Calcium sequestration by the sarcoplasmic reticulum in heart failure

Matthew A. Movsesian a,*, Robert H.G. Schwinger b

a Salt Lake City VA Medical Center, Internal Medicine and Pharmacology, University of Utah School of Medicine, Salt Lake City, Utah, USA
b Laboratory of Muscle Research and Molecular Cardiology, Clinic III of Internal Medicine, University of Cologne, Cologne, Germany

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1. Introduction

Myocardial contraction and relaxation are dependent upon the rise and fall of cytosolic [Ca\textsuperscript{2+}] in cardiac myocytes. The release and sequestration of Ca\textsuperscript{2+} by the sarcoplasmic reticulum are the principal mechanisms through which this occurs. During relaxation, Ca\textsuperscript{2+} is actively transported from the cytosol into the sarcoplasmic reticulum; during contraction, this sequestered Ca\textsuperscript{2+} is passively released into the cytosol through ryanodine-sensitive Ca\textsuperscript{2+} channels.

Because of the dependence of contraction and relaxation upon ATP-dependent Ca\textsuperscript{2+} sequestration by the sarcoplasmic reticulum, the possibility that an impairment in this process contributes to the pathophysiology of heart failure has been the focus of a large body of research over the past two decades. While there seems to be a general agreement that ATP-dependent Ca\textsuperscript{2+} sequestration by the sarcoplasmic reticulum is impaired in failing human myocardium, there remains a fairly intense controversy regarding the molecular aetiology of this impairment. In this review, the rationale underlying the experimental approaches to this issue in animal models and human tissues and the conclusions that can be drawn from their results are examined.

2. Molecular mechanisms of Ca\textsuperscript{2+} transport and its regulation

Ca\textsuperscript{2+} accumulation by cardiac sarcoplasmic reticulum occurs through the activity of SERCA2, a 105 kDa Ca\textsuperscript{2+}-

and Mg\textsuperscript{2+}-dependent ATPase that transports Ca\textsuperscript{2+} from the cytosol to the lumen of the sarcoplasmic reticulum [1–3]. The K\textsubscript{m} of SERCA2 for Ca\textsuperscript{2+} in sarcoplasmic reticulum-enriched vesicles isolated from human left ventricular myocardium is 0.63–0.68 \textgreek{m}M, a value which falls within the range of cytosolic Ca\textsuperscript{2+} concentrations that have been measured in intact cardiac myocytes during relaxation and contraction (0.1–1.0 \textgreek{m}M), while the Hill coefficient is 1.6–1.7 [4,5]. The rate of ATP-dependent Ca\textsuperscript{2+} transport by the sarcoplasmic reticulum is thus sensitive to small changes in cytosolic [Ca\textsuperscript{2+}] concentrations within the physiologic range. When cytosolic [Ca\textsuperscript{2+}] rises to a value exceeding K\textsubscript{m} following its release from the sarcoplasmic reticulum, the rate of Ca\textsuperscript{2+} sequestration by sarcoplasmic reticulum rises to a near-maximal level. As Ca\textsuperscript{2+} sequestration progresses during relaxation, the reduction in cytosolic Ca\textsuperscript{2+} lowers the rate of Ca accumulation until a low diastolic cytosolic Ca\textsuperscript{2+} is restored. It is important to appreciate, therefore, that SERCA2 activity determines not only the rate and extent of relaxation, but also the rate and amplitude of contraction, since these are determined by the amount of Ca\textsuperscript{2+} sequestered by the sarcoplasmic reticulum and the Ca\textsuperscript{2+} gradient between the sarcoplasmic reticulum and the cytosol at the time Ca\textsuperscript{2+} release occurs.

The activity of SERCA2 is modulated through its interaction with phospholamban, a pentamer comprised of five identical 6 kDa (52 amino acid) monomers [6,7]. In its unphosphorylated form, phospholamban binds SERCA2 and inhibits Ca\textsuperscript{2+} transport activity, principally by decreasing affinity (increasing K\textsubscript{m}) for Ca\textsuperscript{2+} but also probably by decreasing V\textsubscript{max} [8]. Phosphorylation of phospholamban by

* Corresponding author. Cardiology Division, 4A-100 SOM, University of Utah Health Sciences Center, 50 North Medical Drive, Salt Lake City, UT 84132, USA. Tel. (+1-801) 581 7715; Fax (+1-801) 581 7735; E-mail:matthew.movsesian@hsc.utah.edu

