Characterization of Cardiac Sarcoplasmic Reticulum Dysfunction during Short-Term, Normothermic, Global Ischemia

Stephen Krause and Michael L. Hess
From the Departments of Medicine (Cardiology) and Physiology and Biophysics, Virginia Commonwealth University, Medical College of Virginia, Richmond, Virginia

SUMMARY. It has been proposed that breakdown of the excitation-contraction coupling system plays a pivotal role in myocardial dysfunction during the course of acute ischemia. We tested this hypothesis by characterizing the function of the sarcoplasmic reticulum at pH 7.1 and 6.4 after 7.5, 15, and 30 minutes of canine normothermic global ischemia. At pH 7.1, whole heart homogenate sarcoplasmic reticulum demonstrated a 49% depression of oxalate-supported calcium uptake at 7.5 minutes of ischemia, which progressed to 85% at 30 minutes of ischemia. At pH 6.4, control homogenate calcium uptake rates were significantly depressed, accompanied by a further depression in the ischemic groups. Isolated sarcoplasmic reticulum calcium uptake mirrored the effects of the whole heart homogenate. Calcium-stimulated magnesium-dependent ATPase (calcium-ATPase) activity was significantly depressed by both ischemia and acidosis, with a decrease in the coupling ratio (μmol calcium/μmol ATP) at 15 and 30 minutes of ischemia. Acidosis (pH 6.4) significantly shifted the sarcoplasmic reticulum pCa-calcium-ATPase curve to the right, increasing 50% activation from pCa 6.0 to 5.5 and depressing the maximum velocity (pH 7.1 = 2.06 ± 0.14; pH 6.4 = 1.41 ± 0.05 μmol P_i/mg per min; P < 0.01). With ischemia, there was a progressive decrease in maximal activation of the calcium-ATPase enzyme and a progressive shift in calcium sensitivity to a higher concentration. Steady state calcium uptake, in the absence of oxalate, demonstrated a similar depression after 7.5 and 15 minutes of ischemia at pH 7.1 and 6.4, associated with a significant increase in the passive permeability coefficient for calcium. Sarcoplasmic reticulum isolated from the 30-minute ischemic groups could not support steady state calcium uptake. It is concluded that during short-term normothermic ischemia, there is significant and progressive sarcoplasmic reticulum dysfunction which is magnified at pH 6.4, characterized by a decrease in calcium uptake and ATPase activity. There is also an uncoupling of calcium transport from ATPase activity which may be the result in part of an increase in the calcium permeability of the sarcoplasmic reticulum membrane. It is postulated that, during primary myocardial ischemia, this breakdown in sarcoplasmic reticulum function may serve as the source of intracellular calcium overload. (Circ Res 55: 176–184, 1984)

GLOBAL myocardial ischemia and hypoxia are initially characterized by a decrease in developed tension, followed by an increase in resting tension. Subsequently, contracture develops which results in a significant increase in resting tension without the capability to generate active tension (Nayler et al., 1979). This latter phase of irreversible myocardial ischemia has been postulated to be the result of a breakdown of sarcolemmal barriers to calcium, and a resultant intracellular calcium overload (Zimmerman et al., 1967). The initial phases, characterized by a decrease in developed tension and the increase in resting tension, have been postulated to be due to a breakdown in the excitation-contraction coupling system (Nayler et al., 1979; Hess et al., 1980) and have been shown to be reversible with reoxygenation and reperfusion (Nayler et al., 1979; Cobbe and Poole-Wilson, 1980a, 1980b). The mechanisms responsible for these mechanical observations in the early phase of ischemia are not well understood.

The sarcoplasmic reticulum (SR) of cardiac muscle is a potential mediator of the early responses to ischemia and hypoxia, and could contribute to the intracellular calcium overload seen in the final, contracture phase. The SR has been shown to serve as both the source and sink for coupling calcium in the excitation-contraction coupling system (Fabiato and Fabiato, 1975). Depression of SR function during ischemia would result in the loss of regulation of intracellular calcium. This hypothesis gains credence from the earlier work of McCallister et al. (1978), who demonstrated swelling and distortion of the SR T-tubule system after 20 minutes of normothermic ischemia, and by de Leiris and Feuvray (1979), who found extensive SR damage after 30 minutes of acute ischemia. Functionally, there are few studies which indicate damage to the SR enzyme-transport system during ischemia. Schwartz et al. (1973) found a defect in SR calcium release after 12–60
minutes of ischemia, whereas, with 30 minutes of ischemia, Hess et al. (1980) found calcium uptake to be depressed. Feher et al. (1980) found depressed calcium uptake after 60 minutes of normothermic ischemia; however, they also cautioned that the isolated SR fraction was injured out of proportion to the SR calcium uptake in the whole heart homogenate, suggesting either an injury process occurring during isolation, or a selection process for a more damaged SR preparation. These studies indicate that the time course for damage to the SR is more rapid than previously appreciated, and indicate that SR dysfunction may be related to the loss of tension development during the course of ischemia.

