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Review

Sarcoplasmic reticulum Ca^{2+}-ATPase modulates cardiac contraction and relaxation

Konrad F. Frank, Birgit Bölck, Erland Erdmann, Robert H.G. Schwinger*

Clinic III of Internal Medicine, Laboratory of Muscle Research and Molecular Cardiology, University of Cologne, Cologne, Germany

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Abstract

The cardiac SR Ca^{2+}-ATPase (SERCA2a) regulates intracellular Ca^{2+}-handling and thus, plays a crucial role in initiating cardiac contraction and relaxation. SERCA2a may be modulated through its accessory phosphoprotein phospholamban or by direct phosphorylation through Ca^{2+}/calmodulin dependent protein kinase II (CaMK II). As an inhibitory component phospholamban, in its dephosphorylated form, inhibits the Ca^{2+}-dependent SERCA2a function, while protein kinase A dependent phosphorylation of the phospho-residues serine-16 or Ca^{2+}/calmodulin-dependent phosphorylation of threonine-17 relieves this inhibition. Recent evidence suggests that direct phosphorylation at residue serine-38 in SERCA2a activates enzyme function and enhances Ca^{2+}-reuptake into the sarcoplasmic reticulum (SR). These effects that are mediated through phosphorylation result in an overall increased SR Ca^{2+}-load and enhanced contractility. In human heart failure patients, as well as animal models with induced heart failure, these modulations are altered and may result in an attenuated SR Ca^{2+}-storage and modulated contractility. It is also believed that abnormalities in Ca^{2+}-cycling are responsible for blunting the frequency potentiation of contractile force in the failing human heart. Advanced gene expression and modulatory approaches have focused on enhancing SERCA2a function via overexpressing SERCA2a under physiological and pathophysiological conditions to restore cardiac function, cardiac energetics and survival rate.

Keywords: Ca-pump; Calcium (cellular); Contractile function; SR (function)

1. Introduction

The sarcoplasmic reticulum (SR) is an intracellular membrane system in cardiac cells, which plays a predominant role in cardiac excitation–contraction coupling and cardiac contractility. A 1000-fold Ca^{2+}-gradient is maintained across the cardiac sarcoplasmic reticulum membrane by the SR Ca^{2+}-ATPase (SERCA2a). Molecular cloning analyses has identified three SR Ca^{2+}-ATPase genes, SERCA1, 2 and 3, which are spliced alternatively in several isoforms. SERCA1a is mainly expressed in fast-twitch skeletal muscle, while SERCA1b is abundant in fetal and neonatal stages [1]. Four splice variants encode for the SERCA2 isoforms, variant 1 translates in SERCA2a, which is the primary isoform expressed in cardiac and slow-twitch skeletal muscle tissue and variants 2–4 encode for SERCA2b, which is the predominant isoform in nonmuscle (variants 2 and 3) and neuronal cells (variant 4) [2]. SERCA3 isoform is restricted to epithelial and endothelial cell types [3].

In cardiac tissue, the SERCA2a isoform facilitates the storage and distribution of Ca^{2+}-ions in the SR. During systole, the action potential induces a minor Ca^{2+}-influx through sarcolemmal L-type Ca^{2+} channels. This influx initiates a major Ca^{2+}-release from the SR Ca^{2+} stores through the Ca^{2+} release channel or ryanodine receptor (RyR) [4]. Subsequently, Ca^{2+} binds to troponin C and

*Corresponding author. Priv.-Doz. Dr. med. Robert H.G. Schwinger, Klinik III für Innere Medizin der Universität zu Köln, Labor für Herzmuskelphysiologie und Molekulare, Kardiologie, Joseph-Stelzmann-str. 9, 50924 Köln, Germany. Tel.: +49-221-478-3138; fax: +49-221-478-3746.

E-mail address: robert.schwinger@medizin.uni-koeln.de (R.H.G. Schwinger).

