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EPR Study of the Intensity of the Nitric Oxide Production in Rat Brain After Ischemic Stroke

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Abstract Electron paramagnetic resonance of $(DETC)_2$–Fe^{2+}–NO complexes has been used as a method to detect the formation of nitric oxide (NO) in the brain tissues of Wistar rats. The content of nitric oxide in the center of ischemia (left brain hemisphere), in the non-ischemic part of the left hemisphere, and also in the regions conditionally not affected by ischemia, the cerebral cortex of the right hemisphere and the cerebellum of rats was studied in 5, 9, 24 and 72 h after modeling an ischemic stroke. It is found that in the ischemic part of the left hemisphere in 5 h after modeling the ischemic stroke, the NO content in the spin trap and R-conformer compositions decreases by 5 times and 30%, respectively. This decrease in the NO content is found in 9 and 24 h after the stroke. In the cerebral cortex of the right hemisphere and non-ischemic parts of the cerebral cortex of the left hemisphere, the NO content in the composition of the spin trap in 5, 9 and 24 h after the stroke decreases by 40–50%. In the composition of the R-conformer it does not vary. In 72 h, the partial restoration of the NO content in all regions except for the ischemia zone is observed.

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1 Introduction

Under normal conditions nitric oxide (NO) functions through the activation of the soluble form of the enzyme guanylate cyclase (pGC), via the reversible interaction with the enzyme of the respiratory chain cytochrome *c* oxidase, reversible linkage with proteins–enzymes through the mechanisms of S-nitrosylation [1, 2]. It is shown that the change of the NO production in the nervous tissue can result in various pathologies [3, 4]; one of such pathologies is an ischemic insult of brain. At present, the development of brain ischemia and the subsequent occurrence of the stroke are connected with the damages of the brain blood-groove, and also with the disturbances in its regulation by the nitric oxide system [5, 6]. During analyses of NO role in brain ischemia by measuring the activity of NO synthase (NOS) it was found that already in 10 min from the beginning of brain ischemia, the activity of neuronal NOS (nNOS) increases with the maximum in 3 h. Such a response is connected with the activation of the glutamatergic system and is considered as a reason of the earliest damage of the neurons. The activation of inducible NOS (iNOS) is manifested in the problem region in 12 h from the beginning of the ischemia with the reaction peak in 48 h. The inducible enzyme isoform accompanies the development of the inflammatory reaction and provides the damage in the brain with peroxynitrite being the main damaging derivative of NO in this case. Within an hour of the ischemic attack it increases the activity and keeps endothelial NOS (eNOS) for about 1 day. The functioning of the enzyme results in the vasodilation of vessels and promotes the decrease in the damage [7].

According to the above, we should expect the increase in the NO content in the brain tissues and blood within the first 3 days from the beginning of ischemia. However, it is difficult to directly measure the NO content in the brain tissues due to the short lifetime of this molecule and its low concentration, therefore one can judge about the change of the NO content during ischemia according to the indirect data: the increase in cGMP (cyclic guanosine monophosphate) in a cell, the increase in the NOS activity isolated from the ischemic tissues, etc. [6, 8]. It is considered that the content of nitrites and nitrates reflects the activity of the NO system, and their concentration is measured in blood, urine and tissues due to the simplicity of the technique. The situation is complicated by the fact that the mechanisms and the speed of the nitric oxide utilization are not studied.

One can mention two opposite effects of NO: first, the stimulating, positive effect; and secondly, it can produce a toxic, damaging effect and result in the destruction of cells [4, 9]. Most likely, the question is only about the content of nitric oxide in tissues. On this assumption, it is not clear what amount of NO in the tissues of mammalian is considered as small or increased. Therefore, there arises a question about using a modern method of the detection and quantitative determination of the nitric oxide content in the tissues of live organisms, in particular, in different brain regions of animals in the normal conditions and during experimental modeling of pathologies.

Recently, electron paramagnetic resonance (EPR) became one of the most effective methods of detection and quantitative determination of nitric oxide in biological tissues [10, 11]. This happened owing to a technique developed by Vanin

et al. [12], in which they used a method of spin trapping. This method is based on the reaction of a radical (in this case NO) with a spin trap. As a result of this reaction an adduct with a characteristic EPR spectrum is formed. Vanin et al. [10, 12] have applied a complex of Fe^{2+} with diethyldithiocarbamate (DETC) to capture NO and form a steady $(DETC)_2$–Fe^{2+}–NO complex in various tissues of animals. These complexes are characterized by an easily distinguished EPR spectrum with the value of the g-factor $g = 2.035$–2.040 and triplet hyperfine structure.

