Regulation of sarcoplasmic reticulum Ca\textsuperscript{2+} ATPase pump expression and its relevance to cardiac muscle physiology and pathology

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Cardiac sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} ATPase (SERCA2a) plays a central role in myocardial contractility. SERCA2a actively transports Ca\textsuperscript{2+} into the SR and regulates cytosolic Ca\textsuperscript{2+} concentration, SR Ca\textsuperscript{2+} load, and the rate of contraction and relaxation of the heart. In the heart, SERCA pump activity is regulated by two small molecular weight proteins: phospholamban (PLB) and sarcolipin (SLN). Decreases in the expression levels of SERCA2a have been observed in a variety of pathological conditions. In addition, altered expression of PLB and SLN has been reported in many cardiac diseases. Thus, SERCA2a is a major regulator of intracellular Ca\textsuperscript{2+} homeostasis, and changes in the expression and activity of the SERCA pump contribute to the decreased SR Ca\textsuperscript{2+} content and cardiac dysfunction during pathogenesis. In this review, we discuss the mechanisms controlling SERCA pump expression and activity both during normal physiology and under pathological states.

1. Introduction

The molecular mechanisms regulating cardiac contractility have been the subject of intense investigation for several decades. In this regard, sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} transport has received a great deal of attention because of its central role in regulating cardiac function in health and disease. The cardiac SR is an intracellular membrane network that surrounds the contractile machinery. It serves not only as a Ca\textsuperscript{2+} reservoir for Ca\textsuperscript{2+} release but also actively maintains cytosolic Ca\textsuperscript{2+} concentration during contraction–relaxation. During excitation–contraction coupling (EC coupling), Ca\textsuperscript{2+} entry through the L-type Ca\textsuperscript{2+} channel activates Ca\textsuperscript{2+} release from the SR Ca\textsuperscript{2+} stores via the ryanodine receptor.\textsuperscript{1–4} This raises cytosolic Ca\textsuperscript{2+} and initiates muscle contraction, and the free cytosolic Ca\textsuperscript{2+} concentration determines the extent of muscle contraction and therefore force development. Subsequent removal of cytosolic Ca\textsuperscript{2+} by the sarco(endo)plasmic reticulum Ca\textsuperscript{2+} ATPase (SERCA) pump and sarcolemmal Ca\textsuperscript{2+} transporters results in muscle relaxation. The rate of muscle relaxation is largely determined by reuptake of Ca\textsuperscript{2+} into the SR by SERCA2a.\textsuperscript{4–7} In the heart, SERCA2a pump activity is regulated by two small molecular weight proteins: phospholamban (PLB)\textsuperscript{7–14} and sarcolipin (SLN).\textsuperscript{8,11,14–17} The focus of this review is to discuss the mechanisms controlling SERCA pump activity both during normal physiology and under pathological states. A major emphasis will be placed on understanding the role of PLB and SLN in the regulation of SERCA pump.

2. Regulation of SERCA2a expression

2.1. SERCA2a expression during heart development

SERCA2a (encoded by the SERCA2 gene) is the major isoform expressed in developing and in the adult mammalian heart. Although the alternate isoform SERCA2b is expressed in the heart, its levels do not change significantly during heart development. Interestingly, the SERCA1a isoform found in the fast twitch skeletal muscle is never expressed in the heart.\textsuperscript{6,18–20} During heart development, SERCA2a is expressed in the heart tube (10 days post-coitum) even before a functional SR develops and its level increases several fold throughout development. In the adult heart, SERCA2a is the predominant isoform representing the most abundant protein in the SR membrane.\textsuperscript{21–24} The postnatal increase in SERCA pump is accompanied by a shortening of relaxation time in adult heart compared with neonatal ventricle.\textsuperscript{25} In addition, there are chamber-specific differences in the expression levels of
SERCA2a. In rodents, SERCA2a protein level is approximately two-fold higher in atria compared with the ventricles, and the higher SERCA pump levels may account, at least in part, for the shorter duration of contraction in atrial vs. ventricular tissue. The expression levels of SERCA2a are also altered during ageing. Both a decrease in content and activity of SERCA2a has been reported with ageing in animal models and in senescent human myocardium. In senescent rat hearts, reduction in SERCA pump levels (~30–40%) and activity was observed. This decrease was associated with a prolongation of contraction time and depressed myocardial function. Similarly, SERCA2a protein levels were found to be significantly decreased in senescent human myocardium. This decrease in SERCA2a levels was associated with impaired myocardial function at baseline and was further accentuated by hypoxic conditions.

