Sarcoplasmic Reticulum Gene Expression in Cardiac Hypertrophy and Heart Failure

Masashi Arai, Hirosuke Matsui, Muthu Periasamy

In cardiac and skeletal muscle, the sarcoplasmic reticulum (SR) plays a central role in the contraction relaxation cycle of the muscle by virtue of its ability to regulate the intracellular free \( \text{Ca}^{2+} \) concentration. \( \text{Ca}^{2+} \) release from the SR increases \( [\text{Ca}^{2+}] \) (\( \approx 10^{-5} \) mol/L), thus inducing contraction, whereas \( \text{Ca}^{2+} \) uptake by the SR reduces \( [\text{Ca}^{2+}] \) (\( \approx 10^{-7} \) mol/L), producing muscle relaxation. In cardiac muscle, the beat-to-beat rhythm (contraction-relaxation cycle) is tightly controlled by the regulated release and removal of \( \text{Ca}^{2+} \) by the SR. In view of this key role in excitation-contraction coupling, alterations in the SR function are expected to significantly affect cardiac performance. Over the past three decades, varying degrees of defects in the SR \( \text{Ca}^{2+} \) uptake function have been identified in different experimental models of heart disease, and the literature has been reviewed extensively. More recent analyses using animal models and human failing heart samples suggest that alterations in SR function may be primarily due to alterations in the expression level of mRNAs encoding key SR \( \text{Ca}^{2+} \) transport proteins.

The purpose of this review is to highlight recent advances concerning the regulation of SR gene expression during cardiac hypertrophy and heart failure.

Structure of the Cardiac SR

The SR is an intracellular membrane network that is in close contact with the myofibrils and couples with the sarcolemma through transverse tubules (T tubules). The structure of the SR in cardiac muscle is very similar to that described in skeletal muscle (reviewed in Reference 6). As in skeletal muscle, the cardiac SR is composed of two main components: the junctional SR (terminal cisternae) and the longitudinal tubules. However, the structure of the cardiac SR is not as well organized in relation to the T tubule.7 There are relatively few T tubules in cardiac muscle, and they are, in general, of large diameter. They are often coupled on only one side to the terminal cisternae, forming a dyad rather than a triad. Interestingly, many of these single couplings or dyads occur between the terminal cisternae and the sarcolemma. In avian ventricular myocytes, 80% of the terminal cisternae (corbular SR) are not associated with T tubules or sarcolemma,7,8 and only 20% of the terminal cisternae form dyads. These observations suggest that in cardiac muscle, excitation-contraction coupling occurs mostly through diffusible signals. It is believed that \( \text{Ca}^{2+} \)-induced \( \text{Ca}^{2+} \) release accounts for the opening of the SR \( \text{Ca}^{2+} \) release channels in non-junctional SR of the cardiac muscle.9,10

SR \( \text{Ca}^{2+} \) Transport Proteins

The major SR proteins controlling \( \text{Ca}^{2+} \) release, uptake, and storage have been isolated, and their primary structures have been determined (Figure). The contraction of cardiac myocytes is triggered by \( \text{Ca}^{2+} \) release from the SR through a \( \text{Ca}^{2+} \) release channel (also referred to as the ryanodine receptor) located on the junctional SR. The ryanodine receptor from cardiac and skeletal muscle forms a tetrameric structure (recognized as the foot structure) comprised of four monomers of \( \approx 564 000 \) D.11-14 Recent molecular cloning analysis has revealed that two distinct genes encode the cardiac (\( M_c, 564 711 \)) and skeletal muscle (\( M_s, 563 584 \))—specific receptors.15-18 The cardiac ryanodine receptor mRNA is unique to heart muscle and is not expressed in fast- or slow-twitch skeletal muscle.18,19 Muscle relaxation is initiated by ATP-dependent \( \text{Ca}^{2+} \) transport into the SR20,21 by the SR \( \text{Ca}^{2+} \)-ATPase pump (sarco[endo]plasmic reticulum \( \text{Ca}^{2+} \)-ATPase [SERCA]) localized in the longitudinal SR. Five distinct \( \text{Ca}^{2+} \)-ATPase isoforms encoded by three different genes (SERCA1, SERCA2, and SERCA3) have been identified: the adult fast-twitch skeletal muscle isoform (SERCA1a),22 its alternatively spliced neonatal isoform (SERCA1b),22,23 the cardiac/slow-twitch skeletal muscle isoform (SERCA2a),24,25 its alternatively spliced smooth muscle/nonmuscle isoform (SERCA2b),26-28 and an isoform expressed in a broad variety of muscle and nonmuscle tissues (SERCA3).29 Heart muscle primarily expresses the SERCA2a isoform, both in the atrium and ventricle. The function of the SERCA2a-type \( \text{Ca}^{2+} \) pump is modulated by a phosphoprotein, namely phospholamban, with a molecular weight of 25 000.30,31 Phosphorylation of phospholamban by cAMP-dependent protein kinase (in response to \( \beta \)-adrenergic receptor stimulation) significantly increases the \( \text{Ca}^{2+} \) uptake rate.32,33 There are no isoforms of phospholamban, and the same protein is expressed in cardiac and slow-twitch skeletal muscle.34,35 \( \text{Ca}^{2+} \) inside the SR membrane is stored at a high concentration, which is due to binding with a number of \( \text{Ca}^{2+} \)-binding proteins in the lumen of the SR: calsequestrin36 and calreticulin37-39 within the junctional SR, and glycoproteins of 53 and 160 kD (130 kD in cardiac muscle)39,41 within the longitudinal SR. Calsequestrin, a high-capacity moderate-affinity...
Ca\(^{2+}\) storage capacity of SR in striated muscles.42,43 Two distinct isoforms of calsequestrin have been identified: the skeletal muscle isoform,43 expressed in both fast- and slow-twitch skeletal muscle, and the cardiac isoform,44,45 expressed in cardiac and, to a lesser extent, in slow-twitch skeletal muscle.45,46