The mediators of this potential SR dysfunction are not well defined, but one potential candidate is an increasing hydrogen ion concentration. The pH of the myocardium has been found to be in the range of 6.0-6.4 in both global ischemia, using $^{31}$P-nuclear magnetic resonance (Garlick et al., 1979), and during regional ischemia, using a fiberoptic photometric probe (Tait et al., 1982). Cobbe and Poole-Wilson (1980a, 1980b) have correlated the acidosis of both ischemia and hypoxia with a decrease in tension development which becomes irreversible between 15 and 30 minutes, in spite of normalization of the pH. In addition, Mandel et al. (1982) have found a decrease in the rate of formation and decomposition of the high energy phosphate intermediates (E-P) in cardiac SR between pH 6.0 and 6.8. The decrease in the rates of formation and decomposition of E-P with a decline in pH indicate that this would be a viable explanation for a depression in the rates of SR calcium uptake during ischemia. Fabiato and Fabiato (1978) found that a decrease in pH caused a depression of calcium loading and calcium-induced calcium release in the skinned cardiac muscle fiber at pH 6.2-6.6. This is significant, because the amount of calcium released under the acidic conditions was not enough to activate the myofilaments fully.

Based on this background, we designed the following study in an attempt to characterize SR function during the course of short-term (7.5-30 minutes) normothermic global ischemia. Specifically, we sought to answer the following questions: (1) Is the sarcoplasmic reticulum isolated from the ischemic myocardium representative of the SR present in the whole heart homogenate? (2) Is there a defect in the SR calcium transport system in the ischemic myocardium? (3) Does a decrease in pH result in an exaggeration of this defect? (4) Can this defect be localized in the Ca$^{++}$-ATPase, or is it the result of an alteration in the membrane barrier to calcium in the SR? (5) What is the relationship between ischemia and an acidic pH on the calcium transport system of the SR?

### Methods

#### Surgical Procedure

In inducing normothermic global ischemia, we used the following modifications of the method of Hess et al. (1980). Adult mongrel dogs of either sex weighing 20–25 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv). The animals were intubated and ventilated with room air, utilizing a Harvard positive pressure respirator. A median sternotomy was performed and the azygous vein ligated. The superior and inferior venae cavae were isolated. A pericardiotomy was performed and a pericardial cradle created. Normothermic, global ischemia was induced with ligation of the venae cavae, permitting the heart to empty, and then electrical fibrillation was induced with a 9-V AC fibrillator. The aorta then was cross-clamped, a temperature probe inserted into the septum, and, with the use of heating pads, septal temperature was maintained at 37 ± 2°C. The experimental design consisted of sham-operated controls (n = 6) and normothermic global ischemia of 7.5 minutes (n = 5), 15 minutes (n = 6), and 30 minutes (n = 5). Following the ischemic period, the hearts were rapidly excised, placed in ice, normal saline, and the SR was isolated as described below.

#### Analytical Procedures

**Isolation Procedure for Sarcoplasmic Reticulum**

Cardiac SR was isolated by differential centrifugation at 4°C by the method of Hess et al. (1981). A fraction of the 4:1 dilution from the initial homogenization steps was reserved to study SR calcium transport in the whole heart homogenate. After the first centrifugation, the supernatant was filtered through eight layers of cheesecloth. The remaining pellet then was rehomogenized, passed through cheesecloth, and the supernatants combined and centrifuged at 10,400 g$_{max}$ for 15 minutes. The resultant supernatant was filtered through 12 layers of cheesecloth and centrifuged at 51,000 g$_{max}$ for 60 minutes in a Beckman L5-65B ultracentrifuge. The resultant pellet was resuspended in a solution containing 1.0 M KCl and 10 mM imidazole, pH 7.0. This suspension was hand-homogenized, using a glass tissue grinder with a Teflon pestle, and then centrifuged at 198,000 g$_{max}$ for 60 minutes. The resultant pellet, termed sarcoplasmic reticulum, was resuspended in a solution containing 30% sucrose + 10 mM imidazole, pH 7.0, to yield a final protein concentration of 4–8 mg/ml as determined by the method of Lowry et al. (1951). All analytical studies were performed in duplicate.

