BEAT TO BEAT VARIATION OF CONTRACTILE RESPONSES OF SMALL FRAGMENTS OF RAT PAPILLARY MUSCLE

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The amplitude of the contractile response of the myocardial cell is mainly determined by the amount of increase in the calcium ion concentration $[Ca^{++}]_i$ in the myoplasm during excitation of the cell. The main sources of Ca^{++} (contraction activators) in the mammalian myocardium have been shown to be the calcium channels of the sarcolemma, electrogenic sodium-potassium exchange, and the sarcoplasmic reticulum (SR) [1]. If the frequency of excitation is unchanged, the quantity of Ca^{++} entering the cardiomyocytes by the first two of these pathways must evidently be constant, for it depends only on the gradient of the corresponding ions on either side of the sarcolemma and on the kinetics of the channel (or carrier). At the same time, we know that Ca^{++} release from SR can take place spontaneously even in the resting cell [4, 8]. These spontaneous variations in $[Ca^{++}]_i$ are the cause of oscillations of tone (mechanical noise), which can make a considerable contribution to the resting voltage of the preparation [3, 11] and fluctuations of membrane potential, changing the excitability of the cell membrane [9, 13]. As a result, as has been shown in experiments on isolated rat cardiomyocytes, the amplitude of the contractile response fluctuates considerably when the frequency of stimulation remains unchanged [6]. However, under these same conditions the amplitude of contractions of the rate papillary muscle remains unchanged from stimulus to stimulus. The absence of fluctuations of amplitude of the contractile response of the muscle when the frequency of stimulation is unchanged can be explained as follows: mechanical activity of enzymically isolated single cardiomyocytes differs significantly from activity of the intact cells; contraction of the muscle is equal to the sum of the contractile responses of several thousand myocytes, as a result of which the relative contribution of the fluctuations is considerably reduced.

In the present investigation, to determine the possible cause of the differences between contractions of the muscle and of isolated cardiomyocytes mentioned above, contractile responses of small fragments from the surface of the papillary muscles were studied.

EXPERIMENTAL METHOD

Experiments were carried out on papillary muscles isolated from the right atrium of the rat heart. Throughout the experiment the muscles were kept in oxygenated solution of the following composition: NaCl — 150 mM, KCl — 4 mM, $MgCl_2$ — 0.5 mM, Tris-HCl — 10 mM, $CaCl_2$ — 2.5 mM, pH 7.4, temperature 35 ± 1°C. The diameter of the muscle was about 0.4 mm and its length 2.5-3 mm. Preloading of the muscle corresponded to the maximal amplitude of the contractile response and ranged between 1 and 2 mN. The muscle was stimulated by an electric field with pulses with a duration of 3 msec and an amplitude of twice the threshold value. The apparatus used to study mechanical activity of the papillary muscle is shown schematically in Fig. 1. The 6MKh1B mechanotrons were used as strain gauge transducers. One end of the muscle was fixed immovably, while the other end was connected to the strain gauge transducer T_1, which recorded the contractile response of the muscle. The rigidity of T_1 together with its rod C_1 was only 40 N/m, and the relative shortening of the muscle during excitation varied from 2 to 5% of its initial length. The low rigidity of T_1-C_1 was chosen in order to maximize movement of the internal segments of the muscle, the shortening of which was measured by means of T_2 and T_3. To record displacement of two points on the muscle surface, T_2 and T_3 were

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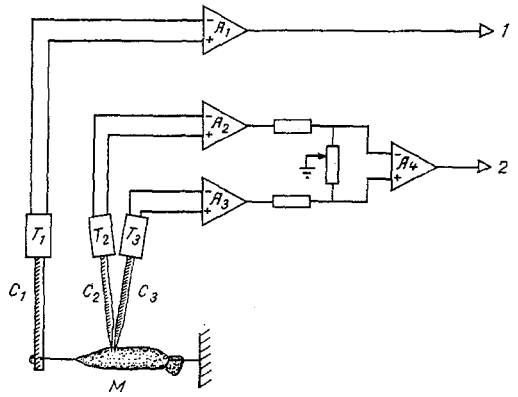

Fig. 1. Apparatus for studying mechanical activity of the papillary muscle. T_1, T_2, T_3) Strain gauge transducers; A_1, A_2, A_3, A_4) amplifiers; M) muscle; R) resistance compensating difference in sensitivities of T_2 and T_3. Devices C_1, C_2, and C_3, connecting strain gauge transducers to muscle, are shaded. 1) Force of contraction; 2) local contractile response.

