Frequency-Dependent Effects of Low-Intensity Ultrasound on Activity of Isolated Heart

N. N. Petrishchev, T. D. Vlasov, M. M. Galagudza, Yu. N. Makov*, and C. M. Minasyan

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 9, pp. 273-276, September, 2003
Original article submitted February 27, 2002

The experiments with low-intensity ultrasound stimulation of isolated rat heart revealed frequency-dependent effects of ultrasound in a frequency range of 45-298 kHz on cardiac activity.

Key Words: *isolated heart; ultrasound; frequency-dependent effects; intraventricular pressure; arrhythmogenic effects*

We previously demonstrated the effects of low-intensity and low-frequency (45 kHz) ultrasound (US) on isolated rat heart, which manifested in an increase in left ventricle developed pressure [1]. By contrast, no such effects were observed in a high-frequency (megahertz) ultrasound range [4]. Evidently, the effect of US stimulation of biological objects (tissues, organs, and individual cells) depends on the main parameters of US stimulation, although specific character of this dependence should be determined experimentally. Specifically, one can expect potentiation of the biological effects with increasing US intensity, and it was corroborated in the experiments [1].

Until now, the effect of another important US parameter (frequency) on the efficiency of heart stimulation remained unclear for some reasons. First, the character of this effect is difficult to predict because of complexity and variability of possible ways of US action. Second, few previous studies of the effect of US stimulation on isolated heart tested only some frequencies in a megahertz range [4-6].

This paper describes the dependence of cardiac activity of rat isolated heart on frequency of US stimulation in the range of 45-298 kHz.

Department of Pathophysiology, I. P. Pavlov State Medical University, St. Petersburg; Department of Acoustic, M. V. Lomonosov Moscow State University. ***Address for correspondence:*** vlasov@spmu.rssi.ru. Vlasov T. D.; makov@acs364.phys.msu.su. Makov Yu. N.

MATERIALS AND METHODS

Perfusion of isolated heart was described in details elsewhere [1].

The experiments were carried out in acoustic setup containing a reservoir with an US stimulator incorporated into its bottom and driven by a G3-123 sine voltage generator in the range of 45-298 kHz. Before the tests, the space distribution of ultrasound intensity in the filled bath was analyzed. The heart was located in the area of maximum ultrasound intensity. The mean US intensity was ~0.3 W/cm^2. Theoretical estimation showed that such US produced no significant thermal effects.

The experiments were carried out with the hearts subdivided into two groups. In control group (n=5) the hearts were perfused without US stimulation for 120-140 min, which was equal to duration of US stimulation in experimental group (n=8), where the hearts were stimulated with US intensity of about ~0.3 W/cm^2. Since preliminary experiments revealed a pronounced increment of the cardiotropic effect of US in the frequency range of 250-298 kHz, further study of frequency-dependent effects was performed with the following non-uniformly spaced frequencies (in kHz): 45, 100, 150, 200, 250, 260, 270, 280, 290, and 298.

Duration of US stimulation at any frequency was 5 min, and the period between stimulation was 5 min. The sequence of stimulation frequencies was chosen stochastically. Systolic (SIVP), diastolic (DIVP) and

0007-4888/03/1363-0239$25.00

pulse (PIVP) intraventricular pressures and heart rate (HR) were recorded in the initial state (before immersion of the heart into the bath) and at minute 10 after immersion. In addition, recordings were made on stimulation minute 5 and on minute 5 of the resting period. In control group, the cardiac parameters were recorded at the same moments of time.

The incidence and degree of rhythm abnormalities in the control and experimental groups were assessed according to Lambeth Conventions [9]. The following parameters were analyzed: the number of ventricular premature contractions (VPC), the number of bursts of ventricular tachycardia, and the number of episodes of ventricular fibrillation during US stimulation at a certain frequency.

The results were analyzed statistically using Statistica software and Student's and Wilcoxon's tests.

RESULTS

We studied the effect of US stimulation on hemodynamic indices of isolated heart (HR, PIVP, DIVP, and SIVP) and on the arrhythmogenic factors such as the number of VPC and relative number of ventricular tachycardia bursts.

No significant difference in HR was observed between the control and experimental groups (252±27 and 248±18 bpm, respectively).

