THE EFFECTS OF ACUTE VIBROACOUSTIC MICROVIBRATIONS ON THE RAT HEART RATE, RHYTHM AND STRUCTURE

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EFEKTI AKUTNE PRIMENE VIBROAKUSTIČKIH MIKROVIBRACIJA NA FREKVENCIJU, RITAM I STRUKTURU SRCA PACOVA

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ABSTRACT

The body surface of homeothermic organisms produces constant microvibrations. In the past, many studies were conducted on this topic, and the amplitude of the microvibrations was described as a sensitive marker of muscle tension and body activity. Subsequent studies indicated that the frequency of the microvibrations is an important variable affecting the body. The aim of this research was to examine the effects of the vibroacoustic microvibrations on the rate, rhythm and structure of the rat heart during physiological conditions. Microvibrations of specific frequency and amplitude were induced by a Vitafon-T, four different modes were used, and the effects of the microvibrations on ECG characteristics and the wall structure of the rat heart were examined. After the application of microvibrations (lasting 10–60 min), no statistically significant changes occurred in the heart rate, but the amplitudes significantly increased after 10, 20 and 30 minutes, and increased even more after 60 minutes. No changes in the heart wall structure were found. Acute in vivo application of vibroacoustic microvibrations in the rats did not produce significant effects on the heart rate and rhythm; however, it increased the amplitude of the R wave by 25–32% in the second standard ECG lead but did not lead to structural changes in the rat heart wall.

Key words: *microvibrations, heart rate, rhythm, structure, heart*

SAŽETAK

Površina tela homeoterma konstantno proizvodi vibracije. U prošlosti su sprovedena mnoga istraživanja na temu mikrovibracija, i opisala amplitudu mikrovibracija kao osetljiv parametar mišićne tenzije i telesne aktivnosti. Kasnija istraživanja su frekvenciju mikrovibracija navela kao varijablu od centralnog značaja za njihove efekte na organizam. Cilj istraživanja je bio da ispitamo efekte vibroakustičkih mikrovibracija na frekvenciju, ritam i strukturu srca pacova u fiziološkim uslovima. U istraživanju je korišćen aparat koji proizvodi mikrovibracije, Vitafon-T određene frekvencije i amplitude, i u skladu sa tim koristili smo četiri režima rada u kojima smo ispitivali efekte mikrovibracija na EKG karakteristike i strukturu zida srca pacova. Utvrdili smo da nakon primene mikrovibracija (u trajanju od 10-60 minuta) nije došlo do statistički značajnih promena u broju otkucaja, vrednosti amplitude R talasa su statistički značajno povećane posle 10, 20 i 30 minuta, a visoko statistički značajno povećane posle 60 minuta. Na preparatu zida srca nema promena u odnosu na normalnu strukturu. Akutna primena vibroakustičkih mikrovibracija kod pacova in vivo ne utiče značajno na frekvenciju i ritam srčanog rada, povećava amplitudu R talasa u drugom standardnom EKG odvodu za 25-32 % i ne dovodi do promena u strukturi zida srca pacova.

Ključne reči: *mikrovibracije, frekvencija, ritam, struktura, srce*

INTRODUCTION

Microvibrations were described as a phenomenon by Rohracher (1) who found that the body surface of humans and other homeothermic organisms, in general, constantly produce vibrations. The amplitude of the microvibrations appeared to be a sensitive marker of muscle tension and total body activity. Later, other researchers (2-7) supported the arguments presented by Rohracher. In a healthy organism, either human or animal, the amplitude of these microvibrations is 1–5 international units with a frequency of 6–12 Hz/sec in a state of maximal relaxation. Basic conclusions of the research done by Rohracher et al. are as follows: microvibrations are possible to record on

