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The role of nitric oxide in the mechanisms of stress-protective action of low-intensity extremely high-frequency electromagnetic radiation in acute and chronic stress

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ABSTRACT

BACKGROUND: The study of stress mechanisms is an area of current interest in physiology and medicine. Understanding the role of nitric oxide (NO) in the mechanism of stress-protective action of low-intensity extremely high-frequency electromagnetic radiation (EHF EMR) requires investigating the main components of the stress response.

AIM: To determine the role of NO in the development of the anti-stress effect of low-intensity EHF EMR.

MATERIALS AND METHODS: 140 male Wistar rats weighing 200–220 g were divided into the following seven groups of 20 animals each: intact animals; AS, animals after a single exposure to acute stress; EHF-AS, animals irradiated with EHF EMR for 10 days and exposed to acute stress on day 10; L-NAME-EHF-AS, animals who were administered the NOS inhibitor L-NAME (10 mg/kg, intraperitoneally) for 10 days and irradiated with EHF EMR after 1 hour; HK, animals exposed to chronic hypokinetic stress for 10 days; EHF-HK, animals exposed to EHF EMR and hypokinetic stress for 10 days; L-NAME-EHF-HK, animals who were administered the NOS inhibitor L-NAME (10 mg/kg, intraperitoneally) for 10 days, irradiated with EHF EMR after 1 hour, and exposed to hypokinetic stress after another 1 hour. The levels of middle-weight molecules (MWM), circulating immune complexes (CIC), and malondialdehyde (MDA) were determined in the blood serum.

RESULTS: Acute stress increased the level of malondialdehyde, activating lipid peroxidation processes; in the EHF-AS group, this effect disappeared. NO blockade in the L-NAME-EHF-AS group activated endotoxication processes, the stress cascade, and physiologically active regulatory peptides (increase in the MWM level at 280 nm), oxidative metabolism of proteins and lipids (increase in the CIC and MDA levels), and inhibited immunogenesis. Hypokinetic stress suppressed the stress inhibiting system of the body and physiologically active regulatory peptides (decrease in the MWM level at 280 nm), suppressed immunogenesis, and increased oxidative metabolism of proteins and lipids (increase in the CIC and MDA levels). The stress-protective effect of EHF EMR was observed in the EHF-HK group. NO blockade in the L-NAME-EHF-HK group activated endotoxication processes, stress cascade and stress inhibiting systems of the body, physiologically active regulatory peptides (increase in the MWM level at 254 and 280 nm), oxidative metabolism of proteins and lipids (increase in the CIC and MDA levels), and inhibited immunogenesis.

CONCLUSIONS: NO blockade with L-NAME amplifies the effects of acute and hypokinetic stress under the exposure to low-intensity EHF EMR. Normal NO levels are necessary for the stress-protective effect of EHF EMR to develop during acute and hypokinetic stress.

Keywords: malondialdehyde; circulating immune complexes; middle-weight molecules; laboratory rats; low-intensity extremely high-frequency electromagnetic radiation; acute stress; chronic stress.

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Роль оксида азота в механизмах стресс-протекторного действия низкоинтенсивного электромагнитного излучения крайне высокой частоты при остром и хроническом стрессе

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АННОТАЦИЯ

Актуальность. Исследование механизмов стресса – актуальное направление в физиологии и медицине. Для понимания роли оксида азота (NO) в механизме стресс-протекторного действия низкоинтенсивного электромагнитного излучения крайне высокой частоты (ЭМИ КВЧ) необходимы исследования основных компонентов стресс-реакции.

Цель — определение роли NO в реализации антистрессорного эффекта низкоинтенсивного ЭМИ КВЧ.

Материалы и методы. 140 крыс-самцов линии Wistar массой 200–220 г разделили на 7 групп по 20 особей: интактная; ОС — животные после однократного воздействия острого стресса; КВЧ-ОС — крыс 10 сут облучали ЭМИ КВЧ, а на 10-е сутки подвергали острому стрессу; L-NAME-КВЧ-ОС — 10 дней вводили внутрибрюшинно ингибитор NOS L-NAME (10 мг/кг) и через 1 ч облучали ЭМИ КВЧ; ГК — 10 сут воздействовали хроническим гипокинетическим стрессом; КВЧ-ГК — 10 сут облучали ЭМИ КВЧ и воздействовали гипокинетическим стрессом; L-NAME-КВЧ-ГК — 10 дней вводили внутрибрюшинно ингибитор NOS L-NAME (10 мг/кг), через 1 ч облучали ЭМИ КВЧ, далее через 1 ч — гипокинетическим стрессом. В сыворотке крови определяли уровень содержания молекул средней массы (МСМ), циркулирующих иммунных комплексов (ЦИК) и малонового диальдегида (МДА).

