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Research Article

Altered Sarcoplasmic Reticulum Calcium Cycling-Targets for Heart Failure Therapy

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ABSTRACT

Heart failure is a complex clinical syndrome with typical symptoms and signs that generally occur on exertion but can also occur at rest (particularly when recumbent), secondary to an abnormality of cardiac structure or function. There are an estimated 23 million people with HF worldwide. The contractile force in cardiomyocytes is determined by the amplitude of the Ca2+ transient generated by SR Ca2+ release via intracellular Ca2+ release channels/ryanodine receptor-2. At the cellular level, the causes of impaired cardiac myocyte relaxation include cytosolic Ca2+ overload, myofilament structural changes or dysfunction, and neurohormonal activation. Alternatively, a decrease in Ca2+ efflux or inadequate Ca2+ reuptake by the sarcoplasmic reticulum during diastole can result in intracellular Ca2+ overload in this condition. SERCA2a is validated to be the major cardiac isoform. SERCA2a is a prime target for modulation of cardiac contractility during heart failure. The dysregulation of SERCA2a is a hallmark of heart failure. Reduction in SR Ca2+ uptake results in systolic dysfunction as well as increased cytosolic Ca2+ level and susceptibility to apoptosis. Alterations in the levels of the ryanodine receptor, triadin, junctin, and calsequestrin may affect Ca2+ storage in the SR, the amount of Ca2+ which is available to be released from the SR and the amount of SR Ca2+ leak during diastole, thus contributing to the pathology of the failing heart. Histidine-rich calcium-binding protein (HRC) regulates Ca2+ homeostasis in the heart. Overexpression of FKBP12.6 reduces Ca2+ leak from the SR during diastole, thereby increasing SR-Ca2+ content, and thus increases the amount of Ca2+ available for release, which in turn increases twitch shortening amplitude. This review aims to introduce altered sarcoplasmic reticulum calcium cycling-targets for heart failure therapy.

Key words: Heart failure; Sarcoplasmic reticulum,; Targets; Proteins

INTRODUCTION

1. Heart failure is a complex clinical syndrome with typical symptoms and signs that generally occur on exertion but can also occur at rest (particularly when recumbent), secondary to an abnormality of cardiac structure or function that impairs the ability of the heart to fill with blood at normal pressure or eject blood sufficient to fulfill the needs of the metabolizing organs [1].

2. There are an estimated 23 million people with HF worldwide. Currently, 5.7 million people in the US have HF, but the projections are worrisome since it is expected that by 2030 more than 8 million people will have this condition, accounting for a 46 % increase in prevalence [2]. Heart failure can be caused by ischaemic, endocrine, immune, inflammatory, infective, genetic, and neoplastic processes, by the failure of the heart to develop properly and even by pregnancy. Rheumatic valvular disease remains a common cause in many developing countries whereas this diagnosis is now uncommon in developed countries; in the latter, a degenerative valvular disease in the elderly is now more common [3]. In the developed world, ventricular dysfunction is the commonest underlying problem and is caused, mainly, by myocardial infarction, hypertension or, often, both infarction and hypertension [4]. Diabetics have a higher prevalence of heart failure. Diabetes accelerates the development of coronary atherosclerosis and is often associated with hypertension [5]. Diabetes is also associated with more autonomic dysfunction and worse renal, pulmonary, and endothelial function, as well as worse functional status and a worse prognosis [6]. Atrial fibrillation can cause heart failure directly as a consequence of the loss of the atrial contribution to cardiac output and reduced diastolic filling as a result of tachycardia [7].

3. The term excitation-contraction (EC) coupling describes the process of converting electrical depolarization of the plasma membrane to contraction of the cardiomyocyte. The release of

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Ca2+ from the sarcoplasmic reticulum (SR) in the cardiomyocyte initiates contraction of the heart during systole. The subsequent reuptake of Ca2+ into the SR or extrusion from the cytoplasm enables relaxation of the heart during diastole [8].

4. Pharmacologic therapy which reduces mortality in the treatment of systolic HF includes angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), beta-blockers, and mineralocorticoid receptor, antagonists. Although digitals do not affect mortality, they were reported to reduce hospitalization [9].