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the whole surface of the body; microvibrations originate in striated muscle; and the amplitude of the microvibrations is multiplied (several times) by contraction of striated muscles, but muscle contractions do not influence the frequency of the microvibrations (which is constant during the given recording). Rohracher (8) identified two significant functions of body microvibrations, which are evident with the emission of quite a small amount of energy. Namely, the body of homeothermic animals has a constant temperature, and the muscles are in a state of permanent readiness. Due to these capacities, fast and positive motor functions are generated (4). Further analysis (5) defined the similarity between alpha waves in an ECG and the wave shapes of frequencies of microvibrations, which pointed to the causality of the phenomenon. These findings were criticised (9) and (10); they concluded that the amplitude of the microvibrations varied depending on the quantity of energy and was a sensitive parameter of muscle tension. Out of the two dependent variables that were investigated (amplitude and frequency), the findings on frequency were of central importance for the origins of the theory. This was due to previous knowledge that muscles produced movements of various amplitudes, but the statement that they showed constant periodical movements with constant frequency was especially important. The established significant facts related to the frequency of the microvibrations are that the frequency is always within an approximate scope of 6–12 cycles per second and that the frequency is constant regardless of the method by which it is measured. The aim of our research was to examine the effects of acutely applied vibroacoustic microvibrations on the frequency, rhythm and wall structure of the rat heart during physiological conditions by using a commercial devise that produces microvibrations of a specific amplitude and frequency. Then, the effects of the microvibrations on ECG characteristics in the second standard lead in rats and the histological wall structure of the rat heart were analysed.

MATERIALS AND METHODS

To investigate the acute effects of the vibroacoustic microvibrations on the rat cardiovascular system during physiological conditions, a Vitafon-T apparatus with defined amplitudes (2.8–12.3 μm) and frequencies (30–18K Hz) was used. With respect to the amplitudes and frequencies, four various modes were used and applied to experimental animals in order to examine the effects of the microvibrations on the frequency, rhythm and histological wall structure of the rat heart. Mode 1 had a frequency of 30–60 Hz and an amplitude of 2.8 μm; Mode 2 had a frequency of 1200–4500 Hz and an amplitude of 6.0 μm; Mode 3 had a frequency of 200–1000 Hz and an amplitude of 5.4 μm; Mode 4 had a frequency of 900–18000 Hz and an amplitude of 12.3 μm. The period of impulse modulation was 0.5, 1 and 2 seconds.

Experimental model for investigating the acute effects of vibroacoustic microvibrations of a certain amplitude and frequency on ECG characteristics, heart rate, heart rhythm and amplitude of waves in the second standard lead in rats

The performed experimental work in rats was aimed at defining ECG characteristics, heart rate, heart rhythm and amplitude of waves in the second standard lead after acute exposure to vibroacoustic microvibrations of a certain amplitude and frequency. In this part of the experiment, the investigation was performed on 4 plus 6 rats (this protocol included a total of 10 Wistar albino rats) that served as a control group at the same time. The rats were 6–8 weeks-old males with a body mass of 160–250 g. They were kept in standard laboratory conditions (air temperature 22 ± 1° C, relative humidity 50%, light-dark cycle 12 : 12 hours, and free access to food and water). The study was performed in stages, and the outlined issues were defined by the experimental protocol. The experiments began with individual animal checkups and measurement of weight. After the conditions for the experimental work were fulfilled, the rats were anaesthetised by applying easy evaporating ether as an anaesthetic. Then, the rats were immobilised. This was followed by connection of the rats to a standard one-channel electrocardiograph. The connection was performed with the help of specially modified electrodes. In continuation, emitters of a Vitafon-T apparatus (*Saint Petersburg, Russian Federation*) were applied directly to the rat skin in the region of the heart and kidneys. The vibroacoustic microvibrations of specific amplitudes were emitted, and the frequencies were distributed in four modes. Before the microvibrations began, an initial ECG was recorded, which also served as a control. Exposure to the microvibrations lasted 10 minutes in each mode. The first four rats were additionally phonated until 60 minutes, recording their ECG at 10, 20, 30 and 60 minutes. After each mode was completed, 5- minute rests were introduced. After exposure to the microvibrations was completed, the results were carefully classified, technically processed and analysed.

