Research Letter

The nitrogen oxide synthase effect in endothelium reaction and change in peripheral perfusion under THz radiation of nitrogen oxide occurrence exposure

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Abstract: There is new information about the mechanism of THz radiation of molecular emission and absorption spectrum (MEAS) of 150.176-150.664 GHz nitrogen oxide occurrence exposure. Organized experiments let us establish the fact that a normalizing effect of THz radiation on microvasculature perfusion and functional endothelium condition in male rats under the acute stress condition is not realized after using of nitrogen oxide synthase (NOS) L-NAME. This shows that endogenic nitrogen oxide and endothelial NOS takes part in mechanisms of potentiation of positive correcting effect of MEAS of 150.176–150.664 GHz nitrogen oxide occurrence on defected parameters of skin microvasculature perfusion, and functional endothelium condition in male rats under the acute immobilization stress condition.

Keywords: peripheral perfusion, endothelium, nitrogen oxide synthase, THz waves

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Introduction

Progressing and severity of the cardiovascular system disease significantly determine microcirculatory dynamics and peripheral perfusion of tissues as well [1]. Changes in regional, coronary, cerebral, renal blood flows and circulatory dynamics, also blood circulation failure, are connected with microcirculation defects [2]. Experimental research had shown an appreciable interaction between changes in mechanisms of regulation in peripheral vessels (cutaneous vessels) and heart attack progression [3]. Vascular endothelium plays a significant role in active microcirculatory regulation [4]. Among numerous of vasoactive substances produced by endothelial cells nitrogen oxide plays a significant role. It is a strong vasodepressor and antiaggregant [4-6]. Nitrogen oxide is synthesized constantly on organs and tissues involving special ferments – nitrogen oxide synthase (NOS) [4-6]. The molecular emission and absorption spectrum (MEAS) of nitrogen oxide is in THz band [7].

Electromagnetic waves of THz bands are one of the non-drug methods of physiological regulation [8]. Quite expectedly that the mechanism of THz waves of the MEAS of nitrogen oxide occurrence effect generate the most interest.

This study aimed the research of NOS role in the effect of electromagnetic waves of THz bands of the MEAS of 150.176–150.664 GHz nitrogen oxide occurrence on peripheral perfusion in white rats under the acute immobilization stress.

Material and Methods

The research had been organized in 75 white outbread male rats of 180-220 g. All of the rats had been in the same conditions during the experiment. The experiment had been organized according to Declaration of Helsinki (2006). Rigid fixation of the rats in dorsal position for 3 hours had been used as a model of an acute stress [9]. Blockade of endothelial NOS had been performed by administration of a 4 mg/kg dose of a nonspecific L-NAME inhibitor [5]. The experiment was performed on 5 groups of animals, 15 animals in each one: the 1^{st} – group of control (intact animals), the 2^{nd} – group of compare (male rats under the acute immobilization stress), the 3^{rd} – experimental group (rats under the acute immobilization stress, after the 30-minute THz irradiation), the 4^{th} – experimental group (rats under the acute immobilization stress with NOS - L-NAME inhibitor administration), the 5^{th} - experimental group (rats under the acute stress with NOS - L-NAME inhibitor administration, in complex with the 30-minute THz irradiation of the MEAS of 150.176–150.664 GHz nitrogen oxide occurrence).

The process of the THz irradiation of the MEAS of 150.176–150.664 GHz nitrogen oxide occurrence had been performed with the help of THz wave generator managed by the Medico-technical association of extremely-high frequencies (EHF) radiations (Moscow, Russia) together with NPP-Istok Ltd. and Central research institute of measuring equipment (Saratov, Russia) [10]. 3 cm^2 skin areas above the metasternum had been irradiated. Irradiator had been located 1.5 cm above the body of an animal. The generator radiant power was 0.7 mW, the power density that arrives at the 3 cm^2 skin area

was 0.2 mW/cm². The irradiation dose had been determined by the power density that arrives at the skin area, and the summary time of the irradiation. There had been a 30-minute single irradiation. Laser Doppler flowmetry (LDF) had been organized with the help of a laser analyzer of a blood flow «LAKK-02» (the 2nd edition; research and production enterprise «Lazma», Russia). To immobilize all the animals there had been a combination of Zoletil injected intramuscularly («Virbac Sante Animale», France) in a dose of 0.05 ml/kg and 1 mg/kg of Xylazine («Interchemie», Netherlands). The detector of the blood flow laser analyzer had been fixed by an atraumatic plaster on a back of the foot on a right paw. The standard recording duration was 7 minutes. Thermo-probe had been done according to a standard method: there had been a skin area heated with maximal speed up to 45^{0}C. The skin area had been heating for 40 seconds. The thermo-probe had been performing for 4 minutes.