**SR Gene Expression During Cardiac Muscle Development**

The expression of the major SR proteins during cardiac and skeletal muscle development has been investigated in the rabbit by use of gene-specific probes.19,45,47 The SR Ca\(^{2+}\)-ATPase isoform SERCA2a is the primary transcript in developing atrial and ventricular muscle.19,47 The expression level of SERCA2a gradually increases with cardiac muscle development, but there is no isoform switching during cardiac muscle development. Heart muscle also contains trace amounts of SERCA2b (the smooth muscle/nonmuscle isoform), but its expression does not change significantly with development.19,28 A quantitative reduction in SR Ca\(^{2+}\)-ATPase mRNA level occurs in aged rat hearts (55% reduction in 24-month-old Wistar rats when compared with 1- to 2-month-old adult rats48 and 60% reduction in 30-month-old Fischer 344 rats when compared with 4-month-old adult rats.49 Cardiac muscle expresses only the cardiac ryanodine receptor and cardiac calsequestrin isoforms throughout development.19,43 Calsequestrin mRNA is equally abundant in fetal, adult, or aged rats,46 but in rabbits, its level increases in parallel with SERCA2a mRNA during development from late fetal to 20-week-old adult rabbits.19 The level of ryanodine receptor mRNA increases threefold during transition from the fetal to adult stage.19 The expression of phospholamban mRNA does not change significantly during development in rabbits,19 although it increases severalfold during the fetal to adult transition in mouse hearts (E. Kranias, personal communication). Interestingly, the cardiac-specific isoforms of SR Ca\(^{2+}\)-ATPase (SERCA2a) and calsequestrin are also expressed in developing fast skeletal muscle, and they are completely replaced by the fast skeletal muscle isoforms in adult muscle.19,45 In contrast, the fast skeletal muscle–specific isoforms of the SR Ca\(^{2+}\)-ATPase (SERCA1), ryanodine receptor, and calsequestrin are not expressed in cardiac muscle at any stage. Therefore, during cardiac muscle development, there is a quantitative change in the expression levels of SR Ca\(^{2+}\) transport proteins, but isoform changes noted in developing fast-twitch skeletal muscle do not occur.

**SR Function in Cardiac Hypertrophy and in Heart Failure**

**Animal Models of Cardiac Hypertrophy and Heart Failure**

Several animal models have been developed to investigate both the process of cardiac hypertrophy and the underlying mechanisms altering cardiac contractility (Table 1). One of the best documented models has been excess thyroid hormone–induced cardiac hypertrophy. Thyroid hormone–induced cardiac hypertrophy is associated with increased rates of tension development and tension decline.50,51 Although some of the changes in mechanical properties can be attributed to changes in myosin heavy chain expression,52 a number of studies have indicated that SR function is also altered in this model of cardiac hypertrophy. Suko53 first reported that the rate of Ca\(^{2+}\) uptake and the rate of Ca\(^{2+}\)-dependent ATP hydrolysis by the SR were significantly increased in hearts from hyperthyroid rabbits, whereas the Ca\(^{2+}\) storage capacity and the steady-state level of Ca\(^{2+}\) were unaltered by different levels of thyroid hormone. Intracellular Ca\(^{2+}\) transient ([Ca\(^{2+}\)]\(_i\)) measurements using aequorin also demonstrated that the hyperthyroid state results in a more rapid rise and decay phase of the Ca\(^{2+}\) transient, apparently without altering the peak level of free myoplasmic Ca\(^{2+}\) that was reached during contraction.50 Rohrer and Dillmann42 showed that an increase in Ca\(^{2+}\)-ATPase mRNA is detectable as early as 2 hours after triiodothyronine injection into the rat, suggesting that the increase in the Ca\(^{2+}\)-ATPase mRNA level is associated with enhanced gene transcription. In a recent study, we55 demonstrated that the mRNA levels of SR Ca\(^{2+}\)-ATPase and ryanodine receptor coordinately increase in hyperthyroid hearts but decrease in hypothyroid hearts. However, a switch from cardiac Ca\(^{2+}\)-ATPase (SERCA2) to fast-twitch skeletal Ca\(^{2+}\)-ATPase (SERCA1) did not occur in hyperthyroid hearts.47,56 We further showed that the Ca\(^{2+}\)-ATPase protein level increases in parallel with the mRNA, suggesting that the observed changes in mRNA levels are responsible for altered SR function. Interestingly, calsequestrin mRNA levels remain unchanged both in hyperthyroid and hypothyroid hearts.55 These data demonstrate that in response to thyroid hormone level, the genes encoding SR Ca\(^{2+}\) transport proteins are regulated in a discordant manner. Furthermore, these data suggest that alterations in SR functions are primarily linked to the altered expression of genes encoding SR proteins.

