Chapter 11
Regulation of Cardiac Sarco(endo)plasmic Reticulum Calcium-ATPases (SERCA2a) in Response to Exercise

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Abstract Sarco(endo)plasmic reticulum calcium ATPase (SERCA2a) plays an integral role in Ca^{2+} cycling in the heart. After a myocardial contraction has occurred, SERCA2a is primarily responsible for transporting Ca^{2+} out of the cytosol into the sarcoplasmic reticulum. Consequently, SERCA2a is key in determining relaxation time and inotropy of subsequent contractions. There are ten different SERCA isoforms in the body, where SERCA2a is the isoform expressed in the heart. Both SERCA2a expression and activity are reduced in models of disease. As such, a large body of research has examined SERCA2a and how it might be used as a means to restore heart function in models of disease. In this chapter, we examine various regulatory mechanisms of SERCA2a and how these mechanisms affect SERCA2a and cardiac function. Transcriptional, protein (e.g., phospholamban and

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sarcolipin), hormonal (e.g., thyroid hormone and adiponectin), and posttranslational modification (e.g., nitration, glutathionylation, SUMOylation, acetylation, glycosylation, and O-glcNAcylation) processes as they regulate SERCA2a are discussed. Additionally, exercise and its effect on the regulatory mechanisms of SERCA2a is examined.

Keywords Sarco(endo)plasmic reticulum calcium ATPase • Heart failure • Phospholamban • Sarcolipin • Thyroid hormone • Adiponectin • Posttranslational modifications • Exercise

1 Introduction

1.1 Role of Calcium in Muscular Contractions

Calcium (Ca^{2+}) is a key component of the excitation–contraction coupling (ECC) process in both cardiac and skeletal muscle. When Ca^{2+} is released from the sarcoplasmic reticulum (SR), free intracellular Ca^{2+} in the cytosol increases approximately tenfold. This facilitates Ca^{2+} binding to troponin C, which allows the tropomyosin filament to rotate and expose the myosin-actin binding site [1]. Myosin and actin are then able to interact and initiate cross bridge cycling, where adenosine triphosphate (ATP) hydrolysis moves the myosin along the actin filaments through a series of conformational changes [2]. Cross bridge cycling instigates the power stroke, resulting in muscular contraction. In cardiac muscle, the release of Ca^{2+} is initiated through Ca^{2+} induced Ca^{2+} release, a process where entry of Ca^{2+} through the L-type Ca^{2+} channel causes a further release of Ca^{2+} from the SR [1, 3].

Following contraction, Ca^{2+} must be removed from the cytoplasm to initiate myocardial relaxation. This occurs through four main transporters: the sarcolemmal Ca^{2+} ATPase, the mitochondrial Ca^{2+} uniport, the Na^{+}/Ca^{2+} exchanger, and the Sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA) [4]. In the mammalian heart, SERCA2a is primarily responsible for removing Ca^{2+} from the cytoplasm and subsequently transporting it back into the SR [4]. The amount of SERCA2a Ca^{2+} removal varies between species. For example, SERCA2a accounts for 92 %, 75 %, and 70 % of Ca^{2+} removal in rat, rabbit, and human hearts, respectively [4, 5].

SERCA2a is a ~110-kDa transmembrane protein that is part of the P-type ATPase category. As such, it functions to actively transport Ca^{2+} across the SR membrane and into the lumen through ATP hydrolysis [6]. SERCA2a is comprised of three distinct regions: the cytoplasmic head, the transmembrane helices, and the luminal loops [7]. Together, the transmembrane helices and luminal loops create the transmembrane domain [8], while the cytoplasmic head can be

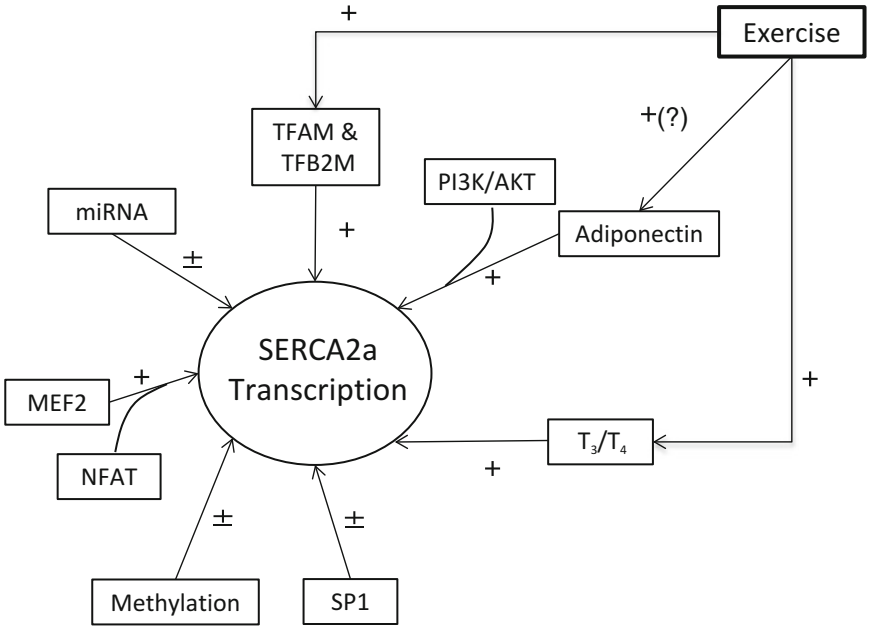

Fig. 11.1 Summary of SERCA2a transcriptional regulation. Factors affected by exercise are also noted. Abbreviations: miRNA, Micro RNA; MEF2, Myocyte enhancing factor-2; NFAT, Nuclear factor of activated T-cells; SP1, Specificity protein 1; T_3/T_4, Thyroid hormone; PI3K/Akt, Phosphatidylinositide 3-kinase/protein kinase 3 pathway; TFAM, Mitochondrial transcription factor A; TFB2M, Mitochondrial transcription factor B2

