The Features of the Stress-Reaction to Repetitive Nanosecond Microwave Pulses

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***Abstract*—The effect of nanosecond repetitive pulsed microwaves on the possibility of triggering a stress response of laboratory mice was investigated. The exposure was carried out every day once per a day during the 5 days' period (4000 pulses per session) with repetition rates of 8, 13, 16 and 22 pulses per second. The source of radiation was a laboratory pulse generator based on a magnetron MI-505 with a carrier frequency of 10 GHz, a pulse duration at half the power level of 100 ns, an output peak power of 180 kW, an electric field strength of 1.5 10^3 V/cm, a peak power density of 1.5 × 10^3 kW/cm^2). It was found that repeated exposure alters the level of corticosterone in the blood serum. The effect depends on the frequency of repetition of microwave pulses. Possible mechanisms of the effect of radiation on the level of corticosterone are considered.**

Keywords—microwave radiation, nanosecond pulses, stress response, corticosterone

I. Introduction

Electromagnetic radiation of the radio frequency range is a factor that affects many functional systems of the body, particularly it can cause stress development [1-3]. The available experimental data demonstrate a changes in the content of hormones of the hypothalamic-pituitary-adrenocortical system after irradiation of laboratory animals (rats, dogs) by electromagnetic radiation with different intensity levels [2]. Most of the research is devoted to the study of biological reactions to microwave effects with intensities of tens and hundreds of mW/cm^2, sufficient for heating the irradiated site of tissues and responsible for triggering mechanisms of influence typical for thermal stress. However, recent small studies [2, 4-7] indicate that exposure to microwaves of nonthermal intensity (mW / cm^2 or less) can also lead to the realization of a stress response, but already by a nonthermal mechanism, in particular by stimulating functional activity adrenal glands, which is accompanied by increasing the secretion of corticosterone [4]. It is also known [8-11] that exposure of rats and mice with low microwaves of nonthermal intensity (up to 1.5 mW/cm^2) exerts influence on intracellular oxidative stress reactions. The main targets of damage in the organism under conditions of oxidative stress are the molecules of proteins, lipids and nucleic acids that undergo oxidative modification and, in the future, are usually unable to perform their functions [8, 12-14]. Thus, the combination of data on the possibility of stress formation in animals with low-intensity radiofrequency influences served as the basis for investigating the effect of a new technogenic factor, nanosecond repetitive pulsed microwaves (RPMs) on the body, as a potential stressor.

Based on the foregoing, the purpose of this study was to evaluate the content of corticosterone as an indicator of the formation of stress in mice organism after nanosecond repetitive pulsed microwaves irradiation.

II. Materials and Methods

The experiments were performed on 30 nonlinear laboratory white male mice weighing 20-25 g. The study was carried out in accordance with the ethical norms of working with laboratory animals and sanitary rules for the arrangement, equipment and maintenance of experimental biological clinics [15]. The animals were kept in cages for 6 individuals under the 12:12 lighting regime at room temperature, constant humidity, on a standard diet with free access to water. The experimental animals were divided into 5 groups with 6 randomly selected individuals in each one. As a control there is a sham group, which was subjected to all manipulations as irradiated, except for the turning the radiation source on.

Irradiation of the organism with pulsed-periodic microwave exposure was performed for 5 days' period daily (4000 pulses per session) with repetition rates of 8, 13, 16 and 22 pulse per second (pps). The duration of the irradiation session varied from 3 to 11 minutes, depending on the repetition frequency of the pulses. The choice of the intensity of RPMs and the pulse repetition rates is due to rather high biological efficiency of RPMs with such parameters, which was demonstrated earlier in

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studies on the effect of RPMs on biological objects at different levels of the organization [10, 11, 16-19].