2.2. Hormonal regulation of SERCA2a expression
Changes in hormonal states can also modify SERCA levels and its Ca\(^{2+}\) transport activity. It has been well documented that thyroid hormone (T4) is a potent regulator of SERCA pump expression and cardiac muscle contractility. Hypothyroidism in animals causes a decrease in SERCA2a protein and an increase in PLB, whereas hyperthyroidism increases SERCA2a levels but reduces PLB expression. The increases in SERCA expression are consistent with an increased velocity of Ca\(^{2+}\) uptake and enhanced cardiac function observed in hyperthyroidism in adult hearts. Interestingly, there is a very close correlation between the Ca\(^{2+}\) dependence of Ca\(^{2+}\) uptake and the ratio of PLB to SERCA2a in hypothyroid, euthyroid, and hyperthyroid hearts, and this determines the contractile parameters of the heart. It is interesting to note that the expression of SERCA and PLB are antithetically regulated, in response to changes in thyroid hormone levels to promote either faster or slower Ca\(^{2+}\) uptake rates.

2.3. SERCA2a levels are altered during cardiac pathophysiology
Because SERCA2a-mediated Ca\(^{2+}\) transport is responsible for efficient muscle relaxation and maintaining SR Ca\(^{2+}\) store, the expression and activity of SERCA pump has been studied extensively in heart failure both using experimental animal models (ranging from pressure overload to pacing-induced heart failure) and in heart tissues from human end stage heart failure. These studies collectively have shown that the SR Ca\(^{2+}\) transport is decreased in heart failure. SERCA2a mRNA and protein levels were found to be decreased in failing human hearts. In some studies, the expression levels of SERCA2a was found to be unaltered despite a decrease in SR Ca\(^{2+}\) transport function. It should be pointed out that there is considerable heterogeneity in the expression level of SERCA in failing hearts, and this could be explained by a variety of factors including animal models studied, methodological differences, and severity of the disease as well as drug treatment, age, and gender. Regardless, the finding that SERCA2a gene transfer can improve muscle function and rescue heart failure provided additional support that SERCA2a-mediated Ca\(^{2+}\) transport plays a critical role in the pathophysiology of heart failure.

2.4. Energetics and SERCA2a activity during disease states
The Ca\(^{2+}\) transport ATPase is the second largest consumer of ATP after myosin ATPase, and therefore SERCA2a activity could be affected by changes in the energetics and ATP supply. It is well known that high ATP concentrations exert many allosteric effects, in that high ATP accelerates ion pumps and passive ion fluxes through membrane channels. In an energy-starved heart, SERCA activity would be decreased because of a decrease in activation energy and this could affect the rate of Ca\(^{2+}\) uptake and muscle relaxation. During heart failure, there is an increased demand for energy because of poor pumping function and increased sympathetic activity. Data show that the total phosphocreatine (PCr) pool is ~60% lower in failing myocardium and thus there is a decrease in the ratio of PCr to ATP. Although ATP levels do not decline drastically, a decrease in PCr supply could limit the rate of ATP regeneration, thus affecting ATP pools around the myofibrillar space. In addition, ATP synthesis by SR-dependant glycolysis provides a ready supply of ATP to SR Ca\(^{2+}\) transport and a decrease in glycolysis could affect SERCA pump activation and enzyme kinetics. The SR Ca\(^{2+}\) ATPase, in fact, seems to be the most sensitive ATPase in response to reduction in free energy released from ATP hydrolysis (ΔG\(_p\)-p), and studies suggest a decrease in ΔG\(_p\)-p may directly translate into defects in EC coupling and contractile function.

The Na-K ATPase is also stimulated by an allosteric effect of ATP and an attenuation of this effect (because of a drop in ATP concentration during ischaemic heart disease) would increase intracellular Ca\(^{2+}\) and could promote Ca\(^{2+}\) extrusion via Na/Ca exchanger but at the same time impair relaxation. Therefore, approaches aimed at improving defective EC coupling without activating the receptor mediator pathways (like β-adrenergic pathway) could be promising. Interestingly, increasing SERCA levels by adenoviral gene transfer in rats with heart failure was associated with an improved energetics with an increase in the PCr/ATP ratio. This finding supports the hypothesis that the reconstitution of EC coupling can actually improve energetics.