further subdivided into three different domains: the actuator domain, phosphorylation domain, and nucleotide domain. Each of these domains plays an integral role in the function of SERCA2a. The transmembrane domain contains two binding sites for Ca^{2+}, and depending on protein conformation, these binding sites can exist in a high- or low-affinity state [9]. The actuator domain, which is the smallest domain, facilitates the major conformational changes that occur as Ca^{2+} is transported into the lumen [9]. Lastly, the interface between the phosphorylation and nucleotide domains form the catalytic site where ATP hydrolysis occurs [10].

SERCA2a's major role in Ca^{2+} transport has made it of primary interest when examining cardiomyopathy. This chapter aims to discuss the regulation of SERCA2a in the heart, its role in heart failure and the mechanisms affecting SERCA2a expression and function. It also examines SERCA2a as a therapeutic target for the prevention or treatment of heart failure. Finally, we describe how exercise may affect cardiac function by regulating SERCA2a function. An overall summary of the mechanisms and pathways affecting SERCA2a can be found in Figs. 11.1 and 11.2.

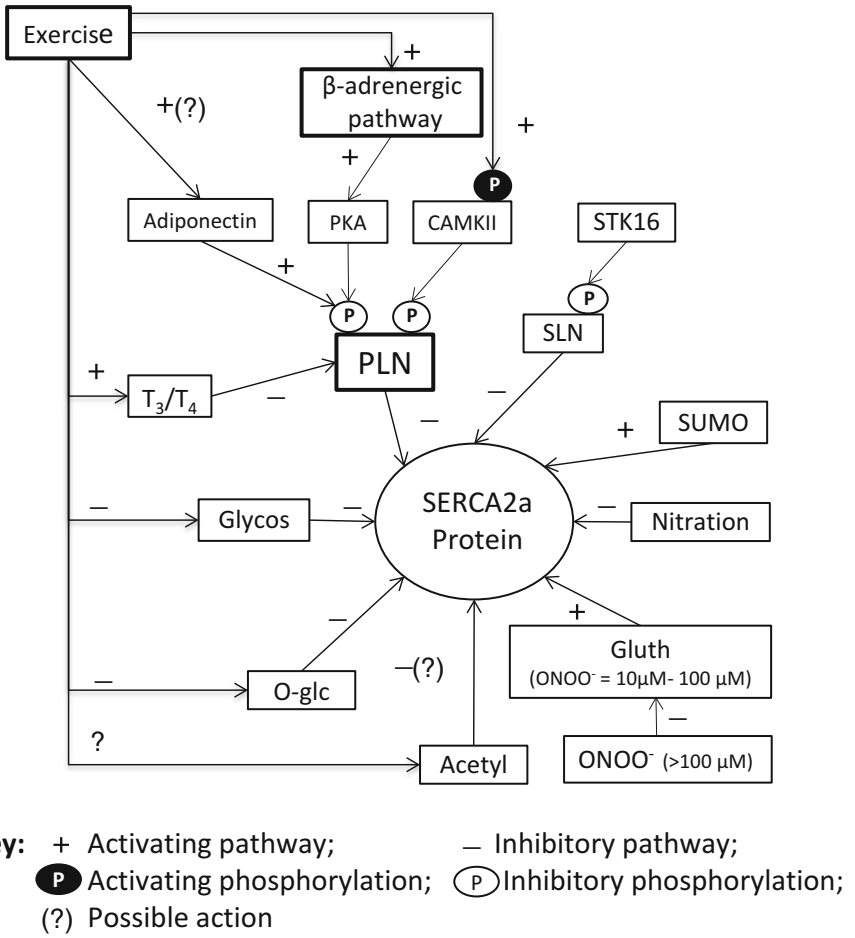

Fig. 11.2 Summary of SERCA2a protein regulation. Factors and pathways affected by exercise are also noted. Abbreviations: T_3/T_4, Thyroid hormone; PLN, Phospholamban; SLN, Sarcolipin; PKA, Protein Kinase A; CAMKII, Ca^{2+}/Calmodulin-dependent protein kinase II; STK16, Serine/threonine kinase 16; SUMO, SUMOylation; Gluth, Glutathionylation; Acetyl, Acetylation; O-glc, O-GlcNAcylation; Glycos, Glycosylation

2 Transcriptional Regulation

Multiple isoforms of SERCA have been identified, all of which are encoded by one of three SERCA genes: ATP2A1, ATP2A2, and ATP2A3. The human *ATP2A2* gene is responsible for encoding the SERCA2a-c isoforms [11], which are expressed in varying quantities throughout the body. *ATP2A2* is located on chromosomal region 12q23-q24.1 and is organized into 22 exons [12, 13]. Alternative splicing at exon 20 produces either SERCA2a or SERCA2b [12], where SERCA2a is expressed predominantly in cardiac and smooth muscle and SERCA2b is expressed in both

muscle and non-muscle cells [14]. In skeletal muscle, this process is partially mediated by a transcriptional factor known as myogenin [15]; however, little research has explored alternative splicing in cardiac muscle. SERCA2c arises from a splice variant inserted between exon 20 and exon 21 and is expressed in confined regions of the cardiomyocyte [9, 12]. The exact role of SERCA2c is yet to be fully elucidated.

