EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Sex-Specific Differences in Changes of Disturbed Functional Activity of Platelets in Albino Rats under the Effect of Terahertz Electromagnetic Radiation at Nitric Oxide Frequencies

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The impact of electromagnetic oscillations of the terahertz band at frequencies of NO radiation and absorption molecular spectrum (150.176-150.664 GHz) for the functional activity of platelets was studied in albino rats exposed to immobilization stress. The effect of these waves was sex-specific: females were more sensitive to radiation than males. Relationship between the terahertz waves and phase of estrous cycle in females was detected: estrus females exhibited maximum sensitivity to these waves.

Key Words: *aggregation; terahertz band; nitrogen oxide; platelets; sex dimorphism*

The problem of stress and increasing incidence of cardiovascular diseases acquire special importance under modern conditions [12].

Microcirculatory disorders caused by impaired thromboresistance of the vascular wall and increased functional activity of platelets make an important contribution to the development of cardiovascular diseases [3,6,9,11,14].

A wide armory of drugs is used for the correction of hemostasis disorders, but drug therapy leads to the development of side effects of different severity. Hence, the search for non-drug methods for correction, *e.g.* low-intensity submillimeter wave radiation [8], is now in progress.

The terahertz frequency band (TFB) attracts special interest because molecular spectra of radiation and absorption of various cell metabolites, such as NO, CO_2, and active oxygen forms, fall within this band [2].

Sex dimorphism (reactions of males and females, different by strength, duration, and significance, to stimuli of the same intensity) is characteristic of the stress reaction [1]. In addition, females are characterized by cyclic changes in ovarian function associated with the development of follicles, ovulation, and corpora lutea formation. Variations of the hormonal profile during different phases of the estrous cycle modulate the course of stress reaction and processes in the hemostasis system [4].

The aim of this study was to detect the characteristic features of platelet reaction to TFB radiation corresponding to frequencies of molecular radiation and absorption spectrum of NO (150.176-150.664 GHz) in females during different phases of the estrous cycle and in males exposed to acute immobilization stress.

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MATERIALS AND METHODS

Platelet-rich plasma samples from 180 outbred albino rats (180-220 g; 60 males and 120 females) were analyzed. Immobilization stress (a single 3-h fixation of animals in the supine posture) [5] served as the model of disorders in the intravascular component of microcirculation. The animals were exposed to TFB waves at NO frequency for 15 or 30 min using a KVCh-NO generator [10].

Platelet aggregation was studied in platelet-rich plasma on a 230 LA "Biola" laser analyzer of platelet aggregation. ADP in a final concentration of 2.5 μM served as the aggregation inductor.

Experimental series I was carried out on 4 groups of male albino rats: 1) control; 2) animals exposed to single 3-h immobilization; 3 and 4) stressed animals exposed to TFB-radiation for 15 and 30 min, respectively.

Experimental series II was carried out on 8 groups of female albino rats (4 groups of estrus females and 4 group of diestrus females), 2 of these groups comprised controls, 2 comprised stressed animals, and 2 groups consisted of stressed animals exposed to TFB for 15 and 30 min.

Hypotheses on the type of distribution (Shapiro—Wilks test) were verified. The greater part of experimental data did not conform to the normal distribution, and hence, the values were compared using Mann—Whitney *U* test.

RESULTS

Functional activity of platelets was elevated in rats of both sexes in a state of acute immobilization stress, which manifested in a statistically significant increase of the maximum size of forming platelet aggregations (PA), maximum rate of the largest PA formation, maximum degree and rate of aggregation (Tables 1, 2).

Exposure of male rats in a state of immobilization stress to TFB in the NO frequency band for 15 min partially restored the impaired platelet aggregation activity (Tables 1, 2). This was paralleled by recovery of the normal maximum size of forming PA by 46% and maximum rate of the largest PA formation by 52% in comparison with stressed animals (Table 1). The maximum rate and degree of aggregation were restored completely and virtually did not differ from the control (Table 2). Exposure to TFB for 30 min led to complete normalization of platelet aggregation, all aggregation parameters in animals exposed to TFB during acute stress did not differ from the control (Tables 1, 2). A possible mechanism of the effect of TFB exposure at frequencies corresponding to the molecular radiation and absorption spectra of NO on functional activity of platelets is activation of endogenous NO. Under these conditions, radiation energy of TFB is spent for increasing the rotatory constituent of molecule energy, which increases its reaction capacity [2]. NO is a potent factor of hemostasis, an antiaggregant [15], and a mediator of stress-limiting system of central and peripheral action [12].