The aim of our study is to measure the dynamics of the NO content by EPR in different brain regions of rats at a focal stroke: separately in the center of the ischemia of the left hemisphere, the remaining part of the left hemisphere; and in the regions conditionally not affected by ischemia, the right hemisphere and cerebellum.

2 Materials and Methods

2.1 Formation of Triple Complex of $(DETC)_2$–Fe^{2+}–NO in Rat Tissue

The main problem in determination of NO concentration in tissues and liquids is its high activity and short living time. These reasons lead to low stationary level of NO. During EPR samples preparation the spin traps method has been used [13]. DETC-Na was introduced into rat's body intraperitoneally in concentration of 500 mg/kg in 2.5 ml water to one animal [14]. The mixture of solutions, 37.5 mg/kg iron sulfate ($FeSO_4 \cdot 7H_2O$, Sigma, USA) and 187.5 mg/kg sodium citrate (all in 1 ml water to one animal), prepared before injection was injected under the skin at three points—left and right legs and withers. In mixture iron sulfate and sodium citrate produce iron citrate. DETC-Na and iron citrate distribute in organism and its interaction generates the water insoluble hydrophobic DETC-Fe complex, which can interact with NO to form the paramagnetic mononitrosyl iron complex (MNIC) $DETC_2$–Fe^{2+}–NO with area detection by EPR spectroscopy.

2.2 Detection of $(DETC)_2$–Fe^{2+}–NO

The experiments have been performed on an EPR spectrometer Bruker ER200SRC at X-band with the following parameters: modulation, 100 kHz; amplitude modulation, 2 G; microwave power, 30 mW; time constant, 200 ms; temperature, 77 K. All experiments were performed in a flack duar and have been conducted without microwave saturation and overmodulation. Microwave parameters were identical for all samples [15]. All measurements were performed on two paramagnetic complexes of an iron ion with nitric oxide. They are $DETC_2$–Fe^{2+}–NO complex based on a spin trap and iron hemoglobin complex with nitric oxide called R-conformer.

The sample was weighed before the experiments. The sample mass was about 100 mg. Amplitude of the EPR spectra was normalized on the sample mass and on the EPR of a standard sample (Fig. 1). 2,2,6,6-Tetramethylpiperidine-1-oxyl (TEMPO) radical solution (8.89 mM/l, 58×10^{17} spins/cm^3, $S = 1/2$, $g \sim 2.0023$) was used as a standard sample [16].

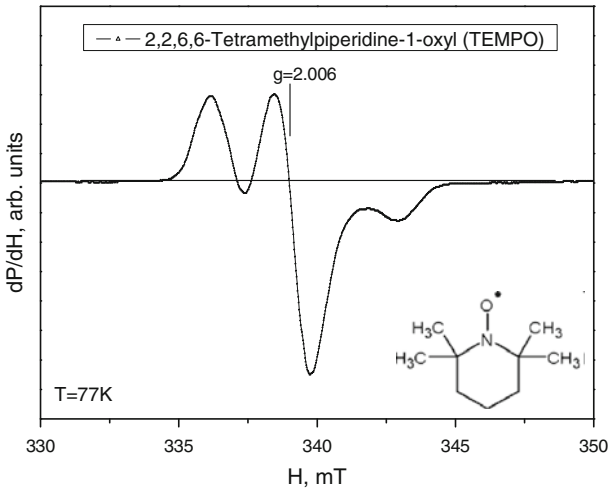

Fig. 1 EPR signal of the reference sample TEMPO radical. Steady-state EPR spectra of a standard sample of TEMPO radical in toluene

2.3 Determination of Paramagnetic Species

EPR is one of the methods to determine the number of NO paramagnetic species in a biological sample [11, 13, 17]. Quantitative EPR as an analytical method yields extensive dynamic range with high sensitivity for measurements of different types of samples. The comparison with a standard sample (1 mM) is one of the procedures to determine the number of paramagnetic particles. Since double integral of the EPR line is proportional to the concentration of paramagnetic species [18], the ratio of the integrals from the investigated and the standard samples will give the needed concentration of the sample [19]. EPR standard must be recorded at the same microwave parameters as the examined sample. For this purpose, the double resonator ER 4105DR (TE 104; nominal frequency, 9.7 GHz; dimensions, [2 × 42] × 23.5 × 11 mm) is usually applied. This resonator has two holes for EPR tubes and these holes are connected with each other and with a microwave power generator [20]. Hence, both the samples have identical microwave parameters.