Alterations in Ca\(^{2+}\) transport have also been extensively investigated in the hemodynamically loaded heart.
Several experimental models have been developed: left ventricular pressure overload induced by aortic banding, right ventricular pressure overload due to pulmonary hypertension induced by monocrotaline or by placing a constrictive band around the pulmonary artery, volume overload induced by creating an aortocaval shunt or by salt intake, and heart failure due to experimental myocardial infarction. Depending on the degree or duration of load, hemodynamic loads produce different types of heart conditions from mild compensated cardiac hypertrophy to the decompensated terminal stage of heart failure.

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Ca(^2+) Uptake Rate</th>
<th>ATP Hydrolysis Rate</th>
<th>E-P</th>
<th>[Ca(^{2+})]: Transient Study</th>
<th>Protein Amount of Ca(^{2+}) ATPase</th>
<th>mRNA Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid hormone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suko (^{53})</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>↔</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Mackinnon et al (^{50})</td>
<td>...</td>
<td>...</td>
<td></td>
<td></td>
<td>Rapid rise and decay</td>
<td>...</td>
</tr>
<tr>
<td>Nagai et al (^{47})</td>
<td>...</td>
<td>...</td>
<td></td>
<td>...</td>
<td>↑</td>
<td>...</td>
</tr>
<tr>
<td>Rohrer and Dillmann (^{54})</td>
<td>...</td>
<td>...</td>
<td></td>
<td></td>
<td>↑</td>
<td>...</td>
</tr>
<tr>
<td>Arai et al (^{26})</td>
<td>...</td>
<td>...</td>
<td></td>
<td></td>
<td>↑</td>
<td>...</td>
</tr>
<tr>
<td>Pressure overload</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AO banding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamers et al (^{64})</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>de la Bastie et al (^{65})</td>
<td>↓</td>
<td>...</td>
<td></td>
<td></td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Siri et al (^{57})</td>
<td>...</td>
<td>...</td>
<td></td>
<td>Prolonged decay</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Komuro et al (^{96})</td>
<td>...</td>
<td>...</td>
<td></td>
<td>↓ in peak [Ca(^{2+})]</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Limas et al (^{63})</td>
<td>...</td>
<td>...</td>
<td></td>
<td></td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Mild AO banding</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>PA banding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gibbs et al (^{90})</td>
<td>...</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Nagai et al (^{47})</td>
<td>...</td>
<td>...</td>
<td></td>
<td></td>
<td>↓</td>
<td>↔↑</td>
</tr>
<tr>
<td>Volume overload</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild salt intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shen et al (^{61})</td>
<td>...</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Aortic insufficiency</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gibbs et al (^{90})</td>
<td>↓</td>
<td>...</td>
<td></td>
<td></td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Aortocaval shunt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bing et al (^{24})</td>
<td>...</td>
<td>↔</td>
<td></td>
<td></td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

"E-P indicates phosphorylated intermediate of the Ca\(^{2+}\)-ATPase; RYR, cardiac ryanodine receptor; SERCA2, sarco(endo)plasmic reticulum Ca\(^{2-}\)-ATPase (cardiac/slow-twitch muscle isoform); PLB, phospholamban; CSQ, cardiac calsequestrin; AO, aorta; PA, pulmonary artery; SHR, spontaneously hypertensive rat; ↑, increase; ↔, same as control; and ↓, decrease."

"No isofrom switch. ↑Same level after 4 weeks (decreased at 1 week)."

E-P indicates phosphorylated intermediate of the Ca\(^{2+}\)-ATPase; RYR, cardiac ryanodine receptor; SERCA2, sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (cardiac/slow-twitch muscle isoform); PLB, phospholamban; CSQ, cardiac calsequestrin; AO, aorta; PA, pulmonary artery; SHR, spontaneously hypertensive rat; ↑, increase; ↔, same as control; and ↓, decrease. No isofrom switch. ↑Same level after 4 weeks (decreased at 1 week).
affinity of the Ca\textsuperscript{2+} pump\textsuperscript{95} or the affinity of the myofibril for calcium\textsuperscript{68,69} does not change during cardiac hypertrophy. A decrease in the density of ryanodine receptor was also reported in the pressure-overload model of severe left ventricular hypertrophy (170 versus 366 receptors per square micrometer of SR in severe hypertrophy versus control).\textsuperscript{70}

