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Decreased expression of cardiac sarcoplasmic reticulum Ca^{2+}-pump ATPase in congestive heart failure due to myocardial infarction

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Abstract

Myocardial infarction in rats induced by occluding the left coronary artery for 4, 8 and 16 weeks has been shown to result in congestive heart failure (CHF) characterized by hypertrophy of the viable ventricular myocardial tissue. We have previously demonstrated a decreased calcium transport activity in the sarcoplasmic reticulum (SR) of post-myocardial infarction failing rat hearts. In this study we have measured the steady state levels of the cardiac SR Ca^{2+}-pump ATPase (SERCA2) mRNA using Northern blot and slot blot analyses. The relative amounts of SERCA2 mRNA were decreased with respect to GAPDH mRNA and 28 S rRNA in experimental failing hearts at 4 and 8 weeks post myocardial infarction by about 20% whereas those at 16 weeks declined by about 35% of control values. The results obtained by Western blot analysis, revealed that the immunodetectable levels of SERCA2 protein in 8 and 16 weeks postinfarcted animals were decreased by about 20% and 30%, respectively. The left ventricular SR Ca^{2+}-pump ATPase specific activity was depressed in the SR preparations of failing hearts as early as 4 weeks post myocardial infarction and declined by about 65% at 16 weeks compared to control. These results indicate that the depressed SR Ca^{2+}-pump ATPase activity in CHF may partly be due to decreased steady state amounts of SERCA2 mRNA and SERCA2 protein in the failing myocardium. (Mol Cell Biochem **163/164**: 285–290, 1996)

Key words: Ca^{2+}-pump ATPase, cardiac SERCA2, myocardial infarction, congestive heart failure, cardiac SR

Introduction

In congestive heart failure (CHF), heart dysfunction is normally preceded by cardiomyocyte hypertrophy and is associated with an increase in cardiac muscle mass to prevent cardiac insufficiency [1]. As a result of myocardial infarction, the heart not only loses a part of its tissue mass but the contractile ability of the surviving myocardium is also altered leading to the progressive development of CHF [2]. Although the functional, biochemical and morphological changes that occur in ischemic myocardium have been studied extensively, the adaptive processes which occur in the surviving tissue of the infarcted heart are incompletely understood. In this regard, it should be pointed out that the active transport of calcium into the lumen of the sarcoplasmic reticulum (SR) has been shown to be altered in different models of cardiac hypertrophy and in heart failure [3–6] but not much is known regarding this abnormality in CHF secondary to myocardial infarction. The exact role of SR in terms of Ca^{2+} uptake and Ca^{2+} release at different stages of CHF has not yet been clearly defined. It also remains to be determined whether changes in SR Ca^{2+} transport activity in failing heart arise from changes at the transcriptional and/or translational levels.

It may be noted that the SR is a membranal tubular network which plays an essential role in the cardiac contraction and relaxation process of the myocardium by raising and lowering the cytoplasmic levels of calcium respectively [7]. The SR Ca^{2+}-stimulated ATPase has been shown to work as a pump in which ATP is hydrolyzed for the transport of calcium [8]. In the heart, the cardiac SR Ca^{2+}-pump ATPase

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(SERCA2) gene is expressed in high levels and encodes the mRNA for two different isoforms. One of the isoforms is expressed abundantly in cardiac muscle and slow twitch skeletal muscle (SERCA2a) whereas the second isoform (SERCA2b) is expressed in a variety of smooth muscle and non-muscle tissues [9]. In the cardiomyocyte, Ca^{2+}-transport into the lumen of the SR is mediated exclusively by the SERCA2a isoform. The present study demonstrates that the level of SERCA2 mRNA and SERCA2 protein content are decreased in the failing hearts secondary to myocardial infarction. Accordingly, it is suggested that changes in the transcriptional rate of the SERCA2 gene may at least in part be responsible for the marked decrease in SR Ca^{2+} stimulated ATPase activity in the failing heart.

