$[Ca^{2+}]_i$ Transients in the Cardiomyopathic Hamster Heart

Joan Wikman-Coffelt, Thomas Stefenelli, Shao T. Wu,
William W. Parmley, and Gaetan Jasmin

Intracellular $[Ca^{2+}]$ transients were studied in isolated hearts of healthy and cardiomyopathic hamsters in late failure perfused with glucose or pyruvate. Hearts of healthy hamsters developed similar pressures when perfused with either glucose or pyruvate, and $[Ca^{2+}]_i$ transients were comparable in amplitude when perfused with either substrate. On the other hand, hearts of cardiomyopathic hamsters in late failure developed normal pressure when perfused with pyruvate but developed depressed pressure (50%) when perfused with glucose. The amplitude of $[Ca^{2+}]_i$ transients fell severely and was associated with a high diastolic $[Ca^{2+}]_i$ in cardiomyopathic hamster hearts when the perfusate was switched from pyruvate to glucose. The high phosphomonoester sugars as evidenced by ^{31}P nuclear magnetic resonance studies and the depressed oxygen consumption in the cardiomyopathic hamster hearts perfused with glucose reflect an inhibition in glycolysis and a subsequent decrease in mitochondrial activity. Without an adequate delivery of substrate to the mitochondria in the cardiomyopathic hamster, the myocardium is no longer capable of maintaining its $[Ca^{2+}]_i$ homeostasis. (*Circulation Research* 1991;68:45–51)

Impaired mechanical performance is the usual cause of congestive heart failure, although diastolic dysfunction may play an important contributory role.[1] Changes in the failing myocardium of the myopathic hamster include a deficient production in cyclic AMP,[2] a decrease in Na^+,K^+-ATPase,[3] a decrease in the function of the sarcoplasmic reticulum,[4] an inhibition in the Na^+/Ca^{2+} exchanger,[5] excessive calcium accumulation,[6,7] fibrosis,[8] high inorganic phosphate,[9] and a depressed phosphorylation potential.[10]

The studies described here were carried out to determine if the decrease in cardiac performance observed in the cardiomyopathic hamster heart perfused with glucose as the substrate[10] was associated with a decrease in the amplitude of calcium transients and an increase in diastolic $[Ca^{2+}]_i$. To assess $[Ca^{2+}]_i$ transients, the fluorescent dye indo 1 and surface fluorometry were used as described previously.[11–14]

From the Departments of Medicine and the Cardiovascular Research Institute, University of California, San Francisco, Calif.

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Address for correspondence: Joan Wikman-Coffelt, PhD, University of California, San Francisco, Room M-1186, 505 Parnassus Avenue, San Francisco, CA 94143.

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Materials and Methods

Animals

Twenty-four Syrian cardiomyopathic hamsters (UM-X7.1 strain, a derivative of the Bio 14.6 strain) in late failure (220–240 days of age) and 24 Syrian age-matched healthy hamsters were studied. Cardiomyopathic hamsters are in severe advanced heart failure at 250 days of age, and all animals die by 280–300 days because of myocardial pump failure or arrhythmias. All animals contained ascites fluid in the abdominal cavity and thrombotic material in the cardiac chambers at the time of death.

Perfusion Conditions

The hamsters were anesthetized with ether, and the hearts were excised rapidly through a midline sternotomy and perfused within 30–60 seconds. The isolated hearts, paced by a Medtronic model 5320 pulse generator (Medtronic Inc., Minneapolis, Minn.) at a constant rate of 220 beats/min, were perfused by a modified Langendorff method.[15] All hearts were perfused at a constant perfusion pressure of 140 cm H_2O for 15 minutes to reach a steady state before beginning the experiment. The hearts (stable for 2 hours[16]) first were perfused with either glucose or pyruvate, and then the media was switched to the alternate. All hearts were perfused with a modified Krebs-Henseleit solution containing (mM) NaCl 117, KCl 4.3, $CaCl_2$ 2.0, $MgCl_2$ 1.2, K_2HPO_4 0.1, $NaHCO_3$