Analysis of SR gene expression revealed that the steady-state levels of SR Ca\textsuperscript{2+}-ATPase mRNA are significantly decreased during pressure-overload hypertrophy in rabbit\textsuperscript{47} and rat\textsuperscript{65,66} models. Phospholamban mRNA is also decreased in these hearts.\textsuperscript{47} Interestingly, the relative amount of Ca\textsuperscript{2+}-ATPase mRNA exhibited a significant negative correlation between the left ventricular weight-to-body weight ratio, an index of cardiac hypertrophy.\textsuperscript{57}

In addition, the relative amount of Ca\textsuperscript{2+}-ATPase protein is also decreased in parallel with the Ca\textsuperscript{2+}-ATPase mRNA level in pressure-overload hypertrophy.\textsuperscript{65,67} These data can be interpreted to suggest that the differences in SR function among normal compensated cardiac hypertrophy or terminal stages of heart failure are not discontinuous or qualitative but merely quantitative. Furthermore, these data suggest that alterations in SR Ca\textsuperscript{2+} uptake function observed in different stages of cardiac hypertrophy can be attributed to a decrease in the number of Ca\textsuperscript{2+} pump sites that is due to a decrease in the steady-state level of SR Ca\textsuperscript{2+}-ATPase mRNA.

The spontaneously hypertensive rat (SHR) is a frequently used model in the study of cardiac hypertrophy. The SHR develops cardiac hypertrophy before the onset of hypertension (prehypertensive stage),\textsuperscript{71} suggesting that cardiac hypertrophy in the SHR is not entirely due to hemodynamic overload. Changes in the density of the L-type Ca\textsuperscript{2+} channel in the plasma membrane have been demonstrated in this animal model.\textsuperscript{72,73} In the aged SHR, intracellular Ca\textsuperscript{2+} transient analysis showed a lower resting [Ca\textsuperscript{2+}], and a prolongation of the time to peak [Ca\textsuperscript{2+}].\textsuperscript{74} A number of studies have demonstrated that an abnormal Ca\textsuperscript{2+} handling exists before and after the onset of hypertension. However, the involvement of the alterations of SR gene expression in the development of abnormal Ca\textsuperscript{2+} cycling still remains to be established.

A number of animal models have been used to study the alteration of Ca\textsuperscript{2+} cycling in failing hearts (Table 2). Perhaps the most widely used model is the hereditary cardiomyopathic Syrian hamster (BIO 14.6 and BIO 53.58 strains),\textsuperscript{75} which displays a thickened ventricular wall resembling hypertrophic cardiomyopathy (BIO 14.6) and a dilated ventricular chamber with a thin wall, resembling dilated cardiomyopathy (BIO 53.58). SR Ca\textsuperscript{2+} uptake by SR vesicles demonstrates that the velocity and capacity of Ca\textsuperscript{2+} uptake in dilated cardiomyopathic hamsters (between 3 and 11 months of age) are significantly lower compared with control and hypertrophic cardiomyopathic hamsters.\textsuperscript{76} Importantly, the ratios of Ca\textsuperscript{2+} uptake velocity to capacity, an estimate of the functional capability of the SR Ca\textsuperscript{2+}-ATPase, were not different between normal and cardiomyopathic hamsters.\textsuperscript{76} These results suggest a decrease either in the volume of SR or in the number of SR Ca\textsuperscript{2+}-ATPase pump sites, with no changes in specific activity of the Ca\textsuperscript{2+}-ATPase enzyme in cardiomyopathic hamsters. In addition, a simultaneous reduction in enzyme activity and gene expression was also demonstrated for the SR Ca\textsuperscript{2+}-ATPase and sarcolemmal Ca\textsuperscript{2+}-ATPase pumps in Syrian hamsters.\textsuperscript{77} These alterations in Ca\textsuperscript{2+}-ATPase gene expression were noted as early as 1 month, at the cardiomyopathic stage.\textsuperscript{77} These data suggest that an abnormality in SR Ca\textsuperscript{2+} transport function could contribute to the systolic Ca\textsuperscript{2+} overload and Ca\textsuperscript{2+} deposition that is observed at the final stages of heart failure. Furthermore, these data also indicate that decreased SR Ca\textsuperscript{2+} uptake is at, least in part, due to the decreased expression of the SR Ca\textsuperscript{2+}-ATPase gene. Heart failure induced by chronic rapid ventricular pacing\textsuperscript{78,79} provides a good model to study the relation between the mechanical properties and Ca\textsuperscript{2+} handling in the failing heart. Isometric length-tension studies of these hearts demonstrate that the peak isometric tension is reduced and that the time to peak tension and the time to 90% decline from peak tension are prolonged when compared with the control condition.\textsuperscript{80,81} In the canine pacing model, myosin isozyme composition (100% V\textsubscript{5} isoform) and myosin ATPase activity were unaltered,\textsuperscript{82} suggesting that other mechanisms including Ca\textsuperscript{2+} cycling are responsible for reduced contractility and distensibility. Indeed, simultaneous intracellular Ca\textsuperscript{2+} transient measurements using aequorin demonstrate that both the time to peak light (peak [Ca\textsuperscript{2+}]) and the time to 80% decline from peak light are also prolonged in this model,\textsuperscript{83} which closely resembles the data from human dilated cardiomyopathic hearts.\textsuperscript{83} It was also reported that SR Ca\textsuperscript{2+}-ATPase activity and SR Ca\textsuperscript{2+} uptake are diminished to half of that of the control muscle.\textsuperscript{82} Importantly, the decrease in SR Ca\textsuperscript{2+} ATPase activity was positively correlated with left ventricular ejection function, an index of degree of myocardial failure.\textsuperscript{82} These data suggest that abnormal [Ca\textsuperscript{2+}] handling is one of the important causes of contractile dysfunction that is seen in pacing-induced heart failure. In addition, deficient production of cAMP\textsuperscript{84} and a defect at the level of the catalytic subunit of adenylyl cyclase\textsuperscript{84} were also observed in these failing hearts, which could modify SR Ca\textsuperscript{2+} uptake function by affecting the state of phospholamban phosphorylation.

