Effect of Physical Training on Calcium Transport by Rat Cardiac Sarcoplasmic Reticulum

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SUMMARY Cardiac microsomes enriched in fragmented sarcoplasmic reticulum (SR) were isolated from hearts of physically trained rats and were compared to those from sedentary rats. Preparations from conditioned rats were found to transport calcium to a greater extent than those from sedentary rats both in the absence and in the presence of 1 mM oxalate. Higher oxalate concentrations abolished the differences in calcium accumulation by cardiac microsomes from conditioned and sedentary rats and indicated a qualitative change in SR from conditioned hearts. We conclude that the increased transport by SR from hearts of conditioned rats may provide one mechanism for enhanced contractile reserve in these hearts.

PREVIOUS studies from one of our laboratories have demonstrated that cardiac function is increased in hearts isolated from conditioned rats when compared to isolated hearts from sedentary rats. Enhanced function was observed under both aerobic and hypoxic conditions. The mechanisms we have found that may partially account for this greater performance are a greater coronary flow and oxygen delivery and increased actomyosin and myosin ATPase activities in conditioned hearts.

In view of a probable role of the sarcoplasmic reticulum (SR) in regulating intracellular calcium ion concentration, the present study was undertaken to determine the effect of physical conditioning on calcium transport by rat cardiac microsomes enriched in fragmented SR. The results suggest that cardiac microsomes from conditioned rats transport calcium to a greater extent than microsomes from sedentary rats, and may provide another mechanism for enhanced contractile reserve in hearts of conditioned rats.

Methods

Male Wistar rats, with initial weights of 200-250 g, were conditioned by swimming. Hearts from rats made to swim 150 minutes 5 days a week for 7-8 weeks were studied. This regimen has been shown to be associated with biochemical and mechanical changes characteristic of physical conditioning. Rats taken from the same initial group as the swimmers were kept at normal cage activity for purposes of this paper, the amount of 45Ca taken up by the group were prepared and analyzed in parallel. For the Schwartz. Sedentary and conditioned rats always were killed at the same time, and the pooled hearts from each group were prepared and analyzed in parallel. For the purposes of this paper, the amount of 45Ca taken up by the SR preparation in the absence of oxalate will be termed calcium storage. Other authors have used the terms "binding" or "accumulation." Troethemamine (Tris)-oxalate was included to make measurements of calcium uptake in order to maintain Ca2+ inside the membrane vesicles at a low level, i.e., that determined by the calcium oxalate solubility product of 2 x 10^-8 M. Calcium storage and calcium uptake were measured at 25°C by the Millipore filtration method. Reaction mixtures contained 5 mM MgATP, 0.12 mM KCl, and 40 mM histidine at pH 6.8. The level of ionized calcium was adjusted by the use of various concentrations of ethylene glycol bis(aminomethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) in solutions containing a total 4CaCl2 of 0.025 mM (0.05 μCi/ml) in the final reaction mixture. The range of EGTA concentrations employed was from 3.90 x 10^-4 M (for Ca2+ of 1.5 x 10^-7 M) to 1.5 x 10^-7 M (for Ca2+ of 3 x 10^-8 M). Ionized calcium concentrations were calculated by the equations of Katz et al. in which contents of total calcium, magnesium, ATP, and EGTA at pH 6.8 are taken into account. A binding constant for Ca2+-EGTA of 4.4 x 10^5 M^-1 was used. Protein concentrations were determined by the biuret method with bovine serum albumin as a standard. Calcium uptake was measured with 15-25 μg of microsomal protein/ml in solutions containing 1-5.0 mM Tris-oxalate, and initial rates were calculated from a series of samples filtered at appropriate times after addition of 4Ca-EGTA buffer. Calcium storage was determined with 0.3 mg of microsomal protein/ml. The radioactivity in the protein-free filtrates was measured in a Nuclear-Chicago scintillation spectrometer (Isocap 300).