Experimental model for investigating the acute effects of the vibroacoustic microvibrations of various amplitudes and frequencies on the rat heart wall structure (hematoxylin and eosin staining method)

In this part of the experiment, the effects of the acute vibroacoustic microvibrations on the heart wall structure of the rat were examined by histological analysis. The experimental animals that were previously exposed to the acute vibroacoustic microvibrations were sacrificed, and the heart was extracted. The extraction was performed by surgical opening of the abdomen, accessing the diaphragm and performing a thoracotomy. The diaphragm was incised in an arc from left to right, and then the thorax was opened laterally along the mammillary line. When the thorax was opened, the pericardium on top of the heart was cut, and thus, prepared for isolation. The blood vessels at the heart

base were resected, and the organ was isolated from the thorax and put into an icy physiological solution (-4° C to -10° C) to stop all physiological processes in the myocardium. Then, the heart was placed in the formalin solution and further processed histologically.

Statistical data analysis

In the statistical data processing, the basic methods of descriptive statistics were used (mean value (X), frequencies, percentages, sample mean value, standard deviation (SD) and standard error (SE)). To test the hypothesis and significance, Student's t-test for dependent and independent samples and two-factor analysis of variance (ANOVA) were used. The qualification of connection between variables was determined using Pearson's linear correlation, which defined the proportions between the two variables. It was expressed as a coefficient of correlation. The coefficient of correlation was high if the coordinated interdependence of activities existed. The probability (a) and significance (r) were established using a standard formula. A p of <0.05 was considered statistically significant. Database and analysis of the results were performed using SPSS 10.0 (*SPSS lnc, Chicago, IL, USA*). The research was performed in accordance with the *Helsinki Declaration* and further amendments, and approval of the experimental protocols was obtained by the Ethical Committee of Faculty of Medicine.

RESULTS

Acute effects of vibroacoustic microvibrations of a certain amplitude and frequency on ECG characteristics, heart rate, heart rhythm and wave amplitudes in the second standard lead in rats

After applying the vibroacoustic microvibrations (lasting 10 minutes) in the heart region, no statistically significant changes occurred in the heart rate. The most marked changes occurred after applying Mode 3, but even these changes were not statistically significant (Appendix 1). When analysing the frequency of rhythm disorder in the second standard ECG lead during the application of the vibroacoustic microvibrations, slight disturbances in the cardiac rhythm were recorded in 25% of the rats for Modes 3 and 4 (Modes 3 and 4 each had one rat with arrhythmias). During Modes 1 and 2, no arrhythmias were recorded (Appendix 2). After analysing the heart frequency after application of the vibroacoustic microvibrations (60 minutes long), no statistically significant changes in the heartbeat were found in Mode 1. The most significant changes occurred after 20 minutes, but they were not statistically significant. Analysis of the frequency of the registered disturbances of the heart rhythm in the second standard ECG lead after 60 minutes of microvibrations revealed that the R wave amplitude in Mode 1 had a statistically significant increase after 10, 20 and 30 minutes (p<0.5), and after 60 minutes, the amplitude further increased with greater statistical significance (p<0.01), (Appendix 3). Figure 4 shows anan ECG recording before and after application of the an microvibrations directly on the rat heart.

Acute effects of the vibroacoustic microvibrations of certain amplitudes and frequency on the rat heart wall structure using hematoxylin and eosin staining

Histological processing of the rat heart wall by hematoxylin and eosin staining showed that the various modes of the vibroacoustic microvibrations did not change the normal structure of the heart (Appendices 5 and 6).

Appendix 1

Table 1. The heart rate measured in the second standard ECG lead after a 10- minute application of the vibroacoustic microvibrations in four different modes (n=4 rats).

Heart rate (beats/min, x±SE)		
Mode 1		
Control	422.50±2.50	Student's t-test p=0.229
Experimental group	445.00±14.43	
Mode 2		
Control	443.33±18.55	Student's t-test p=0.742
Experimental group	440.00±26.55	
Mode 3		
Control	444.33±24.03	Student's t-test p=0.667
Experimental group	446.66±26.66	
Mode 4		
Control	433.66±27.28	Student's t-test p=0.423
Experimental group	440.00±30.55	

Appendix 2

Table 2. The frequency of cardiac rhythm disturbances measured in the second standard ECG lead after a 10- minute application of the vibroacoustic microvibrations in four different modes (n=4 rats).