Adriamycin (doxorubicin) is a widely used antineoplastic anthracycline antibiotic, which may provoke severe heart failure (adriamycin cardiomyopathy).\textsuperscript{85} Disintegration of cellular components,\textsuperscript{86} including SR, have been observed, and diverse theories explaining the cause of adriamycin cardiomyopathy have been proposed: free radical production by lipid peroxidation,\textsuperscript{87} inhibition of oxidative phosphorylation in mitochondria,\textsuperscript{88} and inhibition of Na\textsuperscript{+}, K\textsuperscript{+}-ATPase.\textsuperscript{89} However, abnormal accumulation of intracellular Ca\textsuperscript{2+} has been repeatedly reported.\textsuperscript{90-92} A significant reduction in SR Ca\textsuperscript{2+}-ATPase activity was demonstrated in chronically administered adriamycin cardiomyopathy in dogs.\textsuperscript{91} It was also demonstrated that adriamycin produces a dose-dependent increase in the open probability of the Ca\textsuperscript{2+} release channel of SR, which will stimulate the release of intracellular Ca\textsuperscript{2+} stores.\textsuperscript{90} Thus, it is quite likely that adriamycin-induced alteration of the SR Ca\textsuperscript{2+} transport function will lead to intracellular Ca\textsuperscript{2+} overload, which is closely associated with the pump failure seen in adriamycin cardiomyopathy. However, a detailed study of the expression of SR Ca\textsuperscript{2+} transport
TABLE 2. Alteration of Sarcoplasmic Reticulum Ca\(^{2+}\) Transport in Heart Failure

<table>
<thead>
<tr>
<th>Intervention or Heart Disease</th>
<th>ATP Hydrolysis Rate</th>
<th>RYR Function</th>
<th>[Ca(^{2+})], Transient Study</th>
<th>mRNA Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\uparrow)</td>
<td>(\leftrightarrow)</td>
<td>(\uparrow) in peak [Ca(^{2+})]</td>
<td>SERCA2</td>
</tr>
<tr>
<td></td>
<td>(\downarrow)</td>
<td>(\leftrightarrow)</td>
<td>(\downarrow) in time to peak [Ca(^{2+})], and (\downarrow) in time to 80% decline of [Ca(^{2+})]</td>
<td>CSQ</td>
</tr>
<tr>
<td></td>
<td>(\leftrightarrow) in RYR density</td>
<td>(\leftrightarrow)</td>
<td>(\leftrightarrow)</td>
<td>RYR</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>(\leftrightarrow)</td>
<td>(\leftrightarrow)</td>
<td>(\leftrightarrow)</td>
<td></td>
</tr>
<tr>
<td>Hemodynamic load</td>
<td>(\leftrightarrow)</td>
<td>(\leftrightarrow)</td>
<td>(\leftrightarrow)</td>
<td></td>
</tr>
<tr>
<td>AO banding</td>
<td>(\leftrightarrow)</td>
<td>(\leftrightarrow)</td>
<td>(\leftrightarrow)</td>
<td></td>
</tr>
<tr>
<td>Monocrotaline</td>
<td>(\leftrightarrow)</td>
<td>(\leftrightarrow)</td>
<td>(\leftrightarrow)</td>
<td></td>
</tr>
</tbody>
</table>

\(\uparrow\) Increase; \\(\downarrow\) decrease; \\(\leftrightarrow\) same as control; \\(\uparrow\) increase; RV, right ventricle; LV, left ventricle.

*Simultaneous decrease of sarcolemmal Ca\(^{2+}\)-ATPase mRNA. 
†Increase in RV, decrease in nonischemic LV. 
‡Single-channel behavior. 
§Isolated single myocyte. 
[]Trabeculae carneae. 
\*Polymerase chain reaction.
proteins is required to document the role of Ca\(^{2+}\) transport in this type of heart failure.