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any of several protein kinases — among them cAMP-dependent protein kinase, a membrane-associated calmodulin-dependent protein kinase and protein kinase C — blocks the interaction between phospholamban and SERCA2 and relieves this inhibition (Fig. 1) [9–20]. While numerous proteins involved in contraction and relaxation are substrates for cAMP-dependent protein kinase, phosphorylation of phospholamban and the resulting de-inhibition of SERCA2 appears to be the principal molecular mechanism for the inotropic and lusitropic effects of β-adrenergic receptor agonists. The most convincing evidence comes from experiments comparing myocardial responses to isoprenaline in normal mice and in mice in whom the gene for phospholamban has been ablated [21]. In normal mice, rates of contraction and relaxation are relatively low in the absence of isoprenaline and are increased dose-dependently in its presence. In phospholamban-deficient mice, rates of contraction and relaxation are as high in the absence of isoprenaline as they are in normal mice in its presence, and are not increased upon exposure to isoprenaline. These observations are entirely consistent with a mechanism in which unphosphorylated phospholamban inhibits SERCA2 activity, and in which this inhibition is overcome by activation of cAMP-dependent protein kinase and its phosphorylation of phospholamban. (In contrast, the chronotropic response to isoprenaline is preserved in phospholamban-deficient mice, implying that a separate molecular mechanism is responsible for this response.)

Ca\(^{2+}\) accumulation may also be subject to regulation by cytosolic proteins. Inhibitory activity toward SERCA2 has been found in actin-enriched fractions of myocardial cytosol, and this inhibitory activity can be antagonised by muscle albumin [22–24]. Whether these observations have any in vivo relevance is unknown, however, and the molecular mechanisms involved have not been identified.

3. The hypothesis and its rationale

There are several reasons for proposing that an impairment in Ca\(^{2+}\) sequestration by the sarcoplasmic reticulum could contribute to the pathophysiology of heart failure. First, the rate and extent of decline of [Ca\(^{2+}\)] during diastole in cardiac myocytes are diminished in failing human myocardium [25–27]. A decrease in Ca\(^{2+}\) sequestration by the sarcoplasmic reticulum, attributable either to a decrease in the abundance of SERCA2 or a change in its regulation, would offer a simple explanation for this finding. A paradigm for pathologic changes in the abundance and regulation of SERCA2 can be found in the effect of thyroxin on sarcoplasmic reticulum function: Chronic exposure to thyroxin increases the level of SERCA2 and decreases the level of phospholamban in cardiac sarcoplasmic reticulum [28–32]. This combination of effects can explain the increase in the basal rate of ATP-dependent Ca\(^{2+}\) sequestration as well as the decreased stimulation of Ca\(^{2+}\) sequestration by cAMP-dependent protein phosphorylation in sarcoplasmic reticulum-enriched microsomes isolated from the hearts of hyperthyroid animals (and the converse findings in preparations from hypothyroid animals) [33–37].
An impairment in Ca\(^{2+}\) sequestration by the sarcoplasmic reticulum could also explain the abnormal force-frequency relationship observed in failing human myocardium. In normal human myocardium, an increase of the frequency of stimulation is followed by an increase in developed tension, a phenomenon referred to as a positive force-frequency relationship. In failing human myocardium, in contrast, a negative force-frequency relationship exists, such that an increase in the frequency of stimulation is followed by a decrease in developed tension accompanied by a decrease in intracellular Ca\(^{2+}\) cycling [38,39]. This negative force-frequency relationship could be explained by a reduction in the rate of Ca\(^{2+}\) transport sufficient to render the extent of Ca\(^{2+}\) sequestration by the sarcoplasmic reticulum dependent upon the duration of diastole. In such a situation, the decreased duration of diastole at faster heart rates would result in a decrease in end-diastolic Ca\(^{2+}\) content of the sarcoplasmic reticulum, and the amount of Ca\(^{2+}\) available for release upon the subsequent depolarisation (and hence the force generated in systole) would be reduced. The observations that agents that increase cAMP generation in cardiac myocytes can reverse a negative force-frequency relationship, presumably by increasing phospholamban phosphorylation and thereby stimulating SERCA2 activity, while specific inhibition of SERCA2 in normal human myocardium converts the positive force-frequency relationship to a negative one and reduces the maximal amplitude of the [Ca\(^{2+}\)] transient, are evident in support of the plausibility of this hypothesis [40–42].