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starts the crossbridge movement of the myofibrils resulting in force development and contraction. Quick removal of Ca^{2+} into the SR or alternatively the extracellular lumen is essential for cardiac relaxation. In cardiac tissue, this process is facilitated during diastole mainly by the SR Ca^{2+}-ATPase (70–80% of Ca^{2+} removal in higher mammalian species and human myocardium) and to a lesser extent by the sarcolemmal Na^+, Ca^{2+}-exchanger (20–30%) and slower Ca^{2+}-transport systems [5]. In the SR, Ca^{2+} binds predominantly to the SR Ca^{2+} binding protein calsequestrin [6] and other Ca^{2+} binding proteins, such as calreticulin and the histidine-rich-binding protein [7]. Ca^{2+} is stored close to the Ca^{2+} release channel via the proteins triadin and junctin [8–10], which most likely facilitates a faster Ca^{2+} availability in the proximity of the ryanodine receptor during early systole. In addition, a novel protein, named junctate, has been described recently consisting of combined sequences of junctin and aspartyl-β-hydroxylase, which may also influence SR release kinetics [11]. Thus, SERCA2a is the main player in restoring diastolic Ca^{2+} levels and terminating Ca^{2+}-dependent force activation, while several other components, such as the phosphorylation state of the ryanodine receptor and its accessory proteins may regulate the release mechanism at the SR level (Fig. 1).

The function and Ca^{2+} activation of SERCA2a can be modulated by several indirect and direct factors. The most predominant, indirect mechanism being the phosphoprotein phospholamban, which associates with cardiac isoform SERCA2a [12,13]. Phospholamban inhibits the apparent affinity of SERCA2a for Ca^{2+} in its dephosphorylated form. Upon phosphorylation of phospholamban through β-adrenergic stimulation and enhanced cAMP-dependent protein kinase A activity (PKA), the inhibition of phospholamban on SERCA2a is relieved. This results in enhanced relaxation and increased SR Ca^{2+} load. As a direct modulation, SERCA2a is also under the control of Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II), which has been shown to phosphorylate SERCA2a on residue serine-38 and enhance the Ca^{2+} transport capacity of SERCA2a [14,15]. Other direct factors that modulate SERCA2a activity and expression are thyroid hormones [16], insulin [17] and nitrosylation [18].

This review summarizes recent advances in understanding the modulation of SR Ca^{2+} sequestration by SERCA2a focusing especially on the: (a) regulators of SERCA2a

