The Possible Role of Calcium in Excitation-Contraction Coupling of Heart Muscle

By Saul Winegrad, M.D.

The transfer of Ca\textsuperscript{4+} at rest and during contraction has been measured in isolated guinea-pig atria. During contraction, the rate of transfer increases considerably. The increment in the uptake of calcium by the cells of the atria during contraction is closely correlated with the strength of contraction. This relationship is maintained at different frequencies of stimulation and at different concentrations of external calcium.

The importance of calcium in the contraction of heart muscle has been known since the experiments of Ringer. Early work\textsuperscript{2,3} focused primarily on the dependence of the strength of contraction on the concentration of calcium ions in the bathing solution. More recently an antagonism between sodium and calcium ions at the cell surface has been inferred from the observation that the effects of a decrease in extracellular calcium concentration and an increase in extracellular sodium concentration on twitch tension are similar.\textsuperscript{4} The observation that withdrawal of calcium ions from the bathing solution caused rapid disappearance of mechanical but not of electrical activity of isolated heart muscle implicated the calcium ion as the excitation-contraction link.\textsuperscript{5}

In a series of studies on frog ventricular strips, Niedergerke and Lüttgau\textsuperscript{6} showed that changes in external sodium and calcium concentrations very rapidly altered the characteristics of potassium-induced contracture and that these changes in contracture tension could be produced even after the initial potassium depolarization was complete. Figures 1 to 3\textsuperscript{*} are taken from the work of Niedergerke,\textsuperscript{7} and Niedergerke and Lüttgau.\textsuperscript{8} Figure 1\textsuperscript{a} and b shows the effect of replacement of sodium in the bathing solution at the beginning of a potassium-induced contracture. In figure 2\textsuperscript{a±} the ventricle was depolarized by 100 mM potassium in the presence of 10 mM calcium, while in figure 2\textsuperscript{b} the initial depolarization occurred in 0 Ca, 100 mM potassium but after 90 seconds the solution was changed to one containing 10 mM Ca, 100 mM potassium. The effect of the calcium is clearly not limited to processes accompanying depolarization. Similarly the effect of decreased sodium concentration can occur after depolarization (fig. 3). Electrical measurements eliminated increased depolarization as an explanation for the increases in tension seen with elevated calcium or depressed sodium concentrations.\textsuperscript{8}

By use of radioisotopes, Niedergerke and Harris\textsuperscript{9} demonstrated that the changes in extracellular sodium and potassium concentration associated with increased twitch tension were accompanied by increased uptake of Ca\textsuperscript{4+} by the resting tissue (fig. 4\textsuperscript{±}) and by the tissue in potassium-contracture\textsuperscript{10} (fig. 5\textsuperscript{§}).

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\textsuperscript{1}Figure 2 reproduced from Niedergerke: J. Physiol. 134: 584, 1956. By permission of the author and the Journal of Physiology.

\textsuperscript{2}Figure 4 reproduced from Niedergerke and Harris: Nature 179: 1068, 1958. By permission of the authors and Nature.

\textsuperscript{§}Figure 5 reproduced from Niedergerke: Experientia 15: 128, 1959. By permission of the author and Experientia.
Dependence of contracture on sodium chloride concentration. Contractures always induced after a 30-min. period of equilibration in Ringer's fluid during which strip was stimulated at a constant rate. Ringer's fluid and potassium-rich solutions contained 0.5 mM calcium chloride throughout the experiment. Diameter of strip: 0.35 mm. (a) Contracture in the presence of 100 per cent sodium chloride induced by adding 100 mM potassium chloride to Ringer's fluid. (b) Contracture induced by adding 100 mM potassium chloride and simultaneously replacing the sodium chloride content of Ringer's fluid by sucrose. (From Niedergerke and Lüttgau.)

These studies dealt primarily with rapid changes in calcium transfer; a distinction between changes occurring at the "cell surface" and in the "cell interior" based on a difference in time constants of calcium transfer was not clear.

The demonstration by Bianchi and Shanes of an increased uptake of calcium during contraction of skeletal muscle fibers added substantially to the data implicating calcium as the excitation-contraction link. To test the possibility that excitation in myocardial cells is coupled with contraction by a movement of calcium into the cell during depolarization, a series of experiments comparing calcium uptake by guinea-pig atria at rest and under several different conditions of contraction was conducted. Radioisotope technics similar to those of Bianchi and Shanes were used. Tensions were measured with conventional strain gauges. Left atrial appendages from young guinea pigs were used because the tissues are thin, stable over long periods of time, and devoid of spontaneous rhythm.