Finally, as a result of β-adrenergic receptor downregulation, increased activity of β-adrenergic receptor kinase and increased activity of G\(_{\text{q,i}}\), cAMP generation is impaired and cAMP levels are reduced failing human myocardium [43–50]. In view of the importance of cAMP-dependent phosphorylation of phospholamban and the consequent de-inhibition of SERCA2 activity in normal myocardium, alterations in SERCA2 function and its regulation by phospholamban phosphorylation would be expected to contribute to the molecular pathophysiology of heart failure.

### 4. Experimental results in animal models and human tissue

The results of a large number of studies in animal models have lent credence to the validity of this rationale. Decreased rates of ATP-dependent Ca\(^{2+}\) sequestration have been reported in failing myocardium from animal models involving hypertrophic [51–61], ischaemic [62,63], pacing-induced [64], genetic [65–69], diabetic [70], toxic [71–73] and infective [74] aetiologies of heart failure. In several of these studies, decreased mRNA and protein levels of SERCA2 have been observed as well.

Several groups of investigators have attempted to determine whether these findings in animal models are applicable to heart failure in humans. The results of these studies and their interpretation have proven highly controversial (see Table 1). On at least one point opinion seems unanimous: every group that has compared ATP-dependent Ca\(^{2+}\) sequestration in crude tissue homogenates prepared from normal and failing human myocardium has reported a marked diminution in Ca\(^{2+}\) sequestration in failing tissue [75–78]. As to whether this impairment is attributable to changes in the abundance of SERCA2, however, opinion

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<th>mRNA</th>
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<td>SERCA2</td>
<td>phospholamban</td>
<td>Basal sequestration rate or ATPase activity</td>
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Ca\(^{2+}\) sequestration, protein and mRNA correlations in failing human myocardium. Results of studies involving measurement of SERCA2 and phospholamban protein and mRNA levels and/or ATP-dependent Ca\(^{2+}\) sequestration rates in salt-washed microsomes prepared from failing human myocardium are listed. Findings of a reduction relative to normal human myocardium are denoted with the symbol ↓; findings of no difference between normal and failing myocardium are denoted with the symbol πΔ. Solid horizontal lines differentiate groups of investigators. Dotted horizontal lines separate publications from the same group.
has differed sharply between groups of investigators. One group, studying Ca\(^{2+}\) sequestration in more highly purified (i.e., washed in high salt to remove non-intrinsic or loosely bound proteins) microsomes from normal and failing human myocardium, has reported a ~35% decrease in ATP-dependent Ca\(^{2+}\) transport in preparations from failing human myocardium [76,79,80]. They found this decrease was accompanied by a selective decrease in SERCA2 protein levels (both in the salt-washed microsomes and in the myocardium from which they were prepared) and correlated directly with decreases in the force frequency relationship and inversely with changes in the protein level of Na\(^+\)/Ca\(^{2+}\) exchanger. This results are consistent with findings in animal models and are arguably consistent with unanimous reports of decreases in the level of SERCA2 mRNA in failing human myocardium [76–84]. The conclusion of these investigators is that the decrease in Ca\(^{2+}\) sequestration by the sarcoplasmic reticulum is attributable to a decrease in the abundance of SERCA2 that is generated at the level of gene transcription.

In contrast, however, three other groups of investigators have found that Ca\(^{2+}\) transport rates in salt-washed sarcoplasmic reticulum-enriched microsomes and protein levels of SERCA2 and phospholamban are identical in preparations from normal and failing human myocardium [4,77,78,85–88]. Two of these groups have demonstrated that protein levels for SERCA2 in samples of failing myocardium remain at normal levels despite reductions in SERCA2 mRNA levels in these same tissues [77,78,84], and one of these groups has reported as well that the regulation of Ca\(^{2+}\) transport by phospholamban phosphorylation is comparable in salt-washed microsomes prepared from normal and failing human myocardium, which would suggest that the stoichiometry and coupling of these proteins is unchanged in failing human myocardium [85]. The conclusion of these investigators — we are among them — is that Ca\(^{2+}\) transport by the sarcoplasmic reticulum is impaired in failing human myocardium, but that this impairment is not attributable to changes in the level of SERCA2 or its coupling to phospholamban.

