The role of the duty factor in ultrasound-mediated cardiac stimulation

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Abstract: The role of the duty factor (DF) in ultrasound-mediated cardiac stimulation is studied. Five 3-month-old female rats were exposed transthoracically to 3.5-MHz ultrasonic pulses of 2.0-MPa peak rarefactional pressure amplitude, variable DF, and variable pulse repetition frequency. A change in the heart rate was not observed following the 0.25%-DF sequence. A decrease of ~4% in the heart rate was observed following the 0.50%-DF and 1.00%-DF sequences. Outcomes suggest a possible DF threshold for cardiac pacing.

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1. Introduction

An important aspect of therapeutic ultrasound is its ability to produce biological effects noninvasively. There is an increasing use of controlled ultrasound for physical therapy, thrombolysis,[1] hyperthermia,[2] lithotripsy,[3] and hemostasis.[4]

The relevance of studying ultrasound-induced biological effects lies in the fact that they can be used for therapeutic procedures. The biological effect depends on the occurrence of acoustic parameter combinations which must be determined experimentally.[5,6] For example, variations of ultrasonic parameters [e.g., peak rarefactional pressure amplitude (PRPA), frequency, pulse repetition frequency (PRF), duty factor (DF), pulse duration (PD)] can yield different tissue effects that also depend on the tissue type. Ultrasonic studies of *in vivo* effects in hearts of frogs,[2,3] pigs,[6] guinea pigs,[7] mice,[8,9] and dogs[10] have shown that different PRPAs, frequencies, and PDs can cause premature contractions, inotropic, lusitropic, or chronotropic effects in the heart.

A previous study[11] demonstrated that transthoracic ultrasound can promote a negative chronotropic effect without impairing cardiac pumping function and/or damaging cardiac tissue.[11] This effect was achieved using a specific sequence of 1-MHz ultrasonic bursts delivered transthoracically to the heart, progressively decreasing the PRF while maintaining the same DF. The goal of this study is to investigate the DF role in cardiac pacing.

2. Methods

The experimental conditions were approved by the University of Illinois Institutional Animal Care and Use Committee (protocol #10104). Five 3-month-old 250–300-g female Sprague Dawley rats were used. Animals were exposed to 5% isoflurane for induction of inhalation anesthesia in a chamber, and then to 1.5%–2% isoflurane via face mask for maintenance anesthesia. The level of anesthesia was monitored by pedal reflex.

The thoracic region was shaved and depilated to maximize acoustic transmission. Gel was used for acoustic coupling. Rats were placed on a temperature-controlled platform in dorsal recumbency for ultrasonic cardiac exposure. Respiratory rate, heart rate and ECG were continuously recorded prior to and during the pulsed ultrasonic exposure of the heart (Fig. 1), and then recorded 24 h later.

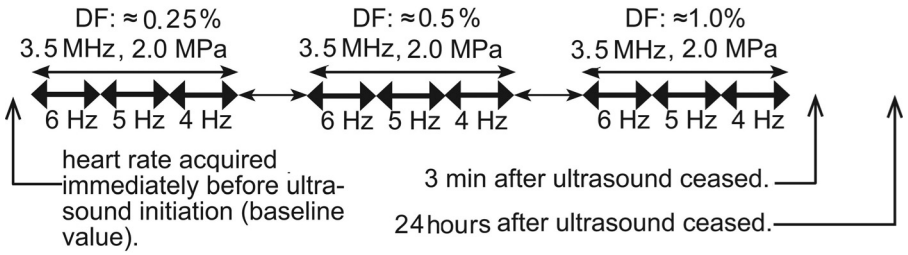

Fig. 1. Time line for the 3.5-MHz 2.0-MPa transthoracic ultrasound stimulation. The time line shows three ultrasonic exposure sequences of 30 s each [each with a different duty factor (DF) of 0.25%, 0.5%, or 1.0%]. During each of the three 30-s sequences, three separate and decreasing PRFs were used, as noted (6, 5, and 4 Hz). The bolded two-ended arrows denote a 10-s duration during which one of the three PRF is constant; the PRF is stated directly below the bolded arrow. Baseline data were acquired prior to the initial ultrasound exposure. Post-exposure data were acquired at 3 min and 24 h after ultrasound exposure.

The 3.5-MHz transducer was driven by a function generator (Agilent 33 250A) and a radio-frequency power amplifier (ENI A150). The function generator and external transmitter produced a timed ultrasonic field, applied through the external surface/chest of the rat. A VisualSonics Vevo 2100 high-frequency ultrasound imaging system was used to monitor the heart via B-mode and M-mode displays. In this study to minimize thermal damage the duty factor ranged between 0.25% and 1.00%. The transthoracic 3.5-MHz ultrasonic bursts of 2-MPa PRPA were applied as shown in Fig. 1: (1) three 10-s intervals, that is, 10 s of 6-Hz PRF, 10 s of 5-Hz PRF, and 10 s of 4-Hz PRF, for a 30-s exposure duration with ≈0.25% DF (0.5-ms PD), (2) three 10-s intervals, that is, 10 s of 6-Hz PRF, 10 s of 5-Hz PRF, and 10 s of 4-Hz PRF, for a 30-s exposure duration with ≈0.50% DF (1-ms PD), and (3) three 10-s intervals, that is, 10 s of 6-Hz PRF, 10 s of 5-Hz PRF, and 10 s of 4-Hz PRF, for a 30-s exposure duration with ≈1.00% DF (2-ms PD). The interval between each sequence was 10 s (no ultrasound exposure) and the total ultrasound exposure duration was 90 s (plus two 10-s no ultrasound intervals). For each duty factor the same PRF sequence (6-5-4-Hz) divided in three sequences of 30 s of ultrasound exposure was used. The heart rate was monitored and recorded during the 10-s interval between sequences. The 10-s no-ultrasound interval was not of sufficient duration to acquire ejection fraction and other physiological parameter data; generally about 3 min is required.

