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CHANGES IN NITROGEN MONOXIDE STABLE METABOLITES LEVEL IN BRAIN TISSUE HOMOGENATES OF CHICK EMBRYOS UNDER THE INFLUENCE OF EXTREMELY HIGH FREQUENCY (EHF) IRRADIATION AND LOW-DOSE RADIATION

Abstract: Changes in nitrogen monoxide stable metabolite level in brain tissue homogenates is associated with changes of activity and quantity of neuronal NO-synthase in brain cells. The activity of this enzyme can be regulated by an external factors such as EHF irradiation and low-dose ionizing radiation altering the level of nitrogen monoxide stable metabolites.

Research shows that EHF irradiation with a frequency of 53.56 GHz as well as ionizing radiation with a dose of 0.048 Gy lead to a significant increase in quantity of nitrogen monoxide stable metabolites.

Keywords: nitrogen monoxide stable metabolites, NO-synthase, prenatal ontogenesis, nitrogen monoxide, EHF irradiation, ionizing radiation.

Introduction

Nitric oxide (NO) acts as a universal regulator of physiological and biochemical processes. Nitrogen monoxide is considered to be an universal neurotransmitter, regulator of the cardiovascular, digestive, urinary, immune, reproductive systems function in the body. The antioxidant properties of nitric oxide appear mainly due to its ability to act as an electron donor in the reduction reactions[1]. Nitric oxide shows a small half-life in vivo (few milliseconds) and diffuses into

[1] Wink D.A. et al. // Antioxid. Redox. Signal. – 2001. – Vjl. 3. – № 2. – P. 203–213.

biological systems with a speed 50 $\mu m \times sec^{-1}$. This characteristic suggests that NO can exhibit its effects within a few microns from the site of its generation[1].

Nitric oxide (NO) is synthesized in the body by removal from L-arginine. This reaction is catalyzed by the enzymes called NO-synthases. There are three types of NO-synthases (NOS): two of them are combined together under the name constitutive NOS, they include neuronal (nNOS) and endothelial (eNOS) synthases which are calcium-dependent, the third one is called inducible (iNOS) synthase which is activated by cytokines[2].

The following enzymes are well understood at present: a) macrophage NO-synthase which has a microbicidal or cytotoxic effect; б) endothelial NO-synthase the main role of which is linked to the fact that NO is a potent vasodilatating agent and is involved in the regulation of the cerebral circulation; в) neuronal NO-synthase – though at present its function and role is not fully understood, the role of NO in the central nervous system under normal conditions is usually associated with the three following processes:

1) participation of NO in the process of interneuronal communication as a neurotransmitter;

2) regulation of cerebral blood flow;

3) establishment of interneuronal synaptic relationships during development of the nervous system.

Neurons containing NO-synthase are seen even during the embryonic period, so is believed that NO can initiate the development of growing axonal and dendritic branches as well as stimulate synapse formation. This area of neurobiological science is still underinvestigated.

The traditional formulation of the statements on the participation of NO in the interneuronal communication is usually limited by the possibility of the synthesis and release of NO from the local regions of the neuron called postsynaptic endings. However the results of scientific research shows that NO-synthase is found throughout the whole neuron body – in perikaryon, axons and dendrites. As far as during the firing of the neuron over the entire length of its processes and body the level of calcium varies cyclically and formation of unique calcium waves is seen, it can be assumed that the synthesis and release of NO can be triggered in any area of the body and the processes of neurons. Thus neurons containing NO-synthase can create an exposure field around itself, they may be considered to be a peculiar field neurons unlike conventional neurons connected to one another in the local areas – synapses[3].

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[1] Gally J.A. et al. // Proc. Natl. Acad. Sci. USA. – 1990. – V. 87. – P. 3547–3551

[2] Bliznetsova G.N. Oxidative stress and nitric oxide system during the postnatal adaptation and development of diseases in farm animals: Abstract. Dis. for the degree of Doctor of Biological Sciences: spec. 03.01.04 biochemistry / G.N.Bliznetsova. – Voronezh, – 2010. – 46 p.

[3] Sosunov A.A. Nitric oxide as an intercellular mediator // Soros Educational Journal. – 2000. – V. 6, – No. 12. – P. 27–34.

Purpose of this research is to determine the effect of EHF irradiation and ionizing radiation on the level change of nitrogen monoxide stable metabolites in brain tissue homogenates.

Materials and methods

To determine the effect of EHF irradiation on the change of the NO-synthase activity in brain neurons setting of 60 hen's eggs was made (30 eggs consisted an experimental group, the remaining 30 eggs served as a control without exposure to radiation).

Before setting of the eggs for incubation an experimental group was exposed to EMR EHF: output power of 30 mW, frequency of 53.56 GHz, the exposure mode of the incubating eggs for 5 minutes at a 500 mm distance from the radiator horn cap to the surface of the egg[1]. To determine the effect of ionizing radiation setting of 90 hen's eggs was made (60 eggs consisted an experimental group, the remaining 30 eggs served as a control without exposure to ionizing radiation).

30 eggs from the experimental group were subjected to radiation treatment before incubation with a dose 0.048 Gy and dose rate 0.0048 Gy/min. This dose is considered to be an optimal in order to ensure maximal stimulating effect. Other 30 eggs from the experimental group were irradiated with a dose of 0.192 Gy and a dose rate of 0.0048 Gy/min[2]. On day 18 of embryonic development the brain was extracted from the embryos of the control and experimental groups. After that the test samples were subjected to a biochemical method in order to establish the presence of nitrogen oxide stable metabolites based on the determination of nitrates using the Griess reagent[3].

Results and discussion

The following results were obtained during the biochemical research: when exposed to EHF irradiation an increase of nitrogen oxide stable metabolites in the brain is seen (19,34 ± 1,93) compared with the control group (13,79 ± 0,94), which suggests a stimulating role of EHF irradiation for the development of NO-ergic system of brain neurons. The following results were obtained during the biochemical research:

1. An increase of the level of nitrogen oxide stable metabolites in brain of chick embryos irradiated with a dose 0.048 Gy (16.02 ± 0.81) compared with control group (13.79 ± 0.94) was seen. Differences of the readings in the control

[1] Deviatkov N.D., Golant M.V., Betshiy O.V. Features of medical and biological applications of millimeter waves. – M.: IRE RAS, – 1994. – P.23–24. S.A. Ilyina, G.F. Bakaushina, V.I. Gayduk and others. Possible role of water in the transmission of radiation exposure EHF on biological objects // Biophysics. –1979. – V. 24. – iss. 3.

[2] Pak V.V. The reaction of the chicken organism to the effects of ionizing radiation / V.V.Pak. – Moscow., – 2001. – P. 166–169.

[3] Metelskaya V.A. The screening method for determining the level of nitric oxide metabolites in serum / V.A. Metelskaya, N.G. Gumanova // Clinical Laboratory Diagnostics. – 2005. – No. 6. – P. 15–18.

and study groups were statistically significant, since $t = 3,21$, with $p \times 0.05$, and the calculated $t >$ table t ($t = 2.176$). These results might indicate the stimulatory effect of ionizing radiation with a dose of 0.048 Gy for the development of a NO-ergic system of neurons in the brain during embryogenesis.

2. Irradiation of the chick embryo with a dose of 0.192 Gy resulted in a slight decrease of the level of nitrogen monoxide stable metabolites in the brain. No statistically significant differences were found.

Conclusion

Thus, it has been shown that NO-ergic system is quite sensitive to the action of physical factors and this enzyme system may be regulated by these factors. Based on obtained data we conclude that ionizing radiation with a dose 0.048 Gy and EHF radiation have a stimulating effect on the development of NO-ergic system of neurons in the brain during embryogenesis of the studied birds.

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