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CONTRACTILE RESPONSE OF THE MYOCARDIUM OF CARDIAC PATIENTS TO CHEMICAL SCARIFICATION OF THE CELL MEMBRANE

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Strips of myocardium from the auricles of the hearts of patients with mitral stenosis (MS) and patients with cardiac septal defects (CSD) were treated with a solution of EDTA (3 mM) to increase the permeability of the cell membrane (scarification). In a 3 mM solution of ethylenehexaminetetraacetic acid (EHTA), against the background of increased permeability of the membrane to the Ca-EHTA complex, whereby the Ca^{2+} concentration in the myofibrils can be regulated between 10^{-9} and 10^{-4} M, a mechanical response of the contractile proteins to a change in Ca^{2+} concentration was recorded. Despite identical threshold concentrations ($5 \cdot 10^{-8}$ M) and saturation concentrations (10^{-4} M) of Ca^{2+}, strips from patients with MS were found to develop a maximal force per unit cross section of the strip only half as high as preparations from patients with CSD, which suggests a probable lesion of the contractile proteins in the hearts of patients with MS. The ratio between the amplitudes of contraction under conditions of complete calcium activation of the contractile proteins and a single isometric contraction for preparations obtained from patients with MS was 8-10 and from patients with CSD 4-5. It is suggested that this is the result of more profound changes in the apparatus of electromechanical coupling of the myocardium of patients with MS.

KEY WORDS: heart failure; calcium ions; contractile proteins.

Comparison of the parameters of isometric contractions of the mycodardium of the atrial auricles of patients with mitral stenosis (MS) and cardiac septal defects (CSD) reveals certain significant differences. On average the time taken to reach the maximum of the isometric contractions has been found to be appreciably longer in the myocardium of patients with MS than in the myocardium of patients with CSD. In MS, moreover, the normal response of the myocardium to an increase in the frequency of stimulation is modified much more often than in CSD, and this is reflected in the total or partial suppression of the Bowditch phenomenon [1, 3].

In view of data indicating a disturbance of the function of the sarcoplasmic reticulum (SR) in cardiac failure [2] it has been concluded that the severer disturbances of the contractile function of the myocardium

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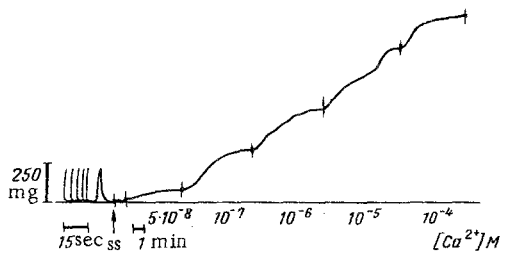

Fig. 1. Contractile response of myocardium of patient with CSD (ventricular septal defect) to change in concentrations of Ca^{2+}. Several isometric contractions shown at beginning of record. ss) Time of addition of scarifying solution. Vertical lines on curve represent artefact of change of solution.

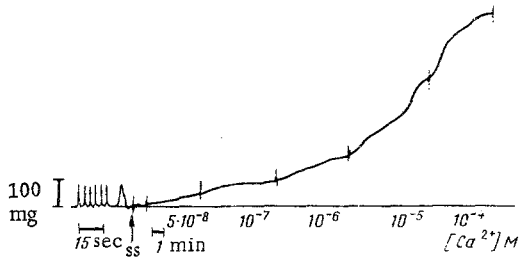

Fig. 2. Contractile response of myocardium of patient with MS. Legend as in Fig. 1.