The RPM source was a laboratory pulse generator based on the magnetron MI-505 (Russia, carrier frequency of 10 GHz, output peak power of 180 kW, pulse duration of 100 ns). During the exposure, the mice were placed in plastic containers of 3 cm in diameter at distances below 20 cm from the transmitting antenna in the form of a rectangular horn. Using attenuators and varying the distance from the transmitting antenna to the irradiated object, a peak power density of 1.5×10^3 kW/cm^2 was provided, with an average power density of 1.5×10^3 mW/cm^2 (nonthermal intensities) [20].

At the end of the irradiation procedures, the mice were withdrawn from the experiment by a one-stage decapitation under CO^2 anesthesia. 1-1.5 ml of blood was collected without addition of anticoagulant into a clean dry tube to obtain serum from decapitated animals. The blood was centrifuged without cooling at 2000 rpm for 15 minutes, the obtained serum was collected in Eppendorf tubes.

The effect of RPMs was assessed by the level of corticosterone in the blood serum of irradiated and sham control mice. The content of hormones was determined using the enzyme-linked immunosorbent sandwich assay with enzyme immunoassay (ELISA) [21]. The procedure for enzyme-linked immunosorbent assay was performed according to the instructions offered by test system manufacturers (DRG, Germany; IDS, UK). In the cells of the microplate, 100 μl of test serum, corticosterone standards with known concentrations and a control solution from the kit were added. 100 μl of the enzyme conjugate was then added to each well of microplate and incubated for 16-20 hours at 2-8 °C. After three times of automatic washing with buffer, 200 μl of the dye substrate solution was added to each well and incubated at room temperature. After 30 minutes, 50 μl of the stopping agent was added. Serum hormone levels were determined spectrophotometrically by measuring the optical density of the samples using a StatFax 303 Plus (USA) flat-panel photometer at 450 nm. The concentration of the hormone was calculated from the calibration curve from the kit.

The results were statistically processed using Statsoft STATISTICA software for Windows 8.0, in which the arithmetic mean of the indicator and its error were calculated. The significance of the differences between the indices of irradiated and sham animals was determined using the nonparametric Mann-Whitney U-test. The statistically significant values of the indicators at the level $p \leq 0.05$ are considered.

III. Results

The experiments showed that after irradiation of the entire body of the mice for 5 days (daily 4000 RPM pulses with an intensity of 1.5×10^3 mW/cm2), the serum corticosterone concentration changed depending on the repetition rate of the pulses (Fig. 1).

After exposure with a repetition rate of 13 and 16 pps, the corticosterone level increased in 3.3 and 2.5 times, respectively, with respect to sham control group. An increase in the content of corticosterone indicates the development of stress in animals as a result of the action of microwave pulses with a repetition rate of 13 and 16 pps.

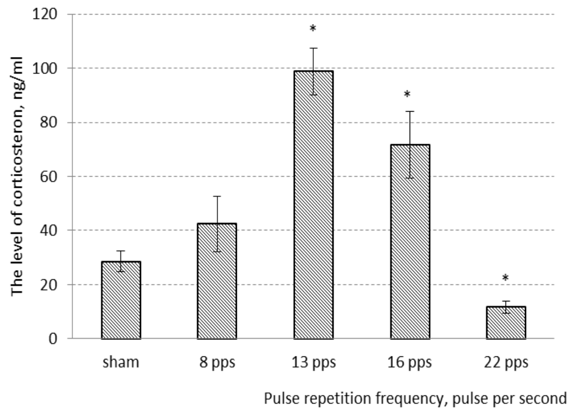

Fig. 1. The level of corticosterone in the mice blood serum after exposure to microwave pules with different pulse repetition frequencies.

At the same time, irradiation of mice with a frequency of 22 pps resulted in a significant decrease (almost 2.5 times) in corticosterone content relative to the level of hormones in sham animals, which could mean an even greater degree of animals' stress, which led to the depletion of the natural pool hormone, similarly to what was observed in another study [22] or activation of stress-limiting systems. The effect of microwave pulses with a frequency of 8 pps did not have a statistically significant effect on cortisone levels in the blood of mice. The result can be explained either by the insensitivity of animals to this mode of exposure, or by the development of adaptive responses to RPMs during a 5-day exposure.