Результаты. Острый стресс повышал уровень МДА, активируя процессы перекисного окисления липидов, у КВЧ-ОС эффект исчезал. Блокада NO в группе L-NAME-КВЧ-ОС активировала процессы эндотоксикации, стресс-реализующую систему и физиологически активные регуляторные пептиды (повышение уровня МСМ при 280 нм), окислительный метаболизм белков и липидов (рост ЦИК и МДА) и ингибировала иммуногенез. Гипокинетический стресс угнетал стресс-лимитирующую систему организма и физиологически активные регуляторные пептиды (снижение МСМ при 280 нм), процессы иммуногенеза и повышала окислительный метаболизм белков и липидов (повышение ЦИК и МДА). В группе КВЧ-ГК наблюдался стресс-протекторный эффект ЭМИ КВЧ. При блокаде NO в группе L-NAME-КВЧ-ГК активировались процессы эндотоксикации, стресс-реализующая и стресс-лимитирующая системы организма, физиологически активные регуляторные пептиды (повышение МСМ при 254 и 280 нм), окислительный метаболизм белков и липидов (рост ЦИК и МДА), ингибировался иммуногенез.

Выводы. Блокада NO L-NAME усиливает последствия острого и гипокинетического стресса в условиях низкоинтенсивного ЭМИ КВЧ. Нормальный уровень NO необходим для реализации стресс-протекторного эффекта ЭМИ КВЧ при остром и гипокинетическом стрессе.

Ключевые слова: малоновый диальдегид; циркулирующие иммунные комплексы; молекулы средней массы; лабораторные крысы; низкоинтенсивное электромагнитное излучение крайне высокой частоты; острый стресс; хронический стресс.

Как цитировать

Раваева М.Ю., Черетаев И.В., Чуян Е.Н., Галенко-Ярошевский П.А., Зеленская А.В. Роль оксида азота в механизмах стресс-протекторного действия низкоинтенсивного электромагнитного излучения крайне высокой частоты при остром и хроническом стрессе // Обзоры по клинической фармакологии и лекарственной терапии. 2024. Т. 22, № 4. С. 377–387. DOI: https://doi.org/10.17816/RCF635404

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BACKGROUND

Our previous studies of microcirculation in rats showed that preventive 10-fold exposure to low-intensity electromagnetic radiation of extremely high frequency (EHF EMR) reduced the severity of hyperemia signs typical of acute stress for 48 h after the stress factor [1]. Simultaneous exposure of the animals to EHF EMR under prolonged mobility restriction alleviated vasoconstriction and blood inflow and outflow disorders caused by stress. In laser Doppler flowmetry, the analysis of the oscillation rhythm structure established that endothelium-dependent, myogenic endothelium-independent and neurogenic components of tissue microcirculatory flow regulation play the key role in the mechanisms of stress-protective action of low-intensity EHF EMR [1, 2].

Meanwhile, additional studies of stress-response links, i.e., immune, peroxidation, and inflammation, are needed for a more complete understanding of the mechanism of stress-protective effect of EHF EMR. Moreover, the role of nitric oxide (NO) in the mechanisms of anti-stress action of EHF EMR has not yet been elucidated.

The study aim was to investigate the level of middle molecules, circulating immune complexes and malondialdehyde in rats under stress of different duration and EHF EMR action, as well as to determine the role of NO in the anti-stress effect of low-intensity EHF EMR.

MATERIALS AND METHODS

The experimental part of the study was carried out at the Department of Human and Animal Physiology and Biophysics in the Center for Collective Use of Scientific Equipment "Experimental Physiology and Biophysics" of Vernadsky Crimean Federal University under Russian Science Foundation (RSF)'s Grant No. 23-24-00332 Tissue Microhemodynamics: Mechanisms of Anti-Stress Action of Low-Intensity Millimeter Radiation and Study Program No. AAAA-A21-121011990099-6 Physiological Mechanisms of Biological Action of Factors of Different Nature and Intensity of the Vernadsky Crimean Federal University.