25, EDTA 0.5, pyruvate 10, or glucose 15, as well as 10 mg/l albumin. The phosphate was too low to be detected by nuclear magnetic resonance (NMR) methods. The medium was bubbled with 95% O_2-5% CO_2 at 25°C, and the temperature was raised to 35°C subsequent to flushing with gases to dissolve maximum oxygen in the perfusate.[15] Temperature was monitored with in-line thermocouples and controlled with specially designed heat exchangers.[17] The oxygen tension of the perfusate was maintained at approximately 650 mm Hg.

Physiological Measurements

Arterial samples were aspirated from the aortic chamber, and venous samples were drawn from a catheter introduced into the right ventricular outflow tract for oxygen measurements (model 165/2 gas analyzer, Corning Glassworks, Medfield, Mass.). Pa_{O_2} was measured, and the myocardial oxygen consumption was calculated as the product of coronary flow and coronary oxygen extraction.[15] Coronary flow was determined by collecting the effluent of the right ventricle for 1 minute.

^{31}P Magnetic Resonance Spectroscopy

^{31}P NMR spectroscopy of the beating isolated perfused heart was obtained on a 5.6-T vertical 76-mm bore magnet as described previously.[18] ^{31}P-NMR spectra were obtained without proton decoupling at 97.3 MHz, using a Model 1180 Nicolet computer (Nicolet Instrument Corp., Madison, Wis.), a pulse programmer, and a high-resolution 20-mm broad-band probe. Pulse angle was 60°, recycle time 1.25 seconds, and spectra width 4,000 Hz. Spectra were obtained by signal averaging 512 free induction decays during a 10-minute period. The signal-to-noise ratio was approximately 30:1. Chemical shifts are referred to the resonance position of phosphocreatine (PCr). The pH was determined from the chemical shift in relation to the resonance of PCr by using a chemical shift titration curve of pH-dependent inorganic phosphate (P_i) to PCr peak difference as described earlier.[18] The peaks characteristic of P_i, PCr, and phosphate groups of adenosine were identified. Each spectrum and the corresponding areas were numerically integrated after the baseline was defined. The phosphate percent then was calculated for P_i, PCr, and β-ATP by dividing each individual peak area by the sum of the areas for all three peaks. The corrected molar ratio of phosphate obtained from ^{31}P-NMR was converted to concentration based on standardized high-performance liquid chromatographic analyses of freeze-clamped tissue as described previously.[10,21] Extracellular space was calculated as described previously[19] by adding K[CoEDTA] to the perfusate at a concentration of 0.6 mM. Millimolar concentration of nucleotides obtained from chromatographic analyses of freeze-clamped tissue then was converted to millimolar cytosolic fraction after adjusting for extracellular space.[18,19]

Surface Fluorometry With Indo 1

Fluorescence excitation was provided by a 100-W mercury vapor lamp, through a 365±10 nm discriminating filter and a custom-made silica fiber-optic cable (Welch Allyn, Inc., Skaneateles Falls, N.Y., designed for assessing indo 1 excitation and emission), onto a circular epicardial area of 38.5 mm^2 of the left ventricular septum, after the free wall of the right ventricle was stripped away.[11] The emitted fluorescence was collected by a coaxial cable of 300 quartz fiber optics, divided by a beam splitter, and then was filtered at 400±5 and 510±12.5 nm. A direct digital readout of intensity values and ratios was recorded on the computer through software, giving 100 points per cardiac cycle, thus allowing for precise determination of maximum and minimum ratios. The fluorescent intensity ratio of F400/F510 also was displayed on the Gould recorder (Gould Instruments, Cleveland, Ohio) simultaneously with left ventricular pressure for monitoring the influx of Ca^{2+} in relation to depolarization as timed by the electrocardiogram.[14]