3. Transgenic approaches to understand the role of SERCA pump in cardiac physiology
To understand whether increased SERCA levels is beneficial to heart function, several groups have developed transgenic (TG) animal models that express higher levels of SERCA pump in the heart. Studies on TG mouse models overexpressing SERCA2a or SERCA1a in the heart demonstrated that SERCA pump overexpression resulted in an increased Ca\(^{2+}\) transport and contractility. Overexpression of the skeletal muscle isoform, SERCA1a, in the mouse heart results in a net increase of SERCA pump by 2.5-fold compared with non-TG (NTG) control hearts. However, the endogenous SERCA2a levels in these mice decreased to 50%, suggesting that there is a limit to how much the SERCA pump can accommodate. Mice with higher levels of SERCA pump show normal in vivo heart function and do not exhibit cardiac pathology. Overexpression of SERCA pump led to an increased SR Ca\(^{2+}\) transport, which in turn increased the rates of cardiac contraction and relaxation. These studies taken together demonstrated...
that increased SERCA expression is well tolerated and could be used to treat conditions such as heart failure where SERCA levels are decreased.

A SERCA2 gene knockout mouse model was developed to examine the effect of decreased SERCA expression.60–62 Disruption of both alleles is lethal and the homozygous null (SERCA2−/−) mice die early in development. There was no compensatory up-regulation of SERCA1a or any other isoform. The heterozygous (SERCA2−/+ ) mice live to term and do not exhibit overt cardiac pathology. SERCA2−/+ mice albeit showed reduced SERCA2 levels (65% of WT hearts), maintained Ca2++ homeostasis and responded to β-adrenergic stimulation.60,61 SERCA2−/−/− mice, when stressed by pressure overload, developed heart failure much more rapidly than WT controls.63 In addition, an isoform-specific (SERCA2a) KO mouse model expressing primarily the high-affinity SERCA2b isoform was developed.64 These mice show serious structural and functional abnormalities and develop cardiomyopathy. Thus, these studies employing TG and gene knockout animal models proved that SERCA pump level is a critical determinant of SR Ca2++ uptake function and changes in its level can modify the contractility.5

4. Phospholamban and cardiac contractility

4.1. Phospholamban is a major regulator of SERCA pump

Phospholamban was first identified in the cardiac microsomes as the principal substrate for cAMP-dependant kinase and the regulator of SERCA pump.7,8,12,65–69 PLB is a 52 amino acid phosphoprotein whose primary structure is highly conserved across species. PLB is expressed at higher levels in the heart compared with slow twitch and smooth muscle tissues. PLB is a principle mediator of the β-adrenergic responses in the heart. It has been well documented that phosphorylation of PLB at Ser −16 and Thr −17 by PKA and CAMKII kinases, respectively, can increase the SERCA pump activity.7,8,12,65–70 Studies have shown that dephosphorylated PLB acts as an inhibitor (brake) on the SERCA pump and that phosphorylation releases inhibition and induces substantial increase in Ca2++ transport to levels increased by four-fold or greater.7,8,12,65–69 Thus, the regulation of SERCA pump by PLB is considered to be the primary mechanism for β-adrenergic-mediated response of the heart, as well for enhanced Ca2++ transport by the SR.

4.2. Regulation of phospholamban expression and phosphorylation

Phospholamban is an inhibitor of SERCA pump. Therefore, an increase or a decrease in PLB level and/or its phosphorylation status can directly impact SR Ca2++ uptake function and muscle contractility. PLB is expressed in both atria and ventricle, but at lower levels in the atria compared with ventricle (Table 1). Like SERCA2a, PLB expression is also altered in a variety of pathophysiologically situations. PLB expression is highly sensitive to thyroid hormone level. PLB expression levels are decreased in hyperthyroidism but increased in hypothyroidism, just opposite of what has been found for SERCA2a. During hyperthyroidism, a decrease in PLB and an increase in SERCA expression lead to increased velocity of Ca2++ uptake and enhanced cardiac function.18,24,34,36,71,72 The opposite was seen in hypothyroidism. Further, PLB expression and its phosphorylation status was shown to be altered in disease conditions.73 Zong et al.73 reported that non-phosphorylated PLB was increased in streptozotocin (STZ)-induced diabetic cardiomyopathy and suggested that an increase in non-phosphorylatable PLB together with a decrease in SERCA pump can significantly affect Ca2++ uptake and muscle relaxation in diabetic cardiomyopathy.