The concentration of endogenous calcium bound to the SR preparations was measured by atomic absorption spectrophotometry after extraction with LaCl3-HCl. For kinetic analysis, linear regression lines were computed by PDP 8e computer according to the method of least squares. Significant differences between means were determined by paired t-test. All solutions were prepared with distilled deionized water. Tris-ATP and EGTA were obtained from Sigma. All other chemicals were Mallinckrodt analytical grade.

Results

Table 1 shows that there were no differences in wet heart weight between sedentary and conditioned rats, whereas the body weights were lower and heart weight to body weight ratios were significantly higher in the condi-
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TABLE 1  Heart and Body Weight Relationships in Conditioned Rats

<table>
<thead>
<tr>
<th></th>
<th>No of hearts</th>
<th>Body wt (g)</th>
<th>Heart wt (mg)</th>
<th>Heart wt/body wt (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>22</td>
<td>390 ± 9</td>
<td>999 ± 20</td>
<td>2.6 ± 0.04</td>
</tr>
<tr>
<td>Conditioned</td>
<td>22</td>
<td>327 ± 6*</td>
<td>972 ± 20</td>
<td>3.0 ± 0.04*</td>
</tr>
</tbody>
</table>

* P < 0.001.

tioned rats (P < 0.001). These findings are typical of male rats conditioned with this swimming and nutritional program. The yield of SR protein varied between preparations, from a mean of 1.9 mg/g when homogenization was accomplished with a Waring Blender to 5.5 mg/g when a Tekmar Tissuemizer was used, but there was no difference in calcium binding or uptake activities with the two techniques, and with each technique the SR yield was the same in hearts of sedentary and conditioned rats. Also, the difference in calcium storage and uptake between preparations from hearts of sedentary and conditioned rats was of the same magnitude whether the SR yield was high or low.

The endogenous calcium of five preparations from sedentary hearts and five preparations from conditioned hearts were 3.94 ± 0.85 (mean ± SE) and 4.27 ± 0.79 nmol/mg, respectively.

CALCIUM STORAGE

The rates at which cardiac microsomes isolated from sedentary and conditioned rats filled with calcium at 3 μM Ca2+ are shown in Figure 1. Calcium storage was a very rapid process that reached equilibrium after 4 minutes in both groups. The microsomes from conditioned hearts showed greater capacity at all times to store calcium than microsomes from sedentary hearts. The mean increase of conditioned over control varied between 20% and 30%. There was no effect of 5 mM azide on calcium storage; this indicates that mitochondrial calcium transport is not being measured under these conditions.

The steady state level of calcium storage by cardiac microsomes increased with increasing calcium ion concentration (Fig. 2). Calcium storage by microsomes from both conditioned and sedentary rat hearts showed saturation kinetics. In the range of free calcium ion concentration studies, 2.5 x 10−7 to 4.5 x 10−6 M, microsomes isolated from conditioned hearts showed a 15-30% greater capacity to store calcium than the microsomes isolated from the sedentary hearts (P < 0.05).

Double reciprocal plots of the pooled data for each group were made. The lines drawn by computed linear regression demonstrated good correlation between the reciprocal of steady state calcium storage and 1/Ca2+ (r = 0.90 in preparations from sedentary hearts and r = 0.83 from conditioned hearts). The calculated calcium concentration at half-maximal calcium storage was 1 x 10−6 M in sedentary hearts and 7.6 x 10−7 M in conditioned hearts. These values were not significantly different.

CALCIUM UPTAKE

The time-dependence of calcium uptake of microsomes from sedentary and conditioned hearts differed at oxalate concentrations of 1-5 mM. The rate of calcium uptake at 3 μM Ca2+ was directly proportional of oxalate concentration and therefore inversely proportional to the free calcium concentration within the vesicles, in accord with earlier findings with dog heart and rabbit skeletal muscle microsomes.14, 15 The rates calculated for uptake experiments are shown in Table 2. With 1 mM oxalate and 3 μM Ca2+ the initial velocity was 42% greater in conditioned hearts than in sedentary hearts, but no differences were
seen when oxalate concentrations were increased to 2.5 and 5 mM.