The LDF-gram analyzing and the results interpretation had been performed according to common methods described in monograph by A.I. Krupatkin and V.V. Sidorov [11]. The first stage of the LDF-gram analyzing was an estimating of an index of a constant component M of an average microcirculatory perfusion of skin (perf.u.) during 7 minutes, there had been determined a standard deviation (SD, perf.u.) of a microcirculatory perfusion relatively to an average of M, coefficient of variations (CV) – percentage proportion of a standard deviation and M. On the second stage there had been performed Fourier analysis. The following amplitude-frequency characteristics had been analyzed: maximal amplitude of a very low frequency waves (endothelial variations, perf.u.), maximal amplitude of a low frequency waves (vasomotor variations, perf.u.), and maximal amplitude of respiratory undulation (respiratory variations, perf.u.), maximal amplitude of pressure pulse and cardiac variations (perf.u.). On the third stage there was a functional thermo-probe analyze. The following parameters had been estimated: initial perfusion (M init., perf.u.), maximal perfusion (M max, perf.u.) and perfusion after the circulation restorage (M rest., perf.u.).

Statistical analysis of the results had been done with the help of Statistica 6.0 soft. There had been Shapiro-Wilk test. The most of the results do not correspond to the normal distribution law, so the Mann-Whitney U-test had been used for the comparison.

Results

In animal models there had been found out that in male rats after 3-hours immobilization there was a statistically valid reduction of the perfusion indices (comparing with the control group). This shows the reduction of microcirculation blood flow (*Table* 1). At the same time there had been a statistically valid reduction of a perfusion standard deviation and coefficient of variations (*Table* 1), this reflects a reduction of a microcirculation blood flow modulation. The results of the amplitude-frequency LDF-gram show that there is a statistically valid decrease of the endothelial variations under the acute immobilization stress in male rats (*Table* 2). This characterizes the decrease of basal endothelial production of nitrogen oxide. There had been also shown a statistically valid decrease of vasomotor variations (*Table* 2), which denotes an increase of peripheral resistance. And there were no statistically valid changes in amplitude of respiratory variations in male rats under the acute immobilization stress, though; there was a statistically valid decrease of amplitude of pressure pulse and cardiac variations (*Table* 2), which shows the reduction of arterial flow into microcirculation vessels. The data got from the thermo-probe denote that in male rats under the acute immobilization stress there was a statistically valid decrease of the initial and maximal perfusion (comparing with the control group) (*Table* 3) and perfusion after the initial blood flow recovery. The reduction of the maximal perfusion in the thermo-probe with fast heating reflects the depression of induced endothelial nitrogen oxide emission.

The experimental results indicate that in male rats under the acute stress and irradiated by THz waves of the MEAS of 150.176–150.664 GHz nitrogen oxide occurrence there is a recovery of perfusion index, which does not (statistically valid) differ from the control group (*Table* 1). In animals of this group there is an increase of a perfusion standard deviation and coefficient of variations (*Table* 1) (statistically valid, comparing with the animals under the acute stress and not irradiated by the THz irradiation). The results of the amplitude-frequency LDF-gram show that after the THz irradiation of the male rats under the acute stress there is a statistically valid increase of the amplitudes of endothelial and vasomotor variations, comparing with the animals under the acute immobilization stress and not irradiated by the THz irradiation (*Table* 2). This reflects an increase of vasodepressive endothelium potency (activation of a basal nitrogen oxide producing) and a reduction of peripheral resistance. There is an increase of amplitude of cardiac variations (*Table* 2), which indicates an increase of arterial flow into the microvasculature. All parameters of the amplitude-frequency LDF-gram analyze of the animals from this group do not differ (statistically valid) from the parameters of the control group (*Table* 2). During the analyzing of the thermo-probe results there had been denoted that in male rats under the acute immobilization stress in the THz irradiation the initial and maximal perfusions are normalized, and the perfusion after the initial blood flow recovery. The maximal perfusions normalization denotes the restoration of the induced secretion of the endothelial nitrogen oxide. All of the thermo-probe parameters of the animals after the THz irradiation under the acute immobilization stress are in the range of the control group variety (*Table* 3).

