CONTRACTURE OF MYOCARDIAL FIBERS OF THE FROG VENTRICLE DURING HIGH-FREQUENCY STIMULATION AFTER BLOCKING OF THE CALCIUM CHANNELS BY MANGANESE IONS

S. I. Zakharov, A. I. Undrovinas, and L. V. Rozenshtraukh

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The strength of contraction of a stimulated (0.5 Hz) strip of frog ventricular myocardium was reduced to 3-5% of its original value by perfusion with Ringer's solution containing 2.5 mM Mn^{2+}; the duration of the action potential under these circumstances was sharply reduced. An increase in the frequency of stimulation to 5 Hz led to the development of a contracture, the magnitude of which reached 30% of the original strength of contraction recorded in normal perfusion solution. The amplitude of the contracture was more than doubled by the addition of ouabain ($2 \cdot 10^{-6}$ g/ml). In analogous experiments with La^{3+} (2.5 mM) contracture did not develop in response to an increase in the frequency of stimulation; ouabain was ineffective in these experiments. It is postulated that the contracture induced in the presence of Mn^{2+} is due to nonelectrogenic Ca^{2+} transport in the muscle fibers.

KEY WORDS: manganese; lanthanum; high-frequency stimulation; contracture.

Mn^{2+} ions depress the mechanical activity of the myocardium by inhibiting the calcium current through the cell membrane [8]. Recently, however, it has been shown for the myocardium of the guinea pig ventricle that, in the presence of Mn^{2+} and in response to a constant frequency of stimulation (0.7 Hz) the heart muscle develops tonic contraction. This contracture is due to the ability of Mn^{2+} to penetrate into the muscle cells and displace Ca^{2+} from the sarcoplasmic reticulum [1].

The object of this investigation was to study the relationship between the frequency of excitation and the effect of Mn^{2+} on mechanical activity of the myocardium of the frog ventricle, which has been shown [7] to be deficient in sarcoplasmic reticulum.

EXPERIMENTAL METHOD

Small strips (15-20 mg) of ventricle from the frog (Rana temporaria) heart were placed in a 1-ml perfusion chamber. One end of the strip was fixed to bipolar stimulating electrodes and the other was connected to a 6MKhlS mechanical–electrical transducer (USSR). The preparation was stimulated by square electrical pulses with a duration of 3 msec, a frequency of 0.5 Hz, and an intensity of 1.5-2 thresholds of excitation. Mechanical activity of the strips was recorded on an oscilloscope (5103 ND-11, Tektronix, USA) equipped with a polaroid camera (C-5, Tektronix, USA). After preliminary perfusion for 30 min with normal Ringer's solution, 2.5 mM Mn^{2+} or 2.5 mM La^{3+} was added to the perfusion fluid. During the action of Mn^{2+} or La^{3+} the frequency of stimulation was increased stepwise to 5 Hz in 30-40 sec. In special experiments ouabain was added up to a concentration of $2 \cdot 10^{-6}$ g/ml to the Ringer's solution containing Mn^{2+} or La^{3+}. The transmembrane action potentials of single cells were recorded by glass microelectrodes with a resistance of 10-30 mΩ.

EXPERIMENTAL RESULTS

Replacing the normal Ringer's solution by solution containing 2.5 mM Mn^{2+} led within 20-30 min to virtually complete cessation of mechanical activity of the preparation; residual contractions in most experiments did not exceed 3-5% of the original amplitude of contraction. The duration of the transmembrane action potential under these circumstances was considerably reduced and was about one-third of the initial value