Numerous factors regulate the transcription of *ATP2A2*. Two of these factors, mitochondrial transcription factors A (TFAM) and B2 (TFB2M), regulate *ATP2A2* transcription in the heart by binding to the -122 to -114 and the -122 to -117 regions, respectively [16]. Myocardial SERCA2a transcription is significantly correlated to TFAM and TFB2M expression, suggesting TFAM and TFB2M play an essential role in the regulation of SERCA2a gene transcription. In fact, overexpression of TFAM and TFB2M in rat myocardial infarction models increased SERCA2a transcriptional activity twofold and prevented stress-induced reductions of SERCA2a mRNA levels [16]. Furthermore, mutation of *ATP2A2* TFAM and TFB2M binding regions significantly reduced SERCA2 gene transcription [16]. In contrast, diabetic hearts and models of heart failure experience a reduction in TFAM levels [17, 18].

Specificity Protein 1 (SP1) is another transcription factor important to the gene regulation of SERCA2. Evidence suggests SP1 promoter sites are essential for full SERCA2a gene transcription [19, 20]. However, SP1 also mediated the decrease of SERCA2a mRNA seen in pressure overloaded hearts [21]. Therefore, SP1 is partially involved in both the basal and pressure-overloaded induced changes in SERCA2a transcriptional activity.

Myocyte enhancing factor-2 (MEF2) also mediates genetic transcription of SERCA2a. MEF2 is a common target for hypertrophic pathways. Although its exact regulation of SERCA2a is yet to be fully elucidated [22], it appears to upregulate SERCA2a transcription in models of hypertrophy [23, 24]. MEF2C works in conjunction with nuclear factor of activated T-cells (NFAT). In ischemic and dilated hearts removed from transplant patients, MEF2C and NFAT protein levels were significantly correlated [25], where ischemic heart saw a significant increase in both proteins [25]. Additionally, in a study by Vlasblom et al. [23], co-transfection of MEF2C and NFAT stimulated SERCA2a promoter sites, but only when both factors were present. This was accompanied by a 2.5-fold increase in SERCA2a mRNA [23]. In contrast to these findings, diabetic patients with heart failure had significantly decreased MEF2C and SERCA2a protein levels when compared to heart failure patients without diabetes [26]. While more research is needed in this area, MEF2C is another factor to consider when examining the transcriptional regulation of SERCA2a.

MicroRNAs (miRNAs; miR) are a class of small, noncoding mRNA molecules that regulate RNA or protein expression [27, 28]. Since various miRNA recognition sites are located within the 3′ untranslated region of SERCA2, it has been suggested miRNA may affect cardiac function through regulation of SERCA2a protein expression. However, the interaction between SERCA2a and miRNAs is complex, with 43–144 miRNAs affecting SERCA2a expression during heart failure [27–29]. Furthermore, binding sites located on 3′ UTR can bind with multiple miRNAs, and miRNAs themselves can bind with up to 10 different sites [29].

Boštjančič et al. [29] identified ten different miRNAs upregulated in infarcted hearts. Of these 10 miRNAs, miR-25 and -185 have been identified as down regulators of SERCA2a specifically [28–30]. MiR-25 is upregulated 270 % in failing hearts [28], whereas reducing miR-25 expression restored end systolic pressure volume and ejection fraction in pressure-overloaded hearts [28]. Furthermore, miR-25 suppression increased total SERCA2a and SUMOylated SERCA2a levels [28].

In comparison, miR-22 and miR-1 are associated with enhanced SERCA2a expression [27, 31]. MiR-22 knockout mice experienced prolonged Ca^{2+} cytosolic decay and a 25 % lower SR Ca^{2+} load than controls [31]. While SERCA2a protein expression was not significantly altered in healthy knockout mice, 1 week of transverse aortic constriction decreased SERCA2a protein content 2.4-fold [31]. Accompanying this decrease was 50 % greater fibrosis levels, impaired fractional shortening, and increased LV end systolic dimensions [31]. MicroRNAs and their regulation of SERCA2a are yet to be fully understood; however, this remains a promising area of research.

Recent studies have begun investigating SERCA2a methylation and the role it may play in SERCA2a expression and function. Methylation is an epigenetic modification that involves the addition of a methyl group to DNA nucleotide [32]. Murine hearts with transverse aortic constriction (TAC) experienced a significant decrease of SERCA2a [33]. This was accompanied by decreased methylation at *ATP2A2* promoter sites and increased methylation at *ATP2A2* repression sites [33]. Moreover, methylation factors were altered after TAC, with displaced demethylases and recruited methyltransferases at the *ATP2A2* promoter regions [33]. Prenatal environment can affect methylation and SERCA2a expression. For example, female mice exposed to estrogen diethylstilbestrol (DES) in utero had increased levels of DNA methylation at the calsequestrin-2 promoter after swim training [34]. Subsequently, SERCA2a and calsequestrin-2 protein expression increased, and cardiac hypertrophy was prevented in mice treated with DES [34].