An explanation for the discrepant findings among these groups of investigators is not apparent. Clearly the two conclusions are mutually incompatible, for which reason one set of data must be right and the other flawed. But discussions between the different investigators have been extensive, and neither side has found a systematic error in the other’s experimental approach. The investigators who have reported low protein levels of SERCA2 in failing myocardium point to the corresponding findings in animal models and the corresponding reductions of mRNA levels in human studies as evidence of the probability their explanation is correct. We would point out, however, that virtually all of the patients included in these studies suffered from ischaemic or idiopathic disease, and that few of the animal models studied are ischaemic and none are idiopathic. Furthermore, humans and their animal models may differ in important ways. In rats, for example, there is a negative force-frequency relationship in normal myocardium [89]. For these reasons, we believe that divergent findings in animal models and failing human myocardium need not be viewed as contradictory. With respect to the corresponding decreases in mRNA levels, we would point out that protein levels are often regulated independently of the level of the encoding mRNA. Large differences in the ratio of mRNA levels to protein content for both SERCA1 and SERCA2 have been observed in different striated muscle [90,91], and levels of Na\(^+\)/K\(^+\)-ATPase and G\(\alpha\) mRNA and protein in cardiac myocytes can be differentially regulated (in the latter example, overexpression of G\(\alpha\) in transgenic mouse hearts using a myosin heavy chain promoter resulted in a 38-fold increase in mRNA level but a mere 2.8-fold increase in G\(\alpha\) protein — i.e., a difference of more than an order of magnitude) [92,93]. Furthermore, a complex post-transcriptional regulation of proteins occurs in rabbits with surgically-induced aortic insufficiency: myocardial protein synthesis rates are increased and myocardial protein degradation rates are decreased initially, but both protein synthesis and protein degradation rates are decreased when myocardial hypertrophy ensues [94]. Ryanodine-sensitive Ca\(^{2+}\) channels are differentially regulated at protein level and mRNA expression in cardiomyopathic and control hearts as well [95]. Perhaps the most directly relevant example comes from a study in which left ventricular hypertrophy was induced in rats by aortic coarctation: eight weeks post-operatively, SERCA2 mRNA levels were reduced by 57%, while SERCA2 protein levels were slightly increased though the SERCA2 mRNA levels were reduced by 57%, while SERCA2 mRNA levels were reduced by 57%, while SERCA2 protein levels were slightly increased though the SERCA2 mRNA levels were reduced by 57%, while SERCA2 protein levels were slightly increased though the SERCA2 mRNA levels were reduced by 57%.
There is preliminary evidence in support of this hypothesis. In studies of ATP-dependent Ca\(^{2+}\) sequestration in crude tissue homogenates, affinity for Ca\(^{2+}\) was significantly lower in preparations from failing myocardium, and this lower affinity was accompanied by a reduced level of phospholamban phosphorylation [96]. In preparations from both normal and failing hearts, addition of cAMP-dependent protein kinase catalytic subunit resulted in an increase in Ca\(^{2+}\) affinity, but the effect was greater in preparations from failing hearts, and the values for K\(_m\) for Ca\(^{2+}\) after maximal stimulation with cAMP-dependent protein kinase were similar in both groups. These findings, which require further confirmation before they can be accepted, are consistent with a situation in which levels of SERCA2 and phospholamban are comparable in normal and failing myocardium, but in which there is greater inhibition of Ca\(^{2+}\) sequestration in failing myocardium because of a lower level of phospholamban phosphorylation (similar evidence suggests there is a decrease in cAMP-mediated phosphorylation of myofibrillar proteins in failing canine and human myocardium [97,98]). It is important to point out, however, that the possible contribution of phosphorylation (or the lack thereof) by other protein kinases referenced earlier in this review [9–20] has not been explored in these studies.

5. Conclusions

We have tried to represent the areas of controversy on the issue of Ca\(^{2+}\) sequestration by the sarcoplasmic reticulum in heart failure objectively and to offer our opinion as to their resolution. We are convinced, however, that the data that have accumulated thus far have raised more questions than they have answered. If, as we believe, levels of SERCA2 and phospholamban protein are maintained in failing myocardium despite a decrease in corresponding mRNA levels, more needs to be learned about the complex mechanisms by which transcription, translation and protein degradation are altered in heart failure. Furthermore, an understanding of the reasons for the different results in animal models and failing human tissue (if these are real) may provide critical information on the molecular pathogenesis of the syndrome. Clearly much important research in this area remains to be done.

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