The differences between calcium uptake by microsomes from the conditioned and sedentary hearts were significant when 25 or 100 μg of microsomes/ml were studied (Fig. 3). At 15 minutes the mean difference between pairs of microsomal preparations for calcium uptake was 26% higher in conditioned than in sedentary hearts with 25 μg of microsomes/ml and was 32% higher in conditioned than in sedentary in the presence of 100 μg of microsomes. When calcium uptake was studied as a function of calcium concentration in the presence of 1 mM oxalate and 0.1 mg of microsomal protein/ml (Fig. 4), the amount of calcium accumulated within 15 minutes at all calcium concentrations between 0.15 to 3 µM was between 17% and 32% higher in preparations from conditioned than from sedentary hearts.

**Discussion**

It is generally believed that SR in cardiac muscle plays an important role in excitation-contraction coupling and in regulating contractility. Many investigators have found calcium transport by fragmented SR to be depressed in hearts in which the contractile state is reduced by myocardial failure, hypertrophy, ischemia,16-19 and cardiomyopathy.20-29 Suko30 reported that the rate of calcium transport was increased in hyperthyroidism and reduced in hypothyroidism, in parallel with observed changes in the contractile state. Our present findings demonstrate an enhanced capacity for calcium storage in microsomes prepared from the hearts of conditioned rats, in accord with previous findings of increased contractile reserve in hearts of conditioned rats and with the faster relaxation rates observed in these hearts.24

The values for calcium storage observed in our present studies are lower than those published by other laboratories for preparations from hearts of other species. Using techniques identical to those we employed for rat SR, we found calcium storage by preparations of SR from dog heart to be 47.5 ± 5.6 nmol/mg at 4 minutes with Ca2+ of 3 x 10^-6 M. These values are similar to those reported by other workers.8,25 The values we obtained for preparations from rats also are consistent with those reported by other workers who used higher calcium concentrations and incubation temperatures. Thus results obtained with our basic methods are similar to those reported by others, so that the low calcium storage capacity and transport rates reported in the present investigation may be due to a species difference between the rat and other animals, as previously noted by Nayler et al.26

In any study of membrane fractions obtained from different types of hearts, the possibility that varying degrees of contamination might lead to false differences must be considered. Although there is no way to ensure complete purity in these preparations, the methods employed for preparing SR have previously been shown to eliminate significant Na+,K+-ATPase activity.19 Several measurements were made in our present study to attempt to identify contaminants. Mitochondrial fragments would be the contaminant most likely to bind calcium. However, calcium uptake in our preparations was not inhibited by
azide, and cytochrome c oxidase activity of our preparations was insignificant. Therefore, it is unlikely that mitochondrial or sarcolemmal contamination influenced the calcium transport properties measured in this study. If SR prepared from sedentary and conditioned hearts differed in purity, then measurements made both in the presence and in the absence of oxalate should differ by the same proportion, regardless of the conditions of the study. However, the response of calcium uptake to increasing concentrations of oxalate differed not only quantitatively but also qualitatively in preparations from sedentary and conditioned hearts (Table 1) as did the findings in the presence and absence of oxalate. Together these data provide strong evidence that the observed differences are due to altered calcium transport characteristics in the SR prepared from conditioned hearts rather than to a systematic difference in contamination of the SR fraction with inert material.

The initial rates of calcium uptake measured in the presence of 1 mM oxalate were greater in the conditioned than in the sedentary group (Table 1), whereas at 2.5 and 5 mM oxalate this difference was abolished. As all levels of calcium and oxalate in the medium were well below the solubility product for calcium oxalate, CaOx concentration inside the vesicle may have been reduced by increasing oxalate concentration without changing CaOx concentration outside of the vesicle. In the absence of oxalate, the internal CaOx concentration will be approximately 1,000 times that in the external medium.19 Internal CaOx in these high ranges inhibits calcium transport,29 and it is possible that the higher concentrations of oxalate abolished differences in internal CaOx which might have accounted for the differences observed between conditioned and sedentary hearts at 1 mM oxalate or when oxalate was absent. Alternatively, the higher calcium content found in vesicles from the hearts of conditioned rats at the higher levels of internal CaOx could arise from lowered calcium permeability.20 Either of these changes could, in the intact heart, allow the SR of conditioned rats to take up calcium more rapidly and to have a greater capacity to store calcium.