The fluorometer was offset for autofluorescence (510 and 400 nm) of the heart before labeling. A standard curve was derived from known concentrations of Ca^{2+} and indo 1 in a balloon in the left ventricular chamber of the unlabeled heart.[11–14,16] The latter was obtained by placing a balloon inside the left ventricular chamber of the nonlabeled heart, adding known concentrations of Ca^{2+} buffered with EGTA, and the free dye (5×10^{-5} M indo 1), exciting at 365 nm, and assessing voltage for the intensity ratios of 510 and 400 nm emission after the emission was filtered by the septum. The fiber optics were placed on the right ventricular side of the septum after stripping away the free wall of the right ventricle.[11] The balloon then was removed and the heart labeled with fluorescent dye. Indo 1-AM was solubilized (6 mg) in a mixture of dimethyl sulfoxide/pluronic F-127 (25% wt/vol) and added to Krebs-Henseleit solution (600 ml) containing 1% calf serum. The heart was perfused 30 minutes with indo 1-AM in Krebs-Henseleit medium as described above except pyruvate was used as the substrate, followed by a 30–45-minute washout with glucose as the substrate. Previous studies in our laboratory have shown the loading is better with pyruvate as the substrate as compared with glucose.[11–14] Loading augments fluorescence of the heart 10-fold. Ca^{2+} estimation was in the range of linearity from 10^{-7} to 10^{-6} M, giving linear ratios between 0.50 and 1.00. The inflection point of the [Ca^{2+}] concentration curve in an intraventricular chamber balloon filtered by myocardial tissue gave a value the same as that obtained theoretically.

Statistical Analysis

Values are reported as mean±SD. To detect significant differences between the groups, Scheffe's test for multiple contrasts by the analysis of variance was