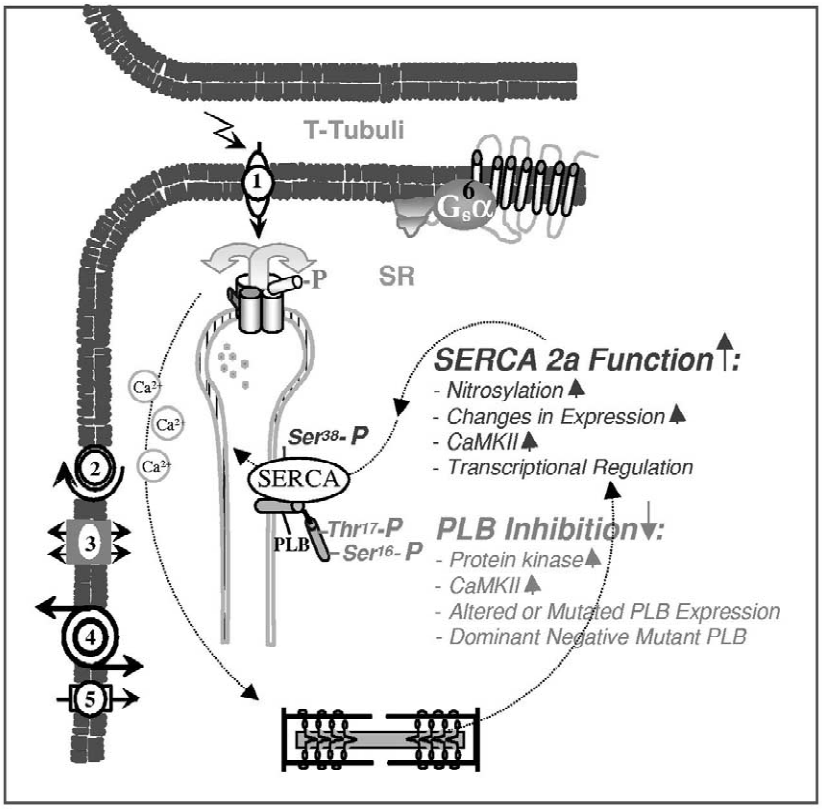

Fig. 1. Schematic representation of myocardial Ca^{2+} homeostasis. During systole, Ca^{2+} enters the myocyte over L-type Ca^{2+} channels (1) and induces a Ca^{2+} release of the ryanodine receptor from the sarcoplasmic reticulum (SR). During diastole Ca^{2+} is subsequently transported into the SR via the SR Ca^{2+}-ATPase (SERCA2a), which is under the inhibitory control of phospholamban (PLB). The intracellular Ca^{2+} concentration is modified by the sarcolemmal Ca^{2+}-ATPase (2), the Na^+/Ca^{2+}-exchanger (3), the Na^+, K^+-ATPase (4), the sarcolemmal Na^+-channels (5), and the β-adrenoreceptor (6). Diastolic SR Ca^{2+} movement is modulated through alterations in SERCA2a function and PLB inhibition.

affinity and transport activation, (b) physiological role of modified SERCA2a activity in genetically engineered and experimentally modulated animal models, and (c) alterations of key modulators of SERCA2a in human tissue in states of diminished cardiac output, such as heart failure.

2. Regulators of SERCA2a activity and expression

Phospholamban (PLB) has been recognized as key regulator of SERCA2a and cardiac contractility. PLB is an integral SR membrane protein, consisting of 52 amino acids, that is expressed predominantly in cardiac muscle and in small amounts in slow-twitch skeletal muscle, smooth muscles and endothelial cells [19–21]. In vitro studies in purified SR membranes have demonstrated that phospholamban can be phosphorylated at three distinct sites: (a) serine-16 by cAMP-dependent protein kinase A, (b) threonine-17 by Ca^{2+}/calmodulin-dependent protein kinase II, and (c) serine 10 by Ca^{2+}-activated phospholipid-dependent protein kinase (PKC) [22–25]. Phosphorylation by each protein kinase increases the apparent affinity of the SR Ca^{2+}-ATPase for Ca^{2+} and thereby, the rate of Ca^{2+}-sequestration into the SR [26]. The inhibitory effects of phospholamban are restored through dephosphorylation by an SR-associated phosphatase [26]. During β-adrenergic stimulation both serine-16 and threonine-17 are phosphorylated, but the cAMP-dependent phosphorylation site (serine-16) appears to be the most important mediator to enhance cardiac contractility [27,28] and has been suggested to be the main regulator of the activity of phospholamban. This was specifically demonstrated in transgenic mouse models that overexpressed several mutant forms of phospholamban lacking these phosphorylation sites [27,29]. In human myocardium, the Ca^{2+}-affinity but not the maximal velocity of SERCA2a was increased via PKA-dependent phosphorylation of phospholamban [30].

