POSSIBLE CONTROL OF INTRACELLULAR CALCIUM METABOLISM BY $[H^+]$: SARCOPLASMIC RETICULUM OF SKELETAL AND CARDIAC MUSCLE[1]

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Received September 28, 1970

SUMMARY

Relaxing systems (sarcoplasmic reticulum) were prepared from cardiac and white skeletal muscles. Employing both dual-beam spectrophotometry with the calcium-chelating dye murexide, and Millipore filtration, it was found that rapid alterations of pH in the range 5.9 to 7.8 significantly influenced the affinity of the reticulum for calcium. By decreasing proton concentration $\frac{1}{2}$ to 1 pH unit (from 6.5) calcium was released in an amount calculated to be sufficient to effect contraction. The release and binding rates at their optimum pH's, respectively, were of the same magnitude.

INTRODUCTION

It is generally thought that contraction of muscle is initiated by calcium ions that are released from internal membranes (perhaps, terminal cisternae) by local current (1-3). Isolated sarcoplasmic reticulum (SR) can vigorously bind and accumulate calcium (4,5); it is also possible that calcium-loaded SR may release calcium. Some factors that have been found to cause calcium release from SR are caffeine (6), monophasic current (7, 8), ADP (9), urea and oleic acid (10). A "trigger" in excitation-contraction coupling should possess properties of rapid calcium release from SR coincidental with phases in the action potential. This report concerns changes of proton concentration that cause a rapid release of calcium from SR. The attractive possibility of this phenomenon in the physiological control of calcium movements is suggested.

[1]Supported by USPHS grants HE 05435 and HE 07906
[2]Career Research Development Awardee (K_3 HE 11,875).

MATERIALS AND METHODS

Relaxing system (sarcoplasmic reticulum; RS) was prepared from skeletal and heart muscle by a minor modification of the procedure employed in this laboratory (11). Five g of muscle were cut into small pieces and, after washing 3 times, were homogenized in 20 ml of 10 mM $NaHCO_3$ with the Polytron, twice each for 10 seconds. The remainder of the procedure was as published (11). The resulting pellet was suspended in 50 mM KCl containing 4 mM Tris-HCl (pH 7.4), to a concentration of 3 to 4 mg/ml. Cardiac RS was used within one day and skeletal muscle RS was used within 3 days. The time course of calcium binding and release was recorded with an Aminco-Chance dual wavelength spectrophotometer using murexide (11,12). Absorption changes were estimated between λ_1 507 mμ and λ_2 542 mμ, in a cuvette containing 3 ml of a reaction mixture, consisting of 40 mM Tris-maleate buffer, 0.2 mM murexide, 8 mM $MgCl_2$ and the sample (0.7-1.2 mg protein) at 30° C. All additions during recording were done by means of a microsyringe injector with vibrator which allowed for a mixing time less than 2 seconds. Using the Millipore filter method, the reaction was started by the addition of 0.2 ml of sample to 0.8 ml of reaction mixture. After filtration of 0.8 ml of the mixture through HA 0.45 μ Millipore filter, both filtrate and filter were used to count ^{45}Ca (11). With this method, however, calcium transport occurring less than 20 seconds after initiation of the reaction could not be measured.

RESULTS

Calcium binding by skeletal muscle RS varied directly with $[H^+]$ (Fig. 1). Thus, increasing the pH (decreasing $[H^+]$) resulted in a diminution of calcium binding. Calcium binding at equilibrium was 166, 130 and 64 nanomoles calcium/mg protein at