One-way analysis of variance for repeated measures was performed to assess how duty factor affected the rat heart. Bonferroni *post hoc* tests were applied to compare the duty factors after ultrasound exposure with the baseline values. Statistical analyses of baseline values were performed on their absolute values. Statistical analyses of post-baseline values were performed on the normalized values, individually normalized to the baseline values for each animal.

The ejection fraction, defined as the ratio of the stroke volume and the end diastolic volume, was used to assess possible cardiac contractility alterations. Because there were no signs of major problems to animals exposed to the pulsed sequence,[11] animals were allowed to recover.

3. Results

Absolute values of heart rate and physiological parameters are listed in Table 1.

Baseline: There were no differences between 0.25%, 0.50%, and 1.00% DFs for heart rate, cardiac output, stroke volume, ejection fraction, and end-diastolic volume.

Heart Rate: There was a statistically significant heart rate effect (decrease of ~4%) as a function of duty factor at 0.50% and 1.00% relative to baseline, but not for 0.25% DF or at 24 h post ultrasound (US) exposure (Fig. 2).

Cardiac output, stroke volume, ejection fraction, and end-systolic volume: At 3 min and 24 h post US exposure, there were no differences relative to baseline.

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Table 1. Absolute values of heart rate and physiological parameters for the rats ($n = 5$) expressed as mean and standard deviation (SD).

		$N = 5$					
Parameter	Units	Baseline Mean ± SD	After 0.25% DF Mean ± SD	After 0.50% DF Mean ± SD	After 1.00% DF Mean ± SD	3-min post US exposure Mean ± SD	24-h post US exposure Mean ± SD
Heart rate	bpm	332 ± 14	325 ± 14	314 ± 20	305 ± 23	309 ± 20	350 ± 23
Cardiac output	mL/min	66 ± 10	[a]	[a]	[a]	52 ± 8	57 ± 7
Stroke volume	μL	175 ± 9	[a]	[a]	[a]	167 ± 7	163 ± 8
Ejection fraction	%	80 ± 5	[a]	[a]	[a]	80 ± 6	76 ± 2
End-diastolic volume	μL	217 ± 9	[a]	[a]	[a]	211 ± 9	214 ± 12

[a]Cardiac output, stroke volume, ejection fraction, and end-diastolic volume values could not be recorded because a time duration of about 3 min is required to acquire such data; the 10-s duration between US sequences was insufficient to acquire such data.

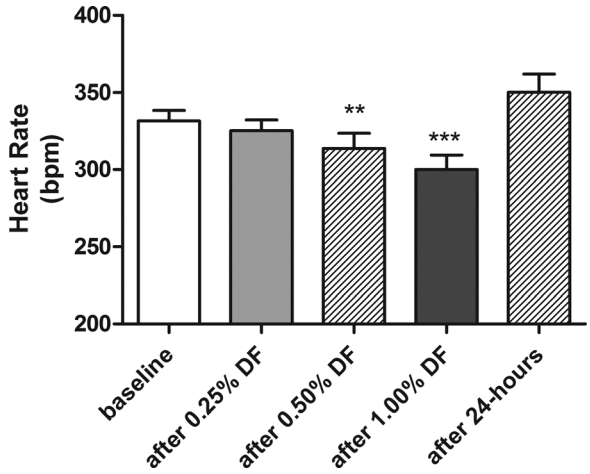

Fig. 2. Mean heart rate values at baseline, after 0.25% DF, after 0.50% DF, after 1.00% DF, and at 24-h post US exposure. The error bar denotes standard deviation ($n = 5$). ** and *** denote, respectively, $p < 0.01$ or 0.001 significant changes relative to baseline.

Table 1 shows the baseline, and 3 min, and 24 h post-exposure values of the physiological parameter of the heart: cardiac output, stroke volume, ejection fraction, and end-diastolic volume. Other than the heart rate at 3 min, these outcomes at 24 h were not significantly different when compared with the baseline values before ultrasound exposure.

4. Discussion

The goal of this study was to investigate the role of the duty factor in ultrasound-mediated cardiac pacing. The negative chronotropic effect was observed after the 0.50%-DF and the 1.00%-DF and heart rate was persistent until ≈45 min after ultrasound application.

The US exposure parameters caused a negative chronotropic effect suggesting that there may be a duty factor effect. Outcomes following the 0.50%-DF and the 1.0%-DF sequences suggest a possible DF threshold for cardiac pacing between duty factors 0.25% and 0.50%. The physiological outcomes after 3-min and 24-h US exposure suggest that there was no cardiac damage.

It is proposed that the negative chronotropic effect arising from the ultrasound application may result from parasympathetic stimulation. One possibility is the Bezold–Jarisch reflex. This reflex is an eponym for a triad of responses (apnea, bradycardia, and hypotension) that depends on intact vagi and is mediated through cranial nervous medullary centers controlling respiration, heart rate, and vasomotor tone.[12]

Another possibility is that the direct ultrasound stimulation of aortic baroreceptors could trigger the baroreceptor reflex, whose output translates into an increased parasympathetic tone and decreased sympathetic tone with consequent bradycardia. To address such issues, additional experiments are being conducted, using the specific set of acoustic parameters proposed here.

5. Conclusion

The 0.50%-DF and 1.00%-DF sequences affected cardiac pacing. Both sequences showed a significant negative chronotropic effect (decrease of ~4%) relative to baseline. The 0.25% DF did not show a significant change in the heart rate. The results are promising for therapeutic application in cardiology as an alternative of leadless pacing of the heart. Additional studies are required to elucidate the physiological mechanisms involved in the production of these effects.

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