The fraction of the heart termed homogenate was prepared by centrifugation at 755 g$_{max}$ for 10 minutes following homogenization of the tissue, as described above. The inclusion of this low speed step removes unfraccionated cells, debris, and nuclei, and results in the post-nuclear supernatant which has been utilized in the characterization of membrane fractions from other muscle (Matlib et al., 1979). This post-nuclear supernatant fraction contained the most sarcoplasmic reticulum from both the control and ischemic groups (homogenate calcium uptakes at 37°C, pH 7.1: nmol Ca$^{++}$/mg protein per min. Control: crude homogenate = 33 ± 6.2; post-nuclear supernatant = 67 ± 5.0; pellet from the 750 g$_{max}$ x 20 minutes centrifugation = 34 ± 5.0; 30-minute ischemia: crude homogenate = 10 ± 3.0; post-nuclear supernatant = 23 ± 2.0; pellet from the 750 g$_{max}$ x 20 minutes centrifugation = 12 ± 2.5). After centrifugation, the supernatant was decanted through eight layers of cheesecloth and a protein determination was performed. Oxalate-supported calcium uptake studies were performed using an incubation medium at 37°C consisting of 18 mM imidazole (pH 7.1 or
6.4), 10 mM KCl, 10 mM K-oxalate, 10 mM NaN₃, 5 mM ATP (pH 7.1 or 6.4), 5 mM MgCl₂, 200 μM CaCl₂, 0.05 μCi/ml ⁴⁵Ca⁺⁺, and a protein concentration of 1 mg/ml. Calcium uptake was determined by filtering aliquots of the incubation medium through 0.45 μm Millipore filters and counting the filtrates in a liquid scintillation spectrophotometer and determining the loss of ⁴⁵Ca⁺⁺ from the total present in the bath. Calcium uptake rates were determined by linear regression analysis of the data from each time point during the incubation period and expressed in terms of μmol Ca⁺⁺/mg per min.

**Isolated Sarcoplasmic Reticulum Studies**

**Calcium Uptake and ATPase Activity**

Oxalate-supported calcium uptake was determined with the same incubation medium described above, except that the protein concentration was 0.15 mg/ml. An aliquot of the filtrate was also utilized to determine the amount of ⁴⁵Ca⁺⁺ released from the hydrolysis of ATP, following the method of Penney (1976). This liberated ⁴⁵Ca⁺⁺ was utilized as an index of the operation of the calcium "pump," and is indicative of the ATPase activity of the sarcoplasmic reticulum, during which time the vesicle was actively sequestering calcium. The ratio of calcium transported to the amount of ATP hydrolyzed, gives the coupling ratio of the calcium "pump."

**pCa-ATPase Activity**

Sarcoplasmic reticulum Ca⁺⁺,Mg⁺⁺-ATPase activity was determined by incubating the purified SR (0.15 mg/ml) at 37°C in a bath with the following composition: 100 mM K⁺, 3.16 mM Na⁺, 3.16 mM Mg⁺⁺, 10 mM EGTA, 20 mM imidazole (pH 7.1 or 6.4), Ca⁺⁺, ranging from 1 mM to 32 mM and an ionic strength of 0.16 M. We determined the ionic concentrations by utilizing the computer program of Fabiato and Fabiato (1979), which simultaneously solves the multiequilibrium equations when the appropriate apparent stability constants are used. The absolute stability constants presented by Fabiato (1981) were utilized. After incubation, aliquots were filtered through 0.45-μm Millipore filters, placed on ice, and the calcium activity determined by linear regression analysis of the data from each time point during the incubation period. Calcium uptake was determined by counting the filtrates in a liquid scintillation spectrophotometer, and the calcium load was not changing as determined by filtration. The passive efflux, Jₚ, was determined by the product of the initial load and the first order rate constant. The initial calcium load was determined by extrapolating the first order efflux curve to the time when the EGTA was added. To demonstrate that the calcium efflux was passive and dependent only on the calcium load, total calcium was varied by the addition of 20, 40, 60, and 80 μM EGTA to the bath prior to the calcium load, which decreased by 49% at 7.5 minutes of ischemia and progressively declined to 78% and 85% depression at 15 and 30 minutes of ischemia, respectively. At pH 6.4 (Fig. 1B), control homogenate calcium uptake was significantly depressed compared with that at pH 7.1 (40 ± 5 nmol Ca⁺⁺/mg per min, P < 0.05), and each period of ischemia demonstrated a further significant depression in calcium uptake rates from control (pH 6.4) (36% depression at 7.5 minutes, 54% depression at 15 minutes, and 80% depression at 30 minutes).

