EFFECT OF CALCIUM CONCENTRATION ON TRANSMEMBRANE POTENTIALS AND CONTRACTION OF MYOCARDIAL CELLS

R. S. Orlov and E. F. Élik

UDC 612.172:612.173.1.015.31:546.41

In a medium with normal Ca concentration an increase in frequency of stimulation shortens the duration of the transmembrane action potential (TAP) and reduces its amplitude. An increase in the Ca concentration in the medium causes an increase in TAP duration at the beginning of stimulation, followed by their more rapid shortening than in the orginal background and a decrease in amplitude in response to a high frequency of stimulation. Removal of Ca from the medium modifies the form and duration of the TAP and the principal parameters of myocardial contraction.

* * *

The principal trigger mechanism of contraction of striated muscles has been shown [2, 5] to be penetration of Ca ions inside the muscle cells. The role of Ca ions for heart muscle is evidently not merely the triggering of contraction, because experiments [4, 6] have shown that the concentration of extracellular Ca influences the velocity of the final phase of depolarization of the transmembrane action potential (TAP) of cells of the frog ventricle and the duration of the plateau phase in Purkinje fibers of the dog myocardium. Participation of Ca ions in TAP generation as well as in activation of contractile elements is interesting from the standpoint of explanation of the links between excitation and contraction of heart muscle cells.

The object of the present investigation was to study the effect of different Ca concentrations on TAP parameters and amplitude of contraction of the isolated ventricle in frogs (Rana temporaria).

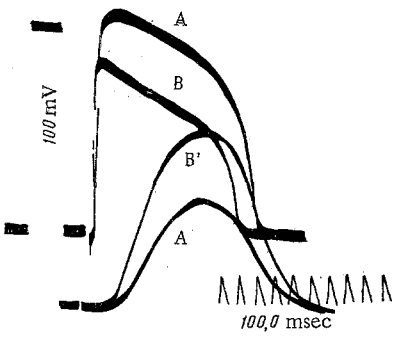

Fig. 1. Changes in TAP configuration and amplitude of contractions in medium containing increased Ca concentration (3 mmoles). A) TAP in normal solution; B) TAP in solution with increased Ca concentration after exposure for 15 min; A') contraction in normal solution; B') contraction in solution with increased Ca concentration. Frequency of stimulation 0.7 Hz.

EXPERIMENTAL METHOD

The heart was perfused with a salt solution [1] while contracting at various frequencies. Square pulses of negative polarity, 10-50 msec in duration, were used for stimulation. TAPs were recorded by movable intracellular microelectrodes filled with 2.5 M KCl, having a tip less than 0.5 μ in diameter and impedance of 20-40 MΩ. Contractions were recorded by a tensometric sensor. In all experiments the responses of the cells to frequencies of 0.2, 0.5, and 0.7 Hz were recorded initially in solutions with normal Ca concentration, and later after the preparation had been kept for 10-15 min in solution with raised or lowered Ca concentration (against a background of stimulation at 0.2 Hz). The recordings were repeated at the same frequencies of stimulation as in the control and after exposure for various periods to a medium with modified concentration of Ca ions. The interval after which the frequency of stimulation was changed was 30 sec. No fewer than 8-10 TAPs and contractions were recorded for each frequency of stimulation. To ensure more complete fixation of Ca in the calcium-free solutions, 1 mmole Chelaton-3 was added.

EXPERIMENTAL RESULTS

In medium with normal Ca concentration a definite connection was found between the duration and amplitude of the TAP and

Department of Normal Physiology, Sverdlovsk Medical Institute (Presented by Academician V. V. Zakusov, Academy of Medical Sciences of the USSR). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 7, pp. 3-8, July, 1969. Original article submitted October 14, 1968.

frequency of stimulation. An increase in the frequency of stimulation shortened the duration of the TAP (mainly at the expense of the plateau phase) and reduced its amplitude. An increase in the Ca concentration in the medium altered the shape of the TAP: a well-marked phase of initial, rapid repolarization appeared. In most preparations the changes in TAP duration occurred in two phases: the duration of the first TAP became longer than in normal solutions, but subsequently they were shortened to a greater degree than in the original solution, particularly at high frequencies of stimulation. In two preparation the TAPs at all frequencies of stimulation were not shortened, but lengthened. The amplitude of the TAP varied depending on the frequency of stimulation. Stimulation at 0.2 Hz increased the TAP amplitude; higher frequencies of stimulation reduced it, and to a greater degree than the same frequencies when applied in a medium with normal Ca concentration (1.08 mmoles). Typical changes in the shape, duration, and amplitude of the TAP in solutions with increased Ca concentration (3 mmoles) compared with the same indices in normal solution are illustrated in Fig. 1.

