ARRHYTHMOGENIC ACTION OF ACOUSTIC CAVITATION ON THE ISOLATED RAT HEART PERFUSED WITH PHYSIOLOGICAL SALINE

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The action of continuous and pulsed focused ultrasound (US) (543 kHz, intensity at focus up to 7.8 W/cm^2) on the pressure developed by the left ventricle and on the electrogram (EG) was studied in experiments on rat hearts isolated by Langendorff's method. In all the experiments US led to extra excitations of the heart, starting with an intensity of 1.35 ± 0.21 W/cm^2 (n = 9). Simultaneously with the extraexcitations of the heart, cavitation bursts were recorded, starting at an intensity of 1.52 ± 0.18 W/cm^2 (n = 6). The action of acoustic cavitation (for 30 sec) was accompanied by a significant decrease in the developed pressure (from 100.8 ± 3.8 to 95.1 ± 4.3 mm Hg; $p < 0.001$), measured 2 min after exposure. No effects of US of below cavitation intensity were found. With the aid of pulses of US (duration 50 msec, intensity up to 5 W/cm^2) the heart could be excited rhythmically if its own automatism was suppressed. Recording the EG showed, however, that during acoustic stimulation the character of excitation changed from one cycle to another. It is considered that acoustic cavitation induces local, reversible microlesions of the myocardium, and that this accounts for its stimulating and arrhythmogenic effect.

It has been shown that the action of US on biological objects, if its temperature effects are excluded, is linked mainly with the effects of acoustic cavitation [2, 7, 10]. The writers showed previously that cavitation leads to reversible inexcitability, depolarization, and contracture of the papillary muscles of the rat heart [1, 13], but the reaction of the whole heart to cavitation has not previously been studied.

The aim of this investigation was to study the effects of cavitation on the work of the isolated heart, perfused with physiological saline.

EXPERIMENTAL METHOD

Rats weighing 250-400 g were anesthetized with pentobarbital (150 mg/kg). The rats were given an intraperitoneal injection of heparin (1000 activity units) 5 min before anesthesia. After isolation of the heart the aorta was cannulated and the heart perfused with physiological saline of the following composition (in mM): NaCl — 145; KCl — 4; $MgCl_2$ — 0.5; $CaCl_2$ — 2.5; HEPES — 5; glucose — 5.5, oxygenated with 100% O_2, at pH 7.4 and 36°C. To suppress automatism, propranolol was added to the solution up to 3 μM. Perfusion was carried out under constant hydrostatic pressure of 110 mm Hg. In some experiments, after cannulation of the aorta, in order to prevent spontaneous activity the heart was damaged by compressing the upper part of the interventricular septum, together with the bundle of His located in it. The heart was stimulated electrically by means of wire electrodes fixed in the myocardium of the ventricle. Stimuli of twice the threshold intensity, with a duration of 3 msec and a frequency of 1 Hz, were generated by an SEN 3101 stimulator (Nihon Kohden, Japan). A rubber balloon filled with water, connected by a catheter to a "Statham 7543" electromanometer was introduced into the left ventricle. By changing the volume of the balloon, the end diastolic pressure in the left ventricle could be established at between 15 and 29 mm Hg. The bipolar EG was recorded from the ventricles by means of flexible wires, insulated along their length. In some experiments two EGs were recorded at once: I) electrodes were placed at a distance

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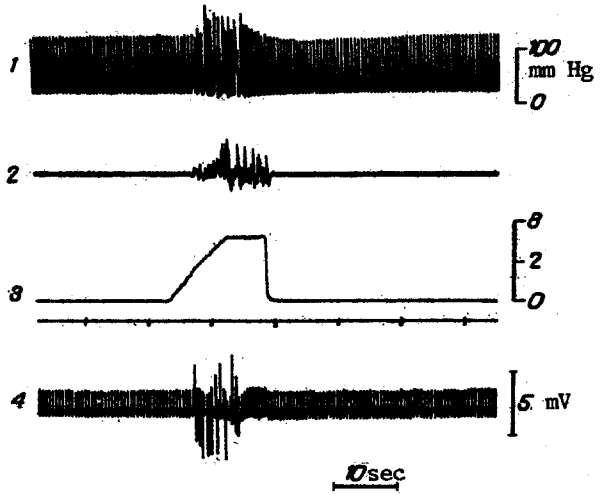

Fig. 1. Action of focused US (543 kHz) on pressure developed by left ventricle (1), and EG-II (4). 2) Amplitude of subharmonic (270 kHz), indicating appearance of cavitation; 3) intensity of US (in W/cm^2).