**Hypertrophic Cardiomyopathy in Humans**

Hypertrophy of cardiac muscle is a phenomenon quite widely observed in various cardiovascular diseases in humans. However, studies that address the subcellular mechanisms of human cardiac hypertrophy are sparse, probably because of the difficulty in obtaining adequate tissue samples. Several studies have been done with heart samples of hypertrophic cardiomyopathy after myectomy.\(^{94,95}\) Simultaneous measurements of tension and the intracellular Ca\(^{2+}\) transient revealed that isometric contraction and relaxation was prolonged, end-diastolic [Ca\(^{2+}\)], was increased, and the intracellular Ca\(^{2+}\) transient was greatly prolonged.\(^{94}\) Furthermore, drugs that increase [Ca\(^{2+}\)], exacerbated these abnormalities, whereas drugs that lower [Ca\(^{2+}\)] or increase cAMP prevented them. cAMP-dependent protein phosphorylation accelerates the Ca\(^{2+}\) uptake by SR Ca\(^{2+}\)-ATPase\(^{30-33}\) and increases the Ca\(^{2+}\) current through the sarcoplasmic Ca\(^{2+}\) ion channels.\(^{96}\) Therefore, abnormalities in SR Ca\(^{2+}\)-ATPase and in sarcoplasmic Ca\(^{2+}\) ion channel function may be involved in abnormal intracellular Ca\(^{2+}\) handling and thus in impaired contractile performance in hypertrophic cardiomyopathy. In fact, Wagner et al\(^{95}\) demonstrated that dihydropyridine binding sites (sarcoplasmic Ca\(^{2+}\) ion channel) in the atrial myocardium from hypertrophic cardiomyopathy patients were increased, suggesting that the transsarcolemmal Ca\(^{2+}\) influx is enhanced in hypertrophic cardiomyopathy.

**Human Heart Failure**

Low-output congestive heart failure is a syndrome characterized by both systolic and diastolic dysfunction.\(^{97}\) The velocity and extent of ventricular contraction and the rate of pressure development are decreased in heart failure.\(^{98-101}\) A number of possible subcellular mechanisms for the observed contractile dysfunction have been explored. These include abnormalities in contractile\(^{102-105}\) and cytoskeletal\(^{106}\) proteins, abnormalities in excitation-contraction coupling,\(^{107-109}\) abnormalities in cardiac metabolism (energy supply),\(^{110,111}\) and abnormalities in the signal transduction system.\(^{112,113}\) We will focus on the abnormalities in intracellular Ca\(^{2+}\) regulation, especially altered Ca\(^{2+}\) transport and altered expression of genes encoding Ca\(^{2+}\) transport proteins in human failing hearts.

**Ca\(^{2+}\) Transport Function in Failing Human Hearts**

The SR Ca\(^{2+}\) transport function in failing and normal human hearts has been investigated by use of tissue homogenates or isolated SR vesicles and radiolabeled \(^{45}\)Ca as a tracer for Ca\(^{2+}\) transport.\(^{107,108,114,115}\) The Ca\(^{2+}\) uptake rate measured in crude homogenates of right ventricular biopsy specimens was considerably lower in dilated cardiomyopathy patients (3.3 nmol/mg per minute for the cardiomyopathic group versus 6.5 nmol/mg per minute for the control group). The rate of Ca\(^{2+}\) uptake showed a modest correlation with several hemodynamic indexes of cardiac performance, i.e., pulmonary capillary wedge pressure, cardiac output, and ejection fraction.\(^{108}\) By contrast, Movsesian et al\(^{115}\) have reported that the maximal Ca\(^{2+}\) uptake rate, the [Ca\(^{2+}\)] at half-maximal Ca\(^{2+}\) uptake, and the Hill coefficient are not different between normal and cardiomyopathic hearts. They further showed that the phospholamban-mediated stimulation of steady-state Ca\(^{2+}\) uptake was not significantly different between normal and failing hearts.\(^{116}\) These data were obtained by use of purified SR vesicles from the left ventricle. Discrepancies between these two studies are intriguing and may be partly due to methodological differences, such as the utilization of whole-muscle homogenate versus purified SR vesicles for the Ca\(^{2+}\) uptake studies. Taken together, these data suggest that the intrinsic Ca\(^{2+}\) uptake activity of SR might not be affected in heart failure but that the amount of Ca\(^{2+}\) pump per cell is diminished.

The activity of the Ca\(^{2+}\) release channel in SR (the ryanodine receptor) has also been examined.\(^{117-119}\) Junctional SR membrane vesicles were incorporated into artificial planar phospholipid bilayers, and the activity of a single channel was recorded under voltage-clamp conditions.\(^{117,118}\) The characteristics of single-channel behavior from heart failure due to dilated cardiomyopathy, ischemic cardiomyopathy, valvular disease, or congenital heart disease were essentially the same as in normal hearts. To quantify the SR Ca\(^{2+}\) release channel indirectly, tension development in chemically skinned fibers was analyzed.\(^{119}\) This study demonstrated that the threshold of Ca\(^{2+}\) release induced by caffeine (an activator of the Ca\(^{2+}\) release channel and of the Ca\(^{2+}\)-induced Ca\(^{2+}\) release) was significantly higher in muscles from patients with dilated cardiomyopathy, suggesting that the gating mechanism of the Ca\(^{2+}\) release channel is impaired.\(^{120}\) These two reports seem contradictory; however, it should be noted that the data generated from single Ca\(^{2+}\) channel measurements may differ from those obtained from the whole muscle (skinned fibers).