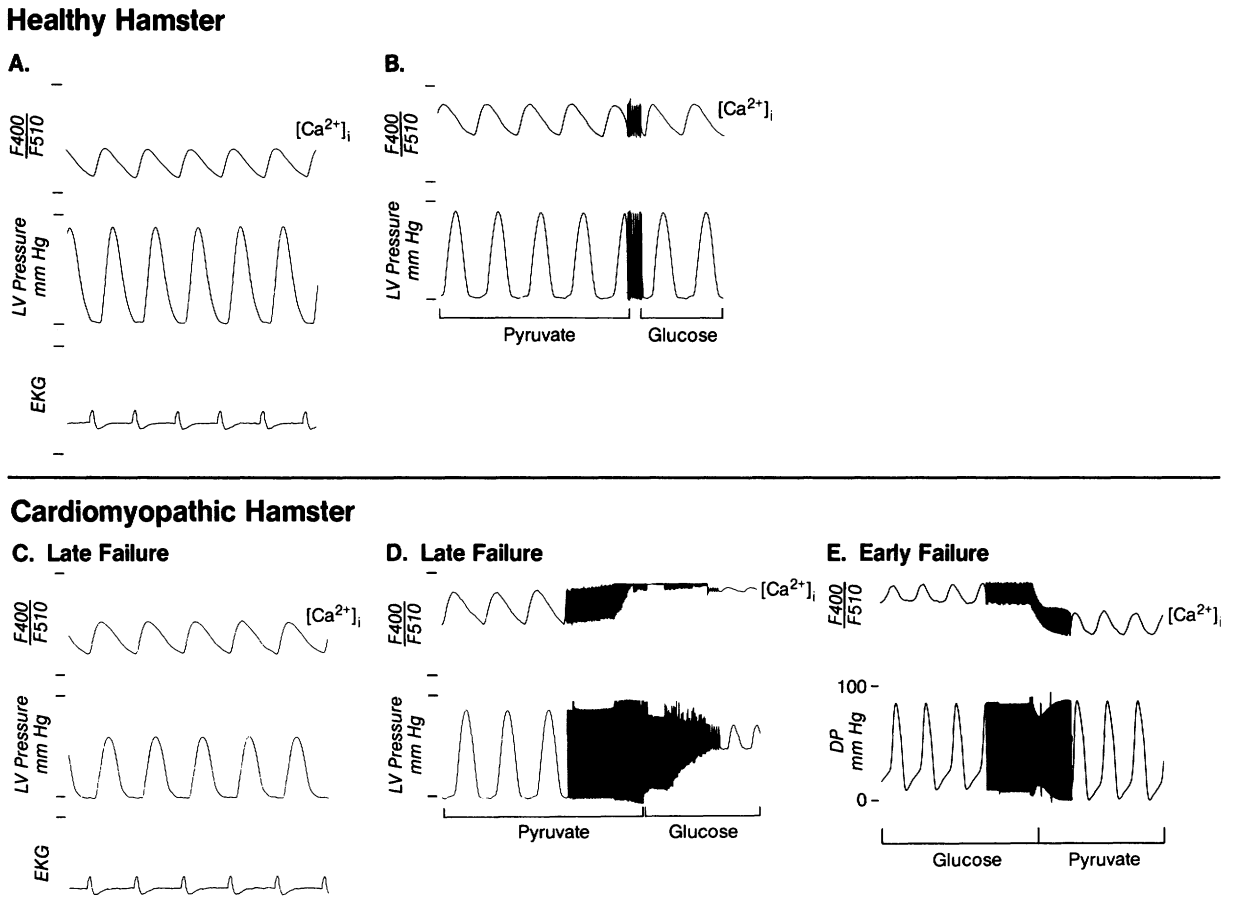

FIGURE 1. Panel A: *Representative calcium-dependent fluorescent changes in hamster heart. The fluorescent intensity ratio of F400/F510 (indicative of $[Ca^{2+}]_i$) is shown on the first channel. Left ventricular (LV) pressure is on the second channel and the electrocardiogram (EKG) on the third channel. The $[Ca^{2+}]_i$ transients peak at half time to peak pressure in the healthy hamster heart, the time it takes the EKG to traverse the heart.* Panel B: *Representative calcium-dependent fluorescent changes in a healthy hamster heart perfused first with pyruvate and then with glucose. The change in substrate causes no alteration in developed pressure, the diastolic calcium, or the amplitude of calcium transients.* Panel C: *Cardiomyopathic hamster heart (late heart failure) perfused with pyruvate.* Panel D: *The same studies carried out in cardiomyopathic hamster hearts in late heart failure did not give similar results. When the perfusate was switched from pyruvate to glucose, there was a large rise in diastolic pressure and diastolic calcium. Concurrently, the amplitude of calcium transients decreased along with developed pressure.* Panel E: *Representative calcium-dependent fluorescent changes in a cardiomyopathic hamster in early failure, perfused first with glucose and then with pyruvate. The change in substrate caused a small increase in developed pressure (DP), a decrease in diastolic calcium, and an increase in the amplitude of calcium transients.*

applied, and Student's t test was used to detect significant differences within a group. The null hypothesis was rejected at the 95% level, considering a value of $p<0.05$ as significant.

Results

Figure 1A is a representative spectrum of fluorescent changes in a normal hamster heart. The ratio that represents free $[Ca^{2+}]_i$ is shown on the first channel. Developed pressure is displayed on the second channel and the electrocardiogram on the third channel. Intracellular calcium peaks at approximately half time to peak pressure and coincides with depolarization as it traverses through the heart. A similar representative tracing of the cardiomyopathic hamster heart perfused with pyruvate is given in Figure 1C. There was no calibration of the dynograph channels for fluorescence emission, because digital voltage readout was recorded on the computer for calculation of intracellular calcium. In Figure 1B, a healthy hamster heart demonstrates no change in calcium transients, diastolic calcium, or developed pressure when the substrate was switched from pyruvate to glucose. On the other hand, the cardiomyopathic hamster heart, in late failure, showed a severe increase in diastolic calcium and diastolic pressure when the perfusate was switched from pyruvate to glucose (Figure 1D). In many cases, the diastolic calcium was so high when the cardiomyopathic hamster heart was perfused with glucose that one could not discern calcium transients because the dye was saturated. In less severe cases of heart failure in the

