Calcium Transport Properties of Cardiac Sarcoplasmic Reticulum From Cardiomyopathic Syrian Hamsters (BIO 53.58 and 14.6): Evidence for a Quantitative Defect in Dilated Myopathic Hearts Not Evident in Hypertrophic Hearts

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Calcium uptake was measured in homogenates and microsomal preparations enriched in sarcoplasmic reticulum vesicles isolated from hearts of hypertrophic (BIO 14.6) and dilated (BIO 53.58) cardiomyopathic as well as control (FIB) Syrian hamsters at 3, 7, 9, and 11 months of age. Calcium uptake studies were done using the Millipore filtration technique under conditions known to restrict transport to the sarcoplasmic reticulum. Steady-state calcium uptake capacity was used as a measure of the relative amounts of sarcoplasmic reticulum in homogenates prepared from individual hearts.

At 3 months of age, there were no differences in calcium uptake in homogenates from control or myopathic hearts. However, by 9 months, although calcium uptake of homogenates from control and hypertrophic hearts was the same, calcium uptake by homogenates from dilated hearts was significantly lower by 3 months of age. The catalytic activity of the sarcoplasmic reticulum transport enzyme was estimated from the ratio of velocity to capacity, which provides a measure of the fractional rate of filling of the sarcoplasmic reticulum with calcium. The velocity-to-capacity ratios were not different at any of the ages in both the homogenate and microsomal preparations. The results of this study demonstrate that a major defect in the dilated cardiomyopathy may be due to a decrease in the volume or number of sarcoplasmic reticulum calcium transport sites rather than a decrease in specific activity of the transport enzyme. (Circulation Research 1988;62:81-85)

The aim of our experiments was to compare the calcium transport properties of the sarcoplasmic reticulum (SR) in hearts from control and cardiomyopathic hamsters. The SR of heart muscle regulates the flow of activating calcium ions to and from the myofilaments. There is ample evidence that the functional properties of SR are depressed in various types of heart failure, including those associated with cardiomyopathy. Although it seems clear that there is a "lesion" in the SR of myopathic hamsters, the nature of this defect is not clear. Early evidence, using a hamster strain that develops a hypertrophic cardiomyopathy, indicated that this muscle disease was associated with a "dilution" of the SR, i.e., a progressive decrease in the amount of SR per unit mass of heart. In addition to this quantitative defect, there may also be qualitative defects in the SR transport enzyme.

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phic and dilated cardiomyopathies result in disease progression to the same end point of congestive heart failure.

The functional properties of SR from hearts with the dilated myopathy have not been previously studied. It was thus of interest to compare properties of the SR from hearts with dilated and hypertrophic myopathies in terms of the qualitative and quantitative defects in SR function described above. Our approach to these questions involved the study of SR vesicles in cardiac homogenates and microsomal fractions during the progression of the disease for up to 11 months. The former approach permitted investigation of the SR population in individual hearts. Our results indicate that the dilated cardiomyopathy is associated with a progressive quantitative defect in the SR, i.e., it appears that in this form of myopathy there are fewer transport sites per unit mass of heart muscle. On the other hand, we found no difference in the calcium transport properties of SR from hypertrophic hearts, which were not different from SR of control hearts.

**Materials and Methods**

Male Syrian hamsters 3, 7, 9, and 11 months of age with either dilated (BIO 53.58) or hypertrophic (BIO 14.6) cardiomyopathy were used for the study. Age-matched inbred normal male golden Syrian hamsters (F1B) served as controls. All the hamsters were obtained from Bio-Breeders Inc., Fitchburg, Mass.

**Homogenate and Sarcoplasmic Reticulum Vesicle Preparation**

Animals were killed by cervical dislocation. The hearts were quickly excised and immersed in ice-cold normal saline. After removing connective tissue, vessels, and atria, each heart was weighed, minced into small pieces, and individually homogenized in 15 volumes of 25 mM imidazole, pH 7.0. Homogenization was carried out at 0–4°C in a Thomas Teflon-glass tissue grinder using a motor driven pestle at 500 rpm with 30–40 passes. Preincubation of homogenates for calcium transport studies was started within 20 minutes of removing the heart. After homogenizing the hearts as described above, a small fraction of the homogenate was kept for calcium transport studies. The remaining homogenates from 2 or 3 hearts of the same strain (about 1 g of wet tissue) were pooled for the preparation of microsomal fractions enriched in SR vesicles by the method of Solaro et al. The measurements of calcium transport were started immediately, and protein determinations were made later by the method of Lowry et al., using bovine serum albumin as the standard. All samples including the standards were preheated in 0.5N NaOH at 90°C for 20 minutes.