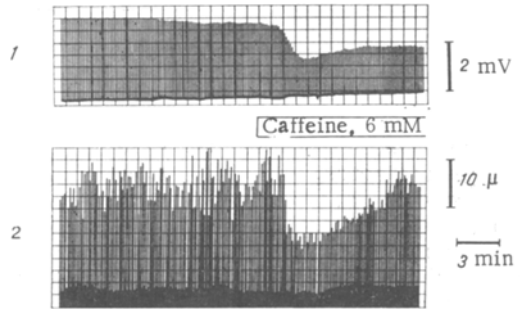

Fig. 2. Action of caffeine on force of contraction (1) and on local contractile response (2) of rat papillary muscle (experiment on December 12, 1985).

used: a glass micropipet with a tip about 1 μ in diameter was fixed to their movable anode. On contact of the micropipets with the muscle surface, signals at the output of A_2 and A_3 were proportional to the displacements of the corresponding points of contact. To obtain a signal at the output of the amplifier A_4 proportional to shortening of the muscle fragment between the points of contact (the local contractile response), the sensitivity of T_2 and T_3 was equalized by changing the position of the midpoint of the potentiometer R. Points of contact of the micropipet were located along the length of the muscle in its central part, 250 μ apart. The high compliance of the micropipet tip (rigidity under 0.5 N/m) enabled the local contractile response to be recorded without distorting mechanical activity of the remaining part of the preparation.

EXPERIMENTAL RESULTS

The results of simultaneous recording of the local contractile response and the force of contraction of the rat papillary muscle during stimulation with a frequency of 0.16 Hz are illustrated in Fig. 2. Clearly the muscle fragment whose contractile response was being measured shortened by approximately 1/10 of its initial length (250 μ), and fluctuations of the local contractile response could amount to one-third of its average value. Similar fluctuations of the force of contraction of the whole muscle were not found. These fluctuations are not the result of a change in the threshold of excitability of the test fragment, for an increase in the strength of stimulation did not affect their magnitude.

Addition of caffeine, which exhausts the calcium reserves in SR [5], to the perfusion fluid led to an abrupt and considerable decrease in fluctuations of the local contractile response (Fig. 2).

In the presence of a 6 mM solution of caffeine, fluctuations of the contractile response decreased from 3.0 ± 0.4 μ (control) to 0.83 ± 0.03 μ ($P < 0.01$). This kind of effect of caffeine indicates that these fluctuations reflect spontaneous cyclic activity of SR.

The results demonstrated the important contribution of spontaneous oscillations of $[Ca^{++}]_i$ to the contractile activity of small fragments of rat myocardium. The evident cause of these fluctuations was dependence of the amplitude of the contractile response of the cardiomyocyte on the phase of spontaneous activity of SR in which the electrical stimulus was applied. A similar dependence of $[Ca^{++}]_i$ was found in [7] in experiments on cells of Purkinje fibers during calcium overloading due to glycoside poisoning.

Incidentally, rat cardiomyocytes possess spontaneous mechanical activity, even in the absence of calcium overloading. The rat myocardium is thus the most suitable object with which to study fluctuations linked with spontaneous activity of SR [10]. It can be postulated that this feature of the rat myocardium is connected with the high sensitivity of the mechanism of Ca-Ca release from SR to Ca^{++} [8].

The absence of fluctuations of amplitudes of the contractile response of the whole papillary muscle under these same conditions can be explained on the grounds that fluctuations in the different fragments composing it are not synchronized and may differ in sign. As a result the ratio of amplitude of the contractile response to the amplitude of fluctuations ought to increase with an increase in weight of the myocardial preparation.

In the resting state oscillations of different regions of the rat papillary muscle likewise are not synchronized [2]. Nonsynchronization also is shown by comparison of the amplitudes of aequorin signals during calcium contracture and of the contractile response of equal magnitude [12]. It has been suggested that these oscillations of $[Ca^{++}]_i$ in the resting state can make a marked contribution to the resting voltage of the preparation [11]. The onset of nonsynchronized oscillations of $[Ca^{++}]_i$ during repetitive stimulation of the muscle must evidently be accompanied by a reduction in the force of its contractions, for in each contractile response virtually none of the cells which spontaneously contracted not long before electrical stimulation will take part. It can be postulated that the decrease in the force of contraction during calcium overloading is similar in nature [14].

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