The relationship between contraction and TAP parameters was investigated with the aid of Bowditch's staircase phenomenon. In all experiments the first contractions after the pause possessed the lowest amplitude, but they corresponded to a TAP of the greatest duration and amplitude. Subsequent contractions were high in amplitude but were accompanied by shortening and a decrease in amplitude of the TAP. The frequency of stimulation modified not only the characteristics of the TAP, but also the amplitude of contraction and the rate of its increase in a staircase series. The amplitude and rate of increase of the contraction were least at a frequency of stimulation of 0.2 Hz. The increase in amplitude of the 10th contraction relative to the first in this case varied from 139 to 300% in different preparations. At a frequency of stimulation of 0.5 Hz the amplitude of the contractions rose by the 10th contraction from 149 to 620% of the amplitude of the first. At stimulation frequencies of 0.7 and 1 Hz the increase in amplitude of the contractions was less than during stimulation at 0.5 Hz, but even so was greater than at the background frequency of 0.2 Hz. The rate of increase in amplitude of the contractions in the staircase series was determined by the formula: $A_n = A_\infty (1-e^{-\alpha n})$, where α represents the time constant of increase, n the second contraction, and A_∞ the amplitude of the 10th contraction expressed as a percentage of the amplitude of the first contraction.

The rate of increase in amplitude of contraction in the staircase series depended on the amplitude of the first contraction. The greater its amplitude, the lower the rate of increase in amplitude, and vice versa. A frequency of stimulation of 1 Hz in most preparations caused alternation of TAP amplitude and duration from the beginning of stimulation, accompanied by alternation of duration and amplitude of the contractions. The first, longest TAP corresponded to the longest possible contraction of low amplitude. Compared with the first, the second contraction was shorter in duration and lower in amplitude. Starting from the third contraction a normal staircase developed against the background of progressive shortening of the TAP.

An increase in concentration of Ca ions in the medium (3 and 5 mmoles) did not change the relationship between the TAP parameters and character of the contractions. Changes occurred only in the rate of increase and the amplitude of contraction in the staircase series. Compared with the background values, the amplitude of contraction was increased. The time constant of increase in amplitude of the contractions was reduced. It was reduced to the greatest degree at a frequency of 0.5 Hz. Recordings of the TAPs and contractions in response to 10 stimuli applied at different frequencies are given in Fig. 2, I and II. Since the amplitude of the TAPs and contractions usually became stabilized after the 7th contraction, the TAP and amplitude of contractions in response to the 7th stimulus at all frequencies used are given in the upper part of the figures.

A definite relationship exists between the parameters of the TAPs and ventricular contractions. At high frequencies of stimulation the TAP duration is reduced and the rate of contractions increased; alternation of amplitude and duration of the contractions accompanies changes in TAP. Besides an increase in TAP duration, an increase in the duration of contraction is also observed. An increase in Ca concentration in the medium does not disturb the relationship between the parameters of the TAP and contraction, although it has an appreciable effect on the duration of the first TAPs recorded after the pause and accelerates the shortening of TAPs in a series of potentials at high frequencies of stimulation.

Experiments to test the action of calcium-free solutions revealed uniform changes in the electrical and contractile properties of the myocardium (Fig. 3). After the preparation had been kept for 1-2 min in a calcium-free solution, spontaneous activity developed at a frequency of 0.5-0.7 Hz, and it persisted throughout the experiment. Because of generation of a spontaneous rhythm, it was impossible to use artificial