Although the changes in calcium storage observed in this study might be considered small, the differences between SR preparations from hearts of sedentary and conditioned rats range between 17% and 42%, depending on the method used. It is of interest that this is in the same range of increase observed in preparations from hyperthyroid hamsters.32 Preparation of SR from fresh recipient human cardiac tissue obtained during transplantation with pig hearts also showed a threefold increase in calcium binding in the presence of caffeine.32 This is in agreement with other studies.32,33 The functional significance of an increased capacity and rate of calcium transport by SR from conditioned hearts remains uncertain and it is difficult to make quantitative extrapolations from studies on isolated subcellular fractions to intact beating hearts in vivo. It is obvious that small biochemical changes might have great physiological effects in hearts contracting at rates of 300–500 beats/min if these changes operate during each beat.

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**Steady State Aldosterone Dose-Response Relationships**

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**SUMMARY** The steady state effects of different infusion rates of aldosterone on plasma concentrations of sodium and potassium, plasma renin activity, sodium space, and mean arterial pressure were determined. Measured amounts of aldosterone were infused continuously into adrenalectomized dogs for 13 weeks. Four rates of aldosterone administration were used: 16 ± 1 µg/day, 48 ± 3 µg/day (approximately the normal secretory rate for 22-kg dogs on a daily sodium intake of 27 mEq, potassium intake of 27 mEq), 91 ± 4 µg/day, and 219 ± 10 µg/day. Each rate of infusion was continued until the dogs were in sodium and potassium balance and measured variables were steady. Decreasing the rate of aldosterone administration below normal led to sharp decreases in plasma sodium concentration and sodium space, while raising the rate above normal had little effect. Plasma potassium concentration varied inversely and significantly with changes in aldosterone administration over the entire range of rates. Plasma renin activity rose extremely rapidly as the level of aldosterone fell below normal and went to zero at aldosterone infusion rates slightly above normal. Arterial pressure increased as aldosterone rose above normal but did not fall below normal at subnormal aldosterone levels, probably because of the pressor effects of simultaneously generated angiotensin II.

**Methods**

Long-term experiments were carried out in a group of five male mongrel dogs whose weights averaged 22.5 kg. Several weeks prior to the start of the study the dogs were adrenalectomized and cannulas were implanted into the aorta and vena cava through the femoral vessels. Following recovery a portable infusion pump designed in our laboratory was connected to the venous cannula, and aldosterone (CIBA) and methylprednisolone (Solu-Medrol, Upjohn) were infused in distilled water continuously 24 hours/day, 7 days a week, for 13 weeks. The rate of flow from the pump was 15 ml/day throughout the study. The rate of aldosterone infusion was increased in four steps, each step lasting at least 2 weeks. Each infusion level was continued until the dogs had reached new sodium and potassium balances and all measured variables were steady. The first rate of aldosterone infusion was 16 ± 1 (SE) µg/day, followed successively by rates of 48 ± 3 µg/day, 91 ± 4 µg/day, and 219 ± 10 µg/day. One dog died after the completion of the second infusion level. At all four levels, 1 mg/day of methylprednisolone was infused along with aldosterone. This dose of synthetic glucocorticoid has essentially no mineralocorticoid effect and was adequate to keep the dogs in good health. Throughout the experiment the dogs were housed in metabolic cages and fed a diet containing 27 mEq/day each of sodium and potassium.

Between 9 a.m. and 12 noon each day during the experiment the dogs were brought into the laboratory in which they had been trained to lie quietly unrestrained. Arterial blood was drawn from the indwelling cannula into syringes containing several microfilters of heparin (1,000 U/ml). Plasma for electrolyte analysis was obtained by immediately centrifuging the blood in a high speed centrifuge for a period of less than 5 minutes, then removing the plasma. All electrolyte determinations were made with an Instrumentation Laboratory model 343 flame photometer. For...
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