

Mechanisms of anti-stress action of low-intensive mm radiation on microcirculation of animals under stress conditions

Marina Ravaeva
Taurida Academy
V.I. Vernadsky Crimean Federal
University
Simferopol, Russia
ravaevam@yandex.ru

Anastasia Furkovskaya
Taurida Academy
V.I. Vernadsky Crimean Federal
University
Simferopol, Russia
doctorhacksaw7@gmail.com

Elena Chuyan
Taurida Academy
V.I. Vernadsky Crimean Federal
University
Simferopol, Russia
Elena-chuyan@rambler.ru

Elena Birukova
Taurida Academy
V.I. Vernadsky Crimean Federal University
Simferopol, Russia
biotema@rambler.ru

Olga Kornilenko
Taurida Academy
V.I. Vernadsky Crimean Federal University
Simferopol, Russia
moarmorq@mail.ru

Abstract — animal studies with laser Doppler flowmetry showed, that there were local and systemic changes in the microcirculation caused by hypokinetic stress, what allowed us to expand our understanding of the pathophysiological processes caused by the influence of a stressing factor on the body. A study of the isolated EHF influence on the microcirculation and its combination with stress-modeling helped us to establish and scientifically substantiate the possibility of using extremely high frequency electromagnetic radiation for correction body disorders caused by stress.

Keywords — stress, hypokinesia, microcirculation, laser Doppler flowmetry, low-intensity electromagnetic radiation of extremely high frequency

I. INTRODUCTION

Due to the high biological efficiency, low-intensity electromagnetic radiation of extremely high frequency (EHF EMR) is widely used for the treatment of a large variety of diseases: gastroenterological, cardiovascular, neurological, oncological, urological, gynecological, dental and some others [1, 2]. Moreover, most of the diseases, for a treatment of which the EHF-therapy is used, are accompanied by hemodynamic disorders. However, the influence of EHF EMR on microcirculation processes has not been studied sufficiently, and many data are very contradictory. That is why the study of the mechanisms of the influence of EHF EMR on tissue microhemodynamics is a fundamental scientific task. The assigned scientific problem can be solved with a new non-invasive method for the microcirculation study - laser Doppler flowmetry (LDF), which allows not only to assess the overall level of peripheral perfusion, but also to identify the features of microcirculation regulation. In addition, the study involves an integrated approach, which includes modern biochemical methods, using a big variety of EHF-exposure modes, monitoring the effects of stress and physiological balance on microcirculation.

This study belongs to the priority area of science development in the Russian Federation, and the results of this project can be used for improvement of medical and veterinary technologies. The results of this study will

significantly expand our understanding of the pathogenetic mechanisms of diseases associated with microhemodynamic disturbances and the fundamental principles of the electromagnetic influence on the human and animal organisms.

II. PROBLEM STATEMENT

It is known, that stress is the way organisms adapt to environmental influences, which contributes, on the one hand, to survival of the body in extreme conditions, and, on the other, to development of pathological processes in the body. Human beings and animals are constantly exposed to stress factors: pain, temperature, emotional, physical, etc. One of the most common stressful factors in the modern world is lack of physical activity or hypokinesia (HK), which causes the development of a number of specific and nonspecific changes in the functioning of almost all organs and systems of the body. In general, stress is a non-specific component of any disease, especially of those, that are accompanied by microhemodynamic disorders (hypertension, coronary heart disease, atherosclerosis, etc.). All this leads to search for and introduction of pharmacology-originated or physical adaptogens. Among the physical factors, the low-intensity EHF EMR exhibits high therapeutic activity in the treatment of acute and chronic microhemodynamic disorders, such as coronary heart disease, myocardial infarction, and hypertension. Despite this, there is no consensus about the mechanisms of EMR EHF therapeutic activity. Therefore, the aim of this study was to identify the early “markers” of pathological changes, which were accompanied by hypokinetic stress, and to study the ways of its correction with low-intensity EHF EMR therapy. Since it is known that numerous protein factors, especially biogenic amines (adrenaline, dopamine, serotonin, bradykinins), and inorganic compounds (CO, NO), play a significant role in the regulation of the microcirculation, the aim of the present study was to establish their role in the mechanisms of EMR EHF influence on the microcirculation during biological balance and stress modeling.