In the isolated SR, after 7.5 minutes of ischemia,

**Steady State Calcium Uptake**

The sarcoplasmic reticulum vesicles were actively loaded in the absence of calcium precipitating anions until steady state was achieved; this was established within 30 seconds after the addition of ATP. The reaction bath contained 104 mM KCl, 20 mM imidazole (pH 7.1 or 6.4), 10 mM NaNO₃, 0.1 mM ATP (pH 7.1 or 6.4), 2 mM MgCl₂, 4 μM added CaCl₂, and 0.2 Ci/ml ⁴⁵Ca⁺⁺ in a total volume of 4 ml at 37°C. The protein concentration ranged from 0.15 to 0.30 mg/ml, depending on the amount needed to observe steady state uptake between 50 and 70% of the total calcium in the bath. The total calcium in the bath was measured by atomic absorption spectrophotometry, using a Perkin-Elmer model 380 instrument, following acid extraction of the incubation medium which was complete except for the deletion of the ⁴⁵Ca⁺⁺ tracer. The total calcium ranged from 9.4 to 10.5 μM. The uptake reaction was initiated by the addition of the ATP, Ca⁺⁺, and Mg⁺⁺ to an otherwise complete bath. The total amount of calcium associated with the sarcoplasmic reticulum was determined by filtering 0.3-ml aliquots of the bath through 0.45 μM Millipore filters. The filtrate was counted in a liquid scintillation spectrophotometer, and the calcium associated with the sarcoplasmic reticulum was calculated from the amount of ⁴⁵Ca⁺⁺ remaining in the filtrate as compared to the total amount in the bath. Steady state occurred when net calcium uptake was not changing as determined by filtration.

**Passive Calcium Efflux**

After steady state was reached, the diffusional calcium efflux was determined by quenching the ATP-dependent calcium fluxes by the addition of EGTA to the bath in a final concentration of 2.5 mM and filtering aliquots of the bath at 0.1-minute intervals for the first 0.6 minute. The volume of EGTA added to the bath was 0.022 ml, which was not enough to have an effect on the final concentration of the other constituents in the bath. The first order rate constant was determined by linear regression of the first order plots of the calcium remaining in the vesicles as determined by filtration. The passive efflux, Jₚ, was determined by the product of the initial load and the first order rate constant. The initial calcium load was determined by extrapolating the first order efflux curve to the time when the EGTA was added. To demonstrate that the calcium efflux was passive and dependent only on the calcium load, total calcium was varied by the addition of 20, 40, 60, and 80 μM EGTA to the bath prior to the addition of the Mg⁺⁺-ATP solution. The decrease in the efflux with a decrease in the calcium load was indicative of a passive rate dependent only on the permeability of the membrane and the calcium load (Feher and Briggs, 1982, 1983).

**Statistical Analysis**

Two sets of statistical comparisons were made. Student's t-test was used when comparing two populations to each other. Comparisons of subsequent interventions to controls were made using a one-way analysis of variance, followed by a Duncan's multiple range test (Walpole, 1974). Differences were considered significant when P < 0.05.

**Results**

Figure 1A presents the effects of ischemia on calcium transport in isolated cardiac sarcoplasmic reticulum (SR) and whole heart homogenate at pH 7.1. At pH 7.1, calcium uptake by the control homogenate fraction was 68 ± 7 nmol Ca⁺⁺/mg per min, which decreased by 49% at 7.5 minutes of ischemia and progressively declined to 78% and 85% depression at 15 and 30 minutes of ischemia, respectively. At pH 6.4 (Fig. 1B), control homogenate calcium uptake was significantly depressed compared with that at pH 7.1 (40 ± 5 nmol Ca⁺⁺/mg per min, P < 0.05), and each period of ischemia demonstrated a further significant depression in calcium uptake rates from control (pH 6.4) (36% depression at 7.5 minutes, 54% depression at 15 minutes, and 80% depression at 30 minutes).
The effects of pH and ischemia on oxalate-supported calcium uptake velocity from isolated cardiac sarcoplasmic reticulum (left) and whole cell-free homogenate (right). Panel A depicts the results at pH 7.1 and panel B depicts the results at pH 6.4. All groups were statistically different from controls. C = control; 7.5 = 7.5 minutes of ischemia; 15 = 15 minutes of ischemia; 30 = 30 minutes of ischemia. Each bar represents the mean ± SEM. In this and subsequent figures, each bar or point represents five or six different experimental preparations with the analytical study performed in duplicate.