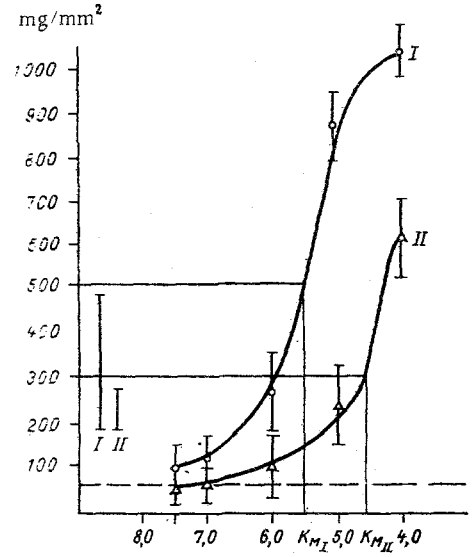

Fig. 3. Calcium-mechanical relationship in atrial myocardium of patients with CSD (I) and MS (II). Abscissa, negative logarithm of Ca^{2+} concentration in DHTA solution; ordinate, force developed (in mg/mm^2 cross section of strip).

in patients with MS are attributable to damage to the apparatus controlling contraction and relaxation in heart muscle. Although the increase in the relaxation time of the myocardium and suppression of the Bowditch phenomenon are evidently directly connected with a disturbance of the function of SR, the reduction in amplitude of isometric contractions may also be connected with a disturbance of the function of the contractile proteins.

It was accordingly decided to study the contractile response of myocardial proteins of the atrial auricles of patients with MS and CSD to different concentrations of Ca^{2+} in order to discover whether the reduction in the force of isometric contractions is due entirely to deficient calcium activation or (and) to a disturbance of the function of the contractile proteins.

EXPERIMENTAL METHOD

Experiments were carried out on thin strips of myocardium (3-4 mm long, cross-section 1-1.2 mm^2) of the trabeculae of the atrial auricles, obtained at biopsy during cardiac surgical operations – mitral commissurotomy (18 preparations) – and correction of congenital heart defects (of the atrial and ventricular septum – 17 preparations). The specimens were placed in a constant temperature transparent plastic chamber with a capacity of 1 mm^3, through which continuously flowed a solution of the following composition (in mM): NaCl 131, KCl 4.5, $NaHCO_3$ 11, KH_2PO_4 0.72, $MgCl_2$ 0.25, $CaCl_2$ (see below), glucose 11 (pH 7.1, temperature 35 ± 0.5°C). The solution was constantly saturated with a mixture of 95% O_2 and 5% CO_2. Mechanical activity was recorded by means of a 6M × 2B mechanotron. To study the length–force relationship the chamber was equipped with a device for controlled stretching of the preparation with an accuracy of 0.01 mm. Supraliminal square pulses, 7 msec in duration and with a period of 3 sec, were applied through massive platinum electrodes from an ÉSL-2 stimulator. Contractions were recorded isometrically. Before the experiment began the strip was "run in" for 1 h until contractions of constant magnitude were obtained. The length–force characteristic was then determined, so that the value of L_{max} could be obtained (the length of strip corresponding to the greatest force of contraction developed).

To study the response of the contractile proteins to various Ca^{2+} concentrations conditions had to be created for maintaining an assigned Ca^{2+} concentration in the myofibrils. This was done by chemical scarification (or destruction) of the cell membranes, so that the substance ethylenehexaminetetraacetate (EHTA), by means of which the Ca^{2+} concentration in the myofibrils could be regulated from 10^{-9} to 10^{-4} M, could be introduced into the sarcoplasm. A solution of the following composition (in mM) was used for scarification: EDTA (ethylenediaminetetraacetate) 3, ATP 5, Tris-buffer 10, KCl 140; temperature 22°C, pH 6.5 ± 0.1; the specimen was kept in the solution for 1 h. On the addition of EDTA to the surrounding solution, the concentrations of Ca^{2+} and sulfates in the myocardial cell increase [4]. This happens because EDTA, which binds bivalent cations by chelation, interacts with the Ca^{2+} of the cell membrane and removes it from the membrane structure, with the result that the permeability of the membrane is considerably increased to substances with relatively high molecular weight (EHTA and ATP, for example). The calcium-mechanical relationship was investigated in a solution of the following composition (in mM): EHTA 3, ATP 5, Tris-buffer 10, KCl 140, $MgCl_2$ 1, and with changing concentration of Ca^{2+} in the following order: 10^{-9}, $5 \cdot 10^{-8}$, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M, pH 6.5 ± 0.1, temperature 22°C. EHTA selectively binds Ca^{2+} ions, but the Ca-EHTA complex thus formed is much less stable than the Ca-EDTA complex, and is able to exchange its Ca^{2+} with the contractile proteins. Under these circumstances EHTA behaves as a buffer and, in a calcium-free medium, it lowers the Ca^{2+} concentration in the contractile proteins to 10^{-9} M. Starting from a threshold concentration ($5 \cdot 10^{-8}$ M), the strips reacted to a change in the Ca^{2+} concentration in the solution by the development of contraction at a speed which depended on the rate of diffusion of the Ca-EHTA complex over the cross section of the strip. The magnitude of the steady-state contraction was taken as a measure of the mechanical response to that particular Ca^{2+} concentration.

