

ACTION OF ULTRASOUND ON STRENGTH OF CONTRACTION AND ACTION
POTENTIAL OF THE PAPILLARY MUSCLE OF THE RAT HEART

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The ability of ultrasound of therapeutic intensity (under 2 W/cm^2) to reduce postinfarction tissue damage and to prevent the development of arrhythmias in the dog myocardium was demonstrated previously [5, 6]. Data showing the defibrillating action of ultrasound (US) also have been obtained [11]. However, no adequate explanation of the therapeutic action of US has yet been found. Several studies of this problem have been made on isolated rat papillary muscles [3, 4, 8]. The workers cited showed that exposure to plane ultrasonic waves of therapeutic intensity increases the force of contraction and modifies rhythm-inotropic relations, and that the action of US is unconnected with any thermal effect. It has been suggested that US acts on binding and release of Ca^{++} ions and, as a result, modifies the inotropic state of the muscle [2]. In the investigations cited above the transmembrane potential of the cardiomyocytes was not measured during US irradiation.

The aim of this investigation was to study the action of US of therapeutic intensities on the membrane potential of the rat papillary muscle.

EXPERIMENTAL METHOD

Experiments were carried out on the papillary muscles of rats weighing 250-400 g, isolated from the right ventricle. The rats were killed by a blow on the head. Isolated muscles were placed in a perfusion chamber, made from transparent plastic, and were stretched between a force transducer and a fixing device. The preload corresponded to I_{max} . The volume of the chamber was 100 ml and the rate of perfusion 50 ml/min. For stimulation an electric field was used with silver electrodes located on both sides of the muscle. Pulses 3 msec in duration were applied at a frequency of 1 Hz. Before the experiment began the muscles were adapted for 1 h or more until the force of contraction became stable. A flat ultrasonic generator of the "Ul'trazvuk T5" medical therapeutic apparatus (USSR) was mounted in the side wall of the perfusion chamber. The muscle was placed opposite the generator and 10 mm away from it. The wall of the chamber opposite to the generator (distance between the walls 40 mm) was covered with mapped ultrasonic absorbing cloth. Frequency US equals 880 kHz, diameter of the emitting disc, 25 mm. The muscle was irradiated as was similar to that described in [2]. Perfusion was carried out with oxygenated (100% O_2) Tyrode solution of the following composition (in mM): NaCl - 150, KCl - 4, MgCl_2 - 0.5, CaCl_2 - 1, Tris-HCl - 10, glucose - 10. The temperature was maintained between 32 and 34°C, pH 7.4. Contraction of the muscle was measured by means of a 6MKh1B mechanical to electrical transducer (USSR) under isometric conditions. Action potentials (AP) were measured by "floating" microelectrodes with a resistance of under 20 MΩ, by means of a WP1 KS-700 microelectrode amplifier (USA). The temperature in the region of the preparation was recorded by an STZ-14V miniature thermistor (USSR). The ultrasonic field was monitored in the zone of the muscle by means of a hydrophone, based on a piezoceramic cylinder, 1 mm in diameter and in height. The above parameters were recorded on a Gould 2800S automatic writer and an oscillograph.

EXPERIMENTAL RESULTS

Ultrasound of therapeutic intensity evoked a positive inotropic effect in the papillary muscles of the rat. The force of contraction began to rise virtually at once and the force

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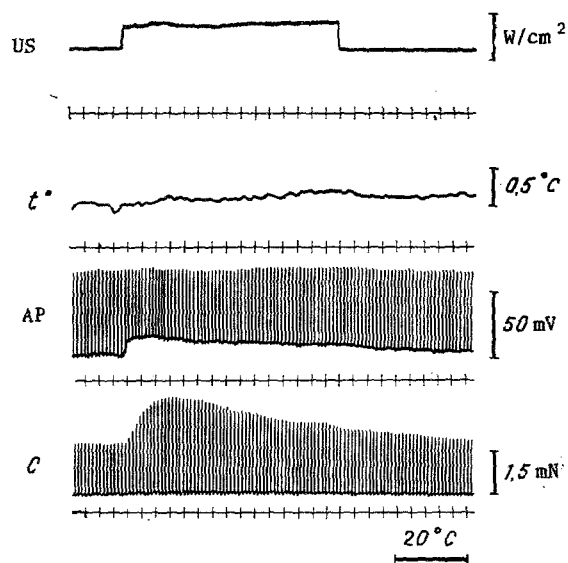