3 Regulation of SERCA2a in Models of Disease

SERCA2a is a key factor in regulating cardiac contractility and relaxation. As such, there is a substantial amount of research examining SERCA2a in both animal and human heart models. Defective SERCA2a functioning via reduced mRNA, protein expression or activity levels leads to abnormal Ca^{2+} handling, reduced SR Ca^{2+} uptake and inefficient energy use [11, 35–37]. These traits are commonly characterized in patients with heart failure and eventually lead to impaired systolic and diastolic function of the heart [11, 37, 38]. In fact, a review of the literature reported significant decreases in SERCA2a mRNA and protein levels in various animal models of heart failure [39]. Moreover, failing human myocardium experienced up to a 60 % decrease in SERCA2a mRNA [39]. Decreases in SERCA2a expression in heart failure is accompanied by diminished activity levels. Arai et al. [35] reported a 50 % reduction in Ca^{2+} reuptake in right ventricular tissue removed from failing

human hearts. However, this effect may be partially mediated by increases in circulating levels of myocardial C-type natriuretic peptide, a molecule known to increase in heart failure [40].

Diabetic hearts also experience a decrease in SERCA2a expression an activity. In diabetic sedentary mice, SERCA2a protein content and maximal SERCA2a activity was decreased by 21 % and 32 %, respectively [41]. This was accompanied by impaired diastolic function [41]. Vasanji et al. [42] reported a decrease in SERCA2a protein content and activity in diabetic hearts, but also noted a significant increase in phospholamban (see Sect. 4 for more details on phospholamban) to SERCA2a protein ratio levels. The underlying mechanisms linking diabetes to SERCA2a activity are not yet fully understood; however it may be partially mediated by reduced enzymatic activity of silent information regulation (SIRT) 1 [43].

Given its integral role in cardiac function, SERCA2a presents a promising target for cardiac treatment. In mice with established diabetic cardiomyopathy, conditional expression of SERCA2a restored cardiac function [44]. Furthermore, activation of SIRT1 in diabetic heart models increased SERCA2a protein and mRNA levels to near control values [43]. In turn, functional parameters of the heart were significantly improved [43]. Transgenic mice overexpressing SERCA1a in the heart demonstrated a 170 %, 50 %, and 66 % increase in maximum Ca^{2+} uptake velocity, peak rate of myocyte shortening, and relengthening, respectively [45]. However, Kalyanasundaram et al. [46] cautioned against using SERCA1a therapy as a heart failure treatment, reporting increased apoptosis, dilated cardiomyopathy, and early mortality in calsequestrin deficient mice over expressing SERCA1a in the heart.

SERCA2a therapy in human models of heart failure has also been well received. Overexpression of SERCA2a by adenoviral gene transfer in human ventricle cardiomyocytes increased SERCA2a protein number and activity, induced a faster contraction velocity, and enhanced relaxation [47]. Likewise, the CUPID trial found that patients with advanced heart failure experienced up to an 88 % risk reduction in adverse event occurrence, such as LV assistive device implant, heart transplant and death, 12 months after receiving an intracoronary infusion of SERCA2a [48]. A 3-year follow-up found those who receive high-dose infusions still had an 82 % risk reduction for recurrent cardiovascular events [49]. Thus, SERCA2a gene therapy for the treatment of the diseased human heart appears to be beneficial.

4 Protein Regulation of SERCA2a

Phospholamban (PLN) is a 52 amino acid protein that has been well established as an inhibitor of SERCA2a activity [50]. PLN binds to SERCA2a and decreases its affinity for Ca^{2+} [51]. This binding occurs when cytosolic Ca^{2+} levels are low and PLN is in a dephosphorylated state. In contrast, phosphorylation of PLN prevents PLN from binding to SERCA2a, allowing SERCA2a to remain active [51]. This occurs through two different mechanisms: Ca^{2+}/Calmodulin kinase (CAMKII) phosphorylation and protein kinase A (PKA) phosphorylation [50, 52]. CAMKII is

a serine/threonine protein kinase that is activated by an increase in cytosolic Ca^{2+} and phosphorylates PLN at the Threonine[17] residue [50]. Similarly, PKA phosphorylates PLN at the Serine[16] residue [5, 52]; however, PKA phosphorylation is governed through β-adrenergic stimulation. When β-Agonists bind to receptors, a signal transduction pathway is activated that increases production of cyclic AMP (cAMP) via adenylate cyclase [5, 52]. This ultimately activates PKA, which phosphorylates PLN [5, 52]. Phosphorylation of PLN through either of these mechanisms can increase SERCA2a activity up to threefold, increasing relaxation velocity and contributing to the positive inotropic and lusitropic effects of β-adrenergic stimulation [5, 53].

Due to its major role in SERCA2a regulation, PLN expression is closely related to SERCA2a activity. In fact, when compared to wild-type littermates, PLN knockout mice experienced significantly greater contraction and relaxation rates, accompanied by an increase in SERCA2a affinity for Ca^{2+} [54]. PLN expression varies in quantity throughout the body's tissues. For example, in the murine heart, PLN is expressed threefold higher in right ventricle tissues compared to right atrial tissues [53]. Consequently, the relative ratio of PLN:SERCA2a is 4.2-fold lower in the atrium and is associated with significantly shortened relaxation and contraction times [53]. Similar findings have been found in the human heart. PLN protein expression is 44 % lower in the right atrium, and time to peak tension, time to relax and total contraction time are significantly decreased when compared to right ventricle tissues [55]. These data suggest that PLN expression, along with phosphorylation, plays a large role in cardiac function though regulation of SERCA2a activity in the heart.

In diseased states, both PLNcontent and phosphorylation in the heart are altered. As mentioned previously, PLN:SERCA2a ratio is increased in diabetic hearts [42]. Furthermore, PLN phosphorylation by both CAMKII and PKA is significantly decreased [42]. In ischemic and ischemia-reperfused hearts, CAMKII PLN phosphorylation is reduced, whereas PKA PLN phosphorylation is diminished in ischemia-reperfused hearts only [56]. Reduced PLN phosphorylation in heart failure may be partially due to lower levels of taurine, a beta-amino acid found in high concentrations in the heart [57].

Sarcolipin (SLN) is a homologue to PLN with high amino acid conservation in the transmembrane domain [58, 59]. Because of this, SLN and PLN likely interact with SERCA2a in a similar manner, although the precise regulatory mechanisms of SLN are not yet fully elucidated. SLN is a 31 amino acid protein and is thought to induce its inhibitory effect by binding directly to SERCA2a and reducing its affinity for Ca^{2+} [51, 60]. However, a study suggests SLN acts by reducing the V_{max} of SECA2a Ca^{2+} uptake, and unlike PLN, it can interact with SERCA2a in the presence of high Ca^{2+} concentrations [61]. SLN also functions by interacting with PLN to create a super-inhibition of SERCA2a [58, 62]. SLN forms a complex with PLN that destabilizes PLN pentamers [50]. This promotes the formation of PLN monomers, the inhibitory form of PLN [50]. Thus, by enhancing the effects of PLN, SLN acts to further reduce SERCA2a activity.

Babu et al. [60] demonstrated SLN's effect by over expressing SLN in rat myocytes through adenoviral gene transfer. These myocytes experienced a 31 % reduction in cell shortening compared to control myocytes [60]. SLN's effect can be relieved through phosphorylation. SLN is phosphorylated by serine/threonine kinase 16 (STK 16) at Threonine^{5}, which promotes dissociation of SLN from SERCA2a and subsequently increases SERCA2a activity [63]. This contributes to the relaxant effect of β-adrenergic stimulation [63]. SLN mRNA is mainly expressed in the atrial tissues of the heart [60]. In fact, Babu et al. [60] found SLN mRNA below detectable levels rat heart ventricle tissues. Similar results have been found in humans, with SLN mRNA being expressed only atrial tissues of the heart [64]. Given the different expression of SLN and PLN in cardiac muscle, it is thought that SLN is responsible for mediating SERCA2a activity where PLN is absent. SLN expression is deregulated in conditions of disease, where SLN mRNA and protein levels can be increased up to 12- and 6-fold, respectively in ventricle tissues [65]. Beyond SLN's role in Ca^{2+} regulation, it is also involved in thermoregulation by promoting SERCA2a uncoupling in skeletal muscle [65]. This interaction is unique to SLN [61].

5 Hormonal Regulation of SERCA2a

Thyroid hormone can be found in two different forms in the body: levothyroxine (T_4) and triiodothyronine (T_3), where T_3 is the active form and T_4 is a prohormone that is converted into T_3 or reverse T_3 [66]. While intracellular T_3 is about 20 times more potent than T_4 [66], administration of both forms has been well documented to affect the expression and activity of SERCA2a [67–71]. This effect occurs through positive regulation of SERCA2a gene transcription [72]. Various animal models have demonstrated thyroid hormone's impact on SERCA2a. For example, hypothyroid conditions imposed on rat and rabbit cardiomyocytes decreased SERCA2a mRNA content to 36–72 % of control levels, whereas hyperthyroid conditions increased SERCA2a mRNA up to 167 % of control levels [68, 70, 71, 73]. Furthermore, administration of T_3 to hypothyroid rat hearts significantly increased SERCA2a mRNA levels 2 h after injection, and normalized it 5 h after injection [71]. Similar results have been found with SERCA2a protein levels, with hypothyroid conditions decreasing protein content 11 %–26 % [69, 73], and hyperthyroid conditions increasing content 34 %–88 % [67, 69].

In addition to its direct regulation of SERCA2a, thyroid hormone further affects SERCA2a activity by regulation of PLN. Thyroid hormone affects PLN in an opposite manner to SERCA2a, where a decrease of T_3 or T_4 upregulates PLN, and an increase downregulates PLN. Reed et al. [73] demonstrates this in mice hearts, where hypothyroidism increased PLN mRNA and protein levels 28 % and 20 %, respectively and hyperthyroidism decreased PLN mRNA and protein levels 13 % and 30 %, respectively. Similar findings have been reported with rat and rabbit hearts. Hyperthyroid conditions induced up to a 50 % decrease in PLN mRNA and

a 25 % decrease in protein levels [68–70]. On the other hand, hypothyroid conditions increased PLN protein content 35 % in rat cardiomyocytes [69].

Coinciding to the changes in SERCA2a and PLN expression, thyroid hormone also induces a change in cardiac function. Chang et al. [67] found administering T_4 to rats with aortic banding eliminated abnormal myocardial functioning and increased contractility, relaxation speed, and cytosolic Ca^{2+} removal when compared to controls. Additionally, both rabbit and rat hyperthyroid hearts have greater Ca^{2+} maximal uptake than euthyroid hearts [68, 69]. A review by Novitzky and Cooper [66] presents thyroid hormone as a possible treatment for patients with "stunned myocardium," a condition where myocardial function is depressed due to global or regional ischemic events. These data demonstrate that through regulation of SERCA2a and PLN, thyroid hormone presents a possible means of reversing cardiac dysfunction and inhibiting cellular damage caused by ineffective Ca^{2+} cycling.

Another hormone responsible for the regulation of SERCA2a is adiponectin. Adiponectin is an adipocyte-derived peptide hormone that is inversely related to traditional cardiovascular risk factors, such as blood pressure, heart rate, and cholesterol and triglyceride levels [74, 75]. It also possesses antioxidant and anti inflammatory qualities [75, 76]. These cardioprotective properties are thought to occur through the modulation of SERCA2a activity [74]. Adiponectin appears to affect SERCA2a through PLN phosphorylation and the PI3K/Akt signaling pathway [74]. In a study by Safwat et al. [74], administration of globular adiponectin significantly restored SERCA2a activity in rats with induced ischemia/reperfusion injury. Additionally, the p-PLN/PLN ratio was significantly increased, suggesting PLN phosphorylation may be the mechanism adiponectin uses to increase SERCA2a activity [74]. Accompanying the increase in SERCA2a activity and PLN phosphorylation was a 139 % increase ($p < 0.05$) in p-Akt/Akt ratio [74]. Adiponectin induced benefits to cardiac function were abolished with administration of LY294002, an inhibitor of PI3K, confirming that PI3K/Akt pathway activation is essential for globular adiponectin to exert its effect on SERCA2a [74].

Other studies examining adiponectinand SERCA2a activity have shown similar results to Safwat et al. [74]. Adiponectin gene therapy significantly increased SERCA expression in skeletal muscle of diabetic rats [77]. This restoration was furthered by a 9 week swimming exercise protocol [77]. Additionally, treatment of H9C2 cardiomyoblasts in an adiponectin-enriched medium significantly increased SERCA2a expression and decreased in inflammatory markers compared to cardiomyoblasts in an adiponectin depleted culture [78]. In contrast, induction of ER stress through tunicamycin treatment reduced SERCA2a expression, adiponectin, and adiponectin receptor 1 by as much as 50 % [78].

6 Posttranslational Modification Regulation of SERCA2a

Various posttranslational modifications have been found to affect the activity of SERCA2a. Some of these modifications are currently being researched, and as such, the process and effects of these modifications are yet to be fully elucidated.

This section will examine current knowledge of nitration, glutathionylation, SUMOylation, acetylation, glycosylation and O-glcNAcylation and their role in SERCA2a function.

Nitration is a chemical process where a nitro group is added to a protein. Nitration inhibits SERCA2a activity through the polyol pathway, a pathway that contributes to oxidative stress in hyperglycemic conditions [79, 80]. In fact, levels of nitrotyrosine on SERCA2a were significantly increased in high-glucose perfused rat hearts compared to hearts perfused with normal glucose levels [80]. Studies on the human heart found nitrotyrosine levels to be nearly doubled in idiopathic dilated cardiomyopathic hearts compared to age matched controls [79]. This was accompanied by a significant positive correlation between time to half relaxation and nitrotyrosine to SERCA2a ratio [79]. This suggests SERCA2a nitration can substantially affect cardiac function [79]. However, nitration's exact role in normal regulation of SERCA2a is yet to be defined.

Glutathionylation is the process where a disulfide bond is formed between the cysteine of a protein and glutathione (GSH) [81]. In SERCA2a, glutathionylation occurs predominately on cysteine^{674} [82–84] and subsequently increases SERCA2a activity and Ca^{2+} uptake [82, 84, 85]. It is well documented that nitric oxide (NO) causes muscle relaxation through cGMP and protein kinase G. However, NO appears to induce relaxation by increasing SERCA2a glutathionylation as well [86]. Exposure to low amounts of oxidative species including NO, Perioxynitrate ($ONOO^-$) and nitroxyl can increase SERCA2a activity 45–60 % in cardiac muscle cells [83–86]. However, it should be noted that NO cannot act alone, and must be combined with the superoxide radical to form $ONOO^-$ before it can react with cytosolic GSH and glutathionylate cys^{674} [82].

While low amounts (10–100 μM) of oxidative species increases SERCA2 activity, transgenic mice exposed to high amounts (>100 μM) experienced a decrease SERCA2 activity [82]. Glutathiolyation can normally be reversed either chemically or enzymatically, but in cases such as atherosclerosis where there is a chronic increase in oxidative species, cys ^{674} is irreversibly oxidized to sulfonylation [82, 84–86]. Consequently, further glutathiolation and activation of SERCA2a is prevented [82, 84]. This causes a subsequent decrease in SERCA2a activity, which can lead to heart failure. Substances known to reverse glutathiolation, such as dithiothreitol, can help prevent SERCA2 inactivation, but are unable to reverse oxidation once it has occurred [85, 86].

SUMOylation and its effect on SERCA2a is a promising area of research when examining SERCA2a protein stability and function. SUMOylation occurs through the binding of small ubiquitin-like modifier 1 (SUMO1) to the lysine ^{480} and lysine^{585} residues of SERCA2a [87, 88]. This modification is thought to have cardioprotective properties [87, 88]. In heart failure, there is a 30 %–40 % decrease in SUMO1, which is accompanied by a decrease in total SERCA2a SUMOylation [87]. Moreover, downregulation of SUMO1 using small hairpin RNA reduced SERCA2a protein levels by 40 % [87]. This is likely due to lower levels of SERCA2a SUMOylation reducing SERCA2a half life from 5.9 to 4.9 days [87]. SUMOylation proved to be a potential therapeutic means when injection of SUMO1 to pressure

overloaded hearts significantly improved cardiac performance and restored SERCA2a function and mRNA expression to almost normal levels [87, 88]. The mechanisms SUMOylation uses to rescue SERCA2a function are yet to be fully elucidated. However, it is thought that SUMOylation competes with other posttranslational modifications, such as ubiquitination or acetylation, to increase SERCA2a's stability and prevent degradation [87]. SERCA2a SUMOylation also appears to increase SERCA2a ATPase activity by increasing its sensitivity to ATP [87].

Acetylation/deacetylation involves the attachment, or removal, of an acetyl group from a molecule. Its precise role in SERCA2a regulation has yet to be examined, although it may regulate SERCA2a in a manner opposite to SUMOylation [89]. Three potential acetylation sites were identified within the nucleotide-binding domain of SERCA2a: lysine^{464}, lysine^{510}, and lysine^{533} [90]. Therefore, acetylation/deacetylation could play a role in cardiac muscle Ca^{2+} cycling [90]. Kho et al. [87] reported increased SERCA2a acetylation in failing hearts, which could be reversed with sirtuin-1 deacetylase, but no data was given to validate this statement. Although current evidence is limited, acetylation/deacetylation is a potential regulator of SERCA2a activity.

Glycosylation occurs when a saccharide is attached to a protein. This can be done enzymatically or nonenzymatically, where the nonenzymatic reaction is better known as glycation. An increase in glycosylation has been associated with a 25–45 % decrease in SERCA2a mRNA and protein levels [91, 92] and a 40 % increase in PLN levels [92]. This combination results in an overall decrease in SERCA2a activity, reducing Ca^{2+} transport into the SR. Since elevated levels of glucose appears to increase SERCA2a glycosylation, this area remains of particular interest when examining the relationship between diabetes and heart disease [91, 92].

O-glcNAcylation is a specific form of glycosylation where a single O-linked *N*-acetylglucosamine is either added or removed from a serine or threonine residue [91, 93, 94]. O-glcNAcylation reduces SERCA2a activity through direct regulation SERCA2a and modification of PLN [91, 93, 95]. High-glucose treated rat cardiomyocytes had substantially increased levels of nuclear O-glcNAclyation, accompanied by a 28–37 % and 25 % reduction in SERCA2a mRNA and protein expression, respectively [91]. Additionally, cardiomyocytes injected with an O-glcNAc-transferase had a 47 % decrease in SERCA2a expression when compared to controls [91]. In contrast, reducing cellular O-glcNAcylation through an adenovirus expressing O-glcNAcase increased SERCA2a protein expression 40 %, reduced PLN protein 50 %, and increased PLN phosphorylation twofold [94]. SERCA2a O-glcNAcylation may be partially mediated by SP1, as SP1 is known to be heavily O-glcNAcylated and is directly involved in the transcription of SERCA2a [20, 96].

7 Regulation of SERCA2a in Models of Exercise

It has been well documented that exercise increases cardiac function through enhanced SERCA2a Ca^{2+} uptake, especially in models of cardiovascular disease [97]. This can occur as a result of direct regulation of SERCA2a transcription and

activity, or through modifications of the regulatory processes discussed earlier in this chapter. Here, we examine the various processes by which exercise regulates SERCA2a.