TABLE 1. Comparative Effects of Glucose and Pyruvate on Hemodynamics and Metabolism

	Glucose	Pyruvate	*p* value
Hemodynamics			
Developed pressure (mm Hg)			
Healthy hamsters	141±10	144±9	NS
Myopathic hamsters	61±14	132±8	0.01
Oxygen consumption (μmol/g dry wt/beat)			
Healthy hamsters	0.24±0.02	0.25±0.02	NS
Myopathic hamsters	0.11±0.01	0.20±0.02	0.01
Coronary flow (ml/min)			
Healthy hamsters	11.2±0.7	11.6±0.8	NS
Myopathic hamsters	7.4±0.5	7.8±0.4	NS
End-diastolic pressure (mm Hg)			
Healthy hamsters	2.1±0.5	1.7±0.9	NS
Myopathic hamsters	20.2±2.4	4.2±1.2	0.01
^{31}P-NMR (standardized by HPLC)			
PME (mM)			
Healthy hamsters	0.80±0.13	0.41±0.11	NS
Myopathic hamsters	5.12±0.07	0.23±0.08	0.01
$[pH]_i$			
Healthy hamsters	7.13±0.04	7.12±0.03	NS
Myopathic hamsters	6.86±0.05	7.11±0.04	0.05
PP ln[(ATP)/(ADP)(P_i)]			
Healthy hamsters	11.1±0.1	11.3±0.2	NS
Myopathic hamsters	9.0±0.2	9.8±0.2	0.05
Surface fluorometry (indo 1)			
$[Ca^{2+}]_i$ ($\times 10^{-7}$ M)			
Healthy hamsters			
Diastole	3.1±0.1	3.2±0.1	NS
Systole	5.8±0.2	6.0±0.2	NS
Myopathic hamsters			
Diastole	6.9±0.6	3.5±0.1	0.01
Systole	8.4±0.5	5.6±0.3	0.01

Because of the shape of healthy hamster hearts, we were not able to succeed in assessing $[Ca^{2+}]_i$ in control hamsters. Values are mean±SD; $n=5$. NMR, nuclear magnetic resonance; HPLC, high-performance liquid chromatography; PME, phosphomonoester sugars; PP, phosphorylation potential.

cardiomyopathic hamster, the increase in developed pressure and calcium transients was less (Figure 1E). These data are summarized in Table 1.

In healthy hamster hearts, there was no significant difference in hemodynamics when the hearts were perfused with glucose versus pyruvate. On the other hand, cardiomyopathic hearts showed a significant difference in developed pressure ($p<0.01$), oxygen consumption ($p<0.01$), and diastolic pressure ($p<0.01$) when perfused with glucose versus pyruvate. Similarly, there was no significant difference in energy metabolites assessed by ^{31}P-NMR in healthy hamster hearts when perfused with glucose versus pyruvate. However, the cardiomyopathic hamster hearts showed a significant difference in phosphomonoester sugars ($p<0.01$), $[pH]_i$ ($p<0.05$), and the phosphorylation potential ($p<0.05$) when perfused with glucose versus pyruvate (Table 1). There was no significant difference in diastolic or systolic calcium in the healthy hamster heart, whereas the cardiomyopathic hamster heart showed a significant difference ($p<0.01$) in both values when perfused with glucose versus pyruvate.

Figure 2A demonstrates premature ventricular contractions in a healthy hamster heart. Contractions 1, 3, and 5 are premature contractions with diminished pressure development. Contractions 2, 4, and 6 are potentiated postextrasystolic contractions. Incomplete relaxation ending in a high diastolic calcium is coupled with a lower pressure on the subsequent premature beat. With increasing prematurity, the diastolic calcium is high at the onset of contraction as at arrow 5, and there is an associated high diastolic pressure as shown at arrow 5 on the fourth channel. During the postextrasystolic pause, there is a more complete relaxation and a low diastolic calcium as shown by arrow 6 (first three channels). This results in a higher peak developed pressure as shown at arrow 6 on the fourth channel.