Calcium influx was determined by soaking the atria for 15 minutes in Ca solution and measuring the amount of Ca remaining in the tissue after the extracellular and loosely bound surface Ca had been washed out in nonisotopic solution. A correction was made for the intracellular Ca lost during the washout. To eliminate the error introduced by the presence of damaged tissue, the edges of the atria cut during dissection were removed from the undamaged tissue at the end of the experiment and their radio-

\[ \text{Figure 1} \]
\[ \text{Figure 2} \]
activity counted separately. The effect of contraction on influx was measured by stimulating the muscles during the last 10 of the 15 minutes in Ca\textsuperscript{45}-Krebs solution and calculating the difference in Ca\textsuperscript{45} content between the stimulated and unstimulated muscles.

The resting influx at external calcium concentrations of 1.25 mM, 2.50 mM, and 3.75 mM was 0.008 ± 0.0010, 0.014 ± 0.0012, and 0.022 ± 0.0046 micromicromole/cm\textsuperscript{2}sec. In figure 6 the influxes are plotted against the external calcium concentration. The data can be best approximated by a straight line that, when extrapolated, misses the origin by a statistically insignificant amount. It would appear, therefore, that in the resting muscle cell, influx of calcium is a function of the external calcium concentration. The resting outflux, in 2.5 mM calcium, calculated by standard desaturation studies, was approximately equal to the influx, suggesting that in the resting atria no net movement of calcium was occurring.

When the atria contracted, the calcium influx increased considerably; the total calcium\textsuperscript{45} content of the stimulated tissue at the end of the experiment rose to as much as 15 times that of the resting controls. Under these circumstances the increment in calcium influx associated with each beat was 0.57 micromicromole/cm\textsuperscript{2} surface area. Contraction was not accompanied by an increase in calcium uptake by the cut edges of the tissue, which themselves did not contract.

It was of interest to determine whether any correlation existed between the size of the calcium influx per beat and the twitch tension. Table 1 consists of the results of experiments performed to study the effect of different external calcium concentrations and different frequencies of contraction on both the calcium uptake and the strength of contraction. Calcium uptake per beat and twitch tension for each condition are expressed in per cent relative to the values at a contraction frequency of 30/min. in 2.5 mM calcium, the latter having been arbitrarily assigned a value of 100 per cent.

The uptake per beat of atria contracting at 6/min. in 2.50 mM calcium is 30 per cent of that of the atria beating at 30/min. (p<0.05). The percentage increase in uptake per beat associated with this change in the rate of contraction does not differ significantly from the percentage increase in twitch tension. When the frequency is changed from 15/min. to 30/min., no significant increase in either twitch tension or uptake per beat occurs. Thus, in these experiments, both calcium influx and contractility apparently exhibit parallel saturation properties.

Muscles stimulated at 15/min. in 1.25 mM

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<tr>
<th>Table 1</th>
<th>Relationship of Ca\textsuperscript{45} Uptake to Twitch Tension</th>
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<td>Calcium uptake (M)</td>
<td>Rate (beats/min.)</td>
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<td>1.25</td>
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*Values ± 1 standard error of mean.

Figure 3
Reversibility and specificity of the action of sodium ions. Ringer's fluid and potassium-rich solutions contained 2 mM calcium chloride throughout the experiment. Diameter of strip, 0.45 mm. (1) Contracture induced by adding 100 mM potassium chloride to Ringer's fluid and simultaneously replacing sodium chloride iso-osmotically with sucrose; (2) reappllication of sodium chloride; (3) replacement of sodium chloride by sucrose. (From Niedergerke and Lüttgau.)
The effect of replacing the sodium chloride of Ringer's fluid by sucrose on the uptake of strontium-89 tracer in heart tissue. Relative amounts of strontium-89 tracer in a ventricle strip during exposure to tracer solutions which contained either 112.5 mM sodium chloride or 5 mM sodium chloride + 202 mM sucrose. The two solutions had identical concentrations of potassium chloride (2 mM) and of tracer Sr$^{89}$-Ca (1 mM) mixture. Vertical bars indicate standard error of the measurement of radioactivity. Sr$^{89}$ is used as an indicator of Ca movement. (From Niedergerke and Harris.)

calcium contract about as vigorously as the atria beating at 6/min. in 2.50 mM calcium. Despite the fact that two important conditions have been changed in opposite directions, the relative uptake per beat and relative tension are still closely correlated. An increase in external calcium concentration from 1.25 mM to 3.75 mM increases twitch tension almost 3-fold ($p<0.05$), and calcium uptake per beat is increased proportionately. An increase in the calcium concentration from 2.5 mM to 3.75 mM is associated with proportionately smaller increases in uptake and twitch tension, but in this comparison both changes are of questionable significance.

When a plot is made of relative twitch tension vs. relative uptake per beat using all the data obtained from different external calcium concentrations and different rates of stimulation (fig. 7), a consistent correlation between the 2 parameters exists. A straight line closely fits the data and its failure to pass through the origin is not statistically significant.

These data demonstrating the increment in calcium influx associated with contraction are consistent with the hypothesis that calcium movement into the cell with depolarization couples excitation with contraction. They suggest, also, that such a link is a factor in determining the strength of the twitch.