III. RESEARCH QUESTIONS

The study had tasks such as:

- 1) identification and differentiation of local and systemic changes in microcirculation indicators under the influence of EHF radiation.
- 2) revealing of the sequence of activation of tissue blood flow regulation mechanisms during under the influence of EHF.
- 3) study the functional changes in tissue microhemodynamics under hypokinetic stress and the possibility of their correction with low-intensity EMR EHF.
- 4) determination of the role of biologically active substances of protein and non-protein nature of endogenous (vascular and extra-vascular) origin in the mechanisms of EHF EMR influence on microcirculation.

The study with uniquely designed experiments and new methods made it possible to expand our understanding of EHF EMR influence on the living things. In addition, the scientific novelty and significance of this study are provided by new opportunities of increasing body resistance to hypokinesia and by prevention of pathological changes in the body accompanied by long mobility restriction with pharmacological correction of this pathology.

IV. PURPOSE OF THE STUDY

The aim of this study was to establish the mechanisms of low-intensity electromagnetic radiation of extremely high frequency (EHF EMR; wavelength = 7.1 mm, power flux density - 0.4 mW / cm²) influence on tissue microhemodynamics of rats under stress.

V. EXPERIMENTAL

The study was performed on 80 white outbred male rats weighing 180-250 g. Selected animals were the same age, characterized by average physical activity and low emotionality by the "open field" test. Strict selection helped form homogeneous groups of animals with the same constitutional features and similar reactions to different factors. After preliminary selection, the animals were divided into 4 groups with 20 rats in each group. The first group of animals was biological control, and animals were kept under standard vivarium conditions. The second group contained rats under experimental conditions, which included modeling of stress-reaction with ten-day mobility restriction (hypokinesia, HK). Rats of the third group were exposed to a 30-minute exposure of EHF EMR everyday. Rats of the fourth group were exposed not only to EHF EMR, but also to HK (HK + EHF).

Conditions of the experimental mobility restriction included using of a special plexiglass cases, made up of 5 cells, sizes of which were 140 x 60 x 60 mm. Animals were placed in that cases for 10 days, and every day spent inside of them for 20 hours. The remaining 4 hours were devoted to feeding and caring for animals, EHF exposure (for HK + EHF group) and registration of microcirculation changes.

EHF exposure was provided by the EHF therapy apparatus of the three-frequency microprocessor EHF-7, 1/5, 6/4, 9 - ND (ND2) (registration certificate No. FSR 2007/00763 dated 09/18/2007, manufactured by Scientific

and Commercial LLC RESLA company, Fryazino, Russia). Generator specifications: operating wavelength 7.1 mm, radiation power flux density 0.4 mW / cm². Animals were impacted for 30 minutes on the occipital-collar region, what, as shown by our previous studies, was the most effective. The study of blood microcirculation parameters was conducted by the LDF method [3-5], described in detail [6].

For conduction of biochemical studies of the catecholamines and serotonin levels, peripheral blood was collected on the 1st, 3rd, 5th, 7th and 9th days of the experiment by the tail vein puncturing. The quantity of catecholamines and serotonin in peripheral blood leukocytes was measured according to the method of B. Falck (Falck, Owman, 1965) [7] in the modification of V.P. Novitskaya (2002) [8]. The method is based on the reaction of monoamines with formaldehyde vapors, what causes forming of fluorescent compounds giving a bright green glow. The installation for recording the luminescent spectra of single leukocytes consists of a ML-4 luminescent microscope with a spectralizing device (FMEL-1K photometric nozzle), a photomultiplier (PMT is a device necessary for converting light into electric current), an analog-to-digital converter, and a personal computer.