Aerobic exercise in hypertensive rats significantly increased cell contractility and Ca^{2+} transport [97]. These changes were partially due to increased PLN phosphorylation and SERCA2a mRNA expression [97]. Similar results were demonstrated in mice with induced heart failure. Aerobic interval training improved atrial myocyte shortening by 89 % and restored SERCA2a function to near control levels [98]. Additionally, genetic mouse models of sympathetic hyperactivity-induced heart failure experienced greater peak Ca^{2+} transient levels and reduced diastolic Ca^{2+} decay time after 8 weeks of aerobic training [99]. These results were enhanced with administration of carvidilol, a beta-blocker used to treat heart failure [99]. Similar benefits have been demonstrated in diabetic models as well. For example, voluntary wheel running in diabetic mice attenuated the decrease in diastolic function and SERCA2a content and activity [41]. Even when disease is absent, cardiac function increases in response to exercise. Kemi et al. [100] reported a 60 % and 50 % increase in fractional myocyte shortening and Ca^{2+} transient amplitude, respectively, in aerobic interval trained mice. With these adaptations was a 25 % increase in SERCA2a protein content [100]. Wisløff et al. [101] report comparable findings in exercise trained mice, where SERCA2a protein content increased 82 % and Ca^{2+} cycling and sensitivity was significantly increased compared to sedentary mice. The effects of exercise training on SERCA2a require a stimulus to be maintained. For example, Carneiro-Júnior et al. [97] noted cardiac adaptations to exercise were reversed to control levels after 4 weeks of detraining. Despite these data, some studies have reported that exercise training in aging models was not able to change age related degradations to SERCA2a and cardiac function [102].

Exercise may partially mediate its effect of SERCA2a through regulation of TFAM and TFB2M [16]. Aerobic fitness is positively correlated with mitochondrial biogenesis [103], and thus, requires an increase in mitochondrial transcription factors such as TFAM and TFB2M. TFAM protein expression was significantly higher in elite athletes compared to moderately active individuals [103], and in male participants, TFB2M mRNA levels nearly doubled after 10 days of exercise training with restricted blood flow [103]. Moreover, exercise training in hyperglycemic mice restored TFAM protein to control levels [104]. Therefore, it is likely exercise affects SERCA2a transcription through regulation of TFAM and TFB2M.

Exercise also affects SERCA2a activity by regulating PLN phosphorylation [105]. In aged, ovariectomized rats, exercise training reversed reduced PLN phosphorylation at $Threonine^{17}$ and normalized SERCA2a activity [105]. Furthermore, aerobic exercise in hypertensive rats significantly increased PLN phosphorylation at both $Serine^{16}$ and $Threonine^{17}$ [106]. This contributed to an improved inotropic and lusitropic response to β-adrenergic stimulation [106]. Exercise's effect on PLN phosphorylation may be partially mediated through CaMKII phosphorylation [100]. Aerobic interval training increases CaMKII $Threonine^{287}$ phosphorylation, indicating activation [100]; this would contribute to the increase in PLN^{Thr17} phosphorylation observed [100]. Accompanying the

increase in PLN phosphorylation, exercise reduces PLN:SERCA2a expression ratios, further enhancing SERCA2a activity [100].

Exercise has varying effects on circulating thyroid hormone, where intense exercise reduces free T_3 (fT_3) and T_3 and increases T_4, free T_4 (fT_4), and thyroid stimulating hormone [107–109]. These changes are acute and most likely due to suppression of T_4 to T_3 conversion [108]; given proper recovery, thyroid hormone levels are restored within 72 h [108]. In contrast, chronic exercise is associated with a significant increase in thyroid hormone levels. Four weeks of treadmill training increased T_3 and T_4 in hypothyroid rats to near control levels [110]. This would suggest a subsequent upregulation of SERCA2a. In fact, 4 weeks of wheel running in adult male rats significantly increased thyroid hormone receptor β1 mRNA nearly twofold, which would augment transcription of downstream thyroid hormone target genes, such as SERCA2a [111]. As such, it is likely exercise partially mediates SERCA2a expression through thyroid hormone regulation.

More research is needed to fully understand how adiponectin responds to exercise; however, studies suggest physical activity increases adiponectin levels. Cross-sectional studies report positive correlations between physical activity levels and adiponectin [112, 113], and intervention studies produce similar results. For example, obese individuals randomized to a controlled physical activity-behavior-diet-based lifestyle intervention for 3 months experienced a 34 % increase in adiponectin concentration [114]. Nevertheless, not all studies agree with these results. In a systematic review by Simpson & Singh [115], less than half the studies on chronic exercise and adiponectin reported significant results. While this does not mean exercise does not regulate adiponectin, more rigorous and long-term studies are needed.

Researchers examining exercise and posttranslational modifications have mainly focused on O-glcNAcylation. Six weeks of exercise training in mice decreased O-glcNAcylation 40–75 %, which was paired with a 30 % increase in SERCA mRNA [93, 96]. Moreover, mice trained at a high running capacity had a significantly lower O-glcNAcylated SERCA2a to total SERCA2a ratio than mice trained at a low running capacity [116]. Swim-trained mice experienced a reduction in O-glcNAcylated SP1, suggesting SP1 may be involved in exercise regulation of SERCA2a [96]. It is possible physical activity reduces O-glcNAcylation through regulation of glucose levels, as O-glcNAcylation increases in the presence of glucose [91]. Research on exercise's effect of other posttranslational modifications if needed to further understand how exercise regulates SERCA2a.

8 Conclusion

Regulation of SERCA2a is governed by numerous factors, such as gene transcription, posttranslational modifications, and endogenous proteins. Given its critical role in heart function and failure, an abundance of research has focused on SERCA2a'a function, regulation, and how it can be used as a therapeutic target. Some of these areas, such as SUMOylation, acetylation, and microRNAs, have yet

to be fully elucidated. Exercise has also presented itself as a possible means of effective therapy, although more research is needed on the exact mechanisms here as well. Overall, SERCA2a remains a promising target to maintain and restore heart function and enhance longevity and quality of life.

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