Materials and methods

Experimental animal model

Myocardial infarction was induced in male Sprague Dawley rats (175–200 g) by occlusion of the left coronary artery as described previously [6, 10]. The animals were anaesthetized with ether and the heart was exposed through the left thoracotomy. The anterior descending branch of the left coronary artery was ligated 2 mm from the origin of the aorta with 6-0 silk suture, the heart was repositioned in the chest and the incision closed with a purse-string suture. A mixture of 95% oxygen and 5% CO_2 was supplied to the animal under positive pressure during the operative procedure. Sham operated animals were treated in the same way except that the artery was not ligated. All of the animals were fed rat chow and water ad libitum until used at 4, 8 and 16 weeks after the operation for mRNA quantitation and biochemical studies. After 4, 8 and 16 weeks of ligating the coronary artery, body weight, left ventricular weight and scar weight of these animals were measured. Ascitic fluid present in the peritoneal cavity was estimated and the wet as well dry weight of the lungs was noted and their wet/dry weight ratio calculated. Only experimental animals with more than 30% scar size of the left ventricle were used in this study.

RNA isolation and analysis

Total RNA was isolated from the left ventricles using the extraction procedure reported by Chomczynski and Sacchi [11]. For Northern blots, 20 μg of total RNA was size fractionated on 1% agarose gel containing 1 M formaldehyde [12] and blotted onto Zeta-Probe GT nylon membranes (BioRad, Canada). For slot blots, 3 or 6 μg of total RNA from each sample were loaded onto the Zeta-Probe GT membranes using a slot blot apparatus (Schleicher and Schuell). The same amount of total RNA was also loaded to detect non-specific binding of probes. For SERCA2a, a 1.7-kb BamH1-Pst1 cDNA fragment containing the 3' protein coding region and the full 3' untranslated region of a rabbit SERCA2a cDNA was used as a molecular probe [13, 14]. For glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 1.63-kb chicken GAPDH cDNA was used [15], whereas for the 28 S rRNA, a 4.8 kb mouse 28 S rRNA EcoRI-SalI fragment was used [16]. The cDNA fragments were labeled with α-[^{32}P]-dCTP using random hexamer labeling kit following the manufacturer's protocol (GIBCO-Life Technologies, Canada). The Zeta-Probe GT nylon membranes were hybridized and washed with the [^{32}P]-labeled cDNA probes or oligonucleotides according to the manufacturer's instructions (Bio-Rad). The filters were then exposed to DuPont Reflection film. After autoradiography, the labeled mRNA bands were quantitated by densitometric scanning using GS-670 imaging densitometer (Bio-Rad, Canada) and the Molecular Analyst (Bio-Rad, Canada) computer analysis software. The mRNA values for SERCA2 were divided by that of 28 S rRNA or GAPDH mRNA to obtain normalized relative SERCA2 mRNA values.

Western blot analysis

SR membrane vesicles were isolated [4, 6] and 50 μg of the membrane proteins separated in 7.5% SDS-PAGE as reported previously [17]. Briefly, gels were electroblotted onto PVDF membranes (Bio-Rad, Canada) as described previously [18] and the membranes were blocked with 5% skim milk and then incubated with a rabbit polyclonal antibody (PAb87) prepared against the first 216 amino acids located in the N-terminal region of the rabbit SERCA2a protein (kindly provided by Dr. A. K. Grover, McMaster University, Hamilton, ON, Canada) [19]. The membranes were washed and incubated with [^{125}I]-labeled protein A. After three washings the membranes were exposed to X-ray film and quantitated by densitometric scanning as described above.

Determination of Ca^{2+}-stimulated and Mg^{2+} ATPase activities

SR vesicles were isolated from control and viable tissue of the left ventricles in experimental hearts as previously described [4, 6]. Total (Ca^{2+} + Mg^{2+})-ATPase and basal Mg^{2+}-ATPase activities were determined in a reaction medium containing 100 mM KCl, 20 mM Tris-HCl, 5 mM $MgCl_2$ and 5 mM NaN_3. Reaction was started by the addition of 5 mM Tris-ATP in the presence of 0.05 to 0.08 mg/ml of SR protein and was terminated with 1 ml of 12% wt/vol cold trichloroacetic acid. Inorganic phosphate liberated during the

reaction was estimated in a protein free filtrate by a spectrophotometric method [4, 6]. The Ca^{2+}-stimulated Mg^{2+}-dependent ATPase activity was reported as the difference between the total and basal Mg^{2+}-ATPase activities.