IV. Discussion

The conducted experiments and analysis of the obtained results made it possible to establish the possibility of forming a stress response to the effect of microwave radiation of a nonthermal intensity. An important parameter of the impact was the pulses repetition frequency, which agrees with the previously obtained results of the RPM effect on different biological objects [10, 11, 16-19]. Multidirectional reactions of the body to microwave irradiation with different repetition rates of pulses are probably related to the effect of radiation on the course of various biochemical and biophysical processes in different cells of the body. A possible primary mechanism of such nonthermal action can be realized, for example, in the form of direct action of an electric field on extended intracellular charged complexes (such as cytoskeleton elements, mitochondrial respiratory chain, etc.) It is also possible that effect is mediated through the oxidative modification of lipids and proteins by the reactive oxygen species (ROS) arising during the action of nanosecond RPMs [11] with subsequent changes in irradiated tissues. The results of our studies, which show multidirectional changes in the functioning of the mitochondrial respiratory chain of mice hepatocytes [17, 19], as well as changes in the state of oxidative and antioxidant cell system, depending on RPM parameters [18] can serve as a confirmation of this assumption.

The mitochondrial reaction to microwave exposure is also possible in brain cells, which is especially important in the aspect of this study, since the central nervous system (CNS) is directly involved in monitoring the endocrine system and has the ability to reverse the negative connection or targeted impact on the central and peripheral endocrine organs. Speaking about targeting, it should be noted that the brain is one of the key targets for electromagnetic radiation. This is confirmed by the results of experiments with the effect of microwave radiation from nonthermal intensities on the brain [5, 23-26]. Such irradiation in the brain reduced the expression of the mitochondrial respiratory chain genes [5] and reduced the activity of succinate dehydrogenase [23] with further normalization of activity after 14 days [24]. In addition, the complex effect of microwave radiation through the damage to mitochondrial membranes of cerebral neurons by the action of active forms of oxygen has been observed [25]. Damage to the membrane promoted an increase in intracellular calcium with further activation of phospholipases and proteases [26]. It was found that microwaves cause activation of the mitochondrial CASP-3 pathway of apoptosis, the signal of which is the inactivation of cytochrome C oxidase [27, 28]. Microwaves can increase the activity of NADH-oxidase, which leads to the accumulation of ROS, which, in turn, damage the mitochondrial membrane. When the last one is being destroyed, there is an accumulation of ROS and, as a consequence, the appearance of a vicious circle [29]. Microwave radiation leads to an increase in intracellular calcium, which leads to the dissociation of ATP synthase and the rest of the respiratory chain complex [30]. All these effects can lead to inhibition of ATP synthesis, including in the central endocrine organs.

Evidence of the RPM effect on neurons in the brain can also be found in studies showed changes in the expression of the early c-fos protein as a marker of neuronal activity in brain structures [31]. In the experiment [31] selective activation of certain brain structures by microwave radiation is shown. In particular, after the RPM action with different pulse repetition frequencies, the neurons of the nuclei of the hypothalamus and the reticular formation are activated. The fact that the results of this study positively correlates with frequency-dependent changes in mice behavioral responses indicating a stress response after brain irradiation confirms the brain involvement in the body's stress response in reaction to microwave irradiation [31]. These reactions demonstrated, mainly, a decrease in the active-search component against the background of an increase in the passive-defensive component of behavior in the "open field".

In general, the data obtained indicate a character of the stress response to the effect of RPMs in the irradiated organism, which differs from the thermal stress that is formed in a complex manner and depends ambiguously on the repetition frequency of the pulses. This makes it difficult to predict possible adverse reactions in different body systems for such exposure. Since there is a high probability of stress, this circumstance must be taken into account in hygienic and ecological practice in order to completely eliminate or minimize undesirable consequences.