**Measurement of Calcium Transport**

To measure calcium uptake by the homogenates and microsomal fractions, the method described by Solaro and Briggs was used. The reaction solutions contained 0.5–0.6 mg homogenate protein/ml or 0.25 mg microsomal protein/ml, 100 mM KCl, 5 mM potassium oxalate, 40 mM imidazole, 5 mM NaCl, 6 mM MgCl₂, 5 mM Na₃ATP, 3 mM creatine phosphate, and 2 units/ml creatine phosphokinase. The reaction mixtures were preincubated for 5 minutes at 37°C, and transport was started by adding the appropriate amounts of a 20-fold concentrated ⁴⁰Ca-EGTA mixture to yield a pCa of 5.0 (150 μM CaCl₂, 145 μM EGTA) at pH 7.0 and 400,000 cpm/ml. The amounts of EGTA and CaCl₂ required were calculated as described by Fabiato and Fabiato. All the experiments were done at 37°C with constant stirring. At various times, a sample was taken and filtered through a Millipore HA, 0.45 μm filter by suction and immediately washed three times with ice-cold 2 mM EGTA and 25 mM imidazole at pH 7.0. Radioactivity trapped on the filter was determined by liquid scintillation spectroscopy.

The rates of calcium transport were determined from slopes of the linear portions of the curve relating calcium uptake to time and were expressed as nanomoles calcium per milligram protein per minute. Steady-state filling of SR vesicles with calcium (capacity) was determined from the average of two points on the plateau of the same curve and were expressed as nanomoles calcium per milligram protein.

Statistical comparisons between the groups were done by the unpaired, two-tailed Student's t test with the significance level being p<0.05. Values given are mean ± SEM.

**Results**

The first set of results (Figures 1A–1C) shows the time course of calcium uptake by homogenates prepared from individual control and myopathic hamster hearts at 3, 9, and 11 months of age. Measurements made early in the progress curve are linear and provide a measure of the velocity of calcium transport. Later time points in which the calcium uptake has reached a steady state provide a measure of the calcium loading or "capacity" for calcium transport. We have previously presented evidence that under the conditions of the measurements, calcium uptake is restricted almost entirely to the SR vesicles in homogenate preparations. As shown in Figure 1A, at 3 months of age SR vesicles in homogenates of hearts from control and myopathic hamsters accumulated calcium with the same velocity and capacity of uptake. This was also true of hearts studied from animals 7 months old (data not shown). However, at 9 months (Figure 1B), velocity and capacity of calcium uptake by SR vesicles in homogenates of hearts from hamsters with dilated cardiomyopathy were significantly lower than those of the controls as well as those of hamsters with hypertrophic myopathy, which were about the same. By 11 months of age, calcium uptake capacity of preparations from the dilated hearts was about half that of the control and/or hypertrophic hearts.

Experiments were also done comparing calcium uptake by SR vesicles in microsomal fractions prepared from homogenates of 2 or 3 pooled hearts from each of the age groups. In general, these calcium uptake measurements gave results similar to those obtained with SR vesicles in the homogenate.

The results presented above show a depression in the

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Ca\textsuperscript{2+} transport properties of SR vesicles in cardiac homogenates prepared from hamsters with the dilated myopathy. This depression could be due to a decrease in the number of transport sites, i.e., a quantitative defect, or to a reduced catalytic activity of the transport enzyme. If the functional capabilities of the transport enzyme are indeed depressed in the homogenates or microsomes from hamsters with dilated cardiomyopathy, then the turnover number or catalytic rate constant should be lower than that of preparations from controls or hypertrophic hamsters. A simplified representation of the transport process is

\[
\text{Ca}^{\text{out}} + E \rightleftharpoons \text{CaE} \rightarrow \text{Ca}^{\text{in}} + E \quad (1)
\]

where \(\text{Ca}^{\text{out}}\) is cytoplasmic \(\text{Ca}^{2+}\), \(\text{Ca}^{\text{in}}\) is \(\text{Ca}^{2+}\) trapped in the SR space, and \(E\) is the Ca-ATPase transport enzyme. Equation 1 represents a Michaelis-Menten mechanism, and accordingly, the velocity \((v)\) of calcium transport is given by

\[
v = k_{\text{cat}}[\text{CaE}] \quad (2)
\]

At high substrate, i.e., at saturating free \(\text{Ca}^{2+}\) concentration of \(p\text{Ca}^5\), \(\text{CaE} = E\) total, and under this condition

\[
k_{\text{cat}} = \frac{v}{[\text{CaE}]} = \frac{v}{\text{capacity}} \quad (3)
\]

As shown in equation 3, we have estimated \(k_{\text{cat}}\) as the ratio of the velocity \((v)\) and capacity for calcium transport. This has been done on the basis of previous studies,\textsuperscript{17} which have shown that the capacity for calcium transport provides a measure of the relative amount of transport enzyme in the various SR preparations. Ratios of velocity and capacity have also been computed that were termed "fractional rate of filling" of SR in skeletal muscle by Salviati et al.\textsuperscript{18}

Results illustrated in Figure 2 show that the ratios of velocity/capacity were nearly the same for homogenate preparations at each of three ages and, in addition, values for controls and for both cardiomyopathic groups were not significantly different at 3, 7, 9, or 11 months of age. The catalytic activity of the transport enzyme, therefore, appears to remain nearly the same with increasing age between 3 and 11 months and was unaffected in either form of cardiomyopathy.