The following procedure is used for determination of the concentration of paramagnetic species: the investigated sample is set to one hole of the resonator and the standard to the other. Note that the position of the sample relative to the center of the resonator strongly influences the intensity of the EPR spectra. The method of positioning the sample in the resonator TE104 described in works of Mazur et al. [20] was used. Using this method we have chosen for each investigated sample the position with the maximum intensity of the EPR signal. In this case the error in the intensity due to changing of the samples (750 samples) and tuning the spectrometer was 8–10%.

The number of spins in the investigated sample, using the comparison method with a standard sample, is given by the following equation [28]:

$$\frac{N^A_{\text{spin}}}{N^B_{\text{spin}}} = \frac{V^A_s D^A Q^B_u \eta^B(g^B) S^B(S^B+1)}{V^B_s D^B Q^A_u \eta^A(g^A) S^A(S^A+1)} \frac{\sqrt{P^B_w} A^A}{\sqrt{P^A_w} A^B} \frac{H^B_{\text{mod}}}{H^A_{\text{mod}}}, \quad (1)$$

where V_s is the volume of the sample, $\eta = 2V_s/V_c$ is the filling factor, S is the spin of the system, g is the g-factor, Q_u is the quality of the resonator, P_w is the microwave power, H_{mod} is the modulation amplitude, A is the double integration value of the EPR spectra.

Equation (1) with account of saving the experimental conditions can be described as:

$$\frac{N^A_{\text{spin}}}{N^B_{\text{spin}}} = \frac{V^A_s \eta^B(g^B)}{V^B_s \eta^A(g^A)} \frac{A^A}{A^B}. \quad (2)$$

EPR spectra of investigated samples were measured without saturation and field modulation. During the experiment, the change in the line width and hyperfine constant was not observed.

2.4 Experimental Protocol: Simulation of Focal Stroke of Rats and Administration of Trapping for Nitrogen Oxide

The work was done with rats from Wistar population with weight of 300–400 g. The animals were kept in vivarium conditions with regulated light 12-h day and 12-h night regime, with free access to water and food. Focal stroke was simulated by modified Chen method, which was published in 1989 [21]. Under hydrochloride anesthesia (400 mg/kg), the left middle brain artery (MBA) and a chain of the frontal vein, which crosses the MBA, were irreversibly coagulated. For stabilization of the damage focus, the ipsilateral carotid artery was irreversible bandaged. The level of coagulation of MBA was chosen such that the necrosis focus forms in the frontoparietal part of the brain cortex, without touching the subcortical structures. All manipulations were done on rats narcotized with hydrochloride (dose, 400 mg/kg). The trapping of nitrogen oxide was introduced in 5, 9, 24, 72 h after stroke simulation of animals. Accumulation of the signal was done for 30 min. The probes of whole blood were taken from a jugular vein by plastic syringe and were immediately frozen in liquid nitrogen. A death lethal injection was performed. The brain fragments were frozen in plastic containers: the region of ischemia of the cortex of the left cerebral hemisphere separately; the remainder of the cortex of the left cerebral hemisphere; the cortex of the right cerebral hemispheres; the cerebella—4 probes of brain from each animal. For each time point in each group 6 measurements were done, the total number of animals was 30.

2.5 Statistical Processing of Experimental Results

Results are presented as $M \pm m$ (average value $\pm$ standard deviation). Statistical processing of data was performed using a Student's t criteria. The differences were significant if $p < 0.05$.

3 Results

3.1 EPR Spectra of the Cerebellum, Cerebral Cortex of the Left and Right Brain Hemispheres in Intact Rats

Before experimental series the EPR spectra from a donor source of NO, sodium nitroprusside (SNP), was measured. Typical triple EPR spectra (Fig. 2) characteristic for NO connected with a spin trap were observed [10, 13, 14]. Figure 3a shows a typical EPR spectrum of the frozen sample of the cerebral cortex of the right hemisphere of a healthy rat after introduction of the nitric oxide trap, $DETC_2 + Fe^{2+}$-citrate. Similar spectra were obtained for other brain regions. By comparing the obtained spectra with the reference EPR spectrum (Fig. 2), the EPR signals of the complex $(DETC)_2$–Fe^{2+}–NO could be unambiguously separated. In all measurements, both the EPR signal from NO in the spin trap composition and that from NO in the R-conformer composition [17, 22] were observed. Figure 4 shows the EPR spectrum of cerebellum in which the latter signal is distinct. Figures 5 and 6 show a typical EPR spectrum of the frozen sample of the cerebral cortex of the left hemisphere of a rat brain: areas without ischemia and an ischemia region after introduction of the nitric oxide trap, $DETC_2 + Fe^{2+}$-citrate. The relative change in the amount of the NO-containing complexes is estimated from the integral intensity of the spectra of $(DETC)_2$–Fe^{2+}–NO. It is found that in the cerebral cortex of the left and right brain hemispheres (separately), under the normal conditions NO is present in the form of $(DETC)_2$–Fe^{2+}–NO in concentration of 340 nM/g of weight in an hour. It is found that in the cerebellum, the NO content in the form of $(DETC)_2$–Fe^{2+}–NO is 395 nM/g of weight in an hour.