A 55% depression in calcium uptake rate occurs at pH 7.1, which increases to 80% with 15 minutes of ischemia. The decrease in uptake is not linearly dependent upon time. At pH 6.4 (Fig. 1B), control calcium uptake by the isolated sarcoplasmic reticulum is significantly depressed (pH 7.1 = 1.38 ± 0.1; pH 6.4 = 0.54 ± 0.08 μmol Ca²⁺/mg per min; P < 0.01). Compared with the pH 6.4 controls, 7.5 minutes of global ischemia caused a 35% depression in calcium transport, a 61% depression at 15 minutes, and an 81% depression at 30 minutes of ischemia. In all cases, the ischemia populations were significantly different (P < 0.05) from the controls and each other, at both pH 7.1 and pH 6.4.

Having demonstrated a depression of calcium transport in the isolated SR that is similar to the depression observed in SR calcium transport in the whole heart homogenates, we sought to determine a mechanism by which a depression in calcium uptake could occur. The major alterations which could result in the efflux of transported calcium prior to the complexing with oxalate.

Figure 2 presents the SR ATPase activity monitored concomitant with calcium uptake in Figure 1. At pH 7.1, control activity was 2.35 ± 0.05 μmol P/ mg per min. After 7.5 minutes of ischemia, enzyme activity had decreased by 34%, and this progressed to a 48% and a 74% depression of enzyme activity at 15 and 30 minutes of ischemia, respectively. At pH 6.4, control ATPase activity was depressed by 61%, compared to the pH 7.1 control. With 7.5 minutes of ischemia, SR ATPase activity at pH 6.4 was depressed by 73% compared to the 7.1 control, and this progressed to an 80% and 85% depression of enzyme activity at 15 and 30 minutes of ischemia, respectively. Table 1 presents the coupling ratios (μmol Ca²⁺ transported/μmol ATP hydrolyzed) at pH 7.1 and 6.4, respectively. At both pH 7.1 and

### Table 1: Coupling Ratios of mol Ca²⁺ Transported/mol ATP Hydrolyzed for Cardiac Sarcoplasmic Reticulum from the Control and Ischemic Groups

<table>
<thead>
<tr>
<th>Time of ischemia</th>
<th>pH 7.1 Coupling ratio (±SEM)</th>
<th>pH 6.4 Coupling ratio (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.58 ± 0.03*</td>
<td>0.56 ± 0.04†</td>
</tr>
<tr>
<td>7.5 Min</td>
<td>0.46 ± 0.03†</td>
<td>0.48 ± 0.03†</td>
</tr>
<tr>
<td>15 Min</td>
<td>0.34 ± 0.03</td>
<td>0.36 ± 0.03§</td>
</tr>
<tr>
<td>30 Min</td>
<td>0.27 ± 0.04</td>
<td>0.22 ± 0.02</td>
</tr>
</tbody>
</table>

*Statistical significance at the level of P < 0.05.
† Between controls and all experimental groups.
§ Between 15 and 30 minutes of ischemia.

**Figure 2.** The effects of ischemia on the Ca²⁺, Mg²⁺-ATPase activity from isolated cardiac sarcoplasmic reticulum at pH 7.1 and pH 6.4. These studies were conducted concomitantly with the calcium uptake studies. The abbreviations are the same as used in Figure 1. Each bar represents the mean ± SEM.
6.4, control coupling ratios were not different from each other. With the onset of ischemia, coupling ratios progressively decreased, with a slightly greater decrease observed at pH 6.4. Thus, an uncoupling phenomenon may partially explain the decrease in calcium transport.