Altered sarcoplasmic reticulum calcium cycling

Regulation of Ca2+ cycling in the heart: The magnitude and timing of the Ca2+ transient, which determines the strength of contraction, is dynamically regulated by phosphorylation of both Ca2+ handling pumps and ion channels. Several kinases, including protein kinase A (PKA), Ca2+/calmodulin-dependent protein kinase (CaMKII) and protein kinase C (PKC) contribute to these effects. Phosphorylation of the Ca2+ channels and pumps by PKA is the downstream event in a signalling cascade that begins with the activation of β-adrenoceptors on the plasma membrane by catecholamines. This allows for the activation of adenylatecyclase by specific G proteins, leading to increased cytosolic levels of cAMP and activation of PKA [10,11].

PKA directly phosphorylates important Ca2+-cycling proteins, including LTCCs, RyR2, and PLB. Activation of the β-adrenoceptorsignalling pathway increases EC coupling gain, thereby increasing the amount of Ca2+ released by RyR2 per amount of trigger Ca2+ entering the cell through LTCC. This signalling pathway, also known as the fight-or-flight response, is highly conserved in evolution and allows for the rapid enhancement of cardiac contractility during exercise or stress [12,13].

There is ample evidence that CaMKII is important in regulating EC coupling in the heart. An increase in heart rate increases CaMKII activity in the heart, which can result in phosphorylation of LTCCs, RyR2, and PLB by CaMKII. The functional effects of phosphorylation of these key Ca2+ handling proteins by CaMKII include an increase in contractile force (for example,a positive force-frequency relationship) [14,15].

PKC-α as a fundamental regulator of cardiac contractility and Ca2+ handling in myocytes. The Ca2+/phospholipid dependent PKC exists as a family of at least 12 distinct isoforms. PKC-α can directly phosphorylate protein phosphatase inhibitor-1 (I-1), augmenting the activity of protein phosphatase-1 (PP1) and causing hypophosphorylation of PLB. Decreased PLB phosphorylation could result in inhibition of SERCA2a and impaired Ca2+ reuptake into the SR [16].

Impaired Ca2+ cycling in heart failure: At the cellular level, the causes of impaired cardiac myocyte relaxation include cytosolic Ca2+ overload, myofilament structural changes or dysfunction, and neurohormonal activation. In many patients with heart failure, excitation-contraction coupling is defective. Several abnormalities of receptors, pumps, and regulatory proteins that are involved in Ca2+ cycling have been shown in these patients. These abnormalities lead to a prolongation of the cytosolic Ca2+ transient time and, in patients with end-stage heart failure, an increase in end-diastolic Ca2+ concentration [13,17].

Such changes, which are often a result of a decrease in the density or function of SERCA2a in the sarcoplasmic reticulum, impair systolic and diastolic function. An increased level of intracellular Na+ in heart failure myocytes can contribute to increased cytosolic Ca2+ loading via NCX. However, up regulation of NCX function, which might also occur in heart failure, could partially compensate for SERCA2a down-regulation concerning diastolic function, but would not affect systolic function [17,18].

In chronic heart failure, sustained activation of the β-AR and downstream signaling pathways have adverse effects on cardiac cells. At the molecular level, β-AR dysfunction is characterized by a reduction of β1-AR density, resulting in an increased β2-AR: β1-AR ratio and uncoupling of β-ARs from G-protein complexes. This desensitization is mediated by elevated G-protein receptor kinase activity. PKA and calcium/calmodulin-dependent protein kinase type II (CaMKII) are key effectors downstream of β-AR stimulation, and their activity might affect target proteins, resulting in inappropriate or maladaptive responses [19,20].

In heart failure, intracellular Ca2+ overload can result from excessive Ca2+ entry into the cytosol, either from the sarcoplasmic reticulum or from outside the cell. Alternatively, a decrease in Ca2+ efflux, or inadequate Ca2+ reuptake by the sarcoplasmic reticulum during diastole can result in intracellular Ca2+ overload in this condition [13,21].

Excessive Ca2+ entry into the cytosol: In cardiac myocytes, Ca2+ influx occurs almost entirely through voltage-activated LTCCs. Ca2+ influx through LTCCs triggers sarcoplasmic reticulum Ca2+ release, which determines the rate and magnitude of myocyte contraction via a process called Ca2+-induced Ca2+ release [13]. LTCC-mediated Ca2+ influx is also associated with cardiac hypertrophic signaling pathways; therefore, abnormalities in LTCC activity and expression can cause cardiac dysfunction [22]. LTCC current caused a compensatory increase in Ca2+ leakage from the sarcoplasmic reticulum via ryanodine receptor 2 (RyR2), resulting in pathological cardiac hypertrophy and heart failure through calcineurin–NFAT signaling [20].