Fig. 1. Change in force of contraction (C), AP, and temperature (t°) near preparation during exposure to ultrasound (US). Amplitude of AP on trace reduced compared with real value, for highest frequency transmitted by automatic writer was 30 Hz.

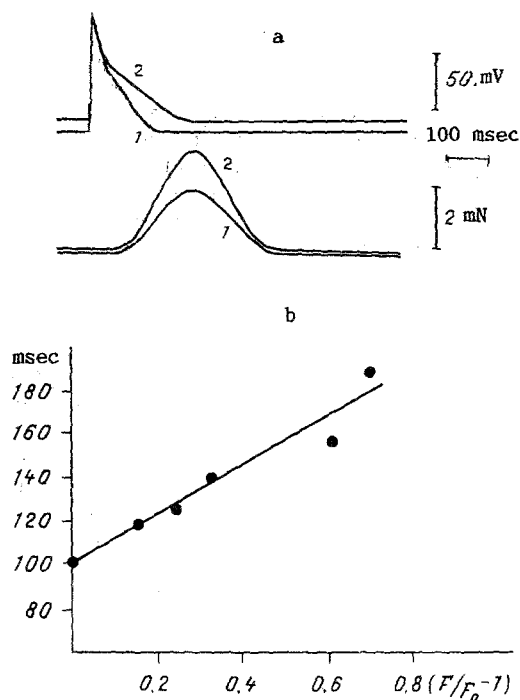


Fig. 2. Correlation between duration of AP at 10% level (AP_{10}) and increase in force during ultrasonic irradiation with an intensity of 0.6 W/cm^2 . a) Traces of AP (top) and force of contraction (bottom) in control (1) and at maximum of inotropic effect of US (2), b) correlation between increase in force (abscissa) and AP_{10} (ordinate); increase of force expressed in units relative to control value.

TABLE 1. Action of US on Duration of Plateau Phase of AP at Levels of 50 and 10% Depolarization and on Value of Resting Membrane Potential

Control, msec		US, msec		Depolarization, mV	US, W/cm ²
AP ₅₀	AP ₁₀	AP ₅₀	AP ₁₀		
44	124	48	170	15	2
25	130	30	165	11	0,6
19	75	24	145	7,5	0,6
40	125	42	155	8	0,4
29	80	33	137	12	0,6