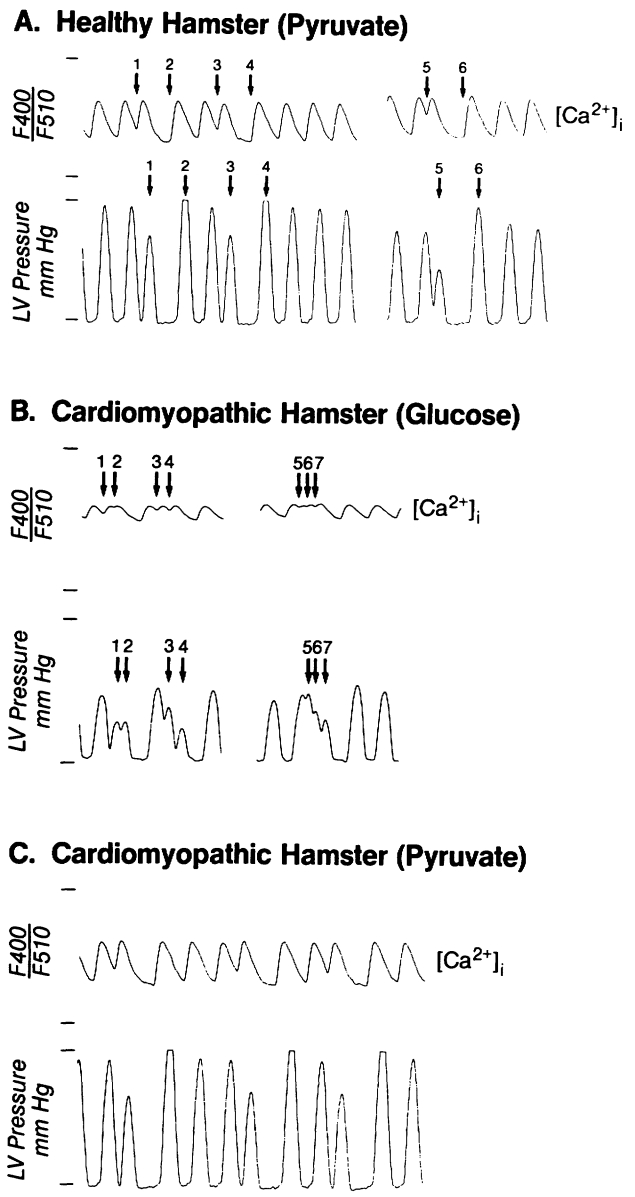

FIGURE 2. Panel A: *Representative $[Ca^{2+}]_i$ tracings of premature ventricular contractions in healthy hamster hearts. The first channel shows the fluorescent intensity ratio of F400/F510, indicative of $[Ca^{2+}]_i$; the second channel shows left ventricular (LV) pressure. Beats 1, 3, and 5 are premature beats, and beats 2, 4, and 6 are potentiated postextrasystolic beats. An increase in diastolic calcium preceding the premature beat is associated with a decrease in developed pressure of those beats (1,3,5).* Panel B: *Representative $[Ca^{2+}]_i$ tracings of coupled ventricular contractions in cardiomyopathic hamster heart (late heart failure). The heart is perfused with glucose. Coupled beats occur in pairs (1-2, 3-4) or in triplets (5,6,7). An elevation in diastolic calcium preceding the premature beat is associated with a marked reduction in developed pressure.* Panel C: *Representative $[Ca^{2+}]_i$ tracings of premature ventricular contractions in cardiomyopathic hamster heart perfused with pyruvate. When depolarization occurred before the heart was fully relaxed, the diastolic $[Ca^{2+}]_i$ remained elevated and the premature contraction was depressed. When relaxation was prolonged, the diastolic $[Ca^{2+}]_i$ fell below control level and the aftercontraction was elevated.*