The experimental data were obtained by statistical analysis of the measured parameters corresponding to various randomly applied US frequencies. Therefore, they reflect the mean frequency-dependent changes of pressure values, which can be affected by the total time-dependent accumulation of US radiation during the experiment.

A significant decrease of PIVP (in comparison with the initial value) was observed only after stimulation with 298-kHz US (86±9 *vs.* 108±7 mm Hg, $p<0.05$). US stimulation at various frequencies produced no significant unidirectional changes in PIVP (Fig. 1). In control group, persistent drop in PIVP was observed during perfusion on minutes 120-140, which resulted from the decrease of SIVP from 106±12 to 88±7 mm Hg and from increase in DIVP. In our experiments this impairment of the systolic and diastolic myocardial functions typical of *ex vivo* preparations was on average 8.3% per hour for PIVP. In the test group, we observed an increase in DIVP (Fig. 1), while SIVP remained stable (114±13 at the start and 112±15 mm Hg at the end of the experiment). Therefore, the drop of PIVP at high frequencies was comparable with the control values.

US stimulation at any frequency significantly increased the number of VPC in comparison with initial number of VPC in the experimental group and with the number of VPC in the control group recorded during the same period (Fig. 2).

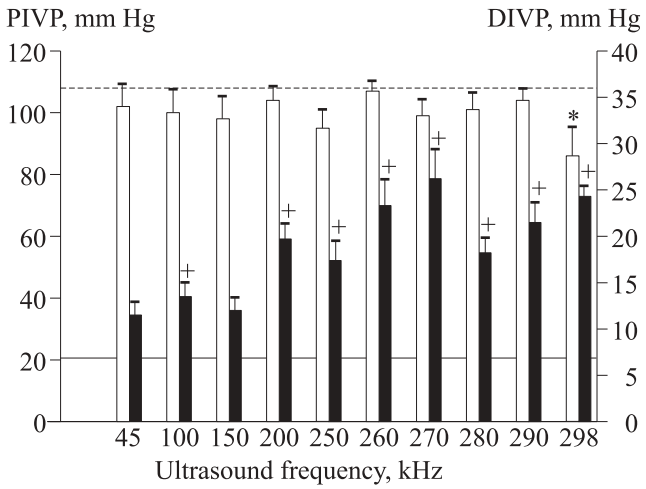

Fig. 1. Effect of ultrasound stimulation on isolated heart. Light and solid bars represent pulse intraventricular pressure (PIVP) and diastolic intraventricular pressure (DIVP), respectively. Solid and dashed lines correspond to DIVP and PIVP before US stimulation. $^{*}p<0.05$ and $^{+}p<0.05$ compared to initial PIVP and DIVP values, respectively.

Initially, the number of VPC in the control and experimental groups did not surpass 1 per minute (0.60±0.08 and 0.70±0.10, respectively). In control group, the number of VPC did not increase during the entire period of observation. However, US stimulation at various frequencies increased the number of VPC to different degree. There were two maxima in VPC number: the first appeared at 150 kHz and the second and more pronounced increase at 270-298 kHz. The number of VPC recorded during US stimulation at 298 kHz was 22.2±3.6 per minute. Between stimulations the number of VPC decreased (in some cases returned to the initial level). However, during resting period after US stimulation at some frequencies (260, 280, or 290 kHz) the number of VPC far surpassed the initial value.

In some cases, short-term episodes of ventricular tachycardia were observed during US stimulation. By contrast, no cases of ventricular tachycardia took place in the control group throughout the experiment. Analysis of the incidence of ventricular tachycardia provoked by US stimulation at various frequencies revealed a distribution similar to the distribution of VPC number over US frequencies (Fig. 2): in 20% cases bursts of ventricular tachycardia appeared at 100, 150, 200, and 280 kHz, in 40% cases at 290 kHz, and in 85% cases at 298 kHz. No cases of ventricular fibrillation provoked by US were observed.