**Discussion**

The purpose of the present study was to compare qualitative and quantitative differences in calcium uptake capabilities of SR vesicles from normal control
and two clinically, as well as morphologically, different types of cardiomyopathy using the Syrian hamster model. Age comparisons were made between these hamsters from 3 to 11 months old. It was of interest to study the dilated cardiomyopathic strain, i.e., BIO 53.58, since it represents a more prevalent form of this disease seen in the clinical setting. Also, calcium uptake by SR isolated from hearts with dilated cardiomyopathy has not previously been described.

The present study demonstrated that both calcium uptake and velocity were markedly depressed by 9 months of age in heart homogenates from the dilated cardiomyopathic group compared with either control or hypertrophic hamster hearts. This suggests that the volume of SR or the number of transport sites is markedly depressed in the dilated myopathy. There is evidence that calcium oxalate capacity provides a reasonable estimate of relative amounts of SR. For example, Briggs et al. showed that relative amounts of SR in fast versus slow muscle fit with the relative calcium oxalate capacities. The finding of no significant difference in calcium uptake capacity by SR from control and hypertrophic (BIO 14.6) hamster hearts is in agreement with the study done by Gertz et al. They found no differences in SR calcium capacity from 10 days through 10 months of age. However, this group did show a significantly reduced SR calcium uptake velocity in the hypertrophic hearts by approximately 7 months of age. In that same year, McCollum et al. demonstrated significantly reduced rates of both SR calcium uptake capacity and velocity in this same hypertrophic (BIO 14.6) strain of hamster by 7 months of age when compared with control animals. An explanation for the discrepancy in results between these two studies and those reported in the present investigation is unclear. Differences in methodological protocols certainly exist between this study and the two previous older studies. In contrast to earlier work where up to 35 hearts were pooled, our measurements were made on preparations either from a single heart or from 2 to 3 pooled hearts in the case of the microsomal preparations. In addition, we homogenized the heart in glass/Teflon homogenizers instead of blade-type homogenizers such as the Sorvall Omnimixer used by Gertz et al. Pagani and Solaro showed evidence that the stability of calcium transport activity of the SR vesicles prepared by hand homogenizers was significantly better than that of vesicles prepared by using the Sorvall Omnimixer. Incubation conditions used in the present study were similar to those used by Gertz et al. except that we used 5 mM oxalate instead of the 10 mM concentration used in their study. Also, over the years, the BIO 14.6 strain of hypertrophic cardiomyopathic hamster has developed a milder form of the disease with a longer life span (personal communication with Dr. Cornelius Van Dongen of Bio-Breeders, Inc., i.e., the hamster supplier). The BIO 53.58 hamsters with dilated cardiomyopathy have a significantly shorter life span and demonstrate a more severely reduced cardiac function at an earlier age than the hypertrophic hamsters (unpublished observations). These differences in the two strains could help explain the discrepancies, but it is presently not known if cardiac SR isolated from hearts of BIO 14.6 hamsters older than 11 months of age would, with time, show similar reductions in calcium uptake capacity and velocity.

The most significant finding of the present study was the demonstration that despite marked decreases in SR calcium uptake and velocity in the dilated hamster hearts at a relatively early age, the ratios of uptake velocity to capacity were not different within all three groups at any of the ages studied. These ratios were used as an estimate of the functional capability of the SR calcium transport enzyme in terms of a "catalytic" rate constant or "fractional rate of filling" of the SR. Therefore, the results presented here suggest that a major defect in the hearts from hamsters with dilated cardiomyopathy is a decrease in either the volume of SR or number of SR calcium transport sites with no change in specific activity of the transport enzyme.

A decrease in the number of SR sites transporting calcium would be in accordance with a slower rate of relaxation of these hearts. A significantly slower relaxation rate, as indicated by the negative left ventricular dp/dt, has been shown in these dilated hearts when compared with control hearts, using the isolated working heart preparation. Also, very similar decreases in cardiac relaxation rates have been demonstrated in diabetic rat heart perfusion studies. In association with these findings other investigators have shown comparable depression of SR calcium uptake capacities and velocities in chronically diabetic rat hearts similar to those found in the dilated myopathic heart SR of the present study. Furthermore, both of these SR parameters were also depressed in pig hearts with so-called pathologic hypertrophy induced by supravalvar banding of the aorta. Alteration in relaxation rate in some of these models could also be related to shifts in the population of myosin heavy chain isoenzymes. In the case of the BIO 53.58 strain, it has been shown that there is a shift in myosin heavy chain population to the slow V3 form, which may be related to a slowed rate of relaxation. However, this is unlikely in the case of pig hearts, in which the heavy chain isoform with slow ATPase activity predominates even in the control situation.

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