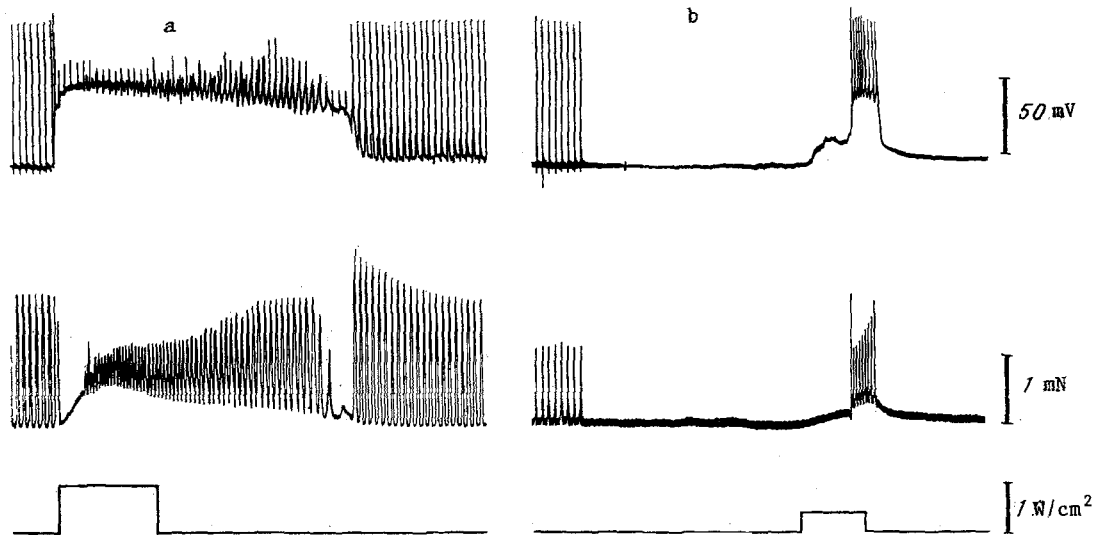


Fig. 3. Illustrations of inexcitability (a) and overexcitation (b) induced by US irradiation. Top - membrane potential, middle - force of contraction, bottom - intensity of US. Amplitude of AP somewhat reduced compared with actual value because of recording made on automatic writer with transmission band up to 60 Hz. In (a) fast waves in initial period of depolarization are stimulation artefacts; in (b), stimulation was stopped before exposure to US.

reached its maximal value after a few tens of seconds. A particular feature of the action of ultrasound was the transient nature of the effect: despite continued irradiation the force of contraction decreased after reaching a peak value (Fig. 1). Sometimes during continuous irradiation, the force of contraction rose to a maximum several times.

The muscles differed in their sensitivity to US. In seven of the 14 experiments the muscle responded by an increase of force to US with an intensity of 0.3-0.6 W/cm², whereas in others the threshold of response exceeded 0.8 W/cm², and two preparations were completely insensitive to the maximal intensity (2 W/cm²). The inotropic response of the muscle also changed. In three experiments the increase of force was more than 100% of the control value, and in the rest it averaged 50%. Monitoring the temperature in the immediate vicinity of the muscle showed that during irradiation with US for 3 min and with an intensity of 1 W/cm², the increase of temperature did not exceed 1°C.

Often a large inotropic response of the muscle to irradiation with US was accompanied by the development of a marked contracture. In the case of preparations highly sensitive to US, irradiation of above threshold intensity caused the development of a strong contracture and loss of excitability. The effects of US on contractility of the rat papillary muscle were reversible.

The effect of US was accompanied by membrane depolarization and by marked lengthening of phase 3, namely the plateau of AP, but the initial part of repolarization of AP showed

little change (Fig. 2a). We were unable to measure the maximal rate of rise of AP accurately because the large electrical artefact in front of AP, but a decrease in the rate of the leading edge of AP was observed against the background of strong depolarization during the action of US. The increase in amplitude of the contractile response against the background of US took place, incidentally, without any marked changes in the shape of contraction. Time to the peak of the contraction was increased, but not significantly, and its total duration was virtually unchanged (Fig. 2a).

Table 1 gives data on the action of US on the duration of the AP plateau at levels of 50% (AP_{50}) and 10% (AP_{10}) on depolarization, obtained on five different muscles. The two columns on the left illustrate parameters of AP in the control, the middle two columns during the peak of inotropic action of US. The two columns on the right show corresponding values of depolarization of the resting membrane potential and threshold intensity of US. Changes in the duration of AP_{10} (phase 3) can be seen to exceed those in AP_{50} by an order of magnitude.

Exposure of the muscle to US led in the first place to rapidly developing membrane depolarization and to lengthening of AP_{10} . The increase in the force of contraction began 1-2 sec after the beginning of the changes in AP, and later, lengthening of AP and the increase of force took place simultaneously. The relationship between the increase of force of contraction and the duration of AP_{10} is shown in Fig. 2b. Switching off the ultrasound was followed by steady restoration of the parameters of AP and contraction.