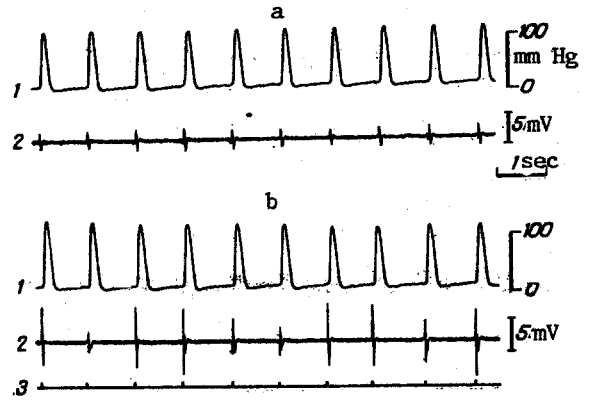

Fig. 2. Stimulation of heart by electrical (a) and acoustic (b) pulses. 1) Pressure developed by left ventricle; 2) EG-II; 3) marker of acoustic stimulation. Parameters of pulses given in text.

of 1 cm apart (one on each side of the heart), II) when the distance between the electrodes was about 1 mm. The heart was sonicated by a focusing US generator with a frequency of 593 kHz. The moment of development of cavitation was determined by the appearance of a signal of a subharmonic (with a frequency of 270 ± 5 kHz), which was recorded by a calibrated hydrophone. The conditions of sonication and the instruments used in the experiments with US were described in detail previously [13]. Since the heart was larger than the region of the focus (an ellipsoid of rotation with hemiaxes of 6.6, 2.7, and 2.7), only the apices of the ventricles were located in the zone of the focus. For this purpose, the heart was immersed to one-third of its vertical height below the level of the liquid in the experimental chamber. The pressure developed by the ventricle, the EG, subharmonic, and voltage applied to the generator were recorded by appropriate units of a "Gould Recorder 2800" automatic writer. Under the influence of US with an intensity of up to 8 W/cm^2 the rise of temperature in the focus did not exceed 1°C. It was measured by a miniature glass-covered thermal resistor about 0.7 mm in diameter.

EXPERIMENTAL RESULTS

A gradual increase in the intensity of US led to the development of cavitation, recorded as the appearance of a subharmonic, and to the development of extraexcitations in the isolated rat heart (Fig. 1). No effects of US could be found in the period before cavitation. We observed a similar effect of cavitation in cases when the intrinsic automatism of the heart was suppressed. It will be noted that (as follows from the shape of the EG) extraexcitations induced by US do not arise in any constant focus, but the location of these foci varies from one contraction to another. Immediately after discontinuation of the US the extraexcitations disappeared.

The experiment showed that the threshold of onset of cavitation (1.52 ± 0.18 W/cm^2) did not differ significantly from the threshold of onset of extraexcitation (1.35 ± 0.21 W/cm^2, $p > 0.05$). It can therefore be tentatively suggested that the cause of the extraexcitations in the experiments was cavitation.

Figure 1 also illustrates the negative inotropic effect of US. However, during the 2 min after discontinuation of US (duration of exposure 15-60 sec) the pressure developed by the left ventricle almost returned to its initial value, at 95.1 ± 4.3 mm Hg, and was only a little below the control values (100.8 ± 3.8 mm Hg, $p < 0.001$).

Besides the effects described above, acoustic cavitation also caused a temporary decrease in the frequency of contractions in the spontaneously contracting heart.

When pulses of US with an intensity of over 2 W/cm^2 were used, extraexcitations of the heart could be induced. The bottom part of Fig. 2 shows that pulses of US with a following frequency of 1 Hz, a duration of 50 msec, and intensity of 2.4 W/cm^2 leads to rhythmic excitation of the heart with suppressed intrinsic automatism. Comparison of the shape of the EG shown in the bottom part of Fig. 2 and the change in EG under the influence of US in Fig. 1 leads to the conclusion that extraexcitations during stimulation by US arise at different points of the heart. The developed pressure and EG during electrical stimulation of the heart are shown in the top part of Fig. 2. As would be expected, in this case the shape of the EG was unchanged from one contraction to another.

Figure 3 shows traces of the developed pressure and EG during electrical stimulation of the heart and during exposure to pulses of US, with a high winding speed. A trace of the EG-II shown in Fig. 3a, b enables the time delay of the appearance of excitation at the recording point relative to the moment of the electrical or acoustic stimulus to be judged. It can be seen that delay of excitation during acoustic stimulation varies sharply and may differ in tens of milliseconds from one stimulus to another. The same point is illustrated by Fig. 3c, d as by Fig. 3a, b, but for EG-I, which carries information about the pathways of spread of excitation over the heart. It can be seen that during acoustic stimulation (Fig. 3d), despite the relative constancy of the developed pressure, the shape of the EG curves varies from stimulus to stimulus. Together with the results in Fig. 3b, this evidently indicates a different character (from stimulus to stimulus) of excitation during exposure to US.

