It can be postulated that membrane-bound Ca^{2+} plays a key role both in the regulation of energy processes and in the stabilization of permeability of the tumor cell MC membrane. What distinguishes tumor MC from liver MC is that even low concentrations of Ca^{2+} lead to an increase in membrane-bound Ca^{2+}. As a result of this, the viscosity of the membrane may be increased and activity of membrane-bound enzymes and ion-transporting systems increased.

The high concentration of membrane-bound Ca^{2+} may be determined by the abnormal phospholipid composition of the tumor MC membranes, which are distinguished by higher affinity for Ca^{2+} [4]. Low activity of ruthenium-sensitive Ca^{2+} transport, in turn, readily explains the long familiar phenomenon of lowering of the steady-state Ca^{2+} level in tumor cells.

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MECHANICAL NOISE OF THE MYOCARDIUM AS AN INDICATOR OF ACTIVITY OF THE SARCOPLASMIC RETICULUM

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Mechanical noise (MN, spontaneous oscillations of tone) in the mammalian myocardium was first described and studied about 10 years ago [8]. During this period research into relations between MN and oscillations of intracellular calcium ion concentration [4, 13], the level of contracture of the preparation [3], fluctuations of membrane potential [10], and also the ability of a myocardial preparation to undergo spontaneous excitation [2] have been published. It has been shown [1, 4, 13] that substances which inhibit activity of the sarcoplasmic reticulum (SPR) lead to disappearance of MN. This suggested that the appearance of MN is linked with activity of SPR. We know that the rate of relaxation of the myocardial contractile response can be used as an indicator of the ability of SPR to bind calcium. It was therefore interesting to compare changes in MN and the rate of relaxation under various conditions modulating activity of SPR, and the investigation described below was undertaken for this purpose.

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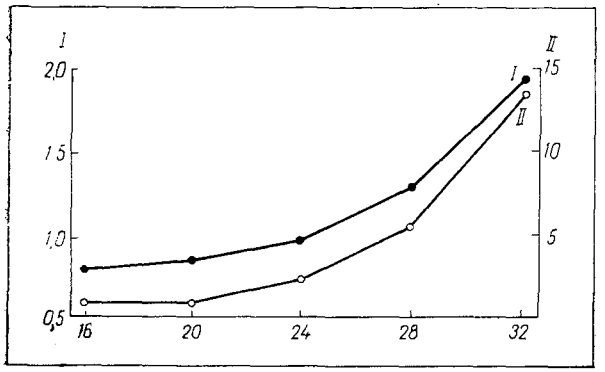

Fig. 1. Effect of temperature on frequency of MN (I) and rate of relaxation of contractile response (II) (experiment on March 30, 1984). Abscissa, temperature (in °C); ordinate: I) frequency of noise (in Hz), II) rate of relaxation (in sec^{-1}).

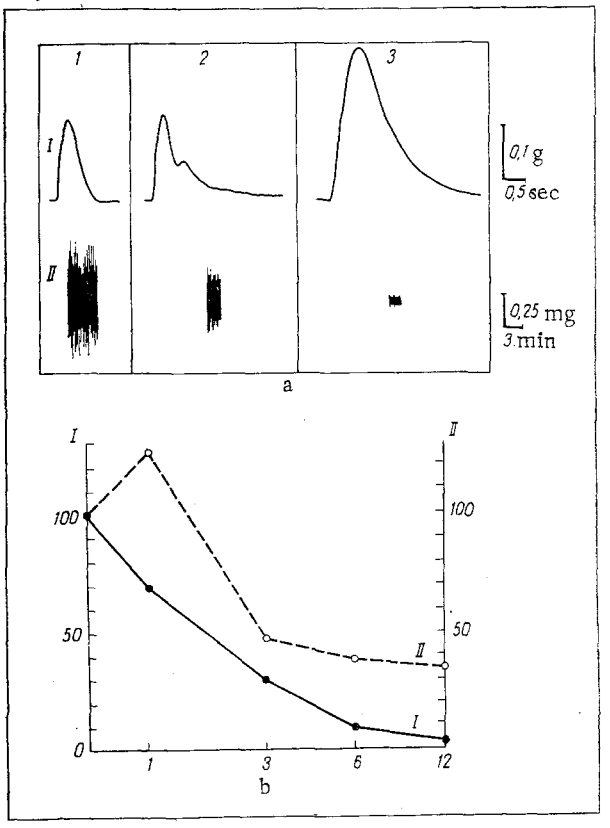

Fig. 2. Action of caffeine on contractile response and MN (experiment on March 1, 1984). Abscissa, dose of caffeine (in mM); ordinate: I) amplitude of noise (in %), II) rate of relaxation (in %). a) Experimental traces of contractile response (I) and MN (II) in control (1) and under the influence of caffeine in doses of 1 mM (2) and 6 mM (3); b) dose–effect curves for the action of caffeine on amplitude of MN (I) and rate of relaxation of contractile response (II). Values of noise amplitudes and relaxation rate normalized relative to corresponding values in control.