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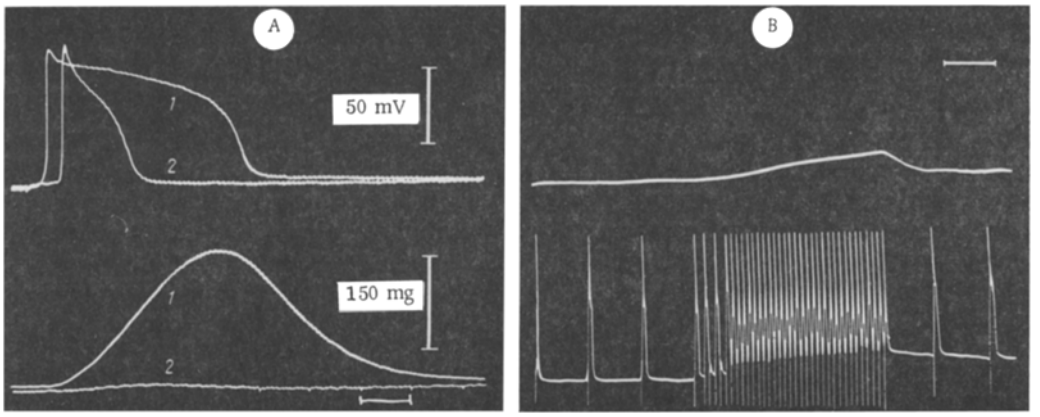

Fig. 1. Action of 2.5 mM manganese on electrical and mechanical activity of myocardial strip from frog's heart. A) Action potential and mechanical response of preparation in normal Ringer's solution (1) and after perfusion for 20 min with solution containing 2.5 mM manganese (2). Top part of figure shows action potential, bottom part contractile response. Time marker 200 msec; B) development of contracture in response to increase in frequency of stimulation (from 0.5 to 5 Hz) after action of manganese for 2 h. Top curve represents contraction, bottom curve action potential. Time marker 2 sec.

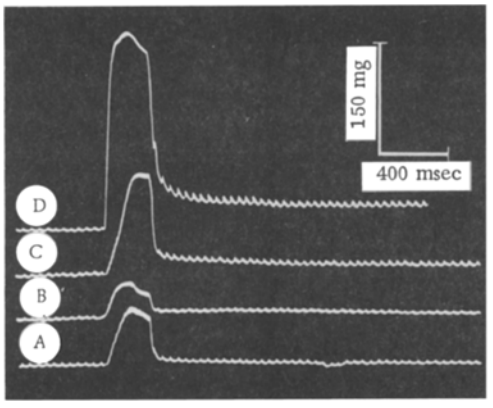

Fig. 2

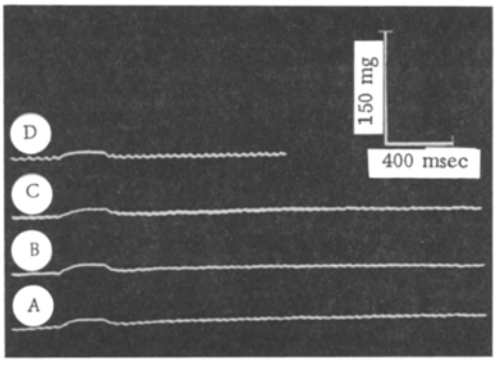

Fig. 3

Fig. 2. Effect of ouabain on amplitude of contracture induced by increase in frequency of stimulation in presence of 2.5 mM manganese. A) Contracture induced after exposure for 10 min to manganese; B) for 20 min to manganese; C) for 10 min to combined action of manganese and ouabain; D) for 20 min to combined action of manganese and ouabain. Amplitude of single contraction in normal Ringer's solution during stimulation of preparation with a frequency of 0.5 Hz shown on right.

Fig. 3. Effect of stepwise increase in frequency of stimulation from 0.5 to 5 Hz on preparation treated with lanthanum and ouabain. A) Increase in frequency of stimulation after exposure to lanthanum only; B, C, and D) increase in frequency of stimulation 10, 20, and 30 min respectively after combined action of lanthanum and ouabain. Amplitude of single contraction in normal Ringer's solution plotted on right. (Time calibration 20 sec).

