Effect of Low-Frequency Low-Intensity Ultrasound on Contractile Function of Isolated Heart

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Stimulation of the isolated heart with low-intensity low-frequency ultrasound produced a positive inotropic effect (at certain ultrasound intensity). This effect manifested in increased systolic and pulse intraventricular pressures and persisted for tens of minutes after the end of sonication.

Key Words: *isolated heart; ultrasound; inotropic effect*

Ultrasound technologies are widely used in medicine with diagnostic, therapeutic, and surgical purposes. The use of ultrasound in diagnostics excludes possible effects and aftereffects on individual cells and whole tissue. The aim of therapeutic procedures is modulation of functional parameters of cells and organs. In surgery ultrasound is used for destruction of tissues, for instance, neoplasms. However, despite more than 30-year history of application of ultrasound technologies in medicine (predominantly in physiotherapy), its effect on functional state of individual organs and, first of all, on the heart, was not studied. Clinicians determined it indirectly via the effects of routine physiotherapeutic procedures [1]. Recent studies began to fill this gap. For example, experiments on isolated frog heart showed that acoustic pressure produced by pulsed ultrasound stimulation (US) during systole decreased blood pressure in the aorta, while US synchronized with the diastole provoked ventricular extrasystoles [2].

However, no significant effect of US on contractile function of the heart was found in the study performed on retrograde perfusion model of isolated rat heart [3]. The characteristic feature of these virtually sole works in this field is the use of ultrasound in the megahertz frequency range. These contradictory results call for further detailed studies of the effects of ultrasound and various modes of sonication on the heart.

We studied changes in contractile function of the left ventricle of isolated rat heart produced by a low-intensity low-frequency US and the dependence of these changes on US intensity. We also studied the arrhythmogenic effects produced by US of various intensities.

MATERIALS AND METHODS

The experiments were carried out on isolated hearts of mature albino rats weighing 230-320 g. The rats were intraperitoneally narcotized with nembutal (50 mg/kg). Heparin (1000 U) was simultaneously injected for prevention of coronary thrombosis. Isolated heart was placed in ice-cold (2-4°C) heparinized Krebs—Henseleit solution and mounted on a Langendorff perfusion setup. The aorta was cannulated and perfused retrogradely with oxygenated Krebs—Henseleit solution at 75 mm Hg (95% O_2 and 5% CO_2, pH 7.4, 37.0± 0.5°C). Electrocardiogram and intraventricular pressure were recorded on a Hellige cardiomonitor connected to a H3031-6 writer. Intraventricular pressure was measured in an isovolumic regime using a latex balloon filled with physiological saline introduced into the left ventricle and connected to a Baxter pressure transducer. After 15-min perfusion the parameters of

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the isolated heart were stabilized (end-diastolic pressure below 10 mm Hg and the mean systolic pressure 85-100 mm Hg). Then the heart was placed into a cylindrical heat-insulated 700-ml vessel with a built-in G3-109 ultrasound generator. The vessel was filled with Krebs-Henseleit solution. The temperature (37°C) was continuously monitored with a Hellige thermistor. Spatial distribution of ultrasound intensity in the filled vessel was analyzed before the test and the heart was placed in the area of maximum ultrasound intensity. Ultrasound frequency 45 kHz was chosen, because the corresponding sound wavelength (3 cm) is comparable with linear size of the heart (≤2.5 cm). Under these conditions, the heart was subjected to positive and negative pressure produced by alternating compression and rarefaction half-waves. This feature distinguished our experiments from previous works [2,3] where ultrasound was used in the megahertz range (wavelength<1.5 mm).

After stabilization of the heart in the vessel for 10 min, US was applied; US intensity was gradually (by 0.05 W/cm^2) increased from 0.05 to 0.25 W/cm^2 with 7-10-min intervals. The amplitude of compression-rarefaction acoustic pressure around the heart at maximum US intensity was 0.86 atm. After application of US of maximum intensity (0.25 W/cm^2) the generator was turned off. The thermal effects produced by unfocused ultrasound of the specified intensity are negligible (rise the temperature only by a few hundredths degree after persistent sonication for several hours). The same parameters at the chosen frequency of 45 kHz were below cavitation threshold.