Study subjects

All applicable international, national and/or institutional principles of animal care and use were followed. All procedures performed in the studies involving animals complied with the ethical standards approved by the legal acts of the Russian Federation, the principles of the Basel Declaration and the recommendations of the Ethics Committee on Bioethics of the Vernadsky Crimean Federal University (Protocol No. 5 of 2022).

The experiment was performed on mature male Wistar rats weighing 200–220 g (Rappolovo Breeding Nursery). The rats were quarantined for at least 14 days. The animals were kept in vivarium conditions with natural light at 18–22 °C on corn cob bedding (Zilubag LLC, Russia), with free access to water and complete pelleted feed (LBK-120, CJSC Tosnensky Mixed Feed Factory, Russia).

For the experiment, 140 male rats of the same age characterized by average motor activity and low emotionality in the open field test (RPC Open Science, Russia), which constitute the majority in the population, were selected from 200 animals. Such selection formed homogeneous groups of animals with close constitutional features, that reacted unidirectionally to the action of one or another factor.

After preliminary selection, the animals were divided into 7 groups of 20 rats each (Table 1): 1) intact (n = 20), where animals were kept under vivarium conditions; 2) AS (n = 20), where animals were exposed to a single action of acute stress (AS); 3) EHF-AS (n = 20), where animals were exposed to EHF EMR for 10 days, followed by AS (on Day 10); 4) L-NAME-EHF-AS group (n = 20), where animals were injected daily with the non-selective NOS inhibitor L-NAME (NG-Nitro-L-arginine Methyl Ester, Hydrochloride, Sigma, USA) at 10 mg/kg into the abdominal cavity for 10 days, and then were exposed to EHF EMR after 1 h; 5) HS group (n = 20), where animals were exposed to chronic hypokinetic stress (HS) for 10 days; 6) EHF-HS group (n = 20), where animals were sequentially exposed first to EHF EMR and then to HS for 10 days; 7) L-NAME-EHF-HS group (n = 20), where animals were injected daily with L-NAME at 10 mg/kg into the abdominal cavity for 10 days, then after 1 h were exposed to EHF EMR, and after another 1 h to HS.

On Day 10 of the experiment, two hours after the end of all activities, the animals were decapitated under ether anesthesia using a guillotine (RPC Open Science, Russia) and their blood was sampled to determine physiological markers of stress-protective effect: middle molecules, circulating immune complexes, malondialdehyde. The blood was collected into vacuum tubes with BD Vacutainer serum separating gel (BD Vacutainer SST II Advance).

Modeling of acute and chronic stress and their combinations

To model acute stress, the animals were placed in a swimming tank; the experiment lasted 1 h. The water temperature was 21–25 °C.

Special plexiglass cases of a suitable size were used in the experiment when creating conditions for restricting the animals' mobility in order to ensure maximum comfort of the rat inside the case and to prevent squeezing of body parts. When modelling hypokinetic stress, the animals were kept in these cases for 10 days, 19–20 h a day. At all other times, feeding and animal care were carried out.

Table 1. Experiment design

Таблица 1. Дизайн эксперимента

Group	Experiment day									
	1	2	3	4	5	6	7	8	9	10
Intact	C	C	C	C	C	C	C	C	C	C
OC	–	–	–	–	–	–	–	–	–	AS
EHF-AS	EHF	EHF	EHF	EHF	EHF	EHF	EHF	EHF	EHF	EHF-AS
L-NAME-EHF-AS	L-NAME-EHF	L-NAME-EHF	L-NAME-EHF	L-NAME-EHF	L-NAME-EHF	L-NAME-EHF	L-NAME-EHF	L-NAME-EHF	L-NAME-EHF	L-NAME-EHF-AS
HS	HS	HS	HS	HS	HS	HS	HS	HS	HS	HS
EHF-HS	EHF-HS	EHF-HS	EHF-HS	EHF-HS	EHF-HS	EHF-HS	EHF-HS	EHF-HS	EHF-HS	EHF-HS
L-NAME-EHF-HS	L-NAME-HS-EHF	L-NAME-HS-EHF	L-NAME-HS-EHF	L-NAME-HS-EHF	L-NAME-HS-EHF	L-NAME-HS-EHF	L-NAME-HS-EHF	L-NAME-HS-EHF	L-NAME-HS-EHF	L-NAME-HS-EHF