(Fig. 1A). The resting tension of the preparation during stimulation at 0.5 Hz remained unchanged during perfusion for several hours with the solution containing Mn^{2+}. An increase in the frequency of stimulation from 0.5 to 5 Hz was accompanied by the development of contracture, the strength of which reached 25-40% of the strength of contraction in normal Ringer's solution. The contracture was maintained throughout this period of high-frequency stimulation and was replaced by relaxation to the initial value of the resting tension when the original frequency of stimulation was restored (Fig. 1B). Contracture was reproduced by repeated high-frequency stimulation; the amplitude of the contracture showed little change in the course of the experiment (1 h). The ability of the preparation to reproduce such a high frequency of stimulation (5 Hz), incidentally, was connected with the fact that the duration of the action potential was considerably shortened by Mn^{2+}. High-frequency stimulation of the preparation when in normal physiological saline without Mn^{2+} caused transformation of the rhythm of excitation, and when the preparation reproduced its highest possible frequency of stimulation, this was accompanied by contracture.

Addition of ouabain ($2 \cdot 10^{-6}$ g/ml) to the perfusion solution containing Mn^{2+} caused an increase in contracture, which reached 300% compared with the contracture recorded in the presence of Mn^{2+} alone (Fig. 2). In some experiments with ouabain the amplitude of contracture exceeded the amplitude of the individual contractions recorded in normal Ringer's solution. Ouabain thus potentiated the tonic contractions arising in response to a high frequency of stimulation in the presence of manganese.

Experiments similar to those described above were carried out with La^{3+} which, unlike Mn^{2+}, completely abolishes movement of Ca^{2+} through the cell membrane [3]. Just like Mn^{2+}, La^{3+} (2.5 mM) inhibited mechanical activity of the myocardial strip, but an increase in the frequency of stimulation and the addition of ouabain to the perfusion solution did not cause tonic contractions (Fig. 3).

The contracture observed in the presence of Mn^{2+} during an increase in the frequency of stimulation to 5 Hz reflects an increase in the intracellular free Ca^{2+} concentration in the myoplasm. Under normal conditions depolarization of the surface membrane of the heart fiber causes an electrogenic inward flow of Ca^{2+}, on account of which the cell in warm-blooded animals receives only part of the Ca^{2+} necessary for contraction [6]. A further increase in the free Ca^{2+} concentration in the cytoplasm can be provided by: 1) the sarcoplasmic reticulum [2], and 2) the electrically neutral energy-dependent flow of Ca^{2+} through the sarcolemma into the cell, of the sodium–calcium exchange type [4, 5].

In the presence of Mn^{2+} the electrogenic Ca^{2+} current is completely inhibited [1], but under these circumstances the myocardium of the warm-blooded animal develops a tonic contraction in response to stimulation at a constant low frequency [1]. The resting tension in the myocardium of amphibians is unchanged under analogous conditions. These differences are evidently connected with the fact that the muscle cells of the amphibian heart have a poorly developed sarcoplasmic reticulum, which is the principal intracellular Ca^{2+} depot [3]. Development of contracture in the frog myocardium in response to an increase in the frequency of stimulation in the presence of Mn^{2+} can be explained on the basis of the hypothesis of the role of sodium–calcium exchange diffusion in the regulation of the contractile act of the myocardial cell [4]. In accordance with this hypothesis Ca^{2+} ions can be transported into the cell by the carrier in exchange for Na^{+} ions, which are removed from the cell. A high frequency of stimulation in the present experiments with Mn^{2+} evidently activates sodium–calcium exchange through an increase in the intracellular Na^{+} concentration. This explanation is confirmed by experiments which showed that manganese contracture was potentiated by ouabain, which also increases the intracellular Na^{+} concentration [4].

Mn^{2+} thus does not prevent sodium–calcium exchange. The absence of contracture during the action of La^{3+} can be explained by the ability of this ion to prevent not only the electrogenic but also the nonelectrogenic energy-dependent transport of Ca^{2+} through the cell membrane [3].

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