) picked out from EPR spectra of hippocampus of an intact rat ("Control"), a rat on the next day ("1 day"), and in 2 days ("2 days") after modeling brain ischemia. The dash-dot lines show areas of complexes (a) $(DETC)_2–Fe^{2+}–NO$ and (b) $(DETC)_2–Cu$ in an observed signal. Average levels of signals of these complexes are shown in percentage. Abscissa shows the magnetic field.

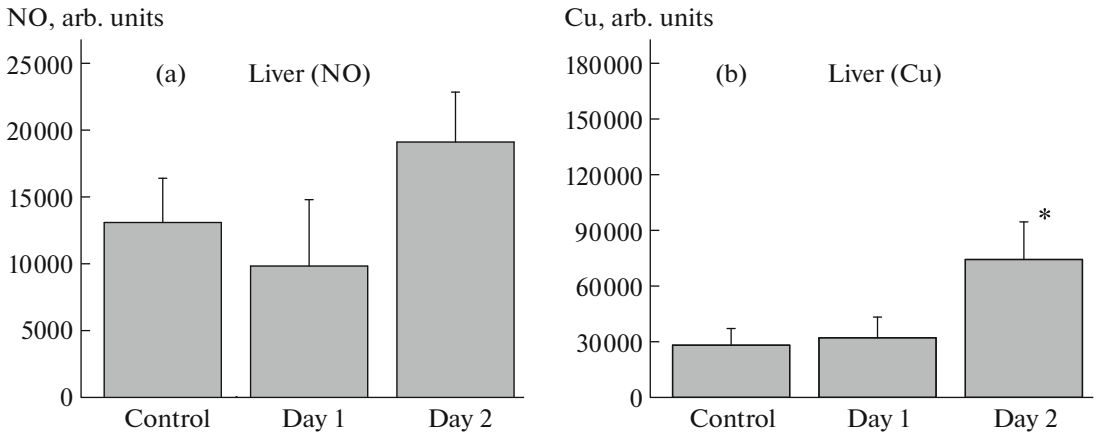

Fig. 4. Relative content of (a) NO and (b) Cu in liver tissues of an intact rat ("Control") and a rat on the next day ("1 day") and in 2 days ("2 days") after modeling brain ischemia. Average values and standard error of the mean are shown; * difference from the control (t-test, $p < 0.05$). Ordinate shows the average integral intensity (arb. units) of the signal of $(DETC)_2$–Fe^{2+}–NO and $(DETC)_2$–Cu complexes.

at a 100-kHz magnetic field modulation, 2 G amplitude modulation, 30-mW SHF emission power, 200-ms time constant, and 77-K temperature in a Bruker finger-type Dewar. Modulation amplitude, amplification, and SHF power were selected on the condition of no overmodulation and no saturation of the EPR signal and kept the same during all measurements.

RESULTS AND DISCUSSION

We have conducted the study of the intensity of NO production and copper content (as a superoxide dismutase indicator) in liver tissues, as well as in the hippocampus of rats after brain ischemia modeling. The modeling of brain ischemia was realized via ligation of common carotids [26, 27], which allows only the brain, not the whole organism, to be subjected to an ischemic effect, as was done in our previous work [25]. An example of the EPR spectrum of liver tissues of intact (control) rats and of rats in 1 and 2 days after brain ischemia modeling is presented in Fig. 1. One can see on these spectra a typical triplet signal from $(DETC)_2$–Fe^{2+}–NO complex with a 2.038-g factor value and a signal from copper [14, 17, 24, 28]. These ($(DETC)_2$–Fe^{2+}–NO) and ($(DETC)_2$–Cu) signals were extracted from the total EPR spectrum for liver tissues (Fig. 2) and hippocampus (Fig. 3).