The phosphorylation status of PLB has been examined in both animal models of heart failure and in end-stage failing human myocardium.74–80 The results were model dependent, where some studies showed enhanced phosphorylation of PLB in heart failure,74,75 whereas others showed a decrease in the phosphorylation status.76,77 Studies on human heart failure samples showed a reduction in PLB phosphorylation at Ser −16, but no change in Thr −17 phosphorylation status, and this finding correlated well with a decreased Ca2++ sensitivity of SERCA2a in failing hearts.77–81 From these studies, one may conclude that PLB-phosphorylation level may vary depending on the severity of heart failure and could be affected by the status of β-adrenergic signaling pathways in these models.

4.3. Mechanism of phospholamban action on SERCA pump

The role of PLB as a regulator of SR Ca2++ transport has long been recognized, but the exact mechanism of action was elusive for quiet sometime. Subsequent purification and reconstitution of PLB in lipid bilayers led to the idea that PLB can act as a channel.7 However, with the advent of

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SERCA2a can be detected in smooth and non-muscle cells at low levels. Unlike PLB, SLN is not detectable in smooth muscle tissues.
gene cloning and TG mouse technology, the role of PLB has been studied extensively using TG and gene KO mouse models.\textsuperscript{7,8,12,65–69} The PLB KO mouse model provided the first convincing evidence that PLB is a regulator of \(\beta\)-adrenergic response in the heart.\textsuperscript{82} In addition, TG mouse models overexpressing different amounts of PLB were studied.\textsuperscript{83–86} These studies suggested that PLB regulates SERCA pump activity by affecting Ca\(^{2+}\) affinity of the pump, but not \(V_{\text{max}}\) of Ca\(^{2+}\) uptake. Further, these studies suggest that the ratio of PLB to SERCA is an important determinant of SR Ca\(^{2+}\) uptake and cardiac contractility. An increase in PLB to SERCA ratio results in maximum inhibition of SERCA pump resulted in depressed cardiac contractility and development of cardiac hypertrophy, whereas a loss of PLB in hearts increases Ca\(^{2+}\) uptake rate and Ca\(^{2+}\) store with increased rates of contraction and relaxation with no obvious pathology. Thus, the loss of PLB in mouse is well tolerated; however, a similar condition in human results in obvious pathology. Thus, the loss of PLB in mouse is well

plotted or due to stronger attraction by the SERCA pump. It was also demonstrated that the pentamer itself is capable of inhibiting the SR Ca\(^{2+}\) ATPase.\textsuperscript{93} Thus, at this time it is not completely clear whether the inhibitory function of PLB depends on the dissociation of pentameric PLB into a

Figure 1 Hypothetical model showing SERCA pump regulation by PLB and SLN. The dephosphorylated phospholamban (PLB) binds to SERCA pump and regulates its activity (top, middle panel). This interaction is disrupted by either phosphorylation of PLB (top, left panel) or in the presence of high calcium concentration (top, right panel). Sarcolipin (SLN) interacts with SERCA in its unphosphorylated form (bottom, middle). This interaction is mainly affected by phosphorylation (bottom, left panel) and is less affected by Ca\(^{2+}\) concentration (bottom, right panel).
monomeric form. Future research in this area will help in our understanding of mechanism of PLB action.

5. Sarcolipin is a novel regulator of SERCA pump

Sarcolipin is a low molecular weight protein (31 amino acids) originally identified to co-purify with the skeletal muscle SERCA1a pump.8,15,94,8,11,12–17 Analyses of SLN expression in the heart show that SLN is expressed predominantly in the atrial compartment and its expression is low in the ventricle (Table 1).5,9,96 It is also found in skeletal muscle tissues, but its expression varies between small and large animals.11,14,94–96 The primary structure of SLN is highly conserved across species and may suggest a similar mechanism of regulation as known for PLB. On the basis of primary sequence, SLN and PLB have similar transmembrane sequences, indicating that they are homologous proteins belonging to the same gene family,15,94 but they differ at their N- and C-terminus. Modelling studies have shown that SLN and PLB interacting with the SERCA pump through their transmembrane domain share considerable homology97–100 and potentially bind to the same regulatory site on the SERCA pump.15,101