Further insight into the role of acidosis and ischemia on SR ATPase activity was obtained by performing pCa-ATPase studies in the absence of oxalate and in the presence of the calcium ionophore A23187 (Pringle and Hidalgo, 1982). In control studies, in the presence of oxalate and/or A23187, no stimulation of the ATPase activity was observed, indicating that both oxalate and A23187 maintained intravesicular calcium levels low enough not to saturate the low affinity calcium binding sites and inhibit activation.

Having established that A23187 has no appreciable effect on ATPase activity at either pH 6.4 or 7.1, we next characterized the function of the ATPase enzyme system from each of the experimental groups at both 6.4 and 7.1. Figure 3 presents the pCa-ATPase relationship of the Ca**-ATPase from cardiac SR at pH 7.1 and 6.4 in the presence of 2 μM A23187 and absence of oxalate. Increasing hydrogen ion concentration results in a significant shift in the pCa-ATPase relationship to the right. Fifty percent activation of the Ca**-ATPase activity occurs at a pCa of 6.0 at pH 7.1, whereas at pH 6.4, there is a shift to the right resulting in 50% activation occurring at pCa 5.5. At pCa 5.25, which corresponds to maximal activation at pH 7.1, the ATPase activity at pH 6.4 is 50% of the pH 7.1 control rate and 75% of the pH 6.4 V_max (pCa 4.50).

With normothermic ischemia, there is a progressive decrease in the maximal activation of the Ca**-ATPase activity at pH 7.1 (Fig. 4) from 2.06 ± 0.14 μmol P_i/mg per min for controls to 0.99 ± 0.15 μmol P_i/mg per min at 30 minutes of ischemia. At pH 6.4 (Fig. 5) the control ATPase activity was 1.41 ± 0.05 μmol P_i/mg per min which decreased to 0.58 ± 0.11 μmol P_i/mg per min at 30 minutes of ischemia. Accompanying this decrease in maximal activation, there is also a progressive shift in calcium sensitivity to a higher concentration as determined by the pCa necessary for 50% activation (EC50), although this shift is only statistically significant following 30 minutes of ischemia at pH 7.1 and 15 minutes of ischemia at pH 6.4 (Table 2). Maximal velocity was taken at pCa 5.25 at pH 7.1, whereas pCa 4.50 was used at pH 6.4. Although it may not appear that the
ATPase rate has reached a maximum at pCa 4.5 at 15 and 30 minutes of ischemia, we feel justified in using this point for comparison for the following reasons. One, at pH 7.1, Vmax is attained at the same pCa (5.25) in each population. It would be unlikely to postulate a shift in the pCa required for maximum activation at one pH (6.4) and not another (pH 7.1). Second, pCa 4.5 is within the known physiological range of calcium available for maximal activation of mammalian heart cells (Fabiato, 1981). Third, a small further increase in activation would result in a lower E50 which is reflected by the decreased calcium load (Ca^2+ + Cai), which is dependent on the free calcium load, Cai, on which the passive efflux is dependent. There is decreased calcium load with ischemia. An explanation for this can be ascertained from the free calcium load, Cai, on which the passive efflux is dependent. There is a marked decrease in Cai, thus, the passive efflux is greatly reduced. These studies indicate that an increase in membrane permeability occurs following ischemia, contributing to the overall decrease in calcium transport by the sarcoplasmic reticulum. However, this increase in permeability is not great enough to account entirely for the decrease in observed coupling ratios.

### Discussion

Our results would appear to document a defect in cardiac sarcoplasmic reticulum (SR) calcium trans-
port during the early phase of myocardial ischemia, and support the hypothesis of Nayler et al. (1979) that the early phase of ischemia and/or hypoxic injury characterized by a decrease in developed tension, followed by an increase in resting tension, results from a breakdown of the excitation-contraction coupling system. A depression in calcium transport was observed in both the whole heart homogenate and the isolated sarcoplasmic reticulum. This observation contrasts with the earlier report of Feher et al. (1980), who noted a disparity between whole heart homogenate SR calcium uptake and calcium uptake by isolated cardiac SR. In contrast to our study, Feher et al. utilized an unfractionated homogenate, a much more severe (60-minute) model of global ischemia and an isopycnic centrifugation technique for SR isolation which requires only 3 hours of centrifugation, thus minimizing the possibility of mechanical injury to the SR during the isolation procedure.