Figure 2B demonstrates coupled ventricular extrasystoles in cardiomyopathic hamster heart perfused with glucose. Extrasystoles are seen in runs of two (arrows 1 to 2 and 3 to 4) and three (arrows 5–7). Incomplete relaxation and high diastolic calcium are accompanied by a high diastolic pressure and a decrease in peak developed pressure. Figure 2C shows premature ventricular contractions in the cardiomyopathic hamster heart perfused with pyruvate. With a premature beat, incomplete relaxation results in a higher diastolic calcium and a lower developed pressure, but not as severe a rise in diastolic pressure. As observed in healthy hamster hearts (Figure 2A), during the postextrasystolic pause, there is a more complete relaxation, a low diastolic calcium, and a subsequently higher developed pressure (Figure 2C).

Discussion

Glycolysis is slow in the heart, limiting delivery of pyruvate to the mitochondria.[10,20,21] A rise in Ca^{2+} and H^+ depresses glycolysis, as observed in ischemia[16,22] and cardiomyopathy.[10,23] The decrease in glycolysis most likely is due to Ca^{2+} and H^+ inhibition of phosphofructokinase,[24,25] a rate-limiting enzyme in glycolysis.[24,25] Phosphomonoester sugars accumulate when glycolysis is inhibited.[26] An accumulation of phosphomonoester sugars in association with an inhibition in glycolysis has been demonstrated with ischemia[16] and heart failure in the cardiomyopathic hamster heart.[12] With an inhibition in glycolysis and limited delivery of pyruvate to the mitochondria, there is a decrease in oxygen consumption[10] and depressed mitochondrial activity.[10] The rise in phosphomonoester sugars, the decrease in the phosphorylation potential, and the low oxygen consumption when the myopathic heart is perfused with glucose as the substrate indicate that glycolysis is inhibited in the cardiomyopathic hamster heart.

The diastolic $[Ca^{2+}]_i$ in the myocardium is influenced by influx and efflux of Ca^{2+}. $[Ca^{2+}]_i$ homeosta-

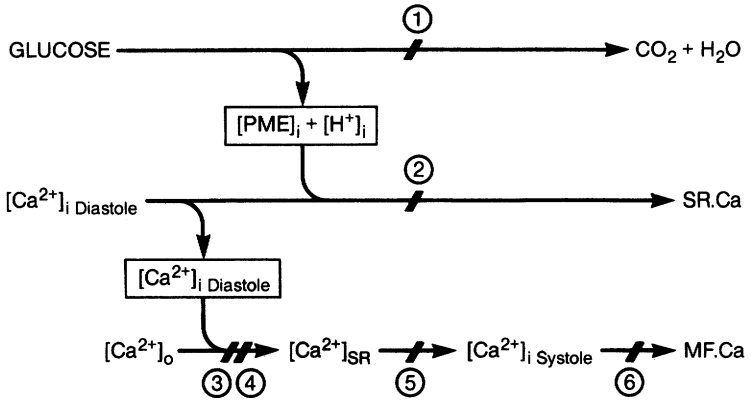

FIGURE 3. *Schematic of a possible mechanism for the depressed cardiac performance associated with an inhibition in glycolysis. The pathway is described in the "Discussion." PME, phosphomonoester sugars; SR, sarcoplasmic reticulum; MF, myofilament.*