The next day after brain ischemia modeling, NO content in liver tissues tended to decrease (Fig. 4a) and decreased significantly in the hippocampus by 50% on average (Fig. 5a). Two days after brain ischemia modeling, NO content recovered in hippocampus and significantly increased (on average by 46%) in liver tissues. Earlier, we obtained a more significant decrease in NO content in brain tissues, and the same in the brain in modeling ischemia of the whole organism via the hypobaric hypoxia method (via conventional ascent to height) [25]. The obtained results show that, in modeling hypoxia of the whole organism, NO production changes in tissues of peripheral organs greater than in brain ischemia. At the same time, changing in the NO production in the brain in modeling of brain ischemia and modeling of the whole-organism ischemia is practically the same. There is a suggestion that, after the burst of NO production in the first hours [30, 31], a depletion of L-arginine, which is expended in the initial minutes or hours after ischemia/hypoxia, occurs [32]. It may be that it is for this reason that the decrease in NO content in tissues in the first 24 h is better expressed in modeling of whole-organism ischemia.

The copper content the next day after modeling of brain ischemia in liver tissues did not change (Fig. 4b), and a tendency toward a decrease in hippocampus was observed (Fig. 5b). Two days after modeling of brain ischemia, the copper content insignificantly increased in hippocampus, and we observed a 2.5-fold average increase in liver tissues. The dismutation of the superoxide with the help of Cu,Zn—COD (superoxide dismutase) cytosolic enzyme is the primary and main protection from free radical oxidation [10, 12]. We have shown that the copper content associated with superoxidedismutase content does not change in a day after ischemia modeling and increases 2 days after ischemia modeling in liver and hippocampus (significant in liver). It shows the preservation of antioxidative protection system activity during this time and even its increase on the second day, which can counteract hypoxic factors. The obtained results can be helpful in the essential background research for creating drugs of diverse therapeutic action [33].

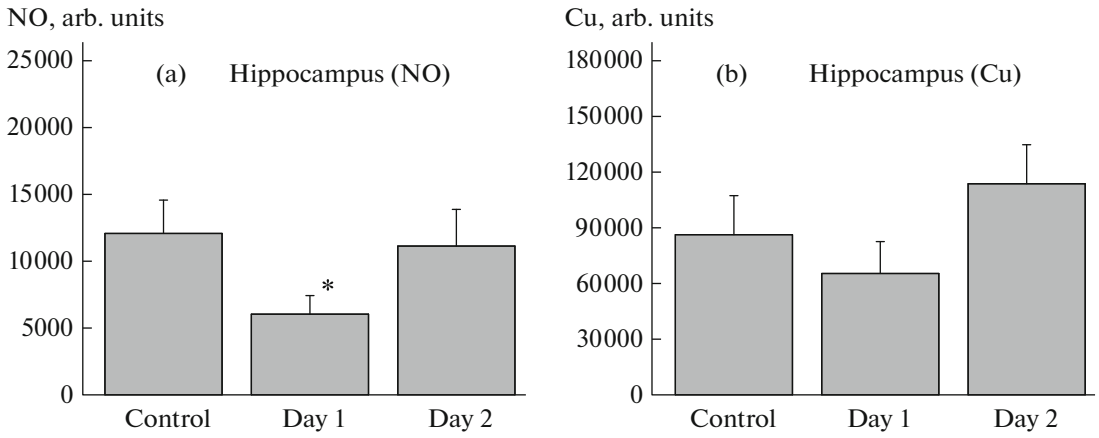

Fig. 5. Relative content of (a) NO and (b) Cu in the hippocampus of an intact rat ("Control") and a rat on the next day ("1 day") and in 2 days ("2 days") after modeling brain ischemia. Average values and standard error of the mean are shown; * difference from the control (t-test, $p < 0.05$). Ordinate shows the average integral intensity (arb. units) of the signal of $(DETC)_2$–Fe^{2+}–NO and $(DETC)_2$–Cu complexes.

CONCLUSIONS

We performed the study of NO production intensity and copper content (as a superoxide dismutase indicator) in liver tissues, as well in the hippocampus of rats after brain ischemia. Modeling of brain ischemia was realized by ligation of common carotids; in this technique, only the brain is subjected to ischemic effect, but not the whole organism, like in hypobaric hypoxia (conventional ascent to height). The obtained results show that, in modeling of ischemia of the whole organism, NO production changes more than in brain ischemia. At the same time, NO production in the brain changes in modeling of brain ischemia and in modeling the whole-organism ischemia in much the same way.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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