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Changes in Free Radical Processes under the Influence of Low-Frequency Electromagnetic Field in Rats

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We studied the effect of a low-frequency (LF) electromagnetic field (EMF) on the state of the antioxidant system of Wistar rats *in vivo*. It was found that changes in activity of antioxidant enzymes and H_2O_2 content in the blood plasma of rats exposed to LF EMF depended on the frequency of EMF. We propose a mechanism of the protective effects of low doses of ROS the generation of which is stimulated by LF EMF.

Key Words: *reactive oxygen species; electromagnetic field; hydrogen peroxide; antioxidant system*

The special interest in the studies of ROS is explained by a wide range of their physiological effects and participation in many pathological processes, as well as the important role of ROS in the aging process [6,9]. The maintenance of the ratio of prooxidant and antioxidant factors in the blood at a certain level is important for the regulation of normal physiological processes in the body: the level of nonspecific and specific immune defense, the level of peripheral vascular tone, self-renewal of cell membranes, the preservation of the mechanism of apoptosis, *i.e.* elimination of functionally and structurally defective cells [9]. Prooxidants, free radicals (superoxide anion radical, nitric oxide, and hydroxyl radical) and reactive molecules (hydrogen peroxide, hypochlorite anion, hydroperoxides, and peroxynitrite), are actively involved in the regulation of many intracellular processes [3], including immune mechanisms, neutralization of xenobiotics, apoptosis, metabolism of biologically active compounds, bone metabolism, and hemoglobin oxidation. Under conditions of imbalance of the prooxidant-antioxidant system with predominance of prooxidant factors, the latter produce damaging effect at the molecular and cellular level accompanied by oxidative stress, a complex of pathological changes in organs and tissues [3,4]. In addition to the widely used approach to reducing the level of ROS, methods of active training of the antioxidant system (AOS) through induction of moderate ROS generation were proposed; this approach leads to an increase in the power of AOS and strengthening of the ROS control system [9] and promotes the general adaptation and biostimulation of the body. In addition, it has been proven [3] that factors leading to ROS generation and causing various disorders in high doses, in low doses, on the contrary, have stimulating, normalizing, and protective effects.

The effect of weak electromagnetic field (EMF) on living systems has been reliably confirmed by experimental data [1,2]. The main contribution to magnetoreception is made by physicochemical processes involving radicals, radical ions, and paramagnetic particles such as ROS [2]. EMF can induce spin triplet-singlet transitions in ion radical pairs, changing their reactivity, in particular, forward and back electron transfer, spin magnetically induced conversion, decay and binding of radicals [2]. The possibility of ROS generation in model aqueous solutions of DNA and proteins under the effect of low-intensity (LF) EMF *in vitro* was shown [5,10]. The molecular mechanisms underlying the influence of weak LF EMF *in vivo* on magnetically dependent biologically important processes involving

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ROS requires detailed study for rational use of the obtained effects in magnetobiology and medicine, in particular, for optimization of the conditions for carrying out physiotherapeutic procedures.

The aim of this work was to study the effect of LF EMF with frequencies from 3 to 60 Hz *in vivo* on the state of the AOS in rats by assessing the levels of hydrogen peroxide in the blood, activities of antioxidant enzymes, and the content of sulfhydryl groups.

MATERIALS AND METHODS

The intensity of free radical oxidation and the state of AOS in the blood of laboratory animals under the action of LF EMF *in vivo* was studied in male Wistar rats aged 2-3 months kept under standard vivarium conditions. All experiments were carried out in compliance with the principles of humane treatment of animals and Good Laboratory Practice and in accordance with Directive 2010/63/EU of the European Parliament and of the Council (September 22, 2010; On the Protection of Animals Used for Scientific Purposes).

The rats were divided into the control (not exposed to EMF, n=10) and experimental group (exposure to EMF with different frequencies, 5 animals for each EMF frequency). Exposure to LF EMF was performed using special equipment; the block diagram is given in [13]. The circuit consisted of a GZ-118 LF signal generator, which is a source of a sinusoidal signal of a precision form, an inductor coil with 1200 turns placed in a shielded chamber. The magnetic induction vector of EMF created by the coil was 30 mT, coil resistivity was 320 Ω, and the voltage across the coil was 14 V. Exposure to EMF was performed at room temperature (22±1°C) for 15 min in the mode of continuous signal generation at the selected frequency. The animals were fixed and placed in an inductance coil inside a chamber. The field strength at the location of the animal was 550±100 A/m, the amplitude was 200 μT, and the frequency range was from 3 to 60 Hz.