sis is regulated, in part, by sarcolemmal Na^+/Ca^{2+} exchanger and indirectly by the Na^+/H^+ exchanger. The Na^+/Ca^{2+} exchanger helps maintain diastolic levels of $[Ca^{2+}]_i$.[27] In the studies described here, the increase in diastolic $[Ca^{2+}]_i$ is associated with an increase in $[H^+]_i$. Kohomoto et al[27] have shown that an increase in $[H^+]_i$ causes an increase in diastolic $[Ca^{2+}]_i$ because of change in $[Ca^{2+}]_i$ buffering and $[Ca^{2+}]_i$ extrusion. Acidosis decreases myofilament sensitivity. The diminution in the affinity of troponin C for Ca^{2+} produces a right shift in the tension–pCa curve.[28] Thus, as the diastolic $[Ca^{2+}]_i$ rose in cardiomyopathic hamster hearts when perfused with glucose, the Ca^{2+} sensitivity of troponin C for $[Ca^{2+}]_i$ most likely decreased. In some cases, diastolic $[Ca^{2+}]_i$ values in glucose-perfused myopathic hearts rose higher than the systolic $[Ca^{2+}]_i$ levels when perfused with pyruvate. Calcium enters the myocardial cell through calcium channels[29] and the Na^+/Ca^{2+} exchanger.[30] A rise in $[Ca^{2+}]_i$, as occurs with a high diastolic $[Ca^{2+}]_i$, causes a negative inotropic effect by a $[Ca^{2+}]_i$ inactivation of the calcium channels and the Na^+/Ca^{2+} exchanger.[28–30] The large increase in diastolic $[Ca^{2+}]_i$ levels in the myopathic hamster heart, when the substrate was switched from pyruvate to glucose, demonstrates that either mitochondrial activity is important for maintaining $[Ca^{2+}]_i$ homeostasis in the heart or glucose has some direct effect on $[Ca^{2+}]_i$ channels and $[Ca^{2+}]_i$ exchange in cardiomyopathic hamster hearts as may occur in brain cells.[31]

In an analysis of $[Ca^{2+}]_i$ transients and developed pressure with premature beats, it can be shown that diastolic calcium and complete relaxation are important to the peak pressure developed on the subsequent beat. As the diastolic $[Ca^{2+}]_i$ increases, it turns off the calcium channels, lowers the amplitude of $[Ca^{2+}]_i$ transients, and thus decreases developed pressure. A high diastolic $[Ca^{2+}]_i$ also may be related to the ability of the sarcoplasmic reticulum to sequester sufficient Ca^{2+} for release on the subsequent contraction. In the cardiomyopathic hamster heart, cyclic AMP is low, diastolic $[Ca^{2+}]_i$ is high, and developed pressure is depressed; thus, there may be an inability of the sarcoplasmic reticulum to sequester Ca^{2+} for ample release on the subsequent beat. The rise in diastolic $[Ca^{2+}]_i$ may incite a constant Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum. There was a direct correlation between diastolic $[Ca^{2+}]_i$ preceding a premature beat and developed pressure on the subsequent beat.

In conclusion, inhibition of glycolysis and the corresponding increase in $[H^+]_i$ in the myopathic hearts in failure cause a disturbance in Ca^{2+} buffering, a severe rise in diastolic $[Ca^{2+}]_i$, a decrease in $[Ca^{2+}]_i$ transients, and depressed cardiac performance. Inhibition of glycolysis results in an intracellular accumulation of phosphomonoester sugars and $[H^+]_i$[12,16] (Figure 3, step 1). During relaxation, $[H^+]_i$ competes with $[Ca^{2+}]_{i\ Diastole}$ for sarcoplasmic reticulum sequestering[28] (Figure 3, step 2). As result, $[Ca^{2+}]_{i\ Diastole}$ rises with a subsequent inactivation of calcium channels and $[Ca^{2+}]_{i\ Systole}$-induced Ca^{2+} release from the sarcoplasmic reticulum[27–29] (Figure 3, steps 3 and 4). The latter prevents a rise in $[Ca^{2+}]_{i\ Systole}$ (Figure 3, step 5) and lowers the availability of $[Ca^{2+}]_{i\ Systole}$ for myofilament binding[28] (Figure 3, step 6). Rigor is partially prevented during the high $[Ca^{2+}]_{i\ Diastole}$ by causing a decrease in myofilament calcium sensitivity.[27,29,32]

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