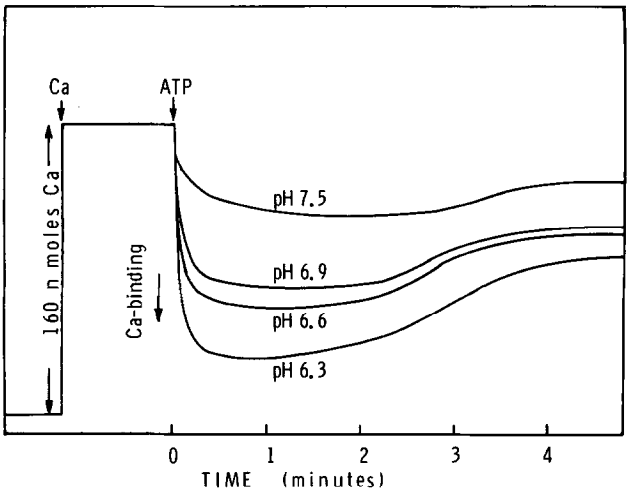

Fig. 1: Time course of calcium binding and release recorded by double-beam spectrophotometry at various pH's. Conditions: 13 mM Tris-maleate at pH indicated, 8 mM $MgCl_2$, 0.2 mM murexide, 26 mM KCl, 0.77 mg RS, at 30°C. Addition = calcium 160 (40 µl) nanomoles, ATP 600 nanomoles (15 µl) (0.2 mM).

pH 6.3, 6.6 and 7.5, respectively. The release process was gradual and almost the same at each pH studied. No differences were found when 100 mM K^+ or 100 mM Na^+ at pH 6.3 or 7.4 was added to the reaction mixture. The lower equilibrium calcium binding at pH 7.5, compared to pH 6.3, suggests that the membrane structure of RS might undergo changes induced by proton concentration, which results in alterations in affinity for calcium. If this change is rapid, calcium might also be quickly released.

Figure 2A demonstrates the effect of pH changes on calcium release from a point when calcium is bound at equilibrium in the skeletal muscle RS. Even one minute after ATP addition, no calcium was released at pH 6.63. By elevating the pH to 7.56 at equilibrium (30 seconds after ATP addition), 46 nanomoles calcium/mg protein were released in the following 5 seconds. This value is similar to the amount of calcium bound in the first 5 seconds after ATP addition, 54 nanomoles calcium/mg protein.

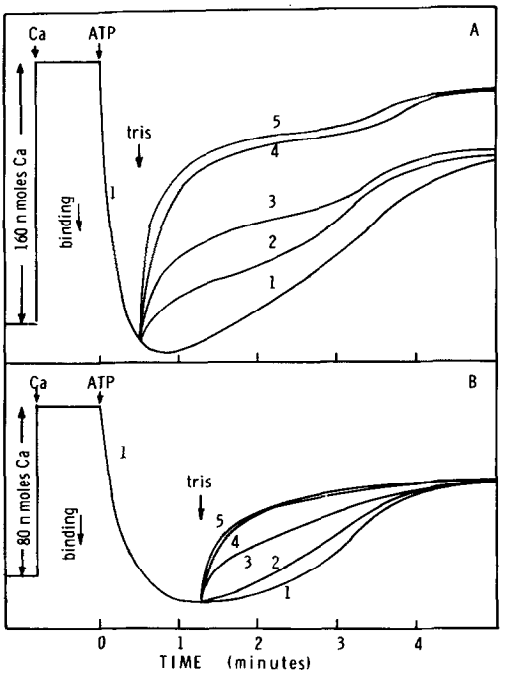

Fig. 2: Calcium binding and release by pH changes. The time courses were recorded by double-beam spectrophotometry. Conditions: 40 mM Tris-maleate, pH 6.63, 8 mM $MgCl_2$, 0.2 mM murexide, 130 mM KCl (total), 1.2 mg RS in (A) and 0.9 mg RS in (B), at 30° C. (A) Skeletal muscle RS: pH was changed by the injection of small increments of 1 M Tris (pH 10.2). Lines: (1) pH 6.63, (2) pH 6.89, (3) pH 7.17, (4) pH 7.40, (5) pH 7.56. Each line was corrected by a control in which pH was changed in the absence of ATP or in the presence of 300 nanomoles EGTA with ATP. (B) Cardiac muscle RS: Lines: (1) pH 6.63, (2) addition of 40 µl of distilled water, (3) pH 6.89, (4) pH 7.17, (5) pH 7.56.

The same phenomenon was observed with cardiac muscle RS (Fig. 2B). The amount of calcium bound at 10 seconds was 30 nanomoles calcium/mg protein, whereas calcium release was 29 nanomoles calcium/mg protein in 10 seconds when the pH was raised to 7.17 after equilibrium calcium binding, 80 seconds after ATP addition. Calcium release caused by the addition of Tris-(hydroxymethyl)-amino-methane was not the effect of this reagent itself, because the same phenomenon occurred using KOH to raise the pH.