Store-operated Ca2+ entry (SOCE, also known as capacitative Ca2+ entry)—a mechanism through which calcium-release-activated calcium (CRAC) channels in the plasma membrane since depletion of intracellular Ca2+ and open to allow influx of Ca2+ from the extracellular reservoir—may be enhanced in pathological remodeling [23].

Reduced Ca2+ efflux from the cytosol: As well as acting as a Ca2+ reservoir, the sarcoplasmic reticulum actively maintains cytosolic Ca2+ concentrations during a contraction–relaxation of cardiac myocytes. In humans and higher mammals, sarcoplasmic reticulum Ca2+ uptake is mainly regulated by SERCA2a. This Ca2+ pump has an important role in restoring sarcoplasmic reticulum Ca2+ levels in diastole and terminating Ca2+ dependent force activation via Ca2+ release into the cytosol through RyR2 in systole. In failing human myocardium, the force-frequency response is blunted, possibly as a result of defective activity or expression of SERCA2a [13,24].

An increase in end-diastolic cytosolic Ca2+ levels and prolongation of Ca2+ transient during diastole were shown in cardiac myocytes isolated from patients with end-stage heart failure. Such abnormalities are associated with the development of enhanced diastolic tension [25].

Targeting SR Ca^{2+} handling proteins as therapy for HF

Sarcoplasmic reticulum calcium uptake proteins as targets for HF Isoforms of the SERCA pump: Invertebrates, three main SERCA proteins are encoded by three different genes (ATP2a1/ SERCA1, ATP2a2/SERCA2, and ATP2a3/ SERCA3). More than ten variants are produced by tissue-dependent alternative splicing at the COOH terminal of the transcripts, which result in specific functions of the variants. SERCA1, expressed in fast-twitch skeletal muscle, is found possessing two major splice variants: SERCA1a (adult form) and 1b (neonatal form) [26].

SERCA2 is expressed abundantly in the adult mammalian heart. SERCA2a is validated to be the major cardiac isoform (~99.9%). Two disparate tissue-specific SERCA2 isoforms (SERCA2a and 2b) are derived from the alternative processing of SERCA2 gene transcripts in the COOH-terminal. SERCA2a, also known as 'cardiac isoform', is the dominant isoform in cardiac muscle and slow-twitch skeletal muscle, while it is also found at lower amounts in smooth muscle and neuronal cells. SERCA2b is traceable in almost all tissues including muscle and no muscle cells as a housekeeping isoform. Recently, a third splice variant (SERCA2c) is reported expressed in the left ventricle, which may be functionally important in (foetal) heart [27,28].

Lately, a new splicing variant (SERCA2d) is discovered in human skeletal muscles, yet more endeavor is needed to explore the corresponding physiological meaning. SERCA3a is known as the major SERCA3 isoform, which has additional five variants (SERCA3b-f) in humans, SERCA3 is widely expressed in many tissues at various concentrations, which is prominent in hematopoietic cell lineages, platelets, epithelial cells, fibroblasts, as well as endothelial cells, but only a minority of SERCA3 is expressed in muscle [29].

Sequestration of Ca2+ from the cytosol into the SR lumen, and thus relaxation of the heart, is mediated by SERCA and its regulatory protein PLN. PLN is a reversible inhibitor of SERCA. Phosphorylation of PLN results in an overall increase in the affinity of SERCA for Ca2+, increasing SR Ca2+ uptake, while dephosphorylation of PLN by an SR-associated phosphatase restores the inhibitory effects of PLN on SERCA [30].

Since PLN and SERCA are major regulators of SR Ca2+ uptake and alterations in the levels or activity of either protein could contribute to decreased SR Ca2+ load, impaired myocyte Ca2+ cycling, and depressed contractile parameters in the failing heart, the expression levels and functional activity of this critical SR Ca2+ handling proteins have been investigated in human heart failure. SERCA mRNA and protein expression levels to be significantly decreased in the failing human heart [31,32].

SERCA protein levels were found to be decreased by a greater proportion than protein levels of PLN, and that PLN was shown to be hypo phosphorylated in the failing myocardium, inhibition of SERCA is predicted to be more pronounced in the human failing myocardium compared to the non-failing myocardium, explaining the observed decrease in SERCA activity in the human failing heart [13,24].