EXPERIMENTAL METHOD

Experiments were carried out on capillary muscles isolated from the right ventricle of rats weighing 300-400 g, which were killed by a blow on the head. The animals' heart was quickly placed in modified Tyrode's solution: NaCl 150 mM, KCl 4 mM, $MgCl_2$ 0.5 mM, Tris-HCl 25 mM, $CaCl_2$ 5.4 mM, glucose 10 mM, pH 7.4. The solution was oxygenated with 100% O_2. Except where indicated to the contrary, the experiments were carried out at room temperature (20-22°C). The papillary muscles (diameter 0.2-0.5 mm) were placed in a plastic experimental chamber with a volume of 7 ml. The muscles were stimulated by an electric pulse with a

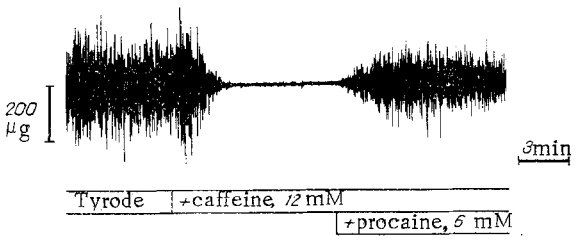

Fig. 3. Competition between caffeine and procaine in their action on MN (experiment on December 7, 1983).

frequency of 42 pulses/min (ESL-2 stimulator, USSR), using silver electrodes. After the force developed by the muscle had become stabilized, the muscle was stretched until the tension in it was 1 g/mm^2. This degree of preloading corresponded to the maximal value of the contractile response of the muscle. MN and the contractile response were recorded by means of low-noise versions of the 6MKhIB mechanotron (USSR). The amplitude of technological noise of the experimental apparatus was 10-15 μg (from peak to peak). MN was obtained from the resting voltage signal after amplification within the frequency band from 0.25 to 5 Hz, using a modification of the U7-1 amplifier (USSR) for this purpose. The data were recorded on a two-channel automatic recorder (Gould Brush 2400, USA) and tape recorder (Hewlett Packard 3964A, USA). The average frequency $f_{1/2}$ of MN was measured from its autocorrelation function, obtained with an SA1-43A analyzer (Honeywell, USA) as described in [11]. The value of $f_{1/2}$ was determined as $(2\pi \cdot t_{1/2})^{-1}$, where $t_{1/2}$ is the delay during which the autocorrelation function falls by 50%. The amplitude of MN was calculated from the amplitude of the autocorrelation function. The rate of relaxation was determined as $(T_{1/2})^{-1}$, where $T_{1/2}$ is the half-relaxation time of the contractile response.

In some experiments, simultaneously with recording MN, the transmembrane potential difference was measured by the usual microelectrode technique.

EXPERIMENTAL RESULTS

A fall of temperature led to a decrease in activity of calcium-dependent ATPase of SPR [12], on account of which the relaxation phase of the contractile response was slowed. Changes in the average noise frequency $f_{1/2}$ and the rate of relaxation of the contractile response depending on temperature are illustrated in Fig. 1. As the results show, these relationships are very similar, suggesting that common mechanisms lie at the basis of these phenomenona.

We know that caffeine, in millimolar concentrations, inhibits Ca^{2+} binding in SPR [6]. As a result the relaxation phase of the contractile response was slowed, and the amplitude of MN reduced. However, low concentrations of caffeine (0.5-1 mM) in these experiments did not slow, but sometimes actually quickened the relaxation phase and, at the same time, always reduced the amplitude of MN (Fig. 2a). For instance, under the influence of 1 mM caffeine the rate of relaxation increased on average by 19 ± 9%, but the amplitude of MN decreased to 58 ± 6% (n = 3). Higher caffeine concentrations reduced both the relaxation rate and the amplitude of MN. Dose–effect curves for the action of caffeine on relaxation rate and on MN amplitude thus diverged appreciably at low caffeine concentrations (Fig. 2b). The inhibitory effect of caffeine on the amplitude of MN was thus detectable at lower concentrations than its slowing action on the relaxation phase of the contractile response. Incidentally, all changes in amplitude of MN took place with an unchanged value of the resting potential and transmembrane potential, and they therefore could not be interpreted as the result of a change in intracellular Ca^{2+} [3]. This suggests that the amplitude of MN is a more sensitive indicator of the action of caffeine on the SPR than the relaxation phase of the contractile response.

The biphasic appearance of the dose–effect curve for the action of caffeine on the rate of relaxation of the contractile response can be explained on the grounds that caffeine, in millimolar concentrations, is a blocker of phosphodiesterase. The decrease in phosphodiesterase activity in the presence of caffeine causes the cAMP level to rise and the inward Na, Ca-current to increase slowly. This evidently ought to mask the slowing effect of caffeine on the relaxation phase.

Using the amplitude of MN as an indicator of SPR activity, it is possible to study the action of various substances on SPR in mice in a state of rest. This method of evaluating

SPR activity under experimental physiological conditions is therefore the only possible method available in cases when the test substance (a local anesthetic, for example) makes the muscle inexcitable.

We know from the literature [5, 7] that some local anesthetics abolish caffeine-evoked contractures of skeletal muscles. Competitive relations between caffeine and local anesthetics also have been demonstrated in experiments on vesicles of SPR isolated from skeletal muscles [9]. Unlike in skeletal muscles, caffeine does not induce any appreciable contracture in mammalian myocardial preparations. All these factors made it impossible to demonstrate the effect of local anesthetics on SPR of the heart under ordinary experimental physiological conditions.

Data showing competitive relations between caffeine and procaine in heart muscle are given in Fig. 3. Procaine, if added to the perfusion fluid a short time after caffeine, restored the amplitude of MN virtually to the control level (before addition of caffeine). Similar restoration of the amplitude of MN was found under the influence of amethocaine (1 mM) and mexiletine (0.5 mM). Unlike the above-mentioned local anesthetics, lidocaine (6 mM) and trimecaine (6 mM) did not restore MN when reduced initially by caffeine. It must be particularly emphasized that none of the local anesthetics had any action on the amplitude of MN in the control (i.e., in the absence of caffeine).

Local anesthetics evidently compete with caffeine in their action on the myocardial SPR. It is interesting to note that lidocaine, which does not abolish caffeine contracture in skeletal muscles [5], likewise does not compete with caffeine in its action on MN in the myocardium. This fact suggests that the mechanism of competition between caffeine and local anesthetics is identical for skeletal and cardiac muscle.

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