After incubation in formaldehyde vapors, blood smears were examined under glycerol immersion with a luminescent microscope at a wavelength of 525 nm at an excitation light length of 405 nm (to study the levels of serotonin) and at a wavelength of 450 nm (to study catecholamines) at an excitation light wavelength of 405 nm. The signal value was calculated in arbitrary units, which is not a true indicator of the absolute amount of substance in the cell, but is directly proportional to its amount. The average amount of serotonin and catecholamines was calculated after measuring the brightness of the glow of ten white blood cells in each blood smear. Autofluorescence of a glass slide without a blood smear was used as a control and subtracted from the average fluorescence of leukocytes. To assess the state of the nitric oxide synthesis system, there was measurement of the blood plasma biochemical parameters, which characterized the intensity of L-arginine exchange through two alternative metabolic pathways (non-oxidative arginase and oxidative NO-synthase). The intensity of non-oxidative metabolism was evaluated by determination of the arginase activity (Arg, nmol / min mg of protein) and the urea content (nmol / mg of protein), which was generated by the work of this enzyme. The intensity of arginine oxidative degradation, during which there was a synthesis of nitric oxide, was evaluated by the activity of various isoenzymes of NO-synthases - calcium-dependent constitutive (pmol / min mg of protein) and calcium-independent inducible (iNOS, pmol / min mg of protein), as well as the level of oxidized stable metabolites of nitric oxide - nitrite- (NO₂⁻), pmol / mg of protein) and nitrate- (NO₃⁻), nmol / mg of protein) anions. The rate of nitrate anion reutilization for nonoxidative resynthesis of nitric oxide was also evaluated by determining the NADP-dependent nitrate reductase activity (nitrate reductase, nmol / min mg of protein). Statistical processing of the results was conducted with the STATISTICA-8.0 package. Since the values distribution of the variables was differed from the normal one, the reliability of the intergroup differences was assessed with the nonparametric Mann-Whitney U test and

the nonparametric Wilcoxon test. The differences were significant at $p \leq 0.05$.

VI. RESULTS AND DISCUSSION

The study showed that tenfold course of EHF exposure had a pronounced effect on the oscillatory parameters of tissue blood flow, leading to an increase of the amplitudes of endothelial (by 47.53%; $p < 0.05$), myogenic (by 56.24%; $p < 0.01$), neurogenic (by 29.03%; $p < 0.05$) and pulse (by 48.37%; $p < 0.05$) oscillations amid a decrease of the amplitudes of respiratory (by 55.9%; $p < 0.05$) rhythms.

The course EHF exposure lead to a decrease in the microvascular tone: in myogenic (by 34.15%; $p < 0.05$), starting from the first session only in the area of EHF exposure localization, neurogenic, starting from the third session in the EHF- exposure area (by 19.32%; $p < 0.05$) and in the distant symmetric region (by 31.34%; $p < 0.05$).

Observations on changes in microhemodynamic circulation under the influence of chronic hypokinetic stress, EHF EMR and their combination showed, that 10-day HK led to dysregulation of tissue microcirculation at all levels, which caused vasoconstriction disorders, impaired blood flow and outflow, led to dominance of shunt blood flow and to decrease of amount of functioning capillaries. Decrease of oscillatory and non-oscillatory microcirculation indicators confirmed that. Thus, the amplitudes of neurogenic rhythms decreased by 14.1% ($p < 0.05$), endothelial rhythms - by 18.3% ($p < 0.05$), myogenic rhythms - by 12.6% ($p < 0.05$) respiratory rhythms by 9.2% ($p < 0.05$) and pulse rhythms by 6.5% ($p < 0.05$) according to the data of the control group. The change of the oscillatory parameters was also seen in the changes of the integral non-oscillatory indicators of tissue blood flow: perfusion level decreased by 30.6% ($p < 0.05$), flux - by 48% ($p < 0.05$) and coefficient of variation - by 39% ($p < 0.05$) according to the data of the control group of rats.