Systolic (SIVP), diastolic (DIVP), and pulse (PIVP) intraventricular pressures were recorded before immersion, after 10-min stabilization in the vessel, and 5 min after the start of exposure at each US intensity. In the control (nonsonicated hearts) the cardiac parameters were recorded at the same time points.

RESULTS

US increased SIVP and PIVP, and this effect was most pronounced at the intensity of 0.15-0.20 W/cm^2, while in the control these parameters decreased with time (Table 1). It is noteworthy that in both groups the initial parameters of myocardium contraction were similar (Table 1).

US with intensity of 0.15 W/cm^2 produced the most pronounced increase in SIVP compared to both values recorded after immersion of the heart into saline (by 15-17% on the average) and corresponding control values (Table 1).

In 50% hearts, increasing US intensity to 0.20 W/cm^2 provoked significant arrhythmias with coupled and multiple polytopic extrasystoles and episodes of ventricular tachycardia. These transient arrhythmias decreased SIVP and PIVP after normalization of the rhythm. In other hearts, US of the same intensity still produced a positive inotropic effect without rhythm disturbances. Further increasing US intensity to 0.25 W/cm^2 led to secondary impairment of myocardial contractility against the background of severe rhythm disturbances (in 3 of 4 hearts) or to primary decrease in SIVP and PIVP without arrhythmia (one heart). After termination of US exposure sinus rhythm recovered in all cases. Contractile activity of the left ventricle by the end of the experiment surpassed the initial value and the corresponding value in the control group. There were no significant differences in DIVP between the groups (Table 1). Thus, our experiments

TABLE 1. Effect of US on Cardiac Indices (mm Hg, *M*±*m*)

Recording period	SIVP		DIVP		PIVP	
	control	US	control	US	control	US
Initial value	96±6	92±8	11±2	10±1	85±5	82±7
After immersion of the heart into a vessel with US generator	89±7	84±5	11±3	11±2	78±6	73±5
US, W/cm^2						
0.05	87±6	88±7	12±2	10±2	75±5	78±6
0.10	84±5	92±6	11±1	12±3	72±5	80±6
0.15	82±6	101±7	11±2	11±1	71±4	90±5
0.20	81±5	94±6	12±2	11±2	69±4	83±5
0.25	80±8	78±7	12±3	11±3	68±5	67±6
After termination of US exposure, min						
10	78±7	99±5	12±2	12±3	66±5	87±4
25	76±7	91±6	12±3	13±2	64±4	78±5

showed that stimulation with an intensity above the threshold US intensity (0.20-0.25 W/cm^2) produced negative effects. These effects manifested in arrhythmia (polytopic extrasystoles and episodes of ventricular tachycardia) followed by secondary inhibition of the contractile function, or in a direct negative inotropic effect. After termination of US exposure sinus rhythm completely recovered and contractile activity of the myocardium increased attaining a submaximum level at optimal US intensity of 0.15 W/cm^2. It can be hypothesized that the positive inotropic effect of ultrasound persisted after termination of US exposure and the duration of aftereffect depends on the duration of US exposure and the number of these procedures. In our experiments the positive inotropic effect of a single US exposure persisted for 40-60 min.

The possible mechanisms of the positive inotropic effect of low-intensity low-frequency ultrasound stimulation observed in our experiments can be related to cardiac events realized at the macro (organ) and micro (cell) levels. It cannot be excluded that the dynamics of cardiac pump function is modulated by external compression-decompression influences. In our experiments this modulation was achieved via periodic acoustic compression and rarefaction applied synchronously to the whole heart due to comparability of its size and US wavelength. On the other hand, recent studies of the dynamic effects of US at the cellular level showed that these effects are caused by changes in stress-deformed state of plasma membranes resulting in microvibrations and micromassage, shift in membrane potential induced by mechanical stress, activation and formation of membrane pores, and increase in membrane permeability [4]. US can increase intracellular concentration of calcium ions in cardiomyocytes, which manifested in enhanced contractile function. Disturbances in calcium homeostasis in pacemaker cells and conducting cardiomyocytes play an important role in arrhythmogenesis. Electromechanical reaction of cells to US stimulation of certain intensity modifies the cardiac contractile function and can provoke arrhythmias.

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