As already stated, high-intensity US (2-3 times above the threshold) evoked strong contracture, depolarization, and inexcitability of the muscle. As a rule, pacemaker activity developed in the depolarized preparation, in the form of slow, self-induced oscillations (Fig. 3a). Pacemaker activity could also be evoked by US in an unstimulated preparation (Fig. 3b). Pacemaker activity against the background of US-induced depolarization began with the development of a normal AP, after which the membrane potential switched to a new and higher level of depolarization, at which spontaneous AP developed.

The results confirmed data [2-4] on the positive inotropic action of US on the force of contraction of the rat myocardium. However, an essential difference of our own results was the transient nature of the effect of US and the contracture which accompanied its action. Like the authors cited above, we do not associate the effect of US with its thermal action. Rapid heating of the myocardium, moreover, leads to the opposite effect namely a decrease in the duration of AP [7].

The action of US was accompanied by membrane depolarization and lengthening of AP_{10} . AP_{10} is known to reflect electrogenic Na-Ca exchange in the myocardium, and activation of Na-Ca exchange under conditions of calcium overloading is accompanied by lengthening of AP_{10} . Meanwhile, factors increasing the Ca current lead to an increase in the duration of AP_{50} [9, 10]. The authors cited demonstrated positive correlation between the duration of AP_{10} and the amplitude of the contractile response. In the present investigation similar correlation was found under the influence of US, suggesting that the inotropic effect is connected with calcium overloading of the muscle. Since the weak sensitivity of AP_{50} to the action of US is evidence of small changes in the Ca current, it can be tentatively suggested that the increase in the force of contraction was largely determined by endogenous Ca. Membrane depolarization under the influence of US can be explained by activation of the electrogenic Na-Ca exchange in the presence of a strong increase in the intracellular Ca concentration. However, there are other possible causes of depolarization notably blocking of the potassium channels of the cell membrane by the action of US.

We have no reason to suppose that the inotropic effect of US is due to injury to the muscle (for example, due to a cavitation process [1]), first, because the effects of US were reversible and, second, as follows from [11], because US with an intensity of 10 W/cm², acting for 2 min, cannot induce appreciable histological changes in the myocardium.

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EFFECT OF LEVAMISOLE (DECARIS) AND SODIUM NUCLEATE ON THE BLOOD ANTIOXIDATIVE SYSTEM OF GUINEA PIGS INHALING PAPRIN

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One of the causes of the decrease in nonspecific resistance of a patient requiring immunocorrection may be long exposure to products of microbiological synthesis and, in particular, paprin [1]. An important role in the mechanism of action of stimulators of immunity is played by activation of phagocytosis. It has been shown, in particular, the decaris and sodium nucleate can increase the phagocytic activity of macrophages and can also stimulate for formation of oxygen radicals by phagocytes [4, 6]. However, excessive production of oxidizing agents may lead to death of the phagocytic cell and to damage to surrounding tissues [8]. It is accordingly interesting to study the state of the antioxidative system (AOS) during administration of immunostimulators, for it protects cellular structures against the damaging action of oxidizing agents and free radicals and is an important component of the mechanisms of nonspecific resistance [11].

The aim of this investigation was to study the effect of decaris and sodium nucleate on the state of the OAS: the thiol-disulfide balance (SH/S-S) of the blood, lipid peroxidation (LPO), the plasma superoxide dismutase (SOD) activity, and glucose-6-phosphate dehydrogenase (G6PDH) and catalase activity of the erythrocytes, on a model of inhalation of paprin.

EXPERIMENTAL METHOD

Experiments were carried out on mature male guinea pigs weighing 250-300 g. All the animals were divided into four groups with eight individuals in each group: intact animals and three experimental groups. The experimental animals were placed in poisoning chambers where they inhaled paprin in a concentration of 3 mg/m³ for 4 weeks, 4 h a day, 5 days a week; the intact animals were placed in similar chambers, ventilated with pure air. The experimental animals of the control group did not receive immunostimulators.

Animals of group 2 were given levamisole (decaris, from Gedeon Richter A. O., Hungary) in accordance with the following schedule: 2.5 mg/kg in 0.15 M NaCl solution, subcutaneously,

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