Rhythm	Disturbance	No disturbance	Chi-squared test
Mode 1			
Control	0 (0%)	4 (100%)	p>0.05
Experimental group	0 (0%)	4 (100%)	
Mode 2			
Control	0 (0%)	4 (100%)	p>0.05
Experimental group	0 (0%)	4 (100%)	
Mode 3			
Control	0 (0%)	4 (100%)	p>0.05
Experimental group	1 (25%)	3 (75%)	
Mode 4			
Control	0 (0%)	4 (100%)	p>0.05
Experimental group	1 (25%)	3 (75%)	

Appendix 3

Table 3. The heart rate, frequency of cardiac rhythm disturbances, and amplitude of R waves measured in the second standard ECG lead after a 60 minute application of the vibroacoustic microvibrations in Mode 1 (n=6 rats).

Mode 1		
Heart rate (beats/min)		
Control	443.33±25.90	Student's t-test K:10' p=0.620 K:20' p=0.394 K:30' p=0.384 K:60' p=0.833
10'	451.66±33.30	
20'	456.66±27.52	
30'	455.00±27.04	
60'	448.33±35.34	
	Repeated measurements ANOVA P=0.646	
Rhythm (yes/no disturbance)		
Control	0/6	Chi-squared test p=0.604
10'	2/4	
20'	2/4	
30'	2/4	
60'	2/4	
R wave amplitude (mm)		
Control	5.50±1.04	**Student's t-test** **K:10' p=0.020** **K:20' p=0.020** **K:30' p=0.020** **K:60' p=0.005**
10'	7.26±1.16	
20'	7.45±1.31	
30'	7.63±1.45	
60'	8.05±1.55	
	Repeated measurements ANOVA p=0.001**	

DISCUSSION

Microvibrations exist in every living organism. The origins of the microvibrations include the pulsating heart activity (infrasound range), vascular activity and muscle activity (sound range). Muscle cells provide microvibrations that are necessary for tissues to maintain their function even in the resting state, which demands considerable energy consumption. The work of Soviet and Russian scientists clarified the knowledge of the existence and effects of the microvibrations on a whole organism. The effect of the so-called hydrodynamic pump was discovered by A. I. Arinchinim who, in his book "Peripheral Heart of Man", claims that muscle fibres tremble along with sound oscillations. His conception is that microvibrations are a physical element by which an organism reduces peripheral resistance in the capillary network and increases venous outflow of blood. The role of the microvibrations in pumping blood and lymph through the blood and lymph vessels in one direction was proven. The frequency of oscillatory motion of smooth muscles in the walls of these vessels improves the efficiency of the venous and lymphatic pumps, and the optimum amplitude of movement-oscillation of the muscles seems to correspond to the diameter of the lumen. As these vessels have different diameters, vibroacoustic vibrations of various shapes, frequencies, amplitudes and length are used to achieve synchronised stimulation of the various vessel types. Consequently, for each diameter of blood and lymphatic vessel, there is not only an optimum frequency, but also optimum characteristics of the waves (energy). Another important characteristic is a reduction in resistance due to blood flow. Vibroacoustic microvibrations reduce the friction between the blood layers at a certain frequency, thus, reducing the viscosity and vascular resistance and leading to an increase in the fractional force, which is a biological stimulus for the production