References

[1] E. A. Pryahin, A. V. Akleev “Elektromagnitnye polya i biologicheskie sistemy: stress i adaptaciya”, Chelyabinsk: Poligraf-Master, 2011, 240 P.

[2] I. A. Cotgreave “Biological stress responses to radio frequency electromagnetic radiation: are mobile phones really so (heat) shocking?” ABB, 2005, vol. 435. I. 1, pp. 227–240.

[3] Y. G. Grigor'ev, A. V. Shafirkin, A. L. Vasin “Biologicheskie effekty mikrovolnovogo izlucheniya nizkoj neteplovoj intensivnosti” Aviakosmich. i ekologich. meditsina., 2005, vol. 39, pp. 3-18.

[4] Y. B. Kudryashov, V. V. Alabovski, S. Y. Perov “Short-wave electromagnetic field effects on adrenal glands functional status in the rats” Vestnik novyh medicinskih tekhnologij, 2015, vol. 1, Public. 1-1.

[5] Y-H. Hao, Z. Li, R-Y. Peng “Effects of microwave radiation on brain energy metabolism and related mechanisms” Military Medical Research, 2015, 2: 4.

[6] K. Sri Nageswari “Mobile Phone Radiation : Physiological & Pathophysiologcal Considerations” Indian J Physiol Pharmacol, 2015, vol. 59(2), pp. 125-135.

[7] S. Thomée, A. Härenstam, M .Hagberg “Mobile phone use and stress, sleep disturbances, and symptoms of depression among young adults - a prospective cohort study” BMC Public Health, 2011, 11:66.

[8] I. Yakymenko, O. Tsybulin, E. Sidorik “Oxidative mechanisms of biological activity of low-intensity radiofrequency radiation” Electromag. Biol Med, 2016, vol. 35(2), pp.186-202.

[9] Y. G. Grigoriev , V. F. Mikhailov , A. A. Ivanov “Autoimmune Processes After Long-Term Low-Level Exposure to Electromagnetic Fields (the Results of an Experiment)” Radiac. biologiya. Radioehkologiya, 2010, vol. 50(1), pp. 22–27.

[10] M. A. Bolshakov, I. R. Knyazeva, V. V. Rostov “Initiation of Free-Radical Oxidation in Albino Mice by Exposure to Pulse Periodic Microwaves and X-Rays” Biophysics, 2005,. vol. 50, Suppl. 1, pp. S104-S109.

[11] I. R. Knyazeva, M. A. Bolshakov, L. P. Zharkova “Analiz pokazatelej okislitelnoj modifikacii lipidov i belkov posle vozdejstviya nanosekundnogo impulsno-periodicheskogo mikrovolnovogo izlucheniya” Trudy 8 Mezhdunar. simpoz. po elektromag. sovmest, Sankt-Peterburg, 2009, pp. 395-398.

[12] V. D. Prokopieva, E. G. Yarygina, S. A. Ivanova “Use of Carnosine for Oxidative Stress Reduction in Different Pathologies” Oxid. Medic. and Cell. Longevity, vol. 2016, Article ID 2939087, 8 pages.

[13] S. Ni, Y. Yu, Y. Zhang “Study of Oxidative Stress in Human Lens Epithelial Cells Exposed to 1.8 GHz Radiofrequency Fields” PLoS ONE, 2013, vol. 8(8): e72370.

[14] N. R Desai, K. K. Kesari, A. Agarwal “Pathophysiology of cell phone radiation: oxidative stress and carcinogenesis with focus on male reproductive system” Reproduc. Biol. and Endocrin, 2009, vol.7:114.

[15] “Euroguide on the accommodation and care of animals used for experimental and other scientific purposes” FELASA: London, UK, 2007, 17 p.

[16] I. R. Knyazeva, M. A. Medvedev, L. P. Zharkova “Pulse-repetitive microwave and x-ray exposure on human erythrocyte” Bulletin of Siberian Medicine, 2009, vol. 1.pp. 24-29.