Statistical analysis

The mRNA values for SERCA2a were divided by that for GAPDH and 28 S rRNA to obtain normalized relative mRNA values. The relative level of the mRNA for SERCA2 was corrected against that for GAPDH and 28 S rRNA in each sample and calculated as percentage of mean values of the corresponding message level in the control groups. The results are presented as mean ± S.E.M. and the statistical comparisons were made by Duncan's new multiple comparisons test [20], a p value of < 0.05 was taken to reflect a significant difference.

Results

In order to gain insight into the mechanisms responsible for the depressed SR calcium transport in failing hearts, CHF was induced in rats by occluding the left coronary artery for 4, 8 and 16 weeks. The left ventricle of experimental rats showed a significant degree of hypertrophy at 4, 8 and 16 weeks of coronary ligation. The left ventricular weight (including the septum) was larger in experimental animals at 16 weeks as compared to controls; however, the left ventricular weight was also increased at 8 and 16 weeks when normalized tissue weight was taken into consideration, as described earlier [6]. The average weight of the scar tissue measured was 0.32 ± 0.12 g in the infarcted hearts; the scar tissue was taken out and the viable tissue of the remaining left ventricle was used in all the experiments. Congestion of lungs and presence of ascites, the indicators of congestive heart failure, were present in the experimental animals (Table 1). The observed changes are consistent with those reported earlier with this experimental model indicating mild, moderate and severe stages of congestive heart failure at 4, 8 and 16 weeks after coronary artery occlusion, respectively [6].

Figure 1 shows the values obtained from Northern blot and slot blot analyses for SERCA2 mRNA levels in the left ventricular viable myocardial tissue of infarcted animals at 4, 8 and 16 weeks after surgery; sham operated animals served as control. The relative SERCA2 mRNA level calculated by normalizing with GAPDH mRNA or 28 S rRNA values obtained from Northern blot analysis indicated a significant reduction of SERCA2 mRNA level after 4 weeks of myocardial infarction compared to the values from control animals (Fig. 1A). A similar trend of decrease of the steady state SERCA2 mRNA values was obtained from slot blot analysis (Fig 1B). These results demonstrate that the level of SERCA2 mRNA was significantly decreased by about 20% in the left ventricles at 4 and 8 weeks whereas about 35% reduction in the level of SERCA2 mRNA was seen at 16 weeks in post-myocardial infarction hearts as compared to sham operated controls. In order to assess if the changes in cardiac SERCA2 mRNA parallel the amount of the SR Ca^{2+}-pump ATPase protein, we performed Western blot analysis of the SR vesicle proteins. The results shown in Fig. 2 for the immunodetection of the SR Ca^{2+}-pump ATPase performed using PAb87 antibody demonstrated that the level of the Ca^{2+}-pump ATPase protein was decreased by 21 ± 9% and 30 ± 7% in the failing hearts at 8 and 16 weeks, respectively. These results demonstrate that the changes in the steady state level of the SERCA2 mRNA closely resembles the trend of changes for the translation of the SR Ca^{2+}-pump ATPase protein.

To confirm that the changes observed for SERCA2 mRNA and protein levels were in fact reflected in alteration of SR Ca^{2+}-pump ATPase activity, the Mg^{2+}-ATPase and Ca^{2+}-stimulated ATPase activities of control and experimental SR preparations were studied at 4, 8 and 16 weeks after coronary occlusion. The Ca^{2+}-stimulated ATPase activity was decreased in experimental hearts at 4, 8 and 16 weeks by about 35, 45 and 65% of control values, respectively. In contrast, no significant change in the Mg^{2+}-ATPase activity was observed at any stage of heart failure (Fig. 3) .

Table 1. Effect of myocardial infarction on growth characteristics of experimental and control rats at 4, 8 and 16 weeks of left coronary artery ligation

	4 Weeks		8 Weeks		16 Weeks	
	Control	M.I.	Control	M.I.	Control	M.I.
Body wt, g	450 ± 13	460 ± 14	519 ± 11	503 ± 14	578 ± 14	555 ± 18
Viable LV wt, mg	782 ± 35	586 ± 30*	932 ± 68	958 ± 45*	1020 ± 60	1305 ± 63*
LV wt/body wt (mg/g)	1.74	1.27*	1.80	1.90	1.76	2.35*
Lung wet/dry wt ratio	3.72 ± 0.30	4.12 ± 0.21*	3.78 ± 0.30	5.16 ± 0.20*	3.79 ± 2.0	5.56 ± 0.21*
Ascites (ml)	N.D.	1.25 ± 0.23*	N.D.	3.50 ± 0.50*	N.D.	5.50 ± 0.30*

M.I. – myocardial infarcted rats; LV – left ventricle. Values are the mean ± S.E.M. of 8–10 experimental animals for each group. *indicates statistical significance ($p < 0.05$) in comparison to sham.