5.1. The role of SLN in cardiac muscle physiology

Unlike PLB, the role of SLN in cardiac contractility is just beginning to be understood.11,17,102–104 The role of SLN as a regulator of cardiac SERCA2a was first studied using adenoviral gene transfer into adult rat ventricular myocytes.102 These studies found that transient overexpression of SLN in the adult rat ventricular myocytes resulted in a decreased Ca\(^{2+}\) transient amplitude and myocyte contractility. More recently, the role of SLN in cardiac function was investigated using TG mouse models overexpressing SLN in the heart.102,104 These studies employed the cardiac-specific \(\alpha\)-MHC promoter, to drive SLN expression both in the atria and ventricle (where SLN levels are relatively absent). SLN overexpression in the ventricle caused a decrease in SERCA2a affinity for Ca\(^{2+}\), a decrease in Ca\(^{2+}\) transient amplitude and shortening, and slowed relaxation at the myocyte level.102,104 This finding is very similar to what has been reported with PLB overexpression and suggests that SLN is also an effective inhibitor of SERCA pump. Overexpression of SLN resulted in a significant decrease in rates of contraction and relaxation when assessed by \textit{ex vivo} work-performing heart preparations. Interestingly, the inhibitory effect of SLN on contractile function was reversed by treatment with isoproterenol.102,104 This suggests that similar to PLB, SLN could also play a role in mediating \(\beta\)-adrenergic response in the atria.

5.2. Sarcolipin effect on SERCA pump is independent of PLB

When SLN is co-expressed with SERCA1a or SERCA2a in HEK293 cells, it decreased the apparent Ca\(^{2+}\) affinity of the SERCA pump.9,10 This indicated that SLN can act as an inhibitor of the SERCA pump. In addition, co-expression of SLN with PLB was shown to induce super-inhibitory effect on SERCA pump. Initially, MacLennan and co-workers proposed that SLN mediates its inhibitory effect via PLB.15,101,105,106 Their earlier studies suggested that SLN can form a binary complex with PLB and that SLN interaction with PLB could prevent PLB polymerization resulting in an increase in the active form, the monomer, thus promoting super-inhibition of SERCA pump.105 A difficulty with this model is that it does not apply to situations where PLB is very low (atria) or non-existent such as in fast-twitch skeletal muscle.

The expression pattern of SLN differs from PLB and if SLN requires PLB for its inhibitory effect then this mechanism is feasible only when SLN and PLB are co-expressed. However, SLN is expressed at high levels in tissues which express either low amount of PLB (as in atria and slow-twitch skeletal muscle) or no PLB as in fast twitch skeletal muscles (Table 1). These observations suggest a possible independent role for SLN, where SLN directly binds to SERCA2a and alters its affinity for Ca\(^{2+}\). The independent role of SLN was recently confirmed by expressing SLN in the PLB null (\(plb^{-/-}\)) background.48 Overexpression of SLN in the \(plb^{-/-}\) hearts caused a decrease in the affinity of SERCA2a for Ca\(^{2+}\), impaired contractility, reduced Ca\(^{2+}\) transient amplitude, and exhibited slower decay kinetics, compared with \(plb^{-/-}\) animals. Furthermore, the inhibitory effect of SLN on Ca\(^{2+}\) transport was reversed by isoproterenol, suggesting that SLN could mediate the \(\beta\)-adrenergic response in the ventricular myocytes from SLN TG/\(plb^{-/-}\). These and other studies suggest that SLN can mediate its inhibitory effect on SERCA2a directly and act as a mediator of \(\beta\)-adrenergic responses in the heart.