[17] I. R. Knyazeva, V. V. Ivanov, L. P. Zharkova “The effect of the repetitive pulsed microwaves on functional activity of isolated mitochondria of mice liver” Vestnik TGU. Biologiya, 2011, vol. 4, pp. 125-135.

[18] M. A. Bolshakov, L. P. Zharkova, V. V. Ivanov “The activity of antioxidant enzymes of liver mitochondria of mice after exposure to nanosecond repetitive pulsed microwave” Vestnik TGU. Biologiya, 2012, vol. 3, pp. 122-136.

[19] I. R. Knyazeva, M. A. Bolshakov, V. V. Ivanov “Response of mice liver mitochondria to repetitive pulsed microwaves and X-rays” Izvestiya vysshih uchebnyh zavedenij. Fizika, 2012, vol. 55, № 10/3, pp. 194-198.

[20] A. I. Klimov, O. B. Kovalchuk, V. V. Rostov “Measurement of Parameters of X band High Power Microwave Superradiative Pulses” IEEE Transactions of Plasma Science, 2008, vol. 36, № 3, pp. 661-664.

[21] P. Tijssen "Practice and theory of enzyme immunoassays" Amsterdam; New York: Elsevier; New York, USA : Sole distributors for the USA and Canada, Elsevier Science Pub. Co, 1985, 502 p.

[22] A. A. Gostyuhina, T. A. Zamoshchina, K.V.Zajcev "Uroven serotonina v syvorotke krovi krys posle fizicheskogo pereutomleniya v usloviyah svetovogo desinhronoza v raznye sezony goda" Sechenov Physiology Journal, 2016, vol. 102, № 9. pp. 1082-1088.

[23] L. F. Wang, R. Y. Peng, X. J. Hu "Studies on the influence of high power microwave radiation on energy metabolism of brian in rats" Chin J Radiol Health, 2006, vol.15, pp. 269–271.

[24] D. Ma, R. Y. Peng, Y. B. Gao "Effects of microwave radiation on structure and energy metabolism in rat hippocampus" Chin J Stereol Image Anal., 2010, vol. 15 pp. 420-424.

[25] R. Caubet, F. Pedarros-Caubet, M. Chu "A radio frequency electric current enhances antibiotic efficacy against bacterial biofilms" Antimicrob Agents Chemother, 2004,vol. 48, pp. 4662–4664.

[26] M. Whiteman, J. S. Armstrong, N. S. Cheung "Peroxynitrite mediates calcium-dependent mitochondrial dysfunction and cell death via activation of calpains" FASEB J., 2004, vol. 18, pp. 1395–1397.

[27] H. Y. Zuo, T. Lin, D. W. Wang, "Neural cell apoptosis induced by microwave exposure through mitochondria-dependent caspase-3 pathway" Int J Med Sci, 2014, vol.11, pp. 426–435.

[28] K. K. Kesari, R. Meena, J. Nirala "Effect of 3G Cell Phone Exposure with Computer Controlled 2-D Stepper Motor on Non-thermal Activation of the hsp27/p38MAPK Stress Pathway in Rat Brain" Cell Biochem Biophys, 2014, vol. 68, pp. 347–358.

[29] S. Shahin, V. P. Singh, R. K. Shukla "2.45 GHz microwave irradiation-induced oxidative stress affects implantation or pregnancy in mice, Mus musculus" Appl Biochem Biotechnol., 2013, vol. 169, pp. 1727–1751.

[30] R. Yang, R. Y. Peng, Y. B. Gao "The effect of microwaves on hippocampal neurons in vitro and its mechanism" Chin J Phys Med Rehabil, 2006, vol. 28, pp.:670–673.

[31] A. V. Kereya, M. A. Bolshakov, M. Y. Hodanovich "Ocenka reakcii mozga myshej na vozdejstvie nanosekundnyh mikrovolnovyh impul'sov po ehkspressii belka c-fos" Radiac. biologiya. Radioehkologiya, 2017, vol. 57. № 2, pp. 179-184.