A second mediator of SERCA2a activation has been proposed to be the Ca^{2+}/calmodulin-dependent protein kinase II. It was demonstrated that phosphorylation of SERCA2a by CaMK II modulates the maximal activity (V_{max}) of the enzyme without changing the apparent affinity of the pump (EC_{50}) [14,15,31] (Fig. 2). Hawkins et al. [32] showed that this phosphorylation was selective and occurred in the cardiac and smooth muscle SR but not in the skeletal muscle SR. Using site-directed mutagenesis, Toyofuku et al. [14] reported serine-38 as the CaMK II phosphorylation site on SERCA2a. On the other hand, Reddy et al. [33] did not observe SERCA2a phosphorylation in canine SR vesicles or purified SERCA2a preparations. It must be noted that in this study [33], there was a protein of approximately the same molecular weight as that of SERCA2a that was weakly phosphorylated in the longitudinal SR and substantially phosphorylated in the junctional SR vesicles; however, the identity of this phosphorylated protein was not determined. The functional consequences of SERCA2a phosphorylation were challenged by Odermatt et al. [34] who were unable to observe this stimulation. The use of inappropriate controls for measuring CaMK II stimulated Ca^{2+}-ATPase and Ca^{2+}-uptake activities was pointed to be a cause for increased V_{max} in the studies of Xu et al. [31] and Toyofuku et al. [14]. Using appropriate controls, Xu et al. [35] not only confirmed their original findings but went a step further to demonstrate stimulation of V_{max} of Ca^{2+}-transport due to selective phosphorylation of SERCA2a in the absence of RyR and PLB phosphorylations. Recent studies [36,37] from Netticadan et al. demonstrated phosphorylation of SERCA2a as well as endogenous CaMK II mediated stimulation of SR Ca^{2+}-uptake in rat hearts, thus, supporting the findings of Xu and colleagues [31,32,35]. Ca^{2+}/calmodulin-dependent activation has also been attributed as the main compensatory mechanism in ischemia induced heart failure [36]. Occlusion of rat coronary arteries inducing an infarct size larger than 30% resulted in significant depressed Ca^{2+}/calmodulin-dependent phosphorylation, reduced activity of the SR-associated Ca^{2+}/calmodulin-dependent protein kinase and increased SR-associated phosphatases. This may in part explain depressed myocardial function in ischemic heart failure. However, co-regulatory processes involving Ca^{2+}/calmodulin as well as PKA-dependent pathways are feasible as well.

In addition to the direct phosphorylation of SERCA2a, other studies [23,24] have demonstrated a Ca^{2+}/calmodulin-dependent phosphorylation of the threonine-17 site in phospholamban, which is accompanied by an increased affinity of SERCA for Ca^{2+} as judged by $^{45}Ca^{2+}$ uptake rates. Data from our laboratory indicate that this Ca^{2+}/calmodulin-dependent phosphorylation of threonine-17 in phospholamban can be modulated by the phosphatase calcineurin B [38]. Since continuous calcineurin activation has been implicated in the development of hypertrophy [39], this phosphatase-dependent activation may be one of the mechanisms by which the hypertrophied heart increases diastolic Ca^{2+}-availability and force of contraction.

3. Lessons from animal models to elucidate the regulatory role of phospholamban

Since SERCA2a is predominantly regulated through phospholamban, its functional role has been studied using transgenic and gene-knockout mouse models. The murine phospholamban gene was ablated by homologous recombination in embryonic stem cells and phospholamban-deficient mice were generated [13]. The phenotypical appearance of these mice was similar to their wild-type controls at the morphological and histological levels [13]. However, the affinity of the SR Ca^{2+}-ATPase, as judged by the EC_{50} values for Ca^{2+}, was increased in the phospholamban