Ca\(^{2+}\) cycling in cardiac muscle can be measured with bioluminescent Ca\(^{2+}\) indicators. Intracellular Ca\(^{2+}\) transients were recorded from thin trabeculae carnea loaded with aequorin\(^{83,120}\) or from an isolated myocyte loaded with fura 2.\(^{121}\) Muscle samples from patients with dilated cardiomyopathy showed high resting [Ca\(^{2+}\)] levels (165±61 nmol/L in failing hearts versus 96±47 nmol/L in control hearts) and low peak [Ca\(^{2+}\)] levels (367±109 nmol/L in failing hearts versus 746±249 nmol/L in control hearts).\(^{121}\) In addition, intracellular Ca\(^{2+}\) transients in dilated cardiomyopathy showed a slower rise of luminescence (time to peak light, 53±5 milliseconds in cardiomyopathy versus 33±6 milliseconds in the control condition) and a slower decline in luminescence (time to 80% decline from peak light, 569±48 milliseconds in cardiomyopathy versus 246±37 milliseconds in the control condition).\(^{83}\) These data indicate that both the Ca\(^{2+}\) release function and Ca\(^{2+}\) uptake (sequestering function) are impaired in dilated cardiomyopathy. Furthermore, tension-independent heat measurements showed a substantial reduction in the amount of heat produced in muscle strips from the failing myocardium.\(^{3,122}\) Tension-independent heat is thought to result from the energy expended for Ca\(^{2+}\) transport in the muscle strips and can provide an estimate of Ca\(^{2+}\) cycled per heart beat. The amount of Ca\(^{2+}\) cycled was 32.2±8.17 nmol/g per beat in control hearts and 16.7±1.72 nmol/g per beat in failing hearts, indicating a 50% reduction in Ca\(^{2+}\) uptake function.\(^{122}\)
This study also suggested that a defect in Ca\(^{2+}\) removal might be one of the principal causes of impaired relaxation in the failing human heart.

**Alterations in SR Gene Expression in Human Heart Failure**

Recent advances in the molecular cloning of SR Ca\(^{2+}\) transport proteins have provided new tools for examining SR function at the molecular level. By use of isoform specific cDNA probes, it is now possible to determine whether abnormal Ca\(^{2+}\) handling in failing human hearts is due to altered expression of genes encoding SR proteins. Several groups, including ours, have recently examined the relative levels of mRNAs (steady-state levels) encoding several SR Ca\(^{2+}\) transport proteins using total RNA isolated from human hearts exhibiting various degrees of failure.\(^{123-126}\) Quantification of steady-state levels of SR Ca\(^{2+}\)-ATPase mRNA revealed that only the SERCA2a mRNA is expressed in failing hearts. However, the steady-state level of expression is greatly reduced in failing hearts from patients with dilated cardiomyopathy,\(^{125-128}\) coronary artery disease,\(^{122,125}\) primary pulmonary hypertension,\(^{124}\) hypertrophic cardiomyopathy,\(^{125}\) valvular disease,\(^{123}\) or congenital heart disease.\(^{125}\) Relative abundance of steady-state mRNA levels in left and right ventricles from hearts of patients of diverse etiology did not show significant differences. Interestingly, the levels of Ca\(^{2+}\)-ATPase mRNA are positively correlated with cardiac functional indexes\(^{125}\) and negatively correlated with the elevated expression level of atrial natriuretic factor (ANF) mRNA\(^{124,125}\) and of brain natriuretic factor (BNF) mRNA in human ventricle.\(^{125}\) Although it remains to be established whether ventricular ANF mRNA or BNF mRNA levels correlate with the severity of heart failure, these studies demonstrate that hearts with higher ventricular ANF mRNA or BNF mRNA levels show the greatest decreases in SR Ca\(^{2+}\)-ATPase expression. These data taken together demonstrate that the steady-state mRNA level of SR Ca\(^{2+}\)-ATPase was significantly reduced in failing human myocardium, regardless of the disease etiology.

The level of phospholamban mRNA has also been examined by Northern blot analysis\(^{124}\) or by the polymerase chain reaction.\(^{126}\) Phospholamban mRNA expression is reduced in heart failure caused by dilated cardiomyopathy,\(^{124,126}\) coronary artery disease,\(^{124}\) and primary pulmonary hypertension.\(^{124}\) The expression level is inversely correlated with the ventricular ANF mRNA level, as noted for Ca\(^{2+}\)-ATPase. In addition, phospholamban mRNA shows a decrease parallel to that of Ca\(^{2+}\)-ATPase in failing hearts, indicating that the expressions of these two genes are coordinately regulated.\(^{124}\)

In contrast, the level of calsequestrin mRNA does not change significantly in heart failure caused by dilated cardiomyopathy,\(^{124,127}\) coronary artery disease,\(^{124,127}\) or primary pulmonary hypertension\(^{124}\) and fails to show a correlation with ANF mRNA levels. This result is somewhat surprising, considering that the SR Ca\(^{2+}\) uptake and release function are modified in failing hearts. This result suggests that the calsequestrin gene is regulated independent of other SR protein genes. Further studies need to be done to understand this discordant regulatory program controlling SR gene expression.