Note. Here and in Tables 2–4: C, control; AS, acute stress; HS, chronic hypokinetic stress; EHF, extremely high-frequency low-intensity electromagnetic radiation's; EHF-AS, a combination of extremely high-frequency low-intensity electromagnetic radiation's and acute stress factors and acute stress factors; L-NAME-EHF-AS, a combination effects of nitric oxide blocker L-NAME, extremely high-frequency low-intensity electromagnetic radiation's and acute stress factors; EHF-HS, a combination of extremely high-frequency low-intensity electromagnetic radiation's and hypokinetic stress factors; L-NAME-EHF-HS is a combination effects of L-NAME nitric oxide blocker, extremely high-frequency low-intensity electromagnetic radiation's and hypokinetic stress.

Примечание. Здесь и в табл. 2–4: C — контроль; AS — острый стресс; HS — хронический гипокинетический стресс; EHF — электромагнитное излучение крайне высокой частоты (ЭМИ КВЧ); EHF-AS — комбинация факторов ЭМИ КВЧ и ОС; L-NAME-EHF-AS — комбинация воздействия блокатора оксида азота L-NAME, ЭМИ КВЧ и ОС; EHF-HS — комбинация факторов ЭМИ КВЧ и ГК; L-NAME-EHF-HS — комбинация воздействия блокатора оксида азота L-NAME, ЭМИ КВЧ и ГК.

Method to determine the serum level of middle molecules

Middle molecules (MM) is an integral biochemical marker of endogenous intoxication [3]. This method includes blood sampling, serum separation from the formed elements by centrifugation, obtaining a protein-free sample by adding 0.5 mL of 10% trichloroacetic acid to 1 mL of serum and centrifugation for 30 min at 3000 rpm. Then, 4.5 mL of distilled water was added to 0.5 mL of supernatant and the optical density of the sample was determined using a spectrophotometer at 254, 275 and 280 nm [4, 5].

Samples were measured vs. control (distilled water) using a PE5400-UV spectrophotometer (Ekros-Analytika LLC, Russia). The MM content was determined by the optical density of the sample, and their level indicated the presence or absence of endogenous intoxication [3].

Method to determine the serum concentration of circulating immune complexes using polyethylene glycol test

The serum concentration of high molecular weight circulating immune complexes (CIC) was determined using the Grinevich method [6] by precipitation of antigen-antibody complexes from serum for 60 min with 3.75% polyethylene glycol prepared with 0.1 M borate buffer (pH 8.4), followed by photometric determination of the optical density of the precipitate. Measurements were performed using a spectrophotometer PE5400-UV (Ekros-Analytika LLC, Russia), determining the optical density of samples (prepared with 0.1 M borate buffer and 3.75% polyethylene glycol) and control (0.1 M borate buffer) in 10 mm cells at 450 nm. Then the difference of optical density was calculated; the result was multiplied by 1000 and the level of CICs in 100 mL of serum was obtained. The response was expressed in units of optical density:

$$E_{cic} = (E_{od} - E_c) \times 1000,$$

where E_{cic} is the density of high molecular weight circulating immune complexes; E_{od} is the optical density of the sample; E_c is the optical density of the control sample.

Method to determine malondialdehyde in serum

Serum MDA was determined according to Uchiyama and Mihara [7]. The method is based on the reaction between malondialdehyde and thiobarbituric acid (TBA), which, at high temperature and acidic pH, proceeds with the formation of a colored trimethine complex containing one molecule of MDA and two molecules of TBA [8].

The test tubes were shaken vigorously until a pinkish white suspension was formed. The tubes were then centrifuged at 3000 rpm (1800 g). Immediately after centrifugation, 3 mL of the supernatant was taken into a clean tube and the optical density of the sample vs. the blank was measured at 540 and 590 nm in a 10 mm cell.

Measurement was performed no later than 1.5 h after centrifugation.