The main result of the present investigation is a demonstration that US can induce extraexcitation of the heart. This property is possessed both by continuous and by pulsed US. It was shown that the threshold intensity of US for the appearance of extraexcitation does not differ significantly from that causing cavitation. This coincidence confirms that the cause of the arrhythmogenic action of US is cavitation arising in the myocardium and (or) the perfusion solution.

The necessary conditions for the onset of cavitation are adequate intensity of US and the presence of a "cavitation nucleus," which may be any particle or air bubble [3, 5, 6]. The last condition does not allow the site of onset of cavitation to be predicted beforehand and it explains the fact that in real experience cavitation bursts arise at different places. The different origin of the extraexcitations in response to the action of US, revealed by the present investigation, thus also indicates indirectly that cavitation bursts are their source.

The results of the present investigation are in agreement with our previous data obtained on the papillary muscle [1], in which acoustic cavitation induced extraexcitation of the preparation, followed by a period of temporary inexcitability. We postulated that this inexcitability is a unique integral reversible response of the preparation to muscle damage. Similar responses have been described in the literature after injury to small isolated fragments of heart tissue [1]. The absence of a period of inexcitability in experiments on the whole heart can be explained by the fact that its mass is much greater (about 1000 times) and the microlesions induced by cavitation in different parts of the heart can cause only extraexcitations.

On the basis of these data we can explain the results of an investigation [11] which demonstrated the ability of US to defibrillate the dog heart in situ. Although in this study no acoustic measurements were undertaken, it can be postulated that the intensity of 10 W/cm^2 (500 kHz) used by these workers led to cavitation (possibly in the transmission medium) and, as a result, to excitation and defibrillation.

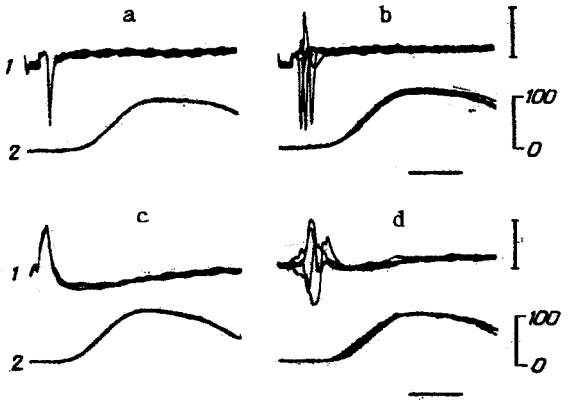

Fig. 3. EG (1) and pressure developed by left ventricle (2) during stimulation by electrical (a, c) and acoustic (b, d) pulses (four superpositions). a, b) EG-II; c, d) EG-I. Calibration of amplification for EG 5 mV, for developed pressure 100 mm Hg; time marker 50 msec.

The arrhythmogenic action of US which we found evidently raises the question of the safety of its use in diagnosis and treatment [2, 12]. However, before extrapolating the results of this study to the heart in vivo, it must be recalled that in our own experiments the heart was perfused with physiological saline saturated with oxygen, and not with blood.

Since the arrhythmogenic ability of US is based on cavitation it is interesting to compare the thresholds of its development for physiological saline oxygenated through a fine filter, and blood in equilibrium with the atmosphere. We undertook experiments of this kind and obtained the following results: in physiological saline cavitation appeared at an intensity of US of 1.4 ± 0.1 W/cm^2. When the chamber above the generator was filled with blood obtained from five rats and the threshold of cavitation was measured, it was found to be 13.3 ± 1.1 W/cm^2 (n = 6). The data given above show that under real conditions, when the heart is perfused with blood, it is much more difficult to induce cavitation. Evidently the relatively high threshold of cavitation in whole blood is linked with its high viscosity [8].

Incidentally, blood in vivo has a much higher cavitation threshold (more than 1.5 kW/cm^2 at 0.75-1.45 MHz [9]) than our own data. These differences are probably due to the fact that in our experiments, during filling of the chamber with blood air bubbles may have entered it and acted subsequently as cavitation "nuclei."

It can be postulated that the response of the myocardium to cavitation revealed by this investigation is the result of injury to cardiomyocytes near the cavitation focus and depolarization of neighboring cells and activation of the mechanically sensitive (stretch-activated) channels recently discovered in the cardiomyocyte membrane [4].

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