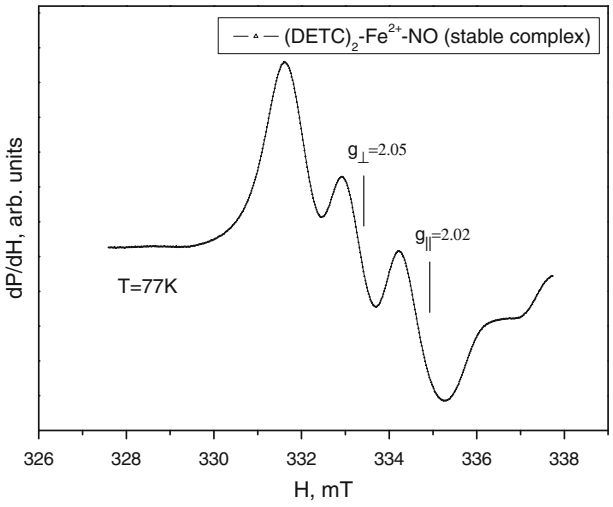

Fig. 2 EPR signal of the reference sample sodium nitroprusside. EPR spectra of $(DETC)_2$–Fe^{2+}–NO stable complex, obtained by reaction of NO with $(DETC)_2$-Fe^{2+} in vivo

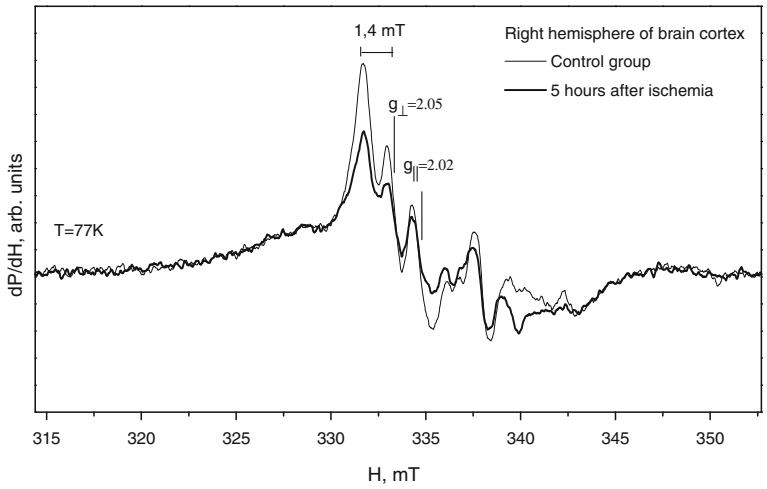

Fig. 3 EPR signal of the cerebral cortex of the right hemisphere of rats injected with MNIC of $(DETC)_2$–Fe^{2+}–NO: healthy rats in a control group (*solid line*) and rats 5 h after the ischemia (*bold line*). $g_{av} = 2.04$

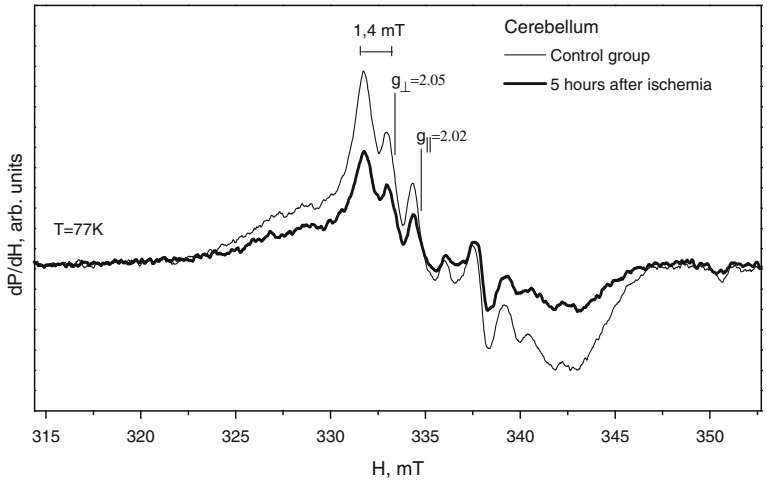

Fig. 4 EPR signal of the cerebellum of rats injected with MNIC of $(DETC)_2$–Fe^{2+}–NO: healthy rats in a control group (*solid line*) and rats 5 h after the ischemia (*bold line*). $g_{av} = 2.04$