MDA concentration was calculated using the following formula:

$$C = \frac{D_{540} - D_{590}}{0.156} \times 16 ,$$

where C is the content of TBA products in the sample, μmol/L; D_{540} is the optical density of the sample at 540 nm; D_{590} is the optical density of the sample at 590 nm; 0.156 is the molar extinction coefficient of malondialdehyde-TBA complex, L/μmol × cm; 16 is the serum dilution factor.

The measurement result is expressed as optical density units.

Statistical analysis of the study results

Statistical processing of the results was performed using Graph Pad Prism 9.5.1. software. Since the distribution of variables in some experimental groups according to the Shapiro–Wilk test differed from normal, the reliability of differences relative to the intact group was determined using the nonparametric Dunn's multiple comparisons test. Differences were considered significant at $p < 0.05$. Data are plotted as mean and standard error of the mean ($M \pm$ SEM).

STUDY RESULTS

Serum level of middle molecules in rats

The literature generally accepts that MMs are stress markers reflecting the pathological state of protein metabolism [9]. MMs can serve as a marker of internal endointoxication and as secondary toxins, significantly disrupting the vital activity of all systems and organs. It is well known that at 280 nm, aromatic non-toxic amino acids as well as phenols, tyrosine, tryptophan, and phenylalanine are detected among the variety of MMs, and at 254 nm, amino acid-free products of incomplete protein degradation with toxic effects can be detected, as well as adenosine diphosphate and adenosine triphosphate, adenine, valine, phenylalanine and derivatives of a number of amino acids [10, 11].

In pathological processes, plasma systems of limited proteolysis activate, an increase in the MM level in the 280 nm spectrum is observed and prevails, and on the contrary, when lipid peroxidation (LPO) and immunogenesis increase, there is an increase in MMs at 254 nm [12]. At 275 nm, MMs containing aromatic tyrosine derivatives are recorded [13].

Based on their diversity, a clear conclusion cannot be made on MMs about any direction of the processes in the body, but only the body intoxication degree can be stated. Thus, some MMs inhibit erythrocyte differentiation, hemoglobin synthesis, DNA synthesis, gluconeogenesis, disrupt the mechanisms of membrane transport of substances, as well as tissue respiration [14].

At 254 nm, only in the L-NAME-EHF-HS group (Table 2) there was a significant increase in the MM level relative to the intact group of rats by 36.7% ($p = 0.04$). This indicates stimulation of LPO and immunogenesis (at 254 nm) with NO blockade, which was not observed in the absence of blockade. In the other experimental groups (AS, HS, EHF-AS, EHF-HS, L-NAME-EHF-HS), no significant changes

Table 2. The levels of middle-weight molecules (in optical density units) in rat blood serum under normal conditions, isolated acute stress, chronic hypokinetic stress, and various combinations of these factors with extremely high-frequency electromagnetic radiation and the nitric oxide blocker L-NAME, $M \pm$ SEM

Таблица 2. Содержание молекул средней массы (е. о. п.) в сыворотке крови крыс в условиях нормы, изолированного острого стресса, хронического гипокинетического стресса, а также различных комбинаций этих факторов с электромагнитным излучением крайне высокой частоты и блокатором оксида азота L-NAME, $M \pm$ SEM

Wave length	Group						
	Intact ($n = 20$)	AS ($n = 20$)	EHF-AS ($n = 20$)	L-NAME-EHF-AS ($n = 20$)	HS ($n = 20$)	EHF-HS ($n = 20$)	L-NAME-EHF-HS ($n = 20$)
254 nm	0.218 ± 0.024, 100.0%	0.224 ± 0.028, 103.0%, $p = 0.98$	0.221 ± 0.025, 102.0%, $p = 1.00$	0.282 ± 0.031, 129.4%, $p = 0.07$	0.178 ± 0.021, 81.7%, $p = 0.84$	0.192 ± 0.020, 88.1%, $p = 0.53$	0.298 ± 0.034, 136.7%, $p = 0.04$
275 nm	0.179 ± 0.018, 100.0%	0.185 ± 0.026, 103.3%, $p = 0.97$	0.195 ± 0.019, 108.9%, $p = 0.93$	0.229 ± 0.033, 127.9%, $p = 0.08$	0.150 ± 0.026, 83.8%, $p = 0.86$	0.167 ± 0.013, 93.3%, $p = 0.92$	0.210 ± 0.026, 117.3%, $p = 0.74$
280 nm	0.174 ± 0.020, 100.0%	0.188 ± 0.023, 108.1%, $p = 0.98$	0.194 ± 0.022, 111.5%, $p = 0.96$	0.240 ± 0.027, 137.9%, $p = 0.03$	0.141 ± 0.016, 81.0%, $p < 0.05$	0.155 ± 0.018, 89.1%, $p = 0.49$	0.227 ± 0.024, 130.5%, $p = 0.04$

Note. p is the significance of differences compared to the intact group, determined using Dunn's nonparametric multiple comparison test.

Примечание. p —достоверность различий по сравнению с интактной группой, установленная с помощью непараметрического критерия множественных сравнений Данна.

Table 3. The levels of circulating immune complexes (in conventional units) in rat blood serum under normal conditions, isolated acute stress, chronic hypokinetic stress and various combinations of these factors with extremely high-frequency electromagnetic radiation and the nitric oxide blocker L-NAME, *M* ± SEM

Таблица 3. Содержание циркулирующих иммунных комплексов (у. е.) в сыворотке крови крыс в условиях нормы, изолированного острого стресса, хронического гипокинетического стресса, а также различных комбинаций этих факторов с электромагнитным излучением крайне высокой частоты и блокатором оксида азота L-NAME, *M* ± SEM

Group	Value
Intact (*n* = 20)	221.7 ± 25.1, 100.0%
AS (*n* = 20)	283.6 ± 35.8, 127.9%, *p* = 0.06
EHF-AS (*n* = 20)	247.9 ± 22.2, 111.8%, *p* = 0.92
L-NAME-EHF-AS (*n* = 20)	323.0 ± 26.2, 145.7%, *p* < 0.01
HS (*n* = 20)	318.6 ± 24.1, 143.7%, *p* < 0.01
EHF-HS (*n* = 20)	261.5 ± 17.2, 118.0%, *p* = 0.82
L-NAME-EHF-HS (*n* = 20)	261.5 ± 17.2, 189.9%, *p* < 0.001

Note. *p* is the significance of differences compared to the intact group, determined using Dunn's nonparametric multiple comparison test.

Примечание. *p* — достоверность различий по сравнению с интактной группой, установленная с помощью непараметрического критерия множественных сравнений Данна.

were found at this wavelength of MM recording compared to the intact group. At 275 nm, none of the studied groups (AS, HS, EHF-AS, EHF-HS, L-NAME-EHF-AS, L-NAME-EHF-HS) significantly differed from the intact group in the MM level.

At 280 nm, the MM level was decreased by 19.0% ($p < 0.05$) in the HS group vs. the intact group. The combination of EHF-HS attenuated this effect to insignificant values relative to the intact group. Under HS, the observed decrease in MM may reflect a decrease in precursor metabolites responsible for the functional activity of the serotoninergic stress-limiting system [15, 16].

At the same recording wavelength, when NO blocker was used in the two groups (L-NAME-EHF-AS and L-NAME-EHF-HS) there was a significant increase in MM substances by 37.9% ($p = 0.03$) and 30.5% ($p = 0.04$), respectively, vs. the intact group. This may be both a manifestation of endotoxicosis (the content of pathological products of protein metabolism with toxic effects increases) and an increase in the level of regulatory peptides affecting various functions of the body. Increased serum levels of MMs may be due to impaired protein breakdown and excretion from the body, or increased formation in tissues [17]. Therefore, NO is necessary for MM normalization. In contrast, NO blockade promotes the accumulation of pathologic protein metabolic products in the form of MMs.

Thus, it can be concluded that NO is a necessary factor for reduction of intoxication under the action of low-intensity EHF EMR, which implements stress-protective effect in AS and HS by reducing/normalizing the pathological serum level of MMs.

Serum level of circulating immune complexes in rats

The study of CIC levels showed that no significant changes in CIC were detected in the AS group compared to the intact animals. It should be noted, however, that in combination with AS, CIC levels were lower than in isolated AS, although these changes were not significant (Table 3).

In the L-NAME-EHF-AS group (Table 3), a significant increase of 45.7% ($p < 0.01$) in CIC levels was observed in preventive use of NO blocker vs. the intact group. This indicates significant impairment of immune system functions, particularly antibody formation, in the absence of NO according to [18]. Thus, the obtained results demonstrated that NO is necessary for the stress-protective effect of preventive exposure to EHF EMR under AS conditions.

CIC levels were increased in rats in the HS group by 43.7% ($p < 0.01$; Table 3) compared to the intact animals. Elevated CIC concentration has been reported to indicate a low level of antibody generation in response to increasing amounts of antigens [18]. The increase in CICs is an indicator of excessive intensity of oxidative processes in the body tissues and a decrease in the immune system ability to inactivate and eliminate CICs. That is, high CIC levels reflect an immune dysfunction.

In the EHF-HS group, this effect disappeared, as the CIC levels here did not significantly differ from the intact group (Table 3). The literature shows no activation of infectious-inflammatory processes under EHF EMR correction either [19, 20]. According to the above data, preventive exposure to EHF EMR attenuated the effect of HS, reducing the CIC and exerting a stress-protective effect.

In contrast, in the L-NAME-EHF-HS group under preventive NO blockade by L-NAME, there was a marked increase in CIC levels by 89.9% ($p < 0.001$) compared to the intact group, which, in accordance with [18], leads to the idea of serious disorders of humoral immunity associated with a decrease in adequate antibody production in response to antigens (Table 3).

The data obtained with respect to HS and L-NAME-EHF-HS groups also indicate a stronger damaging effect of HS on immune system functions compared to AS. As in AS, NO is necessary for the stress-protective effect of preventive exposure to EHF EMR under AS. NO blockade leads to the disappearance of stress-protective effect of EHF EMR in relation to the immune system functions and aggravates the consequences of HS, which is expressed in a significant increase of serum CICs.

CIC production is known to be an important indicator of humoral immune response in inflammation [21]. It represents an important physiological defense mechanism responsible for elimination, destruction of endo- and exogenous antigens, toxic products of protein metabolism, foreign and potentially dangerous viruses and bacteria by the reticuloendothelial system. Normally, CICs formed on the basis of antigens, antibodies and complement components are destroyed by phagocytes. However, in infectious, inflammatory, allergic processes, their destruction rate can be disturbed. As a result, their accumulation and deposition in the perivascular space and renal cortical layer may further activate the complement system and inflammatory reactions, aggravating their course.

Thus, NO in the absence of its blockade is necessary for normal immune response and for the stress-protective action of low-intensity EHF EMR in its preventive usage, one of the mechanisms of which is the reduction of CIC blood level.

Serum malondialdehyde levels in rats

The malondialdehyde (MDA) levels increased in the AS group by 29.5% ($p < 0.05$; Table 4) compared to the intact group, whereas in the serum of the EHF-AS group, this parameter did not significantly differ from the control group. A high MDA level is known to be an indicator of intensive oxidative stress in the body and, in particular, the increase of lipid peroxidation (LPO) products [22, 23].

Thus, AS led to the intensification of oxidative processes in the body and to an increase in LPO products, and in the preventive usage of EHF EMR in the EHF-AS group, the free-radical oxidation was within physiologically acceptable limits.

In the L-NAME-EHF-AS group (Table 4), under preventive usage of NO blocker, MDA levels increased 2-fold, by 101.6% ($p < 0.001$), compared to the intact group. This indicates significant disturbances in oxidative metabolism accompanied by an increase in free radicals according to [22, 23]. Thus, NO was shown to be necessary for the stress-protective effect of preventive exposure to EHF EMR under AS conditions with respect to LPO processes.

MDA levels increased in rats and in the HS group by 86.8% ($p < 0.001$) compared to the intact animals. In the EHF-HS group, this effect disappeared, as the MDA concentration here did not significantly differ from the intact group ($p = 0.90$; Table 4). According to the above data, preventive exposure to EHF EMR attenuated the effect of HS, reducing the MDA level and exerting a stress-protective effect.

In the L-NAME-EHF-HS group, under preventive blockade of NO by L-NAME, an intense more than 2-fold increase in MDA concentration was observed by 113.2% ($p < 0.001$; Table 4) compared to the intact group, which, in accordance with [22, 23], indicates a significant increase in LPO, even more pronounced than in AS. Thus,

Table 4. The levels of malondialdehyde (in μmol/L) in rat blood serum under normal conditions, isolated acute stress, chronic hypokinetic stress, and various combinations of these factors with extremely high-frequency electromagnetic radiation and the nitric oxide blocker L-NAME, $M \pm$ SEM

Таблица 4. Содержание малонового диальдегида (мкмоль/л) в сыворотке крови крыс в условиях нормы, изолированного острого стресса, хронического гипокинетического стресса, а также различных комбинаций этих факторов с электромагнитным излучением крайне высокой частоты и блокатором оксида азота L-NAME, $M \pm$ SEM

Group	Value
Intact ($n = 20$)	1.29 ± 0.11, 100.0%
AS ($n = 20$)	1.67 ± 0.10, 129.5%, $p < 0.05$
EHF-AS ($n = 20$)	1.38 ± 0.12, 107.0%, $p = 0.96$
L-NAME-EHF-AS ($n = 20$)	2.60 ± 0.24, 201.6%, $p < 0.001$
HS ($n = 20$)	2.41 ± 0.13, 186.8%, $p < 0.001$
EHF-HS ($n = 20$)	1.44 ± 0.15, 111.6%, $p = 0.90$
L-NAME-EHF-HS ($n = 20$)	2.75 ± 0.22, 213.2%, $p < 0.001$

Note. p is the significance of differences compared to the intact group, determined using Dunn's nonparametric multiple comparison test.

Примечание. p — достоверность различий по сравнению с интактной группой, установленная с помощью непараметрического критерия множественных сравнений Данна.

NO blockage by L-NAME significantly increases MDA, indicating the intensification of LPO processes in blood. Consequently, a normal NO level is necessary for stress-protective effect of EHF EMR in its preventive usage in AS and HS. One of the mechanisms of this effect is the reduction of LPO processes.

CONCLUSIONS

The present study suggests that NO plays an important role in the stress-protective effect of low-intensity EHF EMR under AS and HS. Relative to the intact group, AS significantly increased only the MDA level by 29.5% ($p < 0.05$), which indicates the intensification of LPO processes. Preventive EHF-exposure had a stress-protective effect, reducing LPO, as in animals in the EHF-AS group the MDA concentration returned to the level typical of the intact animals.

Under the NO blockade, the stress-protective effect of EHF EMR in AS was not manifested, because in the L-NAME-EHF-AS group, the MM level at 280 nm increased by 37.9% ($p = 0.03$), the serum levels of CIC and MDA increased by 45.7% ($p < 0.01$) and 101.6% ($p < 0.001$), respectively, vs. the intact group. This indicates the intensification of endotoxication and components of the stress-realizing system and physiologically active regulatory peptides, inhibition of immunogenesis and increase in oxidative metabolism of proteins, LPO intensification in the absence of NO.

HS-stress significantly decreased MM levels at 280 nm and significantly increased serum CIC and MDA levels by 43.7% ($p < 0.01$) and 86.8% ($p < 0.001$), respectively. This indicates suppression of the components of the stress-limiting system and physiologically active regulatory peptides, inhibition of immunogenesis and increase of oxidative metabolism of proteins, LPO intensification. Preventive usage of EHF (EHF-HS group) had a stress-protective effect in HS, as the MM, CIC and MDA levels returned to the level of intact animals.

Under NO blockade by L-NAME, the L-NAME-EHF-HS group had increased MM levels at 254 and 280 nm by 36.7% ($p = 0.04$) and 30.5% ($p = 0.04$), respectively; CIC and MDA were increased by 89.9% ($p < 0.001$) and 113.2% ($p < 0.001$), respectively. All this indicates the intensification of endotoxication and components of stress-realizing and stress-limiting systems, physiologically active regulatory peptides, inhibition of immunogenesis and increased free-radical oxidation of proteins and lipids in the absence of NO.

Thus, NO is a necessary factor of stress-protective action of low-intensity EHF EMR in its preventive usage in AS and HS. Mechanisms of its action are associated with inhibition of endotoxication, normalization of the level of protein oxidation products and the content of regulatory peptides, activation of humoral immunity, reduction of LPO processes.

ADDITIONAL INFO

Authors' contribution. All authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study. The contribution of each author: M.Yu. Ravaeva, I.V. Cheretaev, E.N. Chuyan, P.A. Galenko-Yaroshevskii, A.V. Zelenskaya — writing an article, data analysis; M.Yu. Ravaeva — editing an article, developing a general concept.

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