Appendix 4

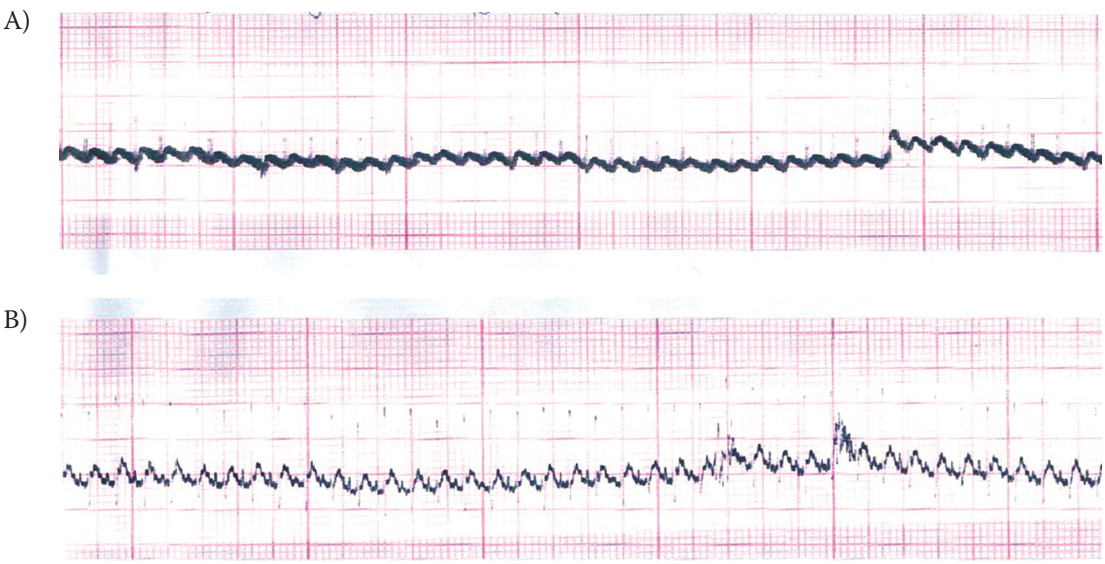
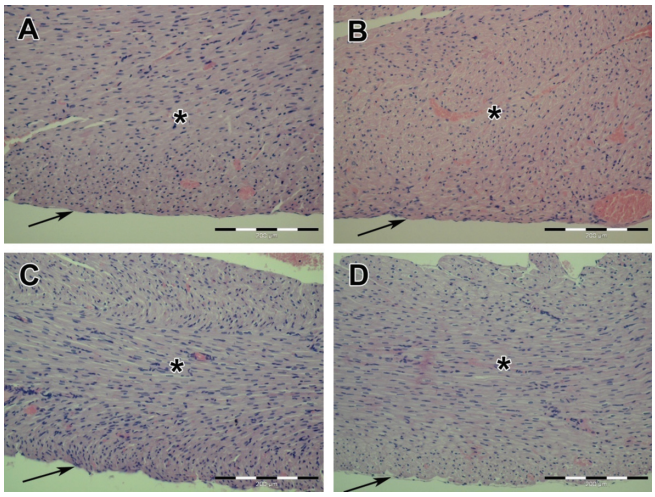
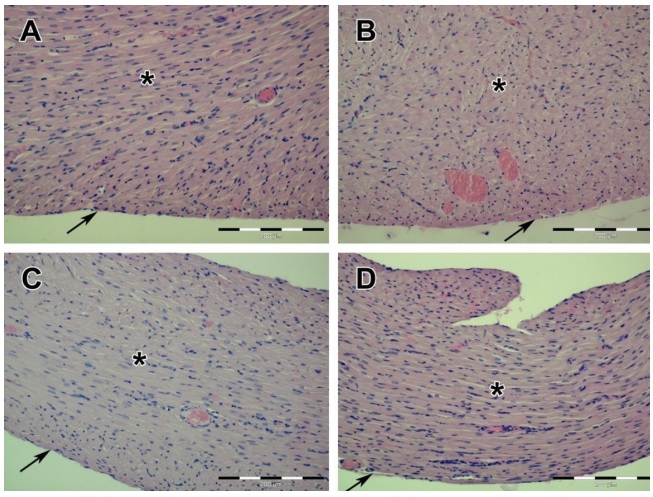


Figure 1. Changes in the R wave amplitude on ECG recordings before (A) and after (B) the application of vibroacoustic microvibrations directly to the rat heart.