In the rats who got the NOS inhibitor L-NAME (under the acute stress) there had been noted more significant changes in the skin microvasculature comparing with the animals under the acute immobilization stress, which had not got the L-NAME. It had been indicated that in male rats under immobilization condition in the L-NAME inhibitor injecting, perfusion index, standard deviation and coefficient of variations are lower (statistically valid) than in animals under the immobilization without L-NAME (*Table* 1). The comparison of the amplitude-frequency characteristics of a blood flow oscillation in microvasculature of the irritated with the L-NAME male rats and in rats irritated with no L-NAME had shown that in animals of the first group the depression of the passive and active mechanisms of microvasculature modulation was more active than in animals of the second group. In male rats irradiated with the L-NAME injection the amplitude of endothelial, vasomotor, cardiac and respiratory variations is lower (statistically valid) than in animals under the immobilization without L-NAME (*Table* 2). During the functional probe of fast heating there had been found out the differences between the animals immobilized with and without L-NAME. So, in animals under the acute immobilization stress on the background of the L-NAME inhibitor injection, the initial and maximal perfusion, and the perfusion after the recovery of the initial flow are lower (statistically valid) than in animals under the immobilization with no L-NAME (*Table* 3). This indicates that the L-NAME injection in the animals with the acute stress-reaction causes more evident depression of an induced endothelial nitrogen oxide secretion.

Table 1. Changes in parameters of skin microvasculature blood flow in animals under the acute stress and in THz irradiation of the MEAS of 150.176–150.664 GHz nitrogen oxide occurrence on the background of the L-NAME injection

Parameters	*Control*	*Acute stress*		*THz irradiation on the background of the acute stress*	
		Without L-NAME injection	With L-NAME injection	Without L-NAME injection	With L-NAME injection
Perfusion index, perf.u.	11.30 (9.90, 13.30)	8.22 (7.20, 8.44), p_1=0.006	5.93 (5.09, 7.14) p_1<0.001, p_2<0.001	11.02 (9.65,11.84), p_1=0.238, p_2=0.029	6.26 (4.53, 7.70), p_1<0.001, p_2=0.032, p_3=0.661, p_4<0.001.
Perfusion standard deviation, perf.u.	1.02 (0.75, 1.26)	0.56 (0.41, 0.72), p_1=0.001	0.27 (0.21, 0.33), p_1<0.001, p_2<0.001	1.23 (0.96, 1.73), p_1=0.183,p_2<0.001	0.32 (0.23, 0.37), p_1<0.001, p_2<0.001, p_3=0.629, p_4<0.001
Coefficient of variations, %	8.61 (7.17, 10.87)	6.69 (5.28, 9.78), p_1=0.040	4.25 (3.05, 6.60), p_1<0.001, p_2=0.034	12.85 (8.43, 16.31), p_1=0.073, p_2=0.002	5.09 (3.24, 7.48), p_1=0.002, p_2=0.069, p_3=0.826, p_4=0.002

Data presented as medians, low and high quartiles, Me (Q_1, Q_3). p_1 – comparing with the group of control; p_2 – comparing with the animals under the acute stress; p_3 – comparing with the animals under the acute stress on the background of the L-NAME injection; p_4 – comparing with the animals under the acute stress after the 30-minute THz irradiation.

Table 2. Amplitude-frequency analyze of a microcirculation in male rats under the acute immobilization stress and irradiated by the THz waves of the MEAS of 150.176–150.664 GHz nitrogen oxide occurrence on the background of the L-NAME injection

Parameters	*Control*	*Acute stress*		*THz irradiation on the background of the acute stress*	
		Without L-NAME injection	With L-NAME injection	Without L-NAME injection	With L-NAME injection
Maximal amplitude of endothelial variations, perf.u.	2.08 (1.65, 2.81)	1.14 (0.72, 1.68), p_1<0.001	0.50 (0.42, 0.71), p_1<0.001, p_2<0.001	2.35 (1.95, 3.39), p_1=0.265, p_2<0.001	0.73 (0.39, 0.97), p_1<0.001, p_2=0.007, p_3=0.392, p_4<0.001
Maximal amplitude of vasomotor variations, perf.u.	1.33 (1.16, 1.87)	1.01 (0.57, 1.33), p_1=0.006	0.38 (0.32, 0.47), p_1<0.001, p_2<0.001	1.54 (1.24, 2.31), p_1=0.336, p_2=0.004	0.48 (0.28, 0.62), p_1<0.001, p_2=0.003, p_3=0.273, p_4<0.001
Maximal amplitude of respiratory variations, perf.u.	0.34 (0.25, 0.46)	0.21 (0.17, 0.35), p_1=0.092	0.13 (0.08, 0.17), p_1<0.001, p_2=0.011	0.38 (0.30, 0.64), p_1=0.247, p_2=0.012	0.13 (0.10, 0.21), p_1<0.001, p_2=0.013, p_3=0.930, p_4<0.001
Maximal amplitude of cardiac variations, perf.u.	0.14 (0.11, 0.29)	0.10 (0.06, 0.17), p_1=0.033	0.05 (0.03, 0.07), p_1<0.001, p_2=0.007	0.20 (0.12, 0.25), p_1=0.678, p_2=0.019	0.05 (0.03, 0.10), p_1<0.001, p_2=0.018, p_3=0.983, p_4<0.001