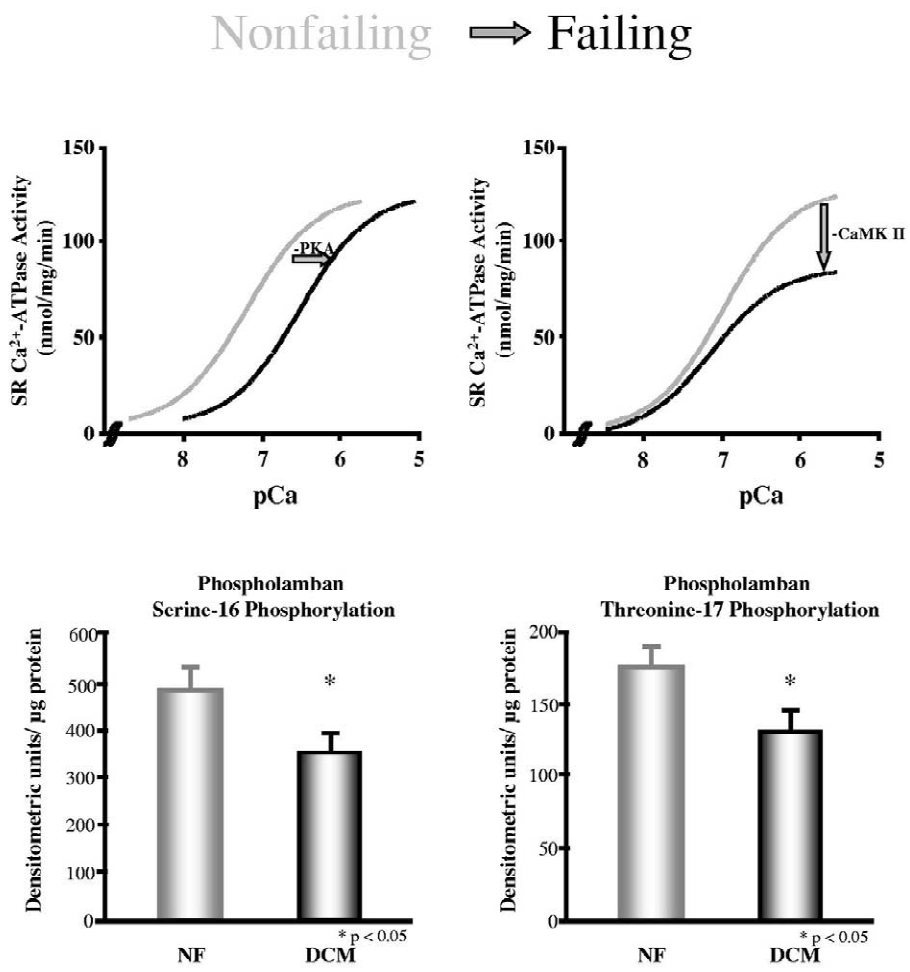

Fig. 2. SR Ca^{2+}-uptake function in nonfailing and failing human myocardium. Nonfailing myocardium is higher phosphorylated at the serine-16 site in phospholamban than compared to failing myocardium, which goes in line with an increased Ca^{2+}-sensitivity of SERCA2a for the SR Ca^{2+}-transport upon protein kinase A stimulation (PKA) (left panel). Stimulation via phosphorylation with Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) induces a significant increase in the V_{max} range of SR Ca^{2+} transport rates in human nonfailing myocardium, and this is associated with increased phosphorylation of the threonine-17 site in phospholamban (right panel).

knockout hearts. These findings were consistent with the previously defined functional modulation of the SR Ca^{2+}-ATPase upon phospholamban phosphorylation in native SR membranes [22]. Physiological measurements in isolated cardiac myocytes exhibited an enhanced myocyte mechanics and parallel increase in amplitude and kinetics of rising and falling phases of Ca^{2+} transients [40]. Furthermore, phospholamban-deficient myocytes exhibited a higher SR Ca^{2+} load [41] and the L-type Ca^{2+} channel currents were significantly larger [42]. The hyperdynamic contractile function, observed in phospholamban-deficient cardiomyocytes, was also apparent at the intact organ level using work-performing heart preparations under identical preload, afterload and heart rate [13]. In addition, isoproterenol stimulation resulted in an attenuated contractile response in isolated myocytes [40], in whole hearts [13] or in intact animals [43]. Interestingly, the attenuation of β-agonist stimulation was not due to alterations in the β-adrenergic signaling pathway or the degree of phosphorylation of other key cardiac regulatory phosphoproteins [44]. In addition, no compensatory mechanisms were observed at the level of the myofilaments, as revealed by the myosin ATPase activity and the Ca^{2+}-dependent force development in skinned fiber experiments [45]. These data from animal studies indicate that phospholamban is a key regulator of basal contractility and a major mediator of the β-agonist responses in the mammalian heart [12].