These data indicate two most important effects of low-intensity US stimulation on isolated heart. On the one hand, US stimulation at various frequencies produces a more pronounced increase in DIVP than in the

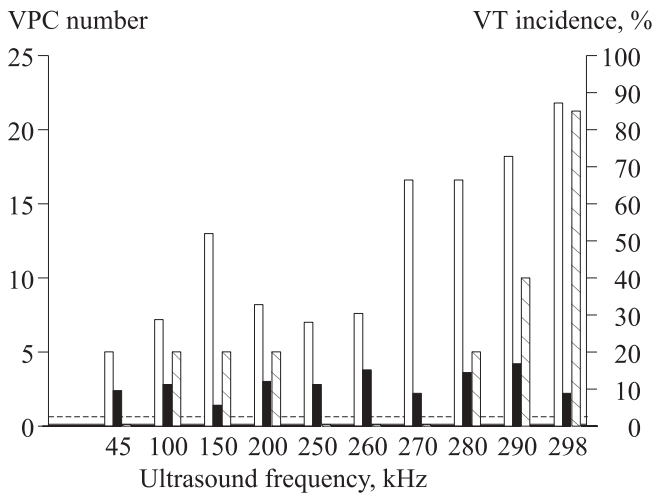

Fig. 2. Effect of ultrasound stimulation on ventricular premature contractions (VPC) and bursts of ventricular tachycardia (VT). Light and solid bars mark the number of VPC on the first minute of stimulation and on the first minute after it, respectively. Dashed bars show the relative number of VT bursts in percentage. Solid line (abscissa) shows the relative number of VT bursts in the control group (not subjected to US stimulation). Dashed line corresponds to the number of VPC without US stimulation.

control group. It is noteworthy that diastolic cardiac function is most sensitive to concentration of sarcoplasmic Ca^{2+} [7]. Logically, predominant disturbance of diastolic myocardial function observed in our study could relate to US-induced increase of Ca^{2+} influx via the ionic channels and probably via newly formed pores [8]. In addition, it can result from abnormalities in the mechanisms responsible for the decrease in intracellular calcium concentration during diastole.

On the other hand, US stimulation revealed a frequency-dependent arrhythmogenic effect manifested by increased incidence of VPC. When analyzing the effects of US stimulation in biologic targets, particular attention is given to the resonance phenomena [3]. In some cases, drawing the stimulation frequency to the natural frequency of a morphological element of the heart (cardiomyocytes, myocardial layer, valve, atrioventricular node, *etc*.) potentiated the observed effect. Similar phenomenon probably took place in our study.

Thus, the reported data demonstrate various features of moderate damaging action of low-intensity US stimulation on isolated heart, and this action depends on stimulation frequency. At present, the sublethal damage to the myocardium is considered as a stimulus inducing strong adaptive response, which improves heart resistance to subsequent sustained and severe damage (phenomenon of myocardium protection) [2]. The reported experiments demonstrated a dependence of basic functional indices of the isolated heart on the parameters of applied US. Further studies should compare the present results with the data reflecting "pure" action of US on the heart under physiological conditions. The present data are important for subsequent studies of US effects in pathologically altered myocardium.

The study was supported by the Russian Foundation for Basic Research (grant No. 01-02-16655).

REFERENCES

1. N. N. Petrishchev, T. D. Vlasov, M. M. Galagudza, *et al.*, *Byull. Eksp. Biol. Med.*, **133**, No. 4, 380-383 (2002).
2. N. N. Petrishchev, E. V. Shlyakhto, T. D. Vlasov, *et al.*, *Ros. Fiziol. Zh.*, **87**, No. 5, 688-705 (2001).
3. V. S. Ulashchik and A. A. Chirkin, *Ultrasound Therapy* [in Russian], Minsk (1983).
4. S. Greenberg, A. Finkelstein, E. Raisman, *et al.*, *Ultrasound Med. Biol.*, **26**, 315-319 (2000).
5. D. Dalecki, C. H. Raeman, S. Z. Child, *et al.*, *Ibid.*, **23**, 275-285 (1997).
6. G. V. Forester, O. Z. Roy, and A. J. Mortimer, *J. Mol. Cell. Cardiol.*, **14**, 475-477 (1982).
7. W. C. Hunter, *Cardiol. Clin.*, **18**, 443-457 (2000).
8. K. Tachibana, T. Uchida, K. Ogawa, *et al.*, *Med. Electron. Microsc.*, **34**, 249-253 (2001).
9. M. J. Walker, M. J. Curtis, D. J. Hearse, *et al.*, *Cardiovasc. Res.*, **22**, 447-455 (1988).