In an analysis of the increment of calcium influx associated with contraction one may consider: (1) the source of this calcium; (2) the mechanism by which it enters the cell; (3) the time in the cardiac cycle during which the added calcium enters the cell; and (4) the possible mode of action inside the cell. Niedergerke's data$^9,10,12$ suggest that superficial sites in the resting cell bind calcium and that certain changes in the composition of bathing solution which produce increased twitch tension are associated with greater binding of calcium by the resting tissue. Moreover, experiments already mentioned show that similar changes in the composition of the bathing solution during a maintained potassium depolarization are rapidly followed by changes in contracture tension. An additional pertinent observation is that of Weidmann,$^{13}$ who demonstrated in turtle ventricle that a sudden increase in the concentration of calcium in the extracellular space during the initial stages of a twitch produced a more rapid rate of tension development and a greater peak tension than occurred at the lower calcium concentration. In addition, the increase in calcium concentration was accompanied by a shortening of the action potential. A hypothesis that incorporates these data with the observed correlation between the rate of calcium influx during a contraction and the size of the contraction would be the following: the calcium that enters the cell with contraction comes from...
superficial sites and from the extracellular fluid; the calcium that enters the cell from the extracellular fluid during depolarization passes through the same superficial sites to which calcium was bound in the resting state.

Weidmann's data\textsuperscript{18} suggest that the increment in calcium influx occurs during the depolarization. In support of this conclusion are the observations that the increased heart rate is associated with a shorter diastole, a shorter action potential but an increased calcium influx per beat, and an increased rate of rise of tension during the twitch.\textsuperscript{14-16} If the influx of calcium is quantitatively related to twitch tension, it might be expected that a greater increment during a shorter action potential would produce a more rapid rise in tension. If the added calcium does enter the cell during the action potential (measured to be 150 msec.), then calcium influx during depolarization may exceed resting influx by as much as 250-fold.

Certain possible relations of the calcium influx to contraction became apparent with a quantitative consideration of the data. If the molecular weight of myosin is about 500,000,\textsuperscript{17} and its concentration in heart muscle is equal to that in skeletal muscle (taken to be 7.6 per cent of wet weight\textsuperscript{18}) then each gram of heart contains $1.5 \times 10^{-4} \text{ mmoles}$. The ratio of the number of calcium ions entering the cell during contraction to the number of myosin molecules already present is about 1/250. A similar ratio results if a comparison is made of the calcium influx to the amount of actin or tropomyosin in the muscle (again assuming that the concentration of actin and tropomyosin in heart muscle is similar to that in skeletal muscle). It is unlikely that only 0.4
The relative calcium uptake per beat of guinea-pig left atrial appendages contracting at 6/min., 15/min., 30/min. in 1.25 mM, 2.50 mM, and 3.75 mM Ca-Krebs solution is plotted against the relative twitch tension produced. All values are expressed relative to calcium uptake and twitch tension at 30/min. in 2.5 mM Ca-Krebs solution.

per cent of the contractile protein is shortening during the strongest contractions of the isolated atrium. Therefore, if calcium does initiate the contraction, each ion must ultimately have an effect on many actin-myosin units. One of the reactions in the contractile process, though not necessarily the one involving calcium, must involve either a chain reaction or the interaction of 1 molecule of ion with as many as 250 molecules or units. The latter could occur by an enzymatic reaction or by a specific type of molecular alignment in which one molecule is in close association with many contractile units.

With respect to this quantitative relationship of calcium taken up during contraction to actomyosin, it is interesting to note the calculation of H. E. Huxley18 that each thick filament in skeletal muscle contains about 400 myosin molecules. If a similar condition exists in the heart, the ratio of calcium ions taken up in a maximal contraction to the number of thick filaments is 1.6:1.

If the calcium that enters the cell with contraction is assumed to achieve immediate uniform distribution in the cell water, the concentration would be $1.2 \times 10^{-6}$ M. Any non-uniform distribution would produce regions within the cell of higher "calcium concentrations." This value of ionized calcium inhibits the relaxing-factor activity in vitro.19 Ebashi has further shown that the relaxing-factor system binds calcium tightly and that procedures which decrease calcium binding proportionately decrease relaxation activity. He has demonstrated that other calcium chelating agents have relaxation activity proportionate to their ability to chelate calcium.20 It is possible therefore that the calcium that enters the cell during excitation inhibits the relaxing system and thereby initiates contraction, or that it activates contraction, relaxation occurring by the removal of the calcium by the relaxing system.

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References

1. Ringer, S.: A further contribution regarding the influence of different constituents of the blood on the contraction of the heart. J. Physiol. 4: 29, 1893.
7. Niederegerke, R.: The potassium chloride con-

Circulation, Volume XXIV, August 1961
19. EBASHI, S.: Personal communication.
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