The phospholamban knockout studies suggested that the relative stoichiometric ratio of phospholamban to SERCA2a may be a critical determinant of the regulation of the cardiac contraction–relaxation cycle. In a second approach, the effects of overexpressing phospholamban through transgenesis in murine hearts was investigated using the cardiac specific α-myosin heavy chain promoter [46]. Phospholamban was overexpressed by two-fold compared to wild-type hearts. Similarly to the phospholamban-deficient model, murine hearts from transgenic animals and wild-type controls were analysed at the molecular, bio-

chemical and physiological levels. Transgenic mice overexpressing two-fold higher levels of phospholamban in the heart showed no morphological abnormalities and no alterations in heart:body weight compared to their wild-type littermates. The affinity of the SR Ca^{2+}-ATPase for Ca^{2+} was decreased in native SR preparations from transgenic hearts and thus, it exhibited opposite properties than the phospholamban-deficient SR preparations [13]. The results may also hold true for changes at the level of the SR Ca^{2+}-release complex. Isolated cardiomyocytes from transgenic animals exhibited decreases in shortening fraction and rates of shortening and relengthening, compared to wild-types controls. The amplitude of the Ca^{2+} signal was also decreased and the rate of decay of the Ca^{2+} transient in Fura-2 loaded transgenic cardiac myocytes was significantly prolonged, consistent with the decreased Ca^{2+} affinity of the SR Ca^{2+}-ATPase. These findings were consistent with depressed contractile parameters at the intact organ and whole animal levels. However, the observed differences in contractile parameters, Ca^{2+} kinetics and depressed left ventricular function in vivo were abolished upon isoproterenol stimulation [46]. These results may be indicative for native SR, there is a fraction of SR Ca^{2+}-ATPase molecules, which is not subject to regulation by phospholamban.

In a third approach, the effects of overexpression of a nonphosphorylatable form of phospholamban, in which the phosphorylation sites serine-16 and threonine-17 were mutated to alanine, was studied [29]. Several transgenic lines were generated, which expressed 1.8-, 2.6-, 3.7-, and 4.7-fold mutated phospholamban over wild-type levels. Cardiac SR Ca^{2+}-uptake experiments of these lines revealed that increasing levels of phospholamban were accompanied by decrease in the apparent affinity for Ca^{2+}. Saturation of the phospholamban inhibitory effect on the affinity of the SR Ca^{2+}-ATPase was achieved at a ratio of phospholamban to SERCA2a of 1 to 2.6, suggesting that only 40% of the SERCA2a pumps are under the inhibitory regulation of phospholamban in wild-type murine hearts [29]. However, it has to be emphasized that alterations in the ratio of phospholamban to SERCA2a was determined in vitro on SDS gels without formally confirming alterations in the ratio in vivo.

In addition to these three approaches on the ratio of phospholamban to SERCA2a, overexpression of mutant forms of phospholamban, such as the mutants L37A, I40A [47], N27A [48] and V49G [49] as so-called superinhibitors of SERCA2a, have revealed that inhibition of SERCA by these mutants resulted in decreased SR Ca^{2+}-uptake, diminished cardiac contractility and in vivo systolic and diastolic function as well as cardiac failure [49]. The inhibition of SERCA2a by these mutant was not dependent on a primarily pentameric or monomeric from of phospholamban.

Based on the studies in genetically engineered phospholamban mouse models, increase in the apparent ratio of phospholamban to SERCA2a may be at least partially responsible for depressed kinetics of Ca^{2+} transients and contractile parameters in the mammalian heart [12].

4. Modified function of SERCA2a in human heart failure

In human heart failure, several lines of evidence suggest that there are alterations in intracellular Ca^{2+}-homeostasis, which may be related to an altered expression, function or regulation of Ca^{2+}-handling proteins. Along these lines, diminished peak and prolonged decay of Ca^{2+} transients have been reported by several groups in cardiomyocytes and papillary muscle strips isolated from hearts of patients with end-stage heart failure [50–52]. These alterations in the rise and fall of the Ca^{2+}-transient have important implications for excitation–contraction coupling and the development of increased diastolic tension in heart failure. Furthermore, these alterations in Ca^{2+}-handling diminish frequency-potentiation, which is one of the potent intrinsic mechanisms inducing cardiac inotropy [53–56]. This goes in line with the finding that inhibition of SERCA activity by CPA initiates, even in nonfailing human myocardium, a negative force–frequency behaviour [30].