Our data add further insight into the effects of acidosis on calcium transport by cardiac SR. Garlick et al. (1979) have demonstrated using $^{31}$P nuclear magnetic resonance to measure intracellular pH, that there is a steady decline in intracellular pH from 7.1 to 6.4 in the first 13 minutes of total global ischemia. Our data suggest that there is a progressive shift in the sensitivity of the Ca++-ATPase curve down and to the right, resulting from a decrease in pH, that has recently been demonstrated in skeletal SR (Inesi et al., 1979). The shift in calcium sensitivity would allow for an increase in the resting levels of intracellular calcium, while the depression in the maximal velocity would result in a depressed calcium transport, which we observe. A mechanism that may cause the shift has been suggested by Mandel et al. (1982), who suggested that, below pH 6.8, there was a prolongation of the rates of formation and decomposition of the phosphorylated enzyme intermediate (E-P) in the Ca++-transport cycle. Since this is considered to be the rate-limiting step, it would have a major influence on the rates of calcium uptake. pH affects not only the mechanism of calcium transport, but, also, the storage of calcium by the SR. Fabiato and Fabiato (1978) indicated that, under moderate acidotic conditions of pH 6.6, cardiac SR did not have the capacity to store adequate amounts of global ischemia and an isolation technique that requires only 3 hours of centrifugation, thus minimizing the possibility of mechanical injury to the SR during the isolation procedure.

<table>
<thead>
<tr>
<th>pH</th>
<th>Time of ischemia</th>
<th>$\text{Ca}_a$ (nmol/mg ± SEM)</th>
<th>$P$ (min$^{-1}$ ± SEM)</th>
<th>$J_p$ (nmol/mg per min ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1</td>
<td>Control</td>
<td>26.94 ± 2.19*</td>
<td>1.62 ± 0.50*</td>
<td>16.01 ± 0.97</td>
</tr>
<tr>
<td></td>
<td>7.5 Min</td>
<td>6.96 ± 2.37</td>
<td>2.46 ± 0.30</td>
<td>3.17 ± 1.02</td>
</tr>
<tr>
<td></td>
<td>15 Min</td>
<td>4.51 ± 2.02</td>
<td>2.47 ± 0.20</td>
<td>2.17 ± 1.20</td>
</tr>
<tr>
<td>6.4</td>
<td>Control</td>
<td>21.69 ± 1.30 NS</td>
<td>1.03 ± 0.12*</td>
<td>21.70 ± 2.45*</td>
</tr>
<tr>
<td></td>
<td>7.5 Min</td>
<td>19.55 ± 4.13</td>
<td>1.63 ± 1.00</td>
<td>12.09 ± 2.44</td>
</tr>
<tr>
<td></td>
<td>15 Min</td>
<td>16.44 ± 1.32</td>
<td>1.78 ± 0.20</td>
<td>8.83 ± 0.94</td>
</tr>
</tbody>
</table>

$\text{Ca}_a + \text{Ca}_b = \text{total intravesicular Ca}^{++}; \text{Ca}_a = \text{free intravesicular Ca}^{++}; \text{Ca}_b = \text{bound intravesicular Ca}^{++}; P = \text{passive permeability coefficient}; \text{Ca}_{\text{bb}} = \text{bound extravesicular Ca}^{++}; J_p = \text{passive Ca}^{++} \text{efflux}; \text{NS} = \text{not significantly different.}$

Statistical significance at the level of $P < 0.05$: *Between controls and all experimental groups. †Between 7.5 minutes and 15 minutes of ischemia.
calcium to activate the myofilaments fully. Our data substantiate these accounts in terms of oxalate-supported calcium uptake, which is depressed 60% with a change in pH from 7.1 to 6.4 in isolated SR and homogenate studies.

Our data also suggest that the effects of acidosis and ischemia on sarcoplasmic reticulum isolated from the ischemic heart are additive. Previous investigators (Schwartz et al., 1973; Hess et al., 1980, 1981; Feher et al., 1980) have studied isolated SR from the ischemic heart at only pH 7.0. Since the data of Garlick et al. (1979) demonstrate an intracellular pH of 6.4 by 5 minutes of global ischemia, the concept of performing studies with tissue preparations from ischemic models at a lower pH (6.4) becomes physiologically relevant. At the onset of ischemia, the cardiac cellular pH is 7.1, whereas, by 15 minutes of ischemia, the intracellular pH has declined to pH 6.4. Thus, if one is to understand the function of the cardiac cell during ischemia, it is necessary to perform the studies under conditions which mimic the ischemic environment. If one compares the decrease in SR calcium uptake from the ischemic group at pH 6.4 over a 15-minute time course against the control at pH 7.1, one finds a tight temporal correlation between the decrease in intracellular pH, loss of SR calcium uptake ability, and the decrease in tension development. This points out the rapidity of ischemic injury to the SR.