Exposure of stressed diestrus female rats to TFB led to complete recovery of platelet aggregation as soon as after 15-min exposure to electromagnetic waves, while in males complete recovery of aggregation was observed only after 30-min exposure. Exposure to TFB led to complete normalization of impaired platelet function, similarly as in

TABLE 1. Platelet Aggregation Parameters in Albino Rats under conditions of Experimental Stress Reaction and Different Modes of Exposure to TFB Waves Corresponding to Molecular Absorption and Radiation Spectrum of NO

Group	Maximum size of forming PA, arb. units			Maximum rate of largest PA formation, arb. units		
	males	females		males	females	
		diestrus	estrus		diestrus	estrus
Control	2.63 (2.20;2.86)	2.65 (2.30;2.87)	3.55 (3.17;3.94)	3.45 (2.47;4.11)	3.62 (2.89;4.15)	5.47 (4.52;6.16)
Stress	6.93* (6.16;8.12)	5.57* (4.62;6.31)	9.45* (7.03;11.05)	12.22* (10.00;15.80)	9.63* (7.30;11.5)	17.31* (12.80;21.3)
Stress+irradiation for 15 min	3.76*+ (3.16;4.50)	2.85+ (2.53;3.34)	3.18+ (2.40;3.63)	5.86*+ (4.46;7.30)	3.93+ (3.06;4.35)	4.64+ (3.01;5.95)
30 min	2.51+o (2.17;2.79)	2.74+ (2.51;2.77)	2.83*+ (2.56;3.21)	3.41+o (2.71;4.30)	3.59+ (3.10;3.68)	3.71*+ (2.87;4.21)

Note. Here and in Table 2: the median, lower and upper quartiles from 15 measurements are presented. $p<0.01$: compared to: *control, +stressed animals, °stressed animals exposed to TFB radiation for 15 min.

TABLE 2. Platelet Aggregation in Albino Rats during Experimental Stress Reaction and Different Duration of Exposure to TFB Waves Corresponding to Molecular Absorption and Radiation Spectrum of NO

Group	Maximum aggregation, %			Maximum aggregation rate, % min		
	males	females		males	females	
		diestrus	estrus		diestrus	estrus
Control	43.9 (32.9;51.0)	37.7 (31.5;43.2)	51.7 (48.5;54.5)	61.0 (44.2;73.6)	56.0 (47.4;65.2)	74.6 (67.5;81.6)
Stress	65.6* (57.0;74.4)	69.3* (61.7;76.8)	77.7* (68.8;87.2)	87.4* (75.1;97.9)	92.8* (79.7;102.0)	105.3* (90.2;123.0)
Stress+irradiation for 15 min	49.5+ (40.6;57.6)	35.7+ (31.0;39.8)	49.3+ (45.4;54.5)	66.0+ (55.1;76.0)	48.6+ (42.3;52.7)	63.7*+ (60.7;68.5)
30 min	42.3+ (35.6;47.5)	38.0+ (34.2;40.2)	48.3+ (43.0;53.6)	56.2+o (46.2;61.3)	48.8+ (41.6;52.9)	64.2*+ (56.9;69.3)

males (Tables 1, 2). Hence, diestrous females are more sensitive to TFB exposure than males.

The 15-min TFB exposure of stressed estrus females led to complete recovery of platelet aggregation, while 30-min TFB exposure reduced functional activity of platelets below the normal, but not restored it. The maximum size of forming PA, maximum rate of formation of the largest PA, and maximum aggregation rate in these animals were significantly ($p<0.05$) lower than in control estrus females (Tables 1, 2).

Hence, stressed albino rat females in the estrus phase are more sensitive to TFB exposure of the NO radiation and absorption molecular spectrum in comparison with the females in the diestrus phase. It was found that estrogens stimulated NO production by the endothelium [13]. Presumably, this fact explains the highest biological effect of TFB exposure in stressed females in the estrus phase.

Sex-specific differences in reactions to TFB exposure necessitate differentiated approach to exposure of male and female patients to TFB waves. Presumably, the mode of exposure should be more mild (fractionated) for females, and introduction sessions of shorter duration [5] should initiate a course of TFB therapy. On the other hand, high sensitivity of the females to TFB waves gives grounds to expect effective use of TFB therapy in gynecology for the treatment of conditions associated with autonomic imbalance.

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