5.3. Sarcolipin as a regulator of Ca\(^{2+}\) transport and contractility of the atria

In all mammals, cardiac contraction–relaxation cycle is regulated by contraction of the atrium followed by the contraction of the ventricle. It is known that the duration of the contraction is shorter in the atrium than ventricles in all mammals, including human. There is no satisfactory explanation why the atrial muscle contract and relax faster. One possible explanation is that in higher mammals, atria express a higher proportion of the fast \(\alpha\)-myosin heavy chain isoform compared with ventricles. However, this does not seem to be a plausible explanation since in rodents (mice and rats) both the atria and ventricle express predominantly \(\alpha\)-MHC isoform. Recent observations suggest that differences in Ca\(^{2+}\) handling at the level of SR may also be responsible for the observed differences between the atria and ventricle.26,27,107 In the mouse heart, the surface area and volume of SR per cell volume are higher in atria, mainly because of higher longitudinal SR. On the other hand, the mammalian atrial myocytes do not have a well-developed T-tubular system and the coupling between \(\text{L}\)-type Ca\(^{2+}\) channels in the sarcolemma and the junctional SR occurs near the periphery of the myocytes.

Unlike in ventricular myocytes, a non-synchronous and biphasic Ca\(^{2+}\)-induced Ca\(^{2+}\) release has been observed in atrial cardiac myocytes. Thus, there are fundamental differences between atrial and ventricular myocytes with regard to Ca\(^{2+}\) transport.

In addition to the structural differences between the atrial and ventricular SR network, biochemical data shows that atria has three to four times lower level of PLB (inhibitor of SERCA), but much higher (approximately two times higher) levels of SERCA pump.26 In principle, lower levels
of PLB could facilitate a faster rate of SR calcium uptake, thus providing more Ca\(^{2+}\) for SR to release. However, there is no direct evidence that the differences in PLB levels are primarily responsible for the observed differences in Ca\(^{2+}\) transport kinetics of the atria. Remarkably, the \(\beta\)-adrenergic response is also known to be different in the atria compared with the ventricle. In a recent study, Kaasik et al.\(^{108}\) examined the relationship between PLB phosphorylation, SERCA function, and contractility in atria and ventricles of rats. This study showed that the amount of phosphorylated PLB is four-fold lower after \(\beta\)-adrenergic stimulation in atrial muscle compared with ventricle. This decrease in phosphorylation corresponds well with decreased PLB levels of the atria. On the basis of this, one would predict a smaller increase in SR Ca\(^{2+}\) uptake in the atria in response to adrenergic activation. On the contrary, Ca\(^{2+}\) uptake in isoproterenol-treated atria showed a much larger increase. In addition, atria responded to isoproterenol with much larger increases in developed tension, contractility, and relaxation rates than ventricular muscle. Thus, the observed increase in both Ca\(^{2+}\) uptake and contractile function could not be explained by low levels of PLB phosphorylation. Therefore, \(\beta\)-adrenergic response in the atria may depend on proteins other than PLB. The fact that, SLN expression is high in the atria suggests that SLN regulation of SERCA pump in the atria may account for the notable contractile difference in atrium vs. ventricle.

5.4. Loss of sarcolipin alters contraction and relaxation of the atrial muscle

To understand the role of SLN in atrial physiology, we generated an SLN null mouse model.\(^{118}\) Ablation of SLN did not affect the growth and survival of the animal. The SLN null mice reached adulthood without any pathology. Ablation of SLN increases the affinity of the SERCA pump for Ca\(^{2+}\), resulting in enhanced rates of SR Ca\(^{2+}\) uptake. In addition, it is also associated with an increase in the \(V_{\text{max}}\) of Ca\(^{2+}\) uptake rates, suggesting that SLN could regulate the kinetics of the ATPase activity at one or more steps. Consistent with the enhanced SR Ca\(^{2+}\) uptake, the rate of atrial muscle relaxation is also faster in the \(sln^{-/-}\) mice. These data provided the first direct evidence that SLN is an important regulator of atrial Ca\(^{2+}\) transport and may be responsible for mediating the \(\beta\)-adrenergic responses in the atrium. This idea is further supported by recent studies from a TG mouse model overexpressing SLN in PLB null background, which demonstrate that the inhibitory effect of SLN can be relieved upon isoproterenol treatment.\(^{109}\) On the basis of the available data, we propose a model (Figure 1) to illustrate the mechanism of SLN action on the SERCA pump. This model advances our current ideas which suggest that (i) the inhibitory function of SLN on SERCA pump is independent of PLB (ii) phosphorylation of SLN at threonine 5 disrupts the interaction of SLN with SERCA pump and relieves its inhibitory effect, and (iii) the inhibitory action of SLN on SERCA pump persists even at high Ca\(^{2+}\), which is in quite contrast to PLB whose inhibitory effect is lost at high calcium. We additionally suggest that a high level of SLN and PLB may have quite different outcome which needs to be better understood (Figure 1).

6. Targeting sarcoplasmic reticulum Ca\(^{2+}\) transport genes to improve contractility

The prospects of gene therapy for heart failure in particular restoring Ca\(^{2+}\) transport have received much attention.\(^{46,53,110–112}\) Data from failing human hearts suggest that a decrease in SR Ca\(^{2+}\) transport is responsible for the negative force frequency seen in the failing hearts. Therefore, recent studies have focused on restoring SERCA pump activity, either by adenoviral mediated gene transfer of SERCA2a or by inhibiting PLB. Adenoviral gene transfer studies showed that overexpression of SERCA2a in failing human ventricular myocytes increased Ca\(^{2+}\) transport and restored contraction and relaxation velocity.\(^{47}\) The negative frequency response was normalized in cardiomyocytes overexpressing SERCA2a.\(^{47}\) Encouraged by these in vitro studies, Hajjar and co-workers developed a catheter-based technique to introduce genes into the myocardium.\(^{46,53,54,111,112}\) SERCA2a gene transfer in a rat model of pressure-overload hypertrophy (where SERCA2a levels were decreased and severe contractile dysfunction was evident) restored both systolic and diastolic dysfunction to normal levels. SERCA2a overexpression decreased left ventricular size and restored the slope of the end-diastolic pressure–dimension relationship to control levels. Further, SERCA2a gene transfer was used to abrogate ventricular arrhythmias in a rat model of ischaemia reperfusion (I/R) injury.\(^{113,114}\) SERCA2a overexpression significantly decreased ventricular arrhythmias during I/R and 24 h later reduced infarct size and improved wall thickening in the anterior wall. These studies suggest that a decrease in diastolic Ca\(^{2+}\) and better handling of intracellular ions are both associated with improved survival of the cardiomyocytes. Therefore, restoring Ca\(^{2+}\) transport by increasing SERCA2a activity appears to be critical for maintaining cardiac inotropy and for preventing the pathological effects of Ca\(^{2+}\) overload.

Phospholamban ablation was shown to prevent structural and functional abnormalities in mouse models.\(^{8,115}\) Therefore, strategies to suppress PLB inhibition and enhance SERCA activity have been tested. An approach to increase SERCA pump activity is to decrease the levels of PLB. Del monte et al.\(^{116}\) showed that adenoviral gene transfer of antisense PLB in failing human cardiomyocytes restored contractility, Ca\(^{2+}\) handling, and the force frequency response. In addition, a pseudophosphorylated mutant PLB peptide (S16EPLN) was tested in BIO14.6 CM hamsters (showing a progressive stage of dilated cardiomyopathy and heart failure). Expression of mutant PLB enhanced myocardial SR Ca\(^{2+}\) uptake and suppressed progressive impairment of left ventricular (LV) systolic function and contractility up to 28–30 weeks.\(^{117}\) However, chronic inhibition of PLB activity may not be beneficial in human hearts, since loss of PLB function leads to dilated cardiomyopathy at a young age in humans.\(^{87,88}\) These experimental studies provide us promising initial results and move us a step forward towards manipulating SERCA pump activity as a therapeutic strategy to rescue contractile function in diseased human hearts. These studies also suggest that maintaining a physiological ratio of SERCA/PLB may be necessary for the hearts ability to meet various physiological demands.
7. Conclusions and perspectives

SERCA2a plays a dominant role in Ca$^{2+}$ removal and contraction–relaxation of the heart muscle. Data suggest that decreases in SERCA pump level and activity directly impact contractility of the heart and contribute to altered relaxation. The regulation of SERCA2a by PLB and SLN plays a critical role in cardiac contractility and in mediating the β-adrenergic response, both in normal and failing hearts. There is overwhelming evidence that alterations in the ratio of PLB to SERCA can profoundly influence the SERCA pump activity and cardiac physiology. However, there remain many gaps in understanding the dynamic regulation of SERCA pump by PLB and SLN in the heart. We also do not know how the effect of PLB and SLN on SERCA pump differs between small and large mammals, because we learned that loss of PLB is well tolerated in mouse but it could be detrimental in man. Future research should be directed towards better understanding the regulations of SERCA pump by PLB and SLN in a chamber-specific manner.

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