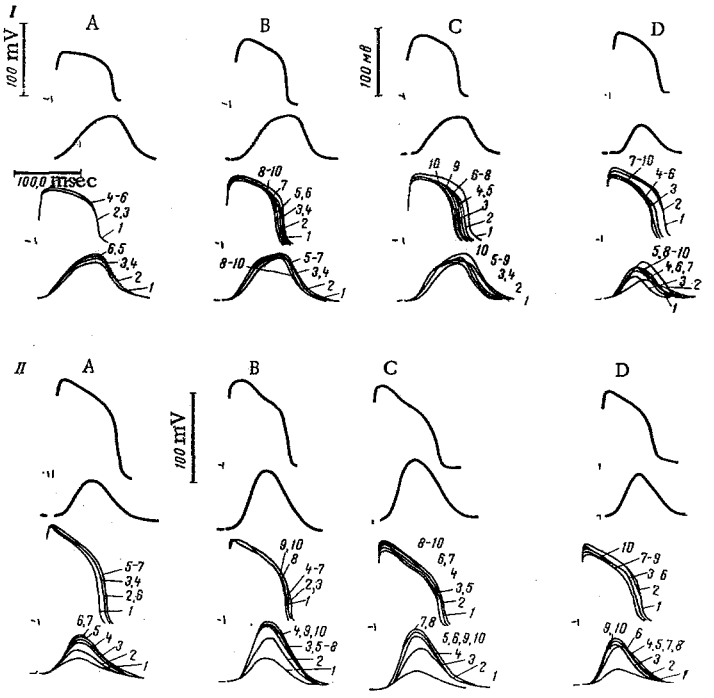

Fig. 2. TAPs and contractions of ventricle. I) In normal solution at different frequencies of stimulation; II) in solution with increased Ca concentration (5 mmoles). Above: TAP and ventricular contractions in response to 7th stimulus at different frequencies of stimulation: A) 0.2 Hz; B) 0.5 Hz; C) 0.7 Hz; D) 1 Hz. Below: superposition of curves of TAPs and contractions from first to 10th stimuli at the same frequencies of stimulation.

stimulation, so that the effect of removal of Ca on the TAP and ventricular contraction had to be studied against the background of "spontaneous stimulation." At a frequency of stimulation of 0.7 Hz, the total duration of the TAP in one preparation (Fig. 3) in normal solution (1.08 mmole Ca) was 1030 msec, decreasing after exposure for 10 min in the calcium-free solution to 960 msec, despite a lower frequency of "spontaneous stimulation" (about 0.5 Hz). During the next 20-40 min of observation the total duration of the TAP fell only slightly, mainly on account of shortening of the plateau phase. Similar results were obtained with other preparations. Only in one case was considerable shortening of the TAP duration detected. It is interesting to note that shortening of the TAPs was accompanied by their aperiodic alternation. More definite changes occurred in the amplitude and velocity of TAP depolarization. Whereas in normal solutions the TAP amplitude varied, depending on the frequency of stimulation, from 115 to 104 mV, after a stay of 10 min in the calcium-free medium it fell to 80-60 mV, and after 40 min it had fallen to 33 mV. The decrease in amplitude of the TAPs occurred simultaneously with lengthening of the depolarization phase, the duration of which increased from 20 msec in normal solutions to 280 msec in the calcium-free medium. Removal of Ca had a much more marked effect on the character of the contractions: after the first minutes the amplitude of isotonic contraction fell progressively until they disappeared completely.

The results of this investigation show that variation of the extracellular Ca concentration causes changes in configuration of the TAP and modifies the rate and amplitude of the contractions. The most marked effect of an increase in Ca concentration in the extracellular fluid was an increase in the duration of the TAPs at the beginning of stimulation and a more rapid shortening of the TAPs and decrease in their amplitude than in the background in response to a high frequency of stimulation. The staircase phenomenon was preserved. Removal of Ca caused changes in the configuration of the TAPs and in the character of myocardial contractions.

The combined changes in configuration and duration of the TAPs and also in the character of the contractile response with changes in the Ca concentration in the medium suggests that Ca provides a link between

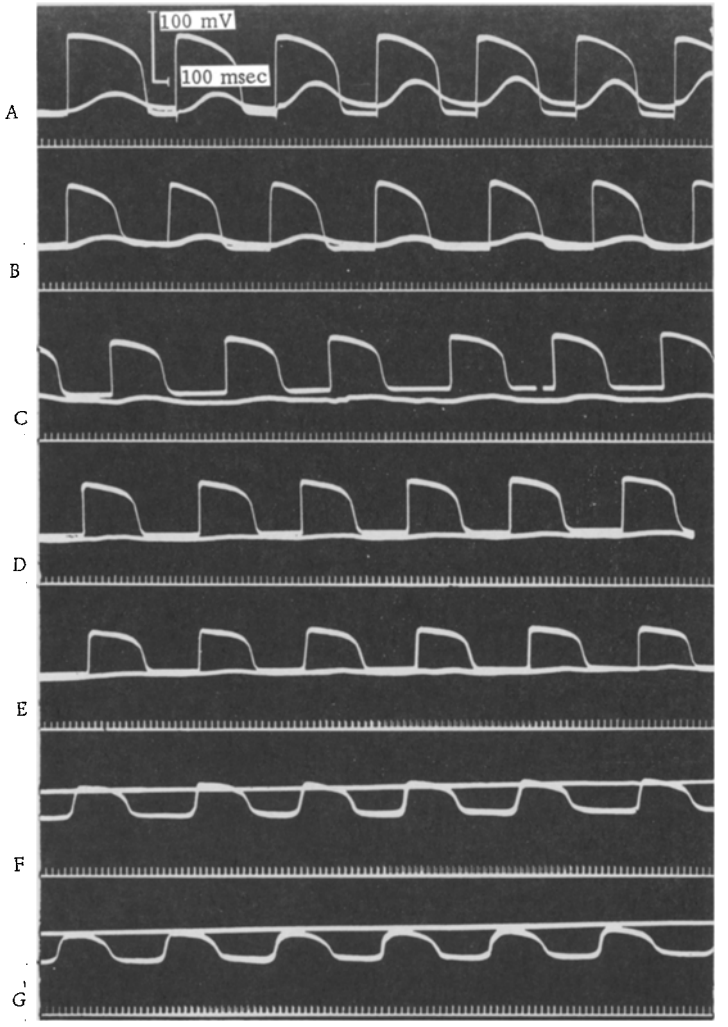

Fig. 3. Effect of calcium-free solution on TAPs and ventricular contractions. From top to bottom: tracing of TAPs, contractions, time marker 100 msec. A) Background, frequency of stimulation 0.7 Hz, Ca concentration in solution 1.08 mmoles; B) after exposure for 12 min in calcium-free solution; C) for 28 min; D, E, F, G) from 57 to 64 min; B, C, D, E, F, G) artificial stimulation absent, rhythm is spontaneous.

the processes of excitation and contraction in the myocardial cells. The effect of an increased Ca concentration can be attributed to the fact that the Ca concentration is increased not only in the surface layers of the cell membrane, but also inside the cell, in the Ca-transport system. It has been shown [4] that an increase in the velocity of contraction of the papillary muscle of the dog is associated with an increase in the velocity of Ca metabolism. On the other hand [3], an increase in the maximum of myocardial tone and the increase in the velocity of contraction if the temperature of the surrounding solution was lowered were observed parallel with an increase in TAP duration. A decrease in the Ca concentration in the medium interfered with this phenomenon. Under normal conditions the duration of the TAP is evidently insufficient to allow access of Ca into the cell in amounts adequate to attain the maximum of the contractile response, as a result of which a staircase phenomenon is observed. In solutions with an increased Ca concentration, saturation of the cells with Ca ions takes place much more quickly. The results of this investigation, together with their explanation, are in harmony with the hypothesis that movement of sodium ions inside and out of myocardial cells may take place in a specialized membrane region together with movement of Ca ions [4]. It has also been concluded [6] that Ca ions participate in the generation of an ion current through the

membrane and they accumulate during stimulation in certain parts of the cell. It can be postulated that the associated movement of Ca and sodium in the same channel of the membrane also exerts an influence on the velocities of movement of potassium ions. When Ca metabolism is increased in solutions with high Ca concentration, the velocity of intracellular displacement of sodium ions is evidently increased also, leading to a more intensive ejection of potassium ions, as revealed by a more marked shortening than in the background of TAPs at high frequencies of stimulation.

LITERATURE CITED

1. A. J. Brady and J. W. Woodbury, J. Physiol. (London), 154, 385 (1960).
2. A. J. Clark, G. H. Percival, and C. P. Steward, J. Physiol. (London), 66, 346 (1928).
3. R. Kaufman and A. Fleckenstein, Pflüg. Arch. Ges. Physiol., 285, 1 (1965).
4. G. A. Langer, Circulat. Res., 17, 78 (1965).
5. M. Moulin and W. Wilbrandt, Experientia, 11, 72 (1955).
6. R. Niedergerke and R. K. Orkand, J. Physiol. (London), 184, 312 (1966).