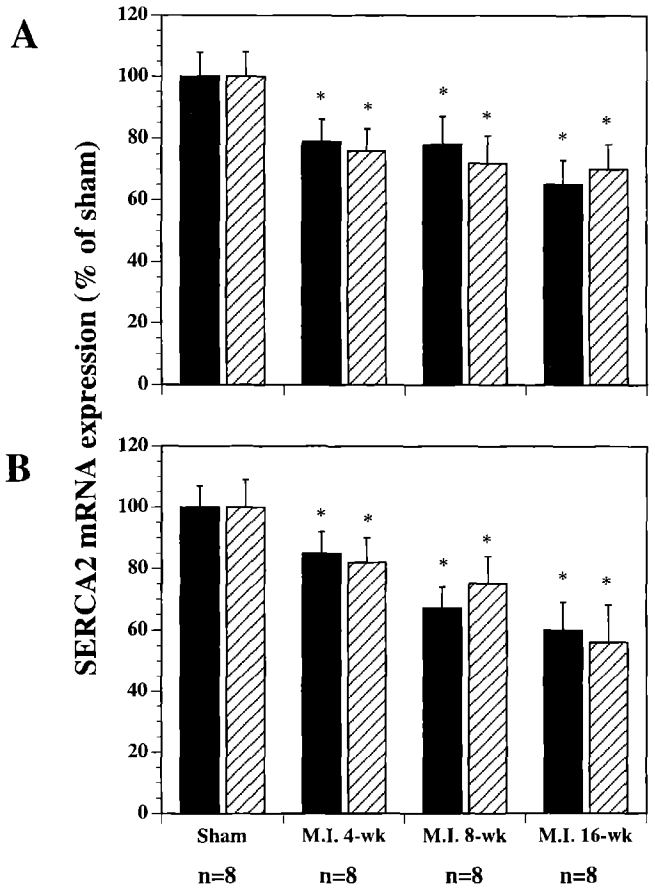

Fig. 1. Cardiac SR Ca^{2+}-pump ATPase mRNA levels estimated by Northern blot (panel A) and slot blot (panel B) analyses. Solid bars represent mRNA values normalized with respect of 28 S rRNA whereas shaded bars represent mRNA values normalized with respect to GAPDH mRNA. Values are the mean ± S.E.M. n – indicates the number of animals for each group. * – indicates statistical significance ($p < 0.05$) between sham and experimental group.

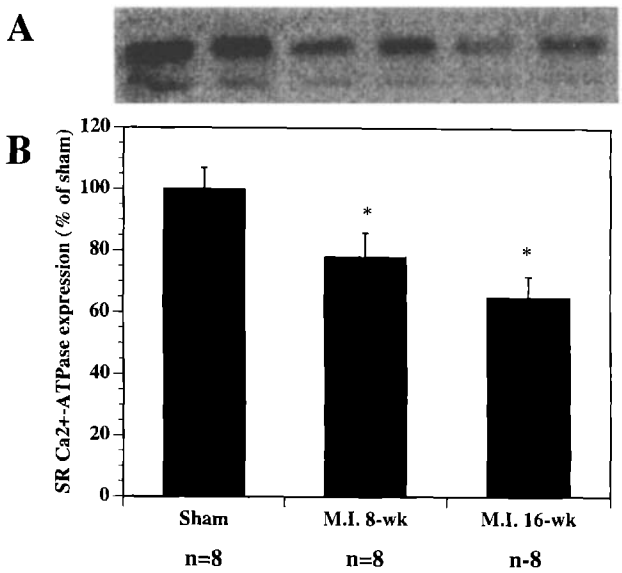

Fig. 2. Cardiac SR Ca^{2+}-pump ATPase protein quantitation by Western blot. Panel A. Representative autoradiography of a Western blot of total SR protein immunodetected with the polyclonal antibody PAb78. Panel B. Solid bars represent the values of the densitometric analysis of the immunodetected protein in 8- and 1 6-week failing left ventricles. Values are the mean ± S.E.M. n – indicates the number of animals for each group. * – indicates statistical significance ($p < 0.05$) between sham and experimental group.