This concept of the accompanying acidosis during ischemia becomes extremely important to the characterization of SR function during ischemia. In both the whole heart homogenate studies and the isolated SR, at pH 7.1, the 15- to 30-minute ischemic groups demonstrated an 80% depression of calcium uptake. However, if one compares the 30-minute ischemic group at pH 6.4 to the pH 7.1 control, a situation analogous to the ischemic myocardial cell, one finds a 93% depression of calcium uptake rate in both the whole heart homogenate and the isolated SR. The increase in intracellular calcium with sub-
cellular pH of 6.4 by 5 minutes of global ischemia, the intracellular source of free calcium as the initiating event for the final, irreversible phase of myocardial ischemia. The formation of rigor complexes most probably involves both a decrease in intracellular ATP concentration and a significant increase in intracellular calcium concentration. The increase in intracellular calcium with subsequent mitochondrial calcium accumulation and resultant decrease in ATP production would appear to be the final common pathway for ischemic contracture.

It has long been assumed that the source for this intracellular calcium overload was the extracellular space owing to the concentration gradient across the sarcolemmal membrane. However, this concept has been challenged by Bourdillion and Poole-Wilson (1982), analyzing calcium influx and efflux in the ischemic rabbit intraventricular septum. These investigators demonstrated no net increase in tissue calcium during the ischemic phase and no change in net efflux. It is only after reperfusion and reoxygenation that an increase in total tissue calcium is observed that can be explained by the influx of calcium from the extracellular to the intracellular compartment. These data strongly support a primary intracellular source of free calcium as the initiating event for the final, irreversible phase of myocardial ischemia. Since it is now recognized that the sarcoplasmic reticulum of cardiac muscle serves as the major source and sink of coupling calcium in the excitation-contraction coupling sequence (Fabiato and Fabiato, 1975), with the progressive decrease in sarcoplasmic reticulum calcium uptake, accompanied by a decrease in the available ATP, free cytosolic calcium concentrations would increase. An increase in intracellular calcium concentration would result in the formation of calcium-tropinin, which, accompanied by a decrease in ATP concentration, would cause formation of rigor complexes. Thus, we would hypothesize that disruption of the sarcoplasmic reticulum during the course of myocardial ischemia serves as a source of the intracel-
lular calcium overload of primary myocardial ischemia.

In summary, we have identified a significant defect in sarcoplasmic reticulum calcium transport beginning at 7.5 minutes of global, normothermic ischemic and progressing to a significant degree of injury by 15–30 minutes of ischemia. This defect is characterized by first a depression of Ca\(^{++}\), Mg\(^{++}\)-ATPase activity, followed by an uncoupling of calcium transport from ATP hydrolysis, and is significantly magnified at pH 6.4. It is hypothesized that this defect in SR function during the early phase of ischemia can result in the breakdown of the excitation-contraction coupling system and that it contributes to the decrease in developed tension and the increase in resting tension observed with hypoxic/ischemic injury. With progression of the ischemic process and further disruption of the sarcoplasmic reticulum, the inability of the sarcoplasmic reticulum to sequester calcium would lead to an increase in the intracellular calcium concentration, rigor complex formation, and ischemic contracture.

References


Fabiato A (1981) Myoplasmic free calcium concentration reached during the twitch of an intact isolated cardiac cell and during calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned cardiac cell from the adult rat or rabbit ventricle. J Gen Physiol 78: 457–497

Fabiato A, Fabiato F (1975) Contractions induced by a calcium-triggered release of calcium from the sarcoplasmic reticulum of single skinned cardiac cells. J Physiol (Lond) 249: 469–495

Fabiato A, Fabiato F (1978) Effect of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. J Physiol (Lond) 276: 233–255

Fabiato A, Fabiato F (1979) Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. J Physiol (Paris) 75: 463–505


INDEX TERMS: Sarcoplasmic reticulum • Calcium • Ischemia • ATPase activity • Acidosis
Characterization of cardiac sarcoplasmic reticulum dysfunction during short-term, normothermic, global ischemia.

S Krause and M L Hess

Circ Res. 1984;55:176-184
doi: 10.1161/01.RES.55.2.176

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1984 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/55/2/176

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at: http://circres.ahajournals.org/subscriptions/