PLN is a critical regulator of basal SR Ca2+ handling and contractility in the heart, due to PLN's ability to modulate SERCA Ca2+ uptake, SR Ca2+ load, and, ultimately, myocyte Ca2+ cycling. Furthermore, increases in the ratio of PLN/SERCA or alterations in the degree of inhibition of SERCA by PLN are not only responsible for impaired Ca2+ homeostasis and depressed contractility but may be critical mechanisms underlying the heart failure phenotype. Thus, increasing the reuptake of Ca2+ into the SR by stimulating SERCA activity may be a promising approach to improve systolic and diastolic function in the failing myocardium. Indeed, clinical trials targeting SERCA are in progress [22, 28,31].

Since naturally occurring heritable mutations have been associated with dilated cardiomyopathy and heart failure, the SERCA2a and PLN genes were screened for the presence of naturally occurring mutations in humans [33,34].

Pharmacological action: β-blockers, ACE inhibitors and aldosterone antagonists are the first-choice drugs applied in the treatment of cardiac diseases, which may prolong life and relieve the symptoms. They cannot correct the underlying causes of contractile dysfunction. Carvedilol is a β-adrenergic receptor blocker that elicits its beneficial effects on cardiac function through the restoration of SERCA2a expression in patients with HF [35]. Even though from then on, drugs used individually or in combination have significant benefits to retain SERCA2a abundance, thereby improve contractility in heart failure, and it is extremely attractive at present. Among these drugs, by blockading the renin-angiotensin system, an angiotensin-converting enzyme inhibitor – enalapril – and an angiotensin II receptor antagonist – losartan – can partially prevent the down-regulation of SERCA2 protein and mRNA content as well as reduction of left ventricular function in a rat model of congestive heart failure [36].

SERCA2a is a prime target for modulation of cardiac contractility during heart failure. The deregulation of SERCA2a is a hallmark of heart failure. Reduction in SR Ca2+ uptake results in systolic dysfunction as well as increased cytosolic Ca2+ level and susceptibility to apoptosis.

Resveratrol, a natural antioxidant agent, utilized in a variety of diseases including heart diseases, is also found to lift SERCA2a expression and cardiac function [37].

Insulin-like growth factor 1 (IGF-1) treatment can regain the amount of SERCA2a in diabetic rat cardiomyocytes, which is mediated by activation of the PI3kinase-AktSERCA2a signaling pathway. Some components extracted from plants reveal to exert cardio protective effects by restoring SERCA2 expression. Oxymatrine, an anti-inflammatory agent extracted from the Chinese herb Sophora japonica, can improve cardiac function via up regulating SERCA2a expression in a rat model of chronic heart failure [38].

Besides, some other drugs that can modulate SERCA activity as an indirect means to improve contractility in heart failure are reported. Glucocorticoid can prevent additional deterioration of SERCA2a activity in piglets with ischemic cardiac dysfunction. Hydralazine, a potential DNA methylation inhibitor, can increase

Table 1: Target for modulation of cardiac contractility during heart failure.

S.No	Pathologic Stimuli	Therapeutic Strategy
1	Excess PKCα activity and expression	Chemical inhibitor
2	Depressed inhibitor activity (I-1)	Active I-1 overexpression
3	Excess PLB dephosphorylation	PLB peptide inhibitor
4	Reduced SUMO1 expression and SUMOylation	SUMO1 overexpression
5	Defective PLB phosphorylation	PLB silencing
6	Defective SERCA2a activity and expression	SERCA2a overexpression

Table 2: Therapeutic strategies for preventing abnormal Ca^{2+} release.

Strategy	Drug
Increasing SERCA activity	
Stimulating SERCA2a activity	Gingerol analogues
Over expressing SERCA2a	(Adenovirus)
Decreasing PLB activity	
Increasing PLB phosphorylation N/A	Not available
Antisense gene therapy	(Antisense)
Gene therapy with rAAV-PLB	(AAV-virus)
Inhibiting PKC-α activity	Not available
Decreasing NCX activity	
NCX antagonist (reverse-mode)	KB-R7943/ SEA0400
NCX antagonist (forward-mode)	SEA0400
Enhancing calstabin2 binding to RyR2	
Increasing RyR2 binding-affinity for calstabin2	JTV519
Overexpression of calstabin2 in myocytes	(Adenovirus)
Reducing PKA phosphorylation of RyR2	
Reducing PKA activity	Beta-blockers
Enhancing PP1/PP2A activity	Beta-blockers

SERCA function via decreasing methylation in the SERCA2a promoter, modulate Ca2+ handling and improve cardiac function in rats with isoproterenol-induced heart failure [39].