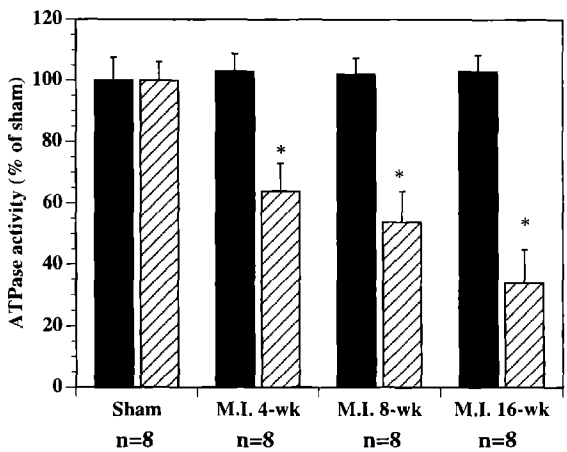

Fig. 3. Total Mg^{2+}-ATPase and Ca^{2+}-stimulated ATPase activities in vesicular SR membranes. Total Mg^{2+}-ATPase and Ca^{2+}-stimulated ATPase activities were measured as described under Materials and methods. The specific activities were expressed as nmoles Pi released per minute per mg of SR protein. The concentration of free Ca^{2+} for measuring Ca^{2+} stimulated ATPase activity was 10 μM. Solid bars represent Mg^{2+}-ATPase activity whereas shaded bars represent the Ca^{2+}-stimulated Mg^{2+}-ATPase activity. Values are the mean ± S.E.M. n – indicates the number of animals for each group. * – indicates statistical significance ($p < 0.05$) between sham and experimental group.

Discussion

The rat model of congestive heart failure secondary to myocardial infarction has been used to study the structural, functional and morphological characteristics of surviving myocardium [6, 21, 22]. The ligation of the left coronary artery resulted in infarction of the left ventricle in experimental animals [6, 22]. CHF in the animals was confirmed by hypertrophy of the remaining myocardium, congested lungs and presence of ascites. These findings are in agreement with previous reports demonstrating increased LVEDP, decreased rate of contraction (+dP/dt) and relaxation (-dP/dt) of failing hearts in animals with CHF [6]. Several investigators have also reported a depression in the SR Ca^{2+} transport activities in different types of failing hearts and have interpreted these results to indicate the inability of the myocardium to relax fully [23, 26, 28, 29]. In fact Ca^{2+}-stimulated ATPase activities and oxalate supported calcium uptake were found to be depressed significantly in experimental hearts, which reflect the abnormal relaxation in this condition of CHF [3–6]. The depressed SR Ca^{2+}-stimulated ATPase activity can be consid-

ered to account for the previously observed decrease in the Ca^{2+}-uptake in hearts failing due to myocardial infarction [6].

In this study we examined the mechanisms for alterations in SR function during congestive heart failure by determining the steady state level of mRNA and protein for the cardiac SR Ca^{2+}-pump ATPase. We demonstrated using mRNA blot analysis that the steady state level of the mRNA for SERCA2 was depressed at early, moderate and severe stages of heart failure. Our results are in agreement with previous reports that demonstrate that the mRNA levels for SERCA2 were decreased in cardiac hypertrophy induced by pressure overload as well as failing hearts of different etiologies [14, 23–30]. It is of particular importance to emphasize that the changes in mRNA and protein expression for the cardiac SR Ca^{2+}-pump ATPase were parallel whereas the SR Ca^{2+}-pump ATPase activity was decreased to a greater extent at mild, moderate and severe stages of congestive heart failure. This would support the hypothesis that changes in mRNA and protein for the SR Ca^{2+}-pump ATPase may be occurring at the transcriptional or post-transcriptional (mRNA stability) levels; however, further studies are necessary to elucidate the molecular mechanisms regulating the expression of the cardiac SR Ca^{2+}-pump ATPase in the hypertrophic and failing heart. It is also possible that the relatively greater magnitude of changes in the SR Ca^{2+}-pump ATPase activity in the failing heart may also be due to mechanisms other than that seen in the SR gene expression in this study.

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