EXPERIMENTAL RESULTS

Strips with a scarified membrane were placed in calcium-free solution containing EHTA-buffer, thus eliminating the microconcentrations of Ca^{2+} that may have remained in the chamber after rinsing. Under these circumstances, in the presence of Mg^{2+} ions and ATP, complete relaxation of the muscle required for recording the background contraction at length L_{max} could be obtained.

The threshold concentration of Ca^{2+} for all the preparations was $5 \cdot 10^{-8}$ M. The rate of development of contraction in the strip depended not only on the rate of diffusion of the Ca-EHTA complex toward the myofibrils, but also on the quality of scarification of the membrane. In well processed specimens the contraction

stabilized toward 6-7 min after the change of solution. The results of experiments carried out on preparations of the atrial auricles of patients with CSD and MS are given in Figs. 1 and 2.

It is interesting to note that in both cases the threshold of activation was $5 \cdot 10^{-8}$ M, and saturation was observed at 10^{-4} M Ca^{2+}. However, the course of the curves of development of contraction differed significantly. The calcium-mechanical relationship and the sensitivity of the contractile proteins of the myocardial preparation obtained from patients with MS and CSD are illustrated in Fig. 3. Dependence of the mechanical response of the Ca^{2+} concentration for preparations of both groups followed an S-shaped curve, in agreement with data in the literature [5]. However, the maximal amplitude of contraction developed in a solution with a Ca^{2+} concentration of 10^{-4} M in preparations of myocardium from patients with CSD was on average twice the maximal amplitude of contraction of the contractile proteins of myocardial preparations from patients with MS. Averaged amplitudes of single contractions obtained with the same myocardial preparations before scarification are shown to the left of the curves. The ratio of the maximal amplitude of contraction of the strip in a solution containing 10^{-4} M Ca^{2+} to the amplitude of a single contraction before scarification of the membrane averaged 4-5 for preparations of myocardium from patients with CSD, but 8-10 for patients with MS, and in some cases it actually reached 12. Significant differences were observed in sensitivity of the contractile proteins to Ca^{2+} ions. The index K_M, numerically equal to the Ca^{2+} ion concentration capable of inducing half of the maximal effect in the preparations, was chosen as the criterion of sensitivity. For strips of myocardium from patients with CSD this index was $5 \cdot 10^{-6}$ M Ca^{2+}, whereas for myocardial preparations from patients with MS its mean value was $5 \cdot 10^{-5}$ M.

To sum up, the following conclusion can be drawn: the magnitude of maximal contraction under conditions of complete calcium activation of the contractile proteins is twice as high for myocardial preparations from patients with CSD as the corresponding values for strips of patients with MS. The sensitivity of myocardial strips of patients with MS to Ca^{2+} is also reduced. These results suggest a probable lesion of the contractile proteins in cardiac failure of rheumatic etiology. The increase in the ratio between the amplitude of contraction in medium with 10^{-4} M Ca^{2+} and the amplitude of isometric contraction for strips from the heart of patients with MS is probably the result of incomplete calcium activation of the contractile proteins in the course of a single contraction, evidence of possible damage to the apparatus of electromechanical coupling in the hearts of patients with MS.

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