3.2 Dynamics of the NO Content in Different Brain Regions After the Stroke

Figures 5 and 6 show typical EPR spectra of frozen samples of the cerebral cortex of the left hemisphere of a rat in normal condition and after the stroke. The irreversible coagulation of the MBA has led to the 5-fold decrease in the NO content

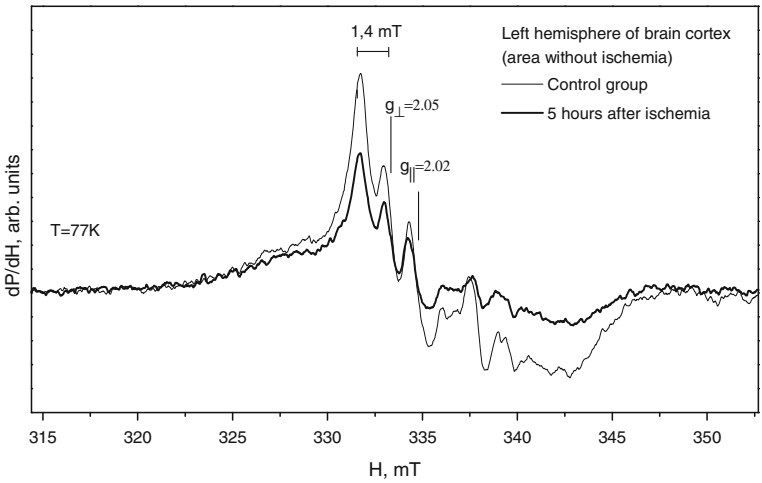

Fig. 5 EPR signal of the cerebral cortex of the left hemisphere (ischemia area) of rats injected with MNIC of $(DETC)_2$–Fe^{2+}–NO: healthy rats in a control group (*solid line*) and rats 5 h after ischemia (*bold line*). $g_{av} = 2.04$

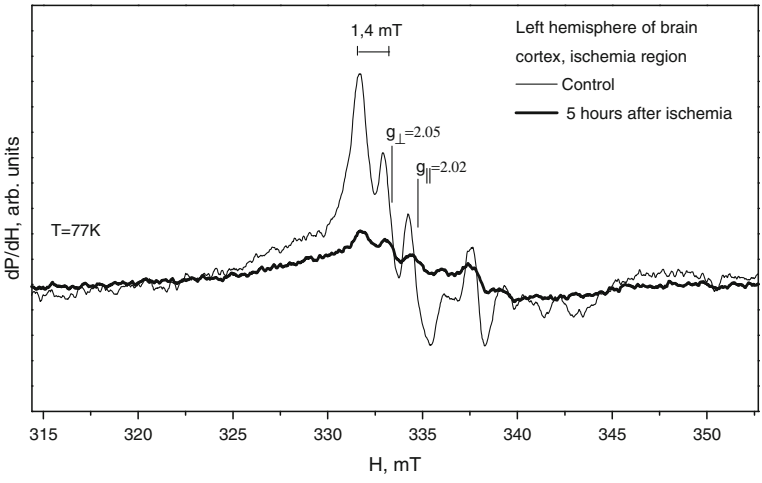

Fig. 6 EPR signal of the cerebral cortex of the left hemisphere (ischemia area) of rats injected with MNIC of $(DETC)_2$–Fe^{2+}–NO: healthy rats in a control group (*solid line*) and rats 5 h after ischemia (*bold line*). $g_{av} = 2.04$

in the center of necrosis in the spin trap composition (Figs. 6, 7). It is necessary to note that in 5, 9 and 24 h after the stroke, the NO content in the spin trap composition in the cerebral cortex of the right hemisphere and non-ischemic parts of the cerebral cortex of the left hemisphere decreased by 40–50% (Figs. 3, 5, 7). In the cerebellum, the NO content in the spin trap composition decreased by 30%