Blood for biochemical studies was collected after decapitation of the animals after preliminary injection of 1 ml aqueous solution of EDTA with a concentration of 7.5%. The following biochemical parameters were measured: activity of antioxidant enzymes catalase, superoxide dismutase (SOD), and glutathione peroxidase (GP) and the content of hydrogen peroxide and SH-groups. The content of hydrogen peroxide was measured by the spectrophotometric method using PerOx kit (Immundiagnostik AG) on a Multiskan microplate reader (Thermo Fisher Scientific). The calibration curve was linear in the range $10^{-5} \div 5 \times 10^{-2}$ M. Catalase activity in blood plasma was measured by recording the rate of hydrogen peroxide destruction at 260 nm [8]. The level of thiol groups in blood plasma (mainly cysteine residues of proteins) was measured by the reaction with 5,5'-dithiobis- (2-nitrobenzoic) acid. The thionitrophenyl anion released during this process has an intense yellow color and absorption maximum at 412 nm. The concentration of this anion is directly proportional to the content of thiol groups in the studied biofluid [12]. SOD activity in blood plasma was measured by the method based on inhibition of quercetin autooxidation in a test system with the generation of superoxide anion radical in the presence of a biofluid (blood plasma) containing the enzyme [15]. GP activity was measured by the method based on assessment of the rate of consumption of the reduced form of glutathione in the reaction with tert-butyl hydroperoxide. Changes in glutathione concentration were assessed by the reaction with 5,5'-dithiobis-(2-nitrobenzoic) acid [8].

The data were analyzed using Statistica 6.0 software (StatSoft, Inc.). The hypothesis of the normal (Gaussian) distribution of the results was tested using the Shapiro—Wilk test and by determining the ordinates of the normalized Gaussian distribution function. Comparison of groups by quantitative characteristics was carried out using a two-sample Student's t test. The differences were considered significant at $p<0.05$.

RESULTS

The concentration of peroxides in the blood plasma of control rats did not exceed 0.041±0.003 mmol/liter (Fig. 1). The most significant increase in this parameter in comparison with the control was recorded in animals exposed to EMF at frequencies of 5 Hz (by 9 times), 8 Hz (by 5.5 times), and 24 Hz (by 2.3 times). Exposure to EMF with frequencies of 3, 20, and 60 Hz only

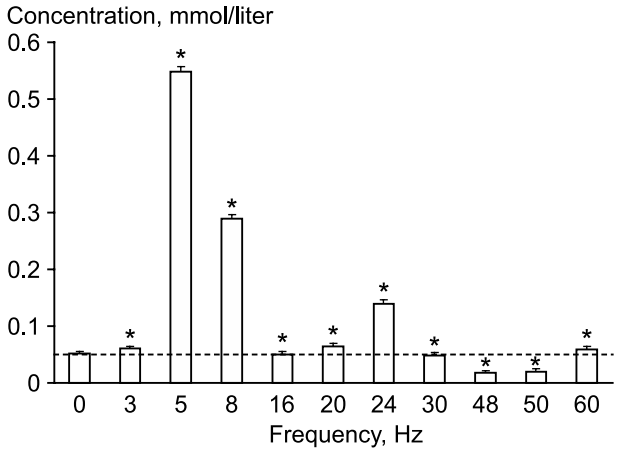

Fig. 1. Effect of LF EMF on the content of peroxides in the blood plasma of rats after *in vivo* treatment. The content of peroxides was measured after 15-min exposure of rats to LF EMF with a frequency range from 3 to 60 Hz. Five rats were used for each frequency. *$p<0.05$ in comparison with the control.

slightly increased the concentration of hydroperoxides in rat plasma, while exposure to 48 and 50 Hz significantly reduced this parameter in comparison with the control. This attested to a significant ($p<0.05$) modulation of ROS content in rat blood after exposure to EMF with the specified frequencies. This result is consistent with the report [1], where a nonlinear dependence of magnetobiological effects on the parameters of weak EMF and frequency selectivity was revealed.

AOS that includes antioxidant enzymes, low-molecular-weight compounds that form a redox buffer, vitamins, albumin, metal ion complexones, *etc*., is responsible for cell protection from the excess of ROS and oxidative damage caused by them [9]. The state of endogenous AOS was assessed by activities of antioxidant enzymes in rat blood plasma: SOD catalyzing dismutation of two superoxide molecules with the formation of H_2O_2 and O_2, catalase catalyzing reduction of the resulting hydrogen peroxide to H_2O and O_2, and glutathione peroxidase that also catalyzes degradation of hydrogen peroxide in peroxisomes.