The opposite dynamics of microcirculation indices developed under EHF influence, so the more the factor was applied, the more the effect grew.

Thus, the amplitudes of microcirculation oscillatory indicators increased: endothelial - by 34% ($p < 0.05$), neurogenic - by 25% ($p < 0.05$), myogenic - by 10% ($p < 0.05$), pulse - by 13%, and the amplitudes of respiratory rhythms decreased by 12% ($p < 0.05$). Non-oscillatory indicators also increased: microcirculation index - by 62% ($p < 0.05$), flux - by 69% ($p < 0.05$), coefficient of variation - by 33% ($p < 0.05$) according to the data of the control group.

Thus, it was found, that EHF exposure causes a significant changes in the activity of all components of the microvascular tone regulation, which was expressed in increasing functional activity of the endothelium, decreasing peripheral resistance, increasing blood flow into the nutritional microvascular bed, and in improvement of venous outflow, which generally showed the perfusion increase.

The EHF influence on the animals, which were under conditions of limited mobility, led to leveling of vasoconstriction caused by hypokinesia and normalization of microcirculation processes. So, after a 10-fold EHF exposure on animals under hypokinesia, there was an increase in the amplitudes of endothelial rhythms by 34.6% ($p < 0.05$),

neurogenic rhythms by 24.1% ($p < 0.05$), myogenic rhythms - by 21.3% ($p < 0.05$), pulse rhythms by 10.8% ($p < 0.05$), microcirculation rate - by 106.3% ($p < 0.05$), flux - by 79% ($p < 0.05$), and the coefficient of variation - by 39% ($p < 0.05$).

It should be noted that after 3 days of EHF exposure the experimental parameters of microcirculation were close to the control data, and on the 5th day they exceeded the control parameters.

The study of the rhythms of the blood flow fluctuations in the bloodstream revealed that endothelium-dependent and myogenic endothelium-independent components of tissue blood flow regulation played the main role in the mechanisms of low-intensity EHF EMR influence.

These effects can be caused by the vasoactive agents, in particular, biogenic amines (adrenaline, dopamine, serotonin, bradykinins), which have specific receptors on vascular endothelium and exhibit various effects after perfusing them into the vascular system.

A number of studies established the role of biologically active substances in the regulation of tissue microcirculation under the low-intensity EHF EMR influence. Thus, the determination of amount of catecholamines (dopamine, norepinephrine and adrenaline) in peripheral blood leukocytes showed that after EHF exposure on intact animals there was decrease of their concentration by an average of 17-20% ($p < 0.05$). Under conditions of HK the catecholamine concentration, on the contrary, increased by 21.94% ($p < 0.01$) by the 5th day, and reached its maximum number by the 7th day: it was amounted to 151.07% ($p < 0.001$), according to the control data. Under the combined influence of HK and EHF the amount of catecholamines became significantly different from the data, received from animals with HK, but without EHF exposure. So, on the 5th day of the experiment there was a significant decrease of catecholamines concentration by 20% ($p < 0.05$). The maximum decrease of their number was observed on the 9th day (by 31%; $p < 0.001$) according to the data received from animals, that were exposed to the isolated HK influence.

Thus, the EHF influence limited the development of the stress response on HK by preventing stress-induced increase of the sympathoadrenal system activity.

Studies of the serotonin concentration in the peripheral blood of rats showed that intact animals had their serotonin ranged from 250.16 ± 17.63 to 271.50 ± 12.88 a.u.

Under the EHF exposure on the 3rd day of experiment serotonin levels in blood leukocytes had tendency to increase ($p > 0.05$), and starting from the 5th day, there were significant differences compared with the control data. By the 9th day of the experiment, the difference between the groups was 37.42% ($p < 0.05$).

Serotonin levels in blood of rats with limited physical activity with or without the systematic EHF exposure had different nature of changes. So on the 3rd day of experiment there was increase of serotonin level (by 32.66% ($p < 0.05$) - observable but not so pronounced, and on the next day amount of serotonin decreased, but was still significantly higher than in the group of animals exposed to the isolated HK influence (by 54%, $p < 0.05$), so EHF exposure made the parameters of the endothelium-dependent and myogenic

endothelium-independent components of the tissue blood flow regulation to increase.

Radioautographic and fluorescence methods demonstrated that serotonin was intensively absorbed by endothelial cells, actively catabolized by mitochondrial monoamine oxidase [4, 5] and stimulated endothelium-dependent vasodilation with the production of nitric oxide [3]. Indeed, the EHF exposure caused changes in the functional activity of microvascular endothelium, which was supported by increasing amplitudes of endothelial vibrations of the basal blood flow and stimulation of NO-synthase activity in pharmacological (there was an increase of skin blood flow level with iontophoretic administration of acetylcholine by 78.76%; $p < 0.05$) and occlusal (there was an increase in the reaction of cutaneous blood flow with registration of post-occlusive reactive hyperemia by 63.28%; $p < 0.05$) samples. Biochemical studies showed that after a 10-fold EHF exposure concentration of constitutive calcium-dependent NO-synthase in human blood plasma increased by 325% ($p < 0.05$) and total NOS by 252% ($p < 0.05$), respectively. The activity of inducible calcium-independent NO-synthase slightly decreased and amounted to 94% of the initial level. Along with this, the circulating pools of nitrite and nitrate anions significantly decreased by 35% and 53% ($p < 0.05$), respectively. In general, after a 10-fold EHF exposure there was observed a more pronounced dominance of the oxygen-dependent pathway over the arginase recycling pathway.

VII. CONCLUSION

In general, almost all regulatory components of vascular and extravascular genesis are involved in the microcirculation reaction to EHF EMR. It is likely that EHF exposure causes such a system response due to a large

amount of targets for EMR, including skin microvessels, blood cells, diffuse neuroendocrine system, as well as nerve endings and peripheral nerves of the skin, the activation of which changes the functional activity of the nervous, immune, endocrine systems, which plays a significant role in the mechanisms of regulation of blood microcirculation processes.

REFERENCES

- [1] A.G. Pakhomov, U.K. Prol, S.P. Mathur, Y. Ak'el, and C.B.C. Campbell, "Search for frequency-specific effects of millimeter-wave radiation on isolated nerve function," *Bioelectromagnetics*, vol. 18, pp. 324–334, 1997.
- [2] F.S. Prato, D. Desjardins, and L.D. Keenlside, "Light intensity and wavelength alters nociceptive effects of magnetic field shielding," *BEMS*, Washington, 2006, pp. 156–158.
- [3] A.I. Krupatkin, and V.V. Sidorov *Laser Doppler flowmetry of blood microcirculation*, M: Medicine, 2005.
- [4] U. Hoffman, A. Yanar, and A. Bolinger, "The frequency histogram – a new method for the evaluation of Laser Doppler Flux Motion," *Microvascul. Res.*, vol 40, pp. 293–301, 1990.
- [5] P. Kvandal, A. Stefanovska, and M. Veber, "Regulation of human cutaneous circulation evaluated by laser Doppler flowmetry, iontophoresis, and spectral analysis: importance of nitric oxide and prostangladines," *Microvascular Research*, vol. 65, pp. 160–171, 2003.
- [6] E.N. Chuyan, and M.Yu. Ravaeva, "The effect of chronic hypokinetic stress on tissue microhemodynamics," *Russian physiological journal named after I.M. Sechenov*, vol. 101, No. 3, pp. 316–325, 2015.
- [7] B. Falck, and C. Owman, "A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic monoamines," *Acta Univ. Lundensis*, No 7, pp. 79–83, 1965.
- [8] V.P. Novitskaya, "Modification of the determination parameters of monoamines in leukocytes on smears of peripheral blood," *Clinical laboratory diagnostics*, No. 1, pp. 24–33, 2002.