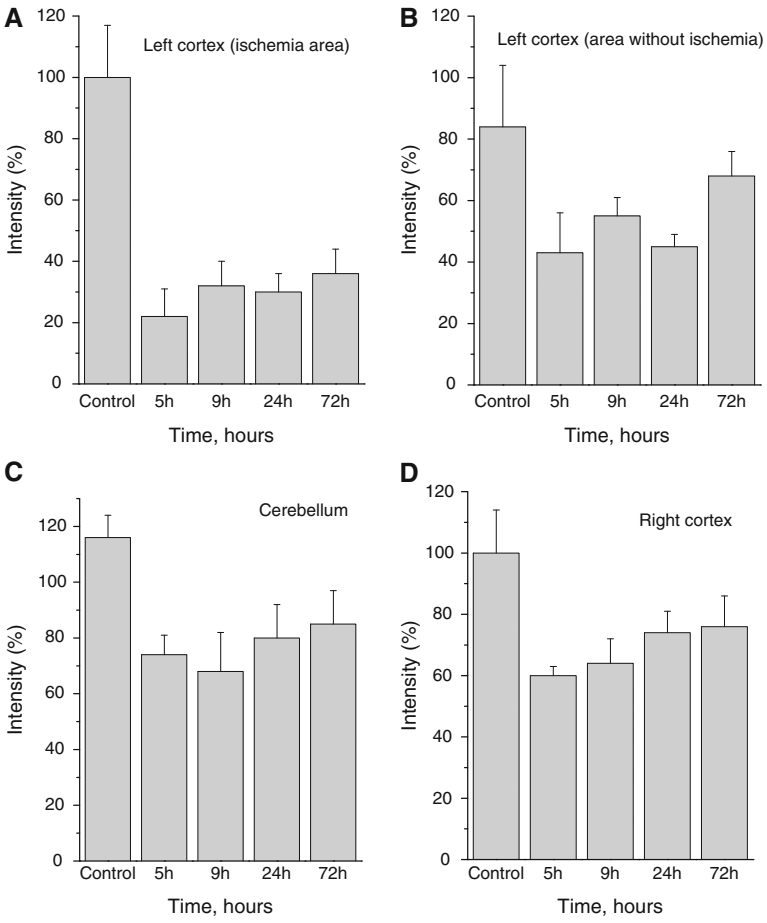

Fig. 7 Relative NO content in the cerebral cortex of the left (**a**, **b**) and left (**d**) hemispheres of the brain and cerebellum (**c**) in the untreated rats (control group) and in rats 5, 9, 24, 72 h after the stroke (*S*). Intensity of the EPR signal is given relative to the signal of the cortex. Content of the NO in right, left brain hemisphere cortex and cerebellum in control group and after 5, 9, 24, 72 h after ischemia

(Figs. 4, 7). Three days after modeling the stroke, in the cerebellum and non-ischemic zone of the cerebral cortex of the hemispheres, partial restoration of the NO content in the spin trap structure was observed. The NO content in the spin trap composition was not restored in the ischemic area of the cerebral cortex of the left hemisphere.

4 Discussion

4.1 Discussion of the Design of the Experiment

The problem of the ischemic stroke is topical worldwide [5, 6, 23]. It is distinguished into two main forms of pang vascular pathology of the cerebrum: total

(global) and focal ischemia [24]. As a rule, stroke develops due to vessel occlusion which leads to the formation of the risk area with the center zone (which has practically no blood supply) and the surrounding penumbra (with the partial blood-groove due to the penetration of the vessels of other vascular regions [23]. Global ischemia appears at the breach of blood flow on large arteries, while focal ischemia is due to the spasm of vessels, supplied by blood of local area of cerebrum [23, 24]. In our experiment, we modeled the focal ischemic stroke by electrocoagulation of the MBA according to the technique developed by Chen [21]. The height of the vessel coagulation was chosen in such a manner that the necrosis center did not affect the brain subcortex structures and did not cause damages in the regulation of the vegetative functions of the organism. To stabilize the damage center, the branch of the frontal vein crossing the MBA in the field of view was coagulated and the ipsilateral carotid was irreversibly tied up.

In a number of investigations it was shown that NO as a free radical becomes an essential factor of the damage and downfall of neurons under ischemia of brain [5–7, 25–27]. The experimental studies of the NO role in ischemic pathology were performed on models of focal and global ischemia of brain, and positive as well as negative effects of NO on the development of the brain infarct was found [24]. Using both inhibitors of NOS and donors of NO also give inconsistent results [25]. It is clear that the level, mechanisms of the NO synthesis and the formation of the final metabolites may strongly depend on whether the brain blood supply is intact. During such operation in our experiments, the center of the ischemia with the volume of 15–25% of the total amount of the cerebral cortex of this hemisphere is predictably located strictly in the frontal–parietal region that allows one to separate it in a sample for the analysis of the NO dynamics at the early stages of the development of the ischemia [26]. The other part of the cerebral cortex of the left hemisphere was investigated separately. The cerebral cortex of the right hemisphere and the cerebellum were chosen as reference samples, considering these regions are conditionally intact with respect to the ischemic hemisphere.

Since the use of a spin trap imposes some restrictions on the protocol of the experiment (for example, it is necessary to wait for the accumulation of a signal for 30 min), it does not make sense to study short time intervals (up to 3 h) from the beginning of ischemia. Taking into account the dynamics of the NOS activation and the limitations of the experimental technique, in 5, 9, 24 and 72 h after modeling the stroke, the nitric oxide trap was introduced into the narcotized animals and in another 30 min euthanasia was performed and the samples for measuring the NO content were taken [6, 7, 28, 29].

4.2 Discussion of the Experimental Results

The direct measurement of the dynamics of the nitric oxide content in the brain after the focal stroke has shown that the NO concentration in a complex with a spin trap in the center zone of ischemia within 3 days after operation decreases statistically significantly. Such a result is quite expected, since in the region with the almost absent blood supply the destructive processes develop very quickly: the metabolism of cells is violated, there are no substrata for the NO biosynthesis, this zone will

undergo necrosis very quickly [23]. However, increase of NO during ischemia was shown by some researchers [25, 30]. On the other hand, the possible reduction of the NO contents under ischemia envisages the use of nitrates for anti-ischemic therapy [31]. Besides, in recent work it was found the reinforcement for deposition NO by vessels walls under hypoksia [27]. It is also possible to expect that different directions of changing contents of NO can depend on the level of ischemia that may influence the activities of NOS of different forms. In our experiments in the cerebral cortex of the left hemisphere without the zone of ischemia, the NO production is also lowered but in 72 h from the beginning of ischemia it increases, this is the time of the maximum activity of the iNOS enzyme and the peak of the development of the inflammation. Surprisingly, the NO production in the conditionally intact brain regions, the cerebral cortex of the right hemisphere and the cerebellum, decreases. By modeling the focal ischemia of the brain on the left, to reduce the variability of the damage size we tied up the general trunk of the carotid located ipsilaterally. The brain of rats has a closed circle of Willis. Probably, the redistribution of the blood-groove in this case partially impoverishes the other brain regions and it is sufficient for the reduction of the NO production. The hyperproduction of NO expected in the first hours of ischemia due to the increase in the perfusion of the regions next to the ischemic ones (reflex Cushing reaction, the increase in the average arterial pressure as a response to brain ischemia) probably was reduced or did not take place at the background of the application of narcosis.

It is interesting to note that the NO content in the R-conformer composition of hemoglobin in the cerebral cortex of the right hemisphere and non-ischemic parts of the cerebral cortex of the left hemisphere, in 5, 9 and 24 h after the stroke did not change in comparison with the intact control. In the cerebellum, the NO content decreased in the R-conformer composition as well (Fig. 4). Why does the dynamics of the NO content change differ in different brain regions not directly affected by ischemia connected with hemoglobin? To answer this question, it is necessary to take into account that the complex on the basis of the spin trap $(DETC)_2$–Fe^{2+}–NO and complexes of heme iron with nitric oxide R-and T-conformers represent different forms of the NO deposition. In the literature, R- and T-conformers are usually interpreted as six- and five-coordinated complexes of heme iron in hemoglobin [32–35]. Probably, the answer to this question is in the compensator change of the blood supply of these brain regions, and, probably, such an answer can be obtained in the study of the mechanisms of NO deposition and extraction in brain tissues [27].

5 Conclusions

The EPR study of the nitric oxide content in the center of ischemia of the left hemisphere, in the remaining part of the left hemisphere, in the regions conditionally not affected by the ischemia: the cerebral cortex of the right hemisphere and the cerebellum, showed the decrease in the NO production in the spin trap structure in 5, 9 and 24 h from the beginning of the stroke in all regions. In 72 h, restoration of the NO content in all regions except for the center zone of ischemia was observed.

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References

1. A.F. Vanin, Biohimia **63**, 924–938 (1998) (in Russian)
2. V.P. Reutov, E.G. Sorokina, Biohimia **63**, 1029–1040 (1998) (in Russian)
3. B. Zhao, Front. Biosci. **10**, 454–461 (2005)
4. V. Calabrese, C. Cornelius, E. Rizzarelli, J.B. Owen, A.T. Dinkova-Kostova, D.A. Butterfield, Antioxid. Redox. Signal **11**, 2717–2739 (2009)
5. V.G. Bashkatova, K.S. Raevskii, Biohimia **63**, 1020–1028 (1998) (in Russian)
6. E.B. Manukhina, H.F. Downey, R.T. Mallet, Exp. Biol. Med. **231**, 343–365 (2006)
7. A.F. Samdani, T.M. Dawson, V.L. Dawson, Stroke **28**, 1283–1288 (1997)
8. H.-Y. Yun, V.L. Dawson, T.M. Dawson, Crit. Rev. Neurobiol. **10**, 291–316 (1996)
9. A. Godecke, J. Schrader, Circ. Res. **94**, e55–e57 (2004)
10. A.F. Vanin, P.I. Mordvintcev, S. Hauschildt, A. Mulsch, Biochim. Biophys. Acta **1177**, 37–42 (1993)
11. V.V. Khramtsov, L.B. Volodarsky, Biol. Magn. Res. **14**, 109–180 (1998)
12. A.F. Vanin, P.I. Mordvintcev, A.L. Kleshchev, Stud. Biophys. **107**, 135–142 (1984)
13. V.D. Mikoyan, L.N. Kubrina, V.A. Serezhenkov, R.A. Stukan, A.F. Vanin, Biochim. Biophys. Acta **1336**, 225–234 (1997)
14. A.I. Ismailova, O.I. Gnezdilov, L.N. Muranova, A.A. Obynochny, V.V. Andrianov, Kh.L. Gainutdinov, A.G. Nasyrova, R.R. Nigmatullina, F.F. Rakhmatullina, A.L. Zefirov, Appl. Magn. Reson. **28**, 421–430 (2005)
15. M.P. Tseitlin, V.S. Iyudin, O.A. Tseitlin, Appl. Magn. Reson. **35**, 569–580 (2009)
16. D. Barr, J. Jiang, R.T. Weber, EPR division, Bruker, Spin Report, 3–6 (2000)
17. V.P. Reutov, V.E. Okhotin, A.V. Shuklin, E.G. Sorokina, N.S. Kosicin, V.N. Gurin, Usp. fiziol. nauk **38**, 39–58 (2007) (in Russian)
18. J.A. Weil, J.R. Bolton, J.E. Wertz, *Electron Paramagnetic Resonance: Elementary Theory and Practical Applications*, 2nd edn. (Wiley-Interscience, New York, 2007), pp. 497
19. H.J.M. Slangen, J. Phys. E: Sci. Instrum. **3**, 775–778 (1970)
20. M. Mazur, M. Valko, R. Klement, H. Mirris, Anal. Chem. Acta **333**, 249–252 (1996)
21. S.T. Chen, C.Y. Hsu, E.L. Hogan, Stroke **17**, 738–743 (1989)
22. V.V. Andrianov, F.G. Sitdikov, Kh.L. Gainutdinov, S.V. Yurtaeva, L.N. Muranova, A.A. Obynochnyi, F.K. Karimov, V.M. Chiglintsev, V.S. Iyudin, Russ. J. Dev. Biol. **38**, 352–356 (2008)
23. E.I. Gusev, V.I. Skvorceva, *Ishemia of brain* (Medicina, Moscow, 2001), 328 p
24. I.V. Viktorov, Vestnik RAMN **4**, 5–10 (2000) (in Russian)
25. K.S. Raevskii, V.G. Bashkatova, A.F. Vanin, Vestnik RAMN **4**, 11–15 (2000) (in Russian)
26. N.S. Samoilenkova, S.A. Gavrilova, V.B. Koshelev, Dokl. acad. nauk **414**, 283–285 (2007) (in Russian)
27. E.B. Manukhina, V.U. Kalenchuk, S.A. Gavrilova, A.V. Goryacheva, I.Y. Malyshev, V.B. Koshelev, Rus. J. Physiol. **94**, 198–205 (2008) (in Russian)
28. J. Garthwaite, C.L. Boulton, Annu. Rev. Physiol. **57**, 683–706 (1995)
29. D. Boehning, S.H. Snyder, Annu. Rev. Neurosci. **26**, 105–131 (2003)
30. S. Sato, T. Tominaga, T. Ohnishi, S.T. Ohnishi, Biochim. Biophys. Acta: Mol. Basis. Dis. **1181**, 195–197 (1993)
31. B. Fink, S. Dikalov, E. Bassenge, Free Radic. Biol. Med. **28**, 121–128 (2000)
32. H. Kon, J. Biol. Chem. **243**, 4350–4355 (1968)
33. A. Szabo, M.F. Perutz, Biochemistry **15**, 4427–4428 (1976)
34. K. Nagai, H. Hori, S. Yoshida, H. Sakamoto, H. Morimoto, Biochim. Biophys. Acta **532**, 17–28 (1978)
35. B. Piknova, M.T. Gladwin, A.N. Schechter, N. Hogg, J. Biol. Chem. **280**, 40583–40588 (2005)