A significant increase in SOD activity in the blood plasma was noted after exposure with EMF at frequencies of 5 and 16 Hz and a decrease at frequencies of 8 and 20 Hz; catalase activity increased after exposure to EMF with frequencies of 3, 5, and 16 Hz and decreased at EMF frequencies of 8, 20, and 50 Hz. Activity of GP in rat plasma before and after exposure to EMF changed insignificantly (Table 1).

The maximum increase in H_2O_2 concentration in rat plasma was observed after exposure to EMF with a frequency of 5 Hz; SOD activity was also increased in comparison with the control group. After exposure to EMF at a frequency of 8 Hz, an increase in the content of H_2O_2 in the plasma was observed, which, apparently, is associated with a significant decrease in activity of catalase that neutralizes H_2O_2 (Table 1). In general, activities of antioxidant enzymes and the content of hydrogen peroxide in the plasma of exposed animals changed in a complex way depending on the frequency of EMF (Fig. 1 and Table 1).

It is known that oxidative modifications primarily affect SH-groups of proteins, leading to inactivation of enzymes and modulation of binding of transcription factors to DNA [7]. The content of SH-groups in rat plasma after exposure to EMF with a frequency of 30 Hz increased by 2 times in comparison with the control ($p<0.05$, Table 1); for other frequencies of EMF, the level of SH-groups in blood plasma changed insignificantly. SH-groups are the targets of all redox-active metabolites and, first of all, ROS [11,14]. ROS can participate in the oxidative modifications of proteins by modulating the total level of glutathione in the cell and the GSH/GSSH ratio. The presence of enzymes with SH-groups differing in biopotential in the cell creates prerequisites for the formation of a differentiated response depending on the level of ROS generated by LF EMF.

The obtained effects can be mediated by a number of mechanisms implemented *in vivo* at the molecular and cellular levels. For instance, exposure to LF EMF induces ROS generation and rapid exchange in hydroxyl and sulfhydryl groups of all organic compounds, including proteins, nucleic acids, and lipids, which can affect the state of the low-molecular-weight elements of AOS, first of all SH- and OH-groups. Moreover, additional effects of ROS in biological systems lead to changes in the structure and properties of nucleic acids and proteins. This can reduce activity of AOS enzymes (catalase, SOD, and GP), thereby reducing the antioxidant potential of the body. Inactivation of antioxidant enzymes *in vivo* disturbs ROS utilization and promotes accumulation of new portions of ROS that modify proteins.

Thus, exposure to LF EMF was followed by the formation of a nonspecific response that improves

TABLE 1. Parameters of Activity of Antioxidant Enzymes and Content of SH-Groups in the Blood Plasma of Rats after Exposure to EMF of Different Frequencies *In Vivo* (*M*±*m*)

EMF frequency, Hz	Catalase activity, mmol/min×liter	GP activity, μmol/liter/min	SOD activity, % inhibition	SH-group, optical density units
Non-exposed control (*n*=10)	258±40	2.89±0.11	50.0±0.7	0.34±0.02
3 (*n*=5)	469±63*	2.74±0.10*	49.8±0.9*	0.31±0.02*
5 (*n*=5)	631±40*	2.70±0.11*	58.5±0.8*	0.35±0.01*
8 (*n*=5)	89±25*	2.66±0.05*	30.8±0.7*	0.26±0.02*
16 (*n*=5)	387±50*	2.60±0.06*	57.5±0.8*	—
20 (*n*=5)	163±20*	2.80±0.09*	34.8±0.9*	—
30 (*n*=5)	176±50*	2.75±0.40*	39.8±0.9*	0.71±0.03*
50 (*n*=5)	129±50*	1.98±0.09*	52.5±3.7*	—

Note. *$p<0.05$ in comparison with the control.

body's adaptation to the changed conditions. The targets of magnetoreception are low doses of radical ion pairs of ROS formed under the action of LF EMF, which is consistent with previous reports [2,5,10]. In this case, the role of antioxidant enzymes, apparently, consists in not only minimization of ROS content in the cell, but also fine adjusting of the level of ROS in accordance with changes in external conditions. The effects of ultra-low doses of ROS can be prolonged and enhanced cooperatively by electrophilic compounds [7]. In this case, the cellular response will be determined by cumulative behavior of all electrophile-sensitive proteins, but not individual protein. The mechanism of the protective effect of small doses of ROS probably consists in redox modifications of SH-groups of sensory proteins, which leads to activation of the tyrosine kinase pathway of the cellular response [9].

The effect of EMF on rat AOS observed in this work provides the basis for further study of possible molecular mechanisms of EMF in *in vivo* models.

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