The alterations in SR function and protein levels, as well as phosphorylation levels of phospholamban in human congestive heart failure have been a matter of debate. Most studies in human heart failure patients revealed that the expression of phospholamban remains unchanged as compared to nonfailing myocardium [54,55,57], although one study reported a downregulation (18%) of phospholamban [58]. Based on the transgenic and gene-targeted mouse model studies, the phospholamban to SERCA2a ratio has been shown to be a major regulator of cardiac contractility [12,29,46]. Thus, any alterations in the expression levels of phospholamban have to be examined in parallel with possible alterations in the expression levels of SERCA2a. Along these lines, it remains controversial whether SERCA2a expression changes in human heart failure. Several studies reported a downregulation of SERCA2a protein [53,58], while others did not observe any significant changes of SERCA2a expression in end-stage failing human myocardium [54,55,57,59]. A decrease in the expression levels of SERCA2a may account for increased PLB to SERCA2a ratios and thus, increased inhibition of the affinity of SERCA2a and prolonged relaxation. This has been deduced from studies using crude membrane preparations or highly purified vesicles [54]. In transgenic and gene-knockout mouse models, changes in SERCA2 expression have been mimicked to evaluate the potential in vivo consequences. The SERCA2a knockout model [60] exhibited in the heterozygous knockout, a 45% reduction in SERCA2a expression levels, which was accompanied by decrease in SR Ca^{2+}-uptake function and cardiac contractility, as judged by the $-dP/dt$ values and mean

arterial pressure. The homozygous SERCA2a knockout is most likely lethal, since it was not observed in heterozygous cross mating [60]. In addition, impaired contractility and hypertrophy was observed in a splice-variant model, in which SERCA2a was replaced by SERCA 2b [61]. On the other hand, increases in the expression of SERCA2a and 2b in transgenic mice have been shown to increase SR Ca^{2+}-function and enhance cardiac contractility [62,63]. In a rat heart failure model using adenoviral gene transfer to overexpress SERCA2a, impaired cardiac function was restored to normal levels and myocardial energetics was not affected by increased SERCA2a expression [64]. These findings underline the importance of SERCA2a for cardiac contractility and SR Ca^{2+} availability.

In addition to possible changes in the protein levels of SERCA2a, the phosphorylation status of phospholamban at serine-16 or threonine-17 sites has been shown to decrease in human heart failure. Phosphorylation at the serine-16 site exhibited a 30% decrease [65,66] and similar changes were observed for the threonine-17 site [66]. A decrease in serine-16 phosphorylation was also observed in rat failing myocardium due to myocardial infarction; however, in this model an unchanged threonine-17 phosphorylation was shown [67]. The decrease in serine-16 phosphorylation levels may reflect decreased expression of β-receptors [68] or increased expression of G_i-protein [69] and represent a downstream alteration in the β-adrenergic signaling pathway. Based on these findings, disruption or alteration of the phospholamban-SERCA2a complex may be a promising target for therapeutic approaches [64,70] (see also Fig. 1).

5. Conclusion

Significant advances have been made in understanding the functional role of SERCA2a by modulation of its accessory phosphoprotein phospholamban. These modulations were obtained by using in vitro systems, experimentally-induced heart failure animal models, transgenic mouse models and human myocardial samples. In initial experimental steps some of the newly gained understanding of how SR function is regulated by SERCA2a is applied in mouse cardiac myocytes and may be utilized in future approaches in higher mammalian and human tissue, to beneficially modulate cardiac function. Furthermore, some promising new data indicate that SR Ca^{2+}-load modulates transcriptional responses in the transition from hypertrophy to heart failure and may be used to alter the progression of this disease. The understanding of the molecular mechanisms of intracellular Ca^{2+} regulation, e.g. SR Ca^{2+} uptake and release mechanisms, prompted the development of gene-mediated transfer mechanisms to deliver the protein of interest into cardiac myocytes, which may be either functionally defective or missing. Gene therapy of heart failure has gained considerable interest, mainly because of continued improvements in vector technology, cardiac gene delivery and better understanding of the molecular basis of heart failure. Upcoming studies will show whether improving Ca^{2+} handling function may prove to be a beneficial target for treating the progression of cardiac failure not only in experimentally induced heart failure, but also in the clinical setting.

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