Appendix 5

Figure 2. Normal wall structure of the rat heart (before the application of vibroacoustic microvibrations). A) left ventricle, B) left atrium, C) right ventricle, D) right atrium; arrow=epicardium; asterisk=myocardium.



Appendix 6

Figure 3. Wall structure of the rat heart after the application of vibroacoustic microvibrations in Mode 1. A) left ventricle, B) left atrium, C) right ventricle, D) right atrium; arrow=pericardium; asterisk=myocardium.

of nitric oxide. Certain effects of applying low frequency sound on the human cardiovascular system have already been indicated. In a study that applied infrasound (below 20 Hz) to astronauts on the Apollo mission, 21 males aged 21–33 years old were stimulated with sounds (between 2 Hz and 12 Hz) between 119–144 decibels in a stimulation chamber. No electrocardiographic disturbances were recorded during the stimulation. The heart rate was higher in 6 people by more than 6 beats per minute during maximum stimulation, while in 5 people, a decreased heart rate was recorded (11). This investigation did not observe any discomforts such as disorientation, mental confusion, or tiredness or decreased sensory abilities in any person. However, the effects of the vibrations and noise on the human cardiovascular and respiratory systems were also investigated in aeronautical workers and helicopter pilots (12, 13). The biological effects of infrasound and low-frequency noise explained by mechanotransduction cellular signalling and its connection with vibroacoustic disease were intensively studied during the last 15 years (14-18). Furthermore, the effects of chronic exposure to low frequency noise on rat pleural mesothelial cells and rat tracheal epithelia were confirmed (19). This attention to low intensity and frequency of vibroacoustics were further studied in the effects of low intensity pulsed ultrasound on integrin-FAK-PI3K/Akt mechanochemical transduction in chondrocytes of osteoarthritic rabbits (20).

In our research, vibroacoustic microvibrations (up to 60 minutes, frequency 30–18 Hz and amplitude 2.8–12.3 μm) were acutely induced in order to evaluate their effects on the cardiovascular system in rats. Analysis of the heart rates in response to the vibroacoustic microvibrations (10 minutes long) did not show statistically significant changes. The most noticeable changes occurred after the application of the microvibrations in Mode 3, but the changes were not statistically significant. When analysing the frequency of disturbances in the heart rhythm in the second standard ECG lead during the vibroacoustic microvibrations, slight changes in the cardiac rhythm were noticed in Modes 3 and 4 in 25 % of the rats (one rat per mode had arrhythmias). No arrhythmias were noted during the application of Modes 1 and 2. When analysing the R wave amplitudes in the second standard lead after the application of the vibroacoustic microvibrations (10 minutes long), a statistically significant increase in the amplitude was present in Modes 3 and 4, while Modes 1 and 2 did not show statistically significant changes. We hypothesise that the increased amplitude of the R waves could be due to vasodilatation induced by the microvibrations on the rat body, which led to increased influx of blood into the heart, i.e., increased cardiac contractions by the Frank-Starling law. Our results could also be due to the direct effect of the microvibrations on cardiac muscle (or tissue), which resulted in the increased contractility. Analysis of the heart rates after application of the vibroacoustic microvibrations (60 minutes long) showed no statistically significant changes in Mode 1. The most prominent changes occurred after 20 minutes of stimulation, but the changes were not statistically significant. Analysis of the frequency of the registered disturbances in the heart rhythm in the second standard ECG lead after application of the microvibrations for 60 minutes showed that the values of the amplitude in Mode 1 were statistically significant after 10, 20 and 30 minutes, and after 60 minutes, the values of the amplitude were highly statistically significant. Consequently, Mode 1 showed that potentially useful effects could appear with the use of low frequencies and amplitudes of microvibrations, although the application of maximum frequencies or amplitudes of microvibrations should be further investigated. Results from the hematoxylin and eosin staining

of the rat heart wall showed that the acute application of the microvibrations did not change the normal histological wall structure. In conclusion, the increase in the amplitudes of the R waves in the second standard ECG lead by 25–32% was not correlated with changes in the heart wall structure. Further research on the detailed physiological mechanisms of the obtained results should be conducted in the future.

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