We confirmed ^{45}Ca release by pH changes from 6.74 to 7.83 in the RS of cardiac muscle by using the Millipore filter method.

Decreasing the pH to 5.94 from 6.74 caused an increase of ^{45}Ca binding, while raising the pH to 7.83 resulted in a marked diminution of binding (Fig. 3).

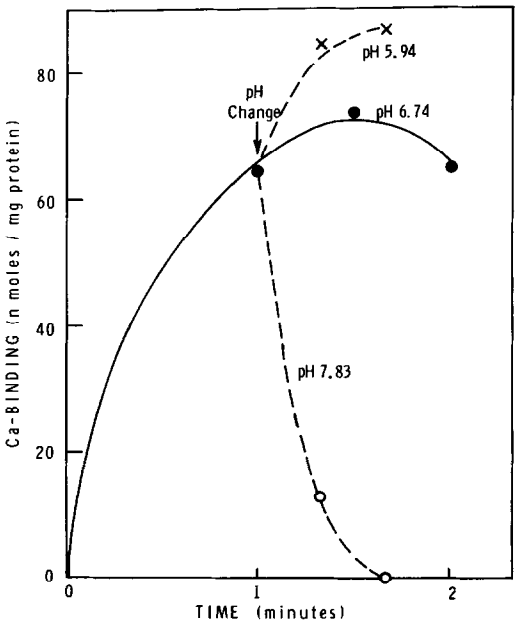

Fig. 3: Release of ^{45}Ca by pH changes in cardiac muscle RS. Conditions: 40 mM Tris-maleate, pH 6.74, 4 mM $MgCl_2$, 10^{-5} M ^{45}Ca (about 20,000 cpm), 0.2 mM ATP, 1.0 mg RS. pH was changed to 7.83 by addition of 20 µl of 1 M Tris (~ pH 10.2) and to 5.94 by 20 µl of 1 M acetate buffer (pH 4.8) to separate test tubes and filtered as described in Methods, at times indicated on abscissa. The filtration procedure takes from 1 - 2 seconds from withdrawal of sample to completion of filtration.

DISCUSSION

It has recently been reported that at pH values of 7.0-7.5, binding was significantly less than at pH 5.6-6.5 (13), while calcium release was more pronounced at pH values of 7.0-8.0, than at pH of 6.5 (10). In the present study we found that the RS rapidly lost its affinity for calcium even in the presence of ATP, when the pH was suddenly raised. The rates of calcium binding at pH 6.6 and calcium release at pH 7.56 in the skeletal preparation are slower than the rates reported by Ohnishi and Ebashi

(14). However, they used a rapid-flow mixing method, while we employed a stationary system with vibrator. It is emphasized, however, that in the present experiments the rate of calcium release at pH 7.6 was the same as the rate of initial calcium binding at pH 6.5. The calcium release exceeds the amount of calcium ions required for contraction of skeletal muscle, assuming that the yield of RS is 4 mg/g muscle and 60-100 nanomoles calcium must be supplied to 1 g of muscle for contraction (15).

It is uncertain whether any rapid pH changes arise during physiological excitation-contraction coupling. Although caution should be applied (16), the results of a recent experiment bear on this point (17). Employing double- and triple-barreled electrodes, intracellular pH of the normal resting skeletal muscle cell was found to be 5.99 at a membrane potential (E_m) of -89 mV. According to the hypothesis of Conway (18), in resting skeletal muscle, the $[H^+]$ activity of intracellular and extracellular fluid is in electrochemical equilibrium. The studies of Carter et al. (17) agree with Conway's suggestion and further show that variations in E_m produced almost instantaneous and predictable changes in intracellular pH, which ranged from 7.69 in the maximally depolarized state to 3.25 in the maximally hyperpolarized state.

Alterations of intracellular $[H^+]$ may also be involved in some pathological conditions. Katz and Hecht (19) suggest, e.g., that in ischemic heart disease, intracellular acidosis may be a cause of heart failure. According to this concept, $[H^+]$ produced during increased anaerobic glycolysis in ischemia, would displace troponin-bound calcium, decreasing the number of actin-myosin interactions. Our data suggest an alternative, that increased $[H^+]$ would increase the affinity of the RS for calcium and decrease release of bound calcium.

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