Table 3. Indices of the functional probe with fast heating of male rats under the acute immobilization stress and irradiated by the THz waves of the MEAS of 150.176–150.664 GHz nitrogen oxide occurrence on the background of the L-NAME injection

Parameters	*Control*	*Acute stress*		*THz irradiation on the background of the acute stress*	
		Without L-NAME injection	With L-NAME injection	Without L-NAME injection	With L-NAME injection
Initial perfusion, perf.u.	10.6 (9.6, 11.5)	7.8 (6.5, 8.2), p_1<0.001	5.5 (4.8, 6.7), p_1<0.001, p_2=0.005	10.1 (9.3, 11.0), p_1=0.361, p_2<0.001	6.5 (6.1, 7.8), p_1<0.001, p_2=0.149, p_3=0.145, p_4<0.001
Maximal perfusion, perf.u.	13.9 (12.4, 14.9)	11.6 (9.5, 14.0), p_1=0.036	9.3 (8.3, 10.1), p_1<0.001, p_2=0.005	13.9 (12.3, 15.0), p_1=0.724, p_2=0.040	9.6 (8.7, 11.1), p_1<0.001, p_2=0.010, p_3=0.467, p_4<0.001
Perfusion after the circulation recovery, perf.u.	11.61 (9.38, 13.47)	8.2 (6.6, 8.72), p_1<0.001	6.9 (5.3, 7.7), p_1<0.001, p_2=0.045	10.7 (9.7, 12.0), p_1=0.633, p_2<0.001	7.8 (6.6, 8.3), p_1<0.001, p_2=0.485, p_3=0.467, p_4<0.001

THz irradiation of the MEAS of 150.176–150.664 GHz nitrogen oxide occurrence in male rats under the acute immobilization stress on the background of the L-NAME injection does not cause any change in perfusion of microvasculature. The perfusion index, standard deviation and coefficient of variations in the animals of this group do not differ (statistically valid) from the parameters of the rats under the acute immobilization stress on the background of the L-NAME injection (*Table* 1). It had been found out, that after

the THz irradiation of the MEAS of nitrogen oxide occurrence on the background of the NOS inhibitor L-NAME injection, there had not been (statistically valid) change in amplitudes of endothelial, vasomotor, cardiac and respiratory variations (comparing of the animals under the acute immobilization stress on the background of the L-NAME injection, which were not irradiated with THz waves) (*Table* 2). This indicates that in the L-NAME injection the THz irradiation does not have an effect on mechanisms of circulation modulation in microvasculature of the male rats under the acute immobilization stress. During the thermo-probe in male rats under the acute immobilization stress on the background of the NOS L-NAME injection and irradiated by the THz waves, there had not been found statistically valid dynamics of the initial and maximal perfusion, perfusion after the microvasculature recovery and capillary blood flow reserve, comparing with the rats under the acute stress on the background of the NOS L-NAME injection without the THz irradiation (*Table* 3).

Discussion

The results of the experiment indicate that THz irradiation of the MEAS of 150.176–150.664 GHz nitrogen oxide occurrence does not realize its positive effect on microvasculature perfusion in male rats under the acute immobilization stress on the background of the NOS inhibitor L-NAME injection. The absence of the effect of the THz irradiation on the reduced amplitude of endothelial variations in animals under the acute stress on the background of the L-NAME injection means that in the blockade of endothelial NOS, the THz irradiation can not normalize basal production of nitrogen oxide. There was no dynamics of thermo-probe parameters in irradiated animals under the acute stress on the background of the L-NAME injection, this shows that in the blockade of NOS the THz waves do not stimulate an inducing nitrogen oxide producing. These facts show that the mechanism of the THz waves realization are directly connected with NO-synthase component of an endogenic nitrogen oxide cycle. The findings are consistent with the results of the recent biochemical researching, which detect the absence of the effect of THz waves on the nitrites concentrations in blood serum of male rats under the immobilization stress conditions in the blockade of NOS [12].

Conclusion

Thus, there had been denoted that endogenic nitroxidergic system, its NOS component as well, has a significant meaning in realization effect of THz irradiation of the MEAS of 150.176–150.664 GHz nitrogen oxide occurrence on peripheral perfusion and its modulation in white male rats in the acute stress.

Conflict of interest: none declared.

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