An analysis of the levels of the cardiac ryanodine receptor (Ca\(^{2+}\) release channel) mRNA revealed a decrease in end-stage heart failure caused by coronary artery disease,\(^{124,128}\) primary pulmonary hypertension,\(^{124}\) and decreased\(^{124}\) or unchanged\(^{128}\) levels of RNA in dilated cardiomyopathy. In addition, Arai et al\(^{124}\) demonstrated that the level of ryanodine receptor mRNA is inversely correlated with that of ANF mRNA. This study also showed that a decrease in the ryanodine receptor mRNA levels correlated with the Ca\(^{2+}\)-ATPase and phospholamban mRNA levels in failing human hearts, suggesting that these genes are coordinately regulated.\(^{124}\) This study concluded that the expressions of mRNAs for Ca\(^{2+}\) uptake and of uptake proteins in SR derived from hearts of patients with end-stage heart failure are decreased in parallel, are related to the severity of heart failure, and are independent of the underlying etiology.

**Conclusion and Perspectives**

The studies reviewed here support a unifying theme: the expression of mRNAs encoding SR Ca\(^{2+}\) transport proteins is altered in a gene-specific manner in cardiac hypertrophy and heart failure both in experimental animal models and in humans. The data are consistent with the hypothesis that abnormal SR Ca\(^{2+}\) handling in hypertrophied and failing hearts is, at least in part, due to altered expression of genes encoding SR Ca\(^{2+}\) transport proteins.

The studies reviewed here do not clarify whether alterations of SR gene expression are etiologic for heart failure or cardiac hypertrophy. Indeed, it is quite likely that these changes are secondary to primary alterations in heart failure or cardiac hypertrophy. However, available evidence reviewed here clearly establishes a correlation between altered Ca\(^{2+}\) transport and contractile dysfunction seen in failing hearts of experimental animals and humans. Furthermore, these studies clearly demonstrate that altered expression of SR genes is the major cause of altered Ca\(^{2+}\) handling seen in heart failure and cardiac hypertrophy. At the present time, however, the mechanisms regulating the expression of these genes are unknown.

Future studies should be aimed toward establishing a direct cause-and-effect relation between SR dysfunction and its relevance to heart failure. These studies are now possible with newly available tools such as transgenic animal models, in which specific gene alterations may be introduced to alter SR Ca\(^{2+}\) transport function.

**Acknowledgments**

This study was supported by funds from the American Heart Association and the National Institutes of Health. Dr Periasamy is an Established Investigator of the American Heart Association. We are grateful to Norman R. Alpert, University of Vermont, for his encouragement and support of this work. We would like to thank Drs Richard A. Walsh, Jeffrey Robbins, Evangelia Kranias, and Junaid Shabbeer for critical reading and helpful suggestions on the manuscript.

**References**


Endo W. Calcium release from the sarcoplasmic reticulum. Physiol Rev. 1977;57:71-108.

Fabiato A. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. Am J Physiol. 1983;245(Cell Physiol 14): C1-C14.


Inui M, Saito A, Fleischer S. Isolation of the ryosodine receptor from cardiac sarcoplasmic reticulum and identity with the feet structures. J Biol Chem. 1987;262:15637-15642.


Otsu K, Willard FH, Khanna VK, Zorzato F, Green NM, MacLennan DH. Molecular cloning of cDNA encoding the Ca(2+)-release channel (ryanodine receptor) of rabbit cardiac muscle sarcoplasmic reticulum. J Biol Chem. 1990;265:13472-13483.


MacLennan DH, Brandle CH, Korczak B, Green NM. Amino-acid sequence of a Ca(2+)-Mg(2+)-dependent ATPase from rabbit muscle sarcoplasmic reticulum, deduced from its complementary DNA sequence. Nature. 1985;316:696-700.


Suko J. The calcium pump of cardiac sarcoplasmic reticulum: functional alterations at different levels of thyroid state in rabbits. J Physiol (Lond). 1973;228:563-582.

Rohrer D, Dillmann WH. Thyroid hormone markedly increases the mRNA coding for sarcoplasmic reticulum Ca2+-ATPase in rat heart. J Biol Chem. 1990;265:17643-17651.


Lamers JHJ, Sinis JT. Defective calcium pump in the sarcoplasmic reticulum of the hypertrophied rabbit heart. Life Sci. 1979;24:2133-2134.


117. Holmgren SRM, Williams AJ. The calcium-release channel from cardiace sarcoplasmic reticulum: function in the failing and acutely ischemic heart. Basic Res Cardiol. 1992;87(suppl 1):255-268.


Sarcoplasmic reticulum gene expression in cardiac hypertrophy and heart failure.
M Arai, H Matsui and M Periasamy

Circ Res. 1994;74:555-564
doi: 10.1161/01.RES.74.4.555

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 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/74/4/555.citation

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/