Istaroxime, a Na+/K+ ATPase inhibitor, can improve Ca2+ cycling, which is essential to the enhancement of Ca2+ uptake into SR, leading to increased SERCA function in cardiac SR vesicles of dogs with heart failure. A clinical trial with the administration of istaroxime is demonstrated to improve pulmonary capillary wedge pressure and decrease heart rate in patients with heart failure via relieving the inhibition of PLN on SERCA2a. In addition, CDN1163, a potent allosteric activator of SERCA, directly binds SERCA, alleviates ER stress and shows potential as a therapeutic for Parkinson's disease, type-2 diabetes and metabolic dysfunction. However, its functions in the heart have not yet been reported. Recombinant adeno-associated viruses (AAV) were used in the first gene therapy clinical trial for HF called Calcium Up-Regulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) [40, 41].

Sarcoplasmic reticulum calcium storage/release proteins as targets for HF: While SERCA and PLN regulate Ca2+ uptake and relaxation of the heart, SR Ca2+sequestration and the subsequent Ca2+ release is facilitated by a massive macromolecular ryanodine receptor complex, strategically localized at the site where a key step in excitation-contraction coupling occurs. (a) four SR junctional proteins, the ryanodine receptor, triadin, junctin, and calsequestrin, associate into a stable quaternary complex at the cardiac SR junctional membrane (b) the interactions between these proteins are Ca2+-sensitive; and (c) this complex regulates SR Ca2+storage and release [42].

Indeed, alterations in the levels of the ryanodine receptor, triadin, junctin, and calsequestrin may affect Ca2+ storage in the SR, the amount of Ca2+ which is available to be released from the SR, and the amount of SR Ca2+ leak during diastole, thus contributing to the pathology of the failing heart [43].

Ryanodine receptor: Ryanodine receptor channels are part of massive macromolecular complexes that play a critical role in excitation-contraction coupling in the heart. Alterations in levels of the ryanodine receptor and accessory proteins that regulate the activity of the ryanodine receptor affect cardiac Ca2+ homeostasis [42,44,45].

Increased SR Ca2+ leak through the ryanodine receptor has been suggested as a significant component of altered excitation-contraction coupling in heart failure. Decreased mRNA expression levels of the ryanodine receptor in human ischemic and dilated cardiomyopathy [46].

PKA hyper phosphorylation of ryanodine receptor channels dissociated the regulatory subunit FKBP12.6 from the channel in canine and human failing myocardium, destabilizing the channel and resulting in the increased opening of the channel and disturbed functional coupling of ryanodine receptors. Furthermore, destabilization and reduced functional coupling of ryanodine receptor channels, as a result of a disturbing interaction with FKBP12.6, may result in defective closure of ryanodine receptor channels, which could be associated with diastolic SR Ca2+ leak, depletion of SR Ca2+ load, and reduced excitation-contraction coupling [47,48].

In light of the evidence that increased SR Ca2+ leak is detrimental and that disrupted interactions between FKBP12.6 and the ryanodine receptor result in SR Ca2+ leak, FKBP12.6 may be a therapeutic target to stabilize the ryanodine receptor [46].

The ryanodine receptor is part of a macromolecular complex and proper formation of a stable Ca2+ release complex at the cardiac SR junctional membrane is required for appropriate regulation of Ca2+ release from the SR. Thus, increasing the regulation of the ryanodine receptor and decreasing spontaneous SR leak may be a promising therapeutic strategy for heart failure. Indeed, normalizing intracellular Ca2+ handling through regulation of expression and function of the ryanodine receptor may prove to be beneficial in cardiac disease [49].

Calsequestrin: Calsequestrin, a high-capacity calcium-binding protein located in the lumen of the SR, is important in SR Ca2+ storage and release. calsequestrin overexpression revealed that exceptionally heavy Ca2+ buffering by calsequestrin strongly depresses fractional SR Ca2+ release, triggering a cascade of molecular events that activate a program of cardiac hypertrophy that may transition to heart failure [50].

Adenoviral-induced inhibition of calsequestrin (antisense) or expression of a mutant calsequestrin (mutation in the calcium-binding domain of calsequestrin), associated with human genetic ventricular tachycardia, revealed a reduced SR Ca2+ load and spontaneous SR Ca2+ release [51]. calsequestrin is a key determinant of the amount of Ca2+ released by the ryanodine receptor channel through stabilization of local luminal Ca2+ in the vicinity of ryanodine receptor channels and modulation of the Ca2+ dependent closure of the channels [46].

