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Investigation of NO and Copper Content in Injured and Non-Injured Areas of the Rats Brain by EPR Spectroscopy 24 Hours and 7 Days after Combined Brain and Spinal Cord Injury

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Nitric monoxide (NO) is one of the key signaling molecules that regulate the physiological functions of the body [1]. Since NO is a chemically highly reactive free radical capable of acting as both an oxidizer and a reducing agent [2], it is assumed that its diverse effects in biological tissues. NO is widespread in the nervous [3], cardiovascular [4] and other functional systems of the body - blood vessel tone, neurotransmission, learning [1, 5]. The involvement of NO in the mechanisms of development of various pathological conditions of the body attracts great interest [6]. It has been shown that the functioning of the NO system is disrupted by ischemia and brain injuries, cerebral ischemia is accompanied by multiple and multidirectional changes in the NO content in the brain and in signal transmission [6, 7]. However, there are contradictions in the information about the role of NO in these processes, which allow us to assert that currently there is no consensus on the role of endogenous NO in the processes occurring with damage to the nervous system [8].
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 [7, 8, 9]. Therefore, we have attempted to detail some biophysical patterns of nitric monoxide formation in case of combined brain and spinal cord injury. We used EPR spectroscopy to study the dynamics of NO in the rat's brain after simulation of combined brain and spinal cord injury. The intensity of NO production by EPR spectroscopy was measured using the spin trap technique [8, 9], 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 [2, 7, 9]. Measurements of the spectra of a complex of biological samples $(DETC)_2$-Fe^{2+}-NO and Cu^{2+}-$(DETC)_2$ was performed on a Bruker X-band spectrometer (9.5320 GHz) EMX/plus.

EPR spectroscopy was used to study the intensity of NO production and copper content in the injured and non-injured areas of the rat brain 1 and 7 days after formation of a combined injury. It was shown that 7 days after the injury, a decrease in NO content was found by 84% in damaged areas of the brain and by 66% in intact areas of the brain. The difference in NO production in the injured and non-injured areas of the brain was also significant. The copper content in the brain remained unchanged a week after the injury simulation. The results demonstrate a significant 30% ($p<0.05$) decrease in NO production in the injured and non-injured areas of the brain as early as 1 day after the formation of a combined injury. The copper content in the brain remained unchanged 1 day after the formation of the combined injury.

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