Calsequestrin is a luminal Ca2+ sensor that inhibits ryanodine receptor channel activity at low luminal Ca2+ and that triadin and junctin are required to mediate the interaction of calsequestrin with the ryanodine receptor. Thus, calsequestrin plays an integral role in excitation-contraction coupling, in combination with the ryanodine receptor, triadin, and junctin, and a role for calsequestrin in the regulation of SR Ca2+ storage and release may be more complex than originally speculated. An impaired ability of the SR to sequester and subsequently release Ca2+ may contribute to the pathology of the failing heart [42,50].

Triadin and junctin: Two anchoring proteins, junctin and triadin, associate with each other, the ryanodine receptor, and calsequestrin, stabilizing the quaternary structure at the junctional SR membrane and regulating SR Ca2+ storage and release [42].

junctin and triadin act not only as structural anchoring proteins between calsequestrin and the ryanodine receptor, but also as important regulators of Ca2+ handling and contractile properties in the heart during excitation-contraction coupling. Naturally occurring heritable mutations in triadin and junctin may also be associated with cardiac disease. Nevertheless, triadin and junctin polymorphisms have not been linked to cardiomyopathies thus far. Further investigation of the expression levels and function of triadin and junctin in the failing heart, as well as the exploration of polymorphisms in triadin and junctin, will provide critical information about whether triadin and junctin may be potential therapeutic targets [52].

Role of Histidine-rich calcium-binding protein on SR Ca2+ cycling: Histidine-rich calcium-binding protein (HRC) regulates Ca2+ homeostasis in the heart. There is debate over whether HRC is located on the cytoplasmic surface of the SR or in the SR lumen. However, the strongest evidence suggests that HRC is a luminal SR protein. The deduced amino acid sequence of HRC revealed structural features that are analogous to calsequestrin, suggesting that HRC may play a similar role to calsequestrin during excitation-contraction coupling, as a Ca2+ storage protein [42, 53].

HRC is an integral regulatory protein in SR Ca2+ sequestration and cardiac function. Cardiac-specific overexpression of HRC in transgenic mice resulted in impaired SR Ca2+ uptake, leading to delayed cardiomyocyte Ca2+ transient decay, cardiac remodeling, and left ventricular dysfunction, which progressed to heart failure [54].

Decreased protein levels of HRC have been reported in human and animal models of heart failure. Identified naturally occurring mutations in the HRC gene of normal individuals, specific HRC polymorphisms have not yet been linked to cardiac disease. Further elucidating the physiological role of HRC in SR Ca2+ cycling, investigating its expression levels in the failing heart, and exploring if naturally occurring mutations exist in HRC and correlate with cardiac disease, have not yet investigated. HRC may be a target for heart failure therapy. Indeed, if HRC is a critical protein involved in SR Ca2+ cycling, targeting HRC may be a potential therapeutic strategy for the treatment of cardiac diseases [55].

Inhibitor-1 is potential target for enhancing SR Ca2+ loading in failing hearts: Dysregulated Ca2+ homeostasis is a hallmark of heart failure that is directly linked to cardiac dysfunction and lethal arrhythmia. It is associated with an imbalance of kinases/phosphatase activities in the corresponding intracellular microdomains, leading to either hypophosphorylation of phospholamban (PLN), hyperphosphorylation of ryanodine receptor (RyR) or a combination of both in failing cardiomyocytes. It has been increasingly recognized that dysregulation of protein phosphatase 1 (PP1) signaling is associated with the imbalance of kinase/phosphatase in the failing heart [56,57].

PP1 inhibition has been thought of as a potential molecular target for restoring impaired Ca2+ cycling mediated by the sarcoplasmic reticulum (SR). In this regard, the role of inhibitor-1 (I-1), an endogenous PP1 inhibitor, has been drawing attention, not only for understanding the mechanism of increased PP1 activity in failing hearts but also for its application as a potential therapeutic target [58].

I-1 is a ubiquitously expressed acid- and heat-stable cytosolic protein that is primarily rich in skeletal and cardiac muscle. When I-1's Thr35 residue is phosphorylated by protein kinase A (PKA), I-1 actively inhibits PP1 activity, potentiates PKA phosphorylation, and enhances cardiac contractility. On the other hand, when the Ser67 or Thr75 residues are phosphorylated by protein kinase C α (PKAα) or the Thr35 residue is dephosphorylated by PP2B, I-1 becomes inactive, increasing PP1 activity and suppression of cardiac contractility [59].

I-1 is also recognized as an important element of β-adrenergic signaling, as well as Gq-receptor coupled signaling (angiotensin II, endothelin, and α-adrenergic signaling). Indeed, the constitutive I-1 expression caused an increase in PLN phosphorylation at Ser16, thereby increasing the amplitude of the Ca2+ transient and decreasing its decay time. Thus, I-1 is considered to be a potential agent for optimizing SR Ca2+ handling in the diseased heart [60].

Role of FK506-Binding Protein FKBP12.6 in heart failure: The FK506-binding protein FKBP12.6 is tightly associated with the cardiac sarcoplasmic reticulum (SR) Ca2+ release channel (ryanodine receptor type 2 [RyR2]), but the physiological function of FKBP12.6 is unclear [61]. Adenoviral gene transfer results in overexpression of FKBP12.6 in rabbit myocytes indicate 3 significant effects of FKBP12.6 overexpression: [1] reduction of RyR2-mediated Ca2+ efflux from cardiac muscle SR; [2] higher SR-Ca2+ load; and [3] increased amplitude of twitch shortening in single myocytes [62].

Cyclic ADP ribose is thought to increase the open probability of RyR, increase peak systolic Ca2+, and increase twitch shortening in guinea pig myocytes. In contrast, tetracaine, a drug that decreases Ca2+ sensitivity of RyR2, had no effects on rat-myocyte shortening in the steady-state. Furthermore, caffeine, a drug that increases the Ca2+ sensitivity of the RyR2, can decrease the peak systolic Ca2+ [63].

Overexpression of FKBP12.6 reduces Ca2+ leak from the SR during diastole, thereby increasing SR-Ca2+ content, and thus increases the amount of Ca2+ available for release, which in turn increases twitch shortening amplitude. FKBP12.6 overexpression may alter not only the Ca2+ efflux through RyR2 but also the degree of cooperative activity of the RyR2 cluster [64].

Rapamycin significantly decreased the rate of Ca2+ uptake at 1 mmol/L in myocytes overexpressing FKBP12.6. Decreased Ca2+ flux through RyR2 observed on overexpression of FKBP12.6 could be reversed by rapamycin. This is consistent with the interpretation that increased cytosolic levels of FKBP12.6 are the cause of altered RyR2 properties with reduced Ca2+ leakage through RyR2 [65].

Effect of Alterations of the β-adrenergic Cascade in SR Ca2+ Cycling: Chronic stimulation of β-adrenergic receptors (β-AR) by elevated plasma catecholamines and the subsequent stimulation of the cAMP/PKA-dependent signaling pathway represents a central factor in the pathogenesis of HF. Three β-AR subtypes have been described in mammalian hearts, the β1-, the β2- and the β3-AR with the β1- and β2-AR subtypes functionally dominating. Both are expressed in cardiac myocytes, couple primarily to the stimulatory G-protein Gs, and mediate the formation of cAMP. In addition, the coupling of the β2-AR to the inhibitory G-protein Gi has been described in several animal species, and in failing human cardiomyocytes. The effects of Gi coupling were evident in the failing human heart from the rescue of β-AR sensitivity after pertussis toxin treatment of isolated myocytes [66,67].

cAMP leads to activation of protein kinase A (PKA) which phosphorylates key regulators of contraction and relaxation as well as of gene transcription in cardiac myocytes. A switch to Gi-coupling, mediated by a PKA dependent phosphorylation of the β2-AR itself, is thought to protect against apoptosis by a number of mechanisms [68,69].

Heart failure is accompanied by an increased global phosphatase activity, as well as SR associated type 1 (PP-1) phosphatase. Overexpression of PP-1 catalytic subunit in the mouse heart, to similar levels as those observed in human failing hearts, caused depressed function and dilated cardiomyopathy [70]. PP-1 activity is regulated by the endogenous I-1 inhibitor. I-1 becomes active upon its phosphorylation at Thr35 by PKA, allowing for amplification of the β-adrenergic responses in the heart. I-1 is also phosphorylated at Ser67 and/or Thr75 by PKC and this result in enhanced phosphatase activity, depressed function as well as attenuation of the stimulatory effects of protein kinase A signaling [71].

Inhibitor-2, another endogenous PP-1 inhibitor, has also been shown to increase cardiac contractility and Ca2+ cycling in a transgenic model and inhibition of PP-1 by inhibitor-2 gene delivery ameliorates heart failure progression in a model of genetic cardiomyopathy [68].

Recently compartmentalization of different components of the cAMP signaling pathway has raised great interest. β-ARs, G-proteins, adenylyl cyclases, PKA anchoring proteins, phosphatases and phosphodiesterases have been reported to be localized in distinct cellular sub-compartments and cause subcellular compartmentalization of cAMP. The topography of cardiac myocytes is altered in heart failure with loss of T-tubules and this could also affect the localization of β-ARs and the β-adrenergic signaling cascade [72].

TPEN activates Ca2+ release through RyR and inhibits the activity of the SR Ca2+ pump: TPEN modifies the calcium homeostasis of cardiac muscle cells via altering the activity of two of the most important SR membrane proteins, the RyR and the SERCA pump. On the molecular level, the precise mechanism of action of TPEN on the RyRs remains elusive [73].

TPEN could act directly on the RyRs, binding to the channel on the cytosolic side and activating the opening of the channel or make it more sensitive to Ca2+. It could also diffuse into the SR and bind to the RyRs on the luminal side thereby activating the channel. Moreover, there is the possibility of an indirect effect of TPEN via a protein other than the RyR or by lowering heavy metal concentrations inside the cell. TPEN has a low affinity for Mg2+ and Ca2+ but a high affinity for several heavy metal ions known to have effects on multiple targets within the cell. Binding of TPEN to Zn2+, an inhibitor of the RyRs in the cardiac cell, might remove this inhibition, thereby increasing the leak through the channel [74].

TPEN somehow altered the spontaneous RyR activity in intact cells to give rise to a larger than usual Ca2+ leak from the SR. Thus, SR depletion would be a secondary effect after RyR activation. This conclusion is strongly supported by the observation of an increased open probability of RyRs reconstituted into lipid bilayers in the presence of TPEN. Thus, the effect of TPEN is somewhat similar to a low concentration of caffeine, which opens the RyRs and produces long-lasting openings. Thereby the SR becomes more leaky and generates more spontaneous Ca2+ sparks which ultimately deplete the SR [75].

Reduced Ca2+ signals during congestive heart failure resulting in hyperphosphorylation of the RyRs due to excessive adrenergic stimulation is thought to render the channels overly sensitive to Ca2+ triggers, resulting in spontaneous Ca2+ leaks ultimately contributing to reduced SR Ca2+ content, attenuated Ca2+ transient amplitudes and finally compromised mechanical performance of the heart. There is a direct interaction of TPEN with the Ca2+ pump, the activity of the SERCA pump was examined in isolated SR vesicle preparations. To estimate the magnitude of the effect, the hydrolytic activity of HSR vesicles was determined at different TPEN concentrations. TPEN inhibits the ATPase activity of the Ca2+ pump [57,76,77].

CONCLUSION

Cardiovascular diseases are the main cause of morbidity and mortality in the world. Decreased SERCA2a content in the SR of patients with failing hearts is responsible for its decreased contractility. In recent years there has been significant progress in understanding Ca2+ handling in the heart and how changes in Ca2+ homeostasis contribute to cardiac disease. Exploring the functional consequences of naturally occurring arrhythmogenic mutations in Ca2+ handling proteins such as RyR2 and CASQ2 has been especially instructive. These studies have led to a better understanding of the control of SR Ca2+ release at the molecular level in normal and disease settings. SERCA protein levels were found to be decreased by a greater proportion than protein levels of PLN, and that PLN was shown to be hypo phosphorylated in the failing myocardium. The magnitude and timing of the Ca2+ transient, which determines the strength of contraction, is dynamically regulated by phosphorylation of both Ca2+ handling pumps and ion channels. Several kinases, including protein kinase A (PKA), Ca2+/calmodulin-dependent protein kinase (CaMKII) and protein kinase C (PKC) contribute to these effects. Histidine-rich calcium-binding protein regulates Ca2+ homeostasis in the heart. I-1 is considered to be a potential agent for optimizing SR Ca2+ handling in the diseased heart. Decreased Ca2+ flux through RyR2 observed on overexpression of FKBP12.6 could be reversed by rapamycin.

Several novel therapies are currently being evaluated in animal models, and the beneficial effects of SERCA2a-targeted therapies in patients with heart failure have been shown in clinical trials. A focus on Ca2+-handling proteins will ultimately guide the future development of novel treatment modalities for patients with heart failure.

ABBREVIATION/ACRONYM

β-AR: β-adrenergic receptors; CaMKII: Ca2+/calmodulin-dependent protein kinase; CHF: Chronic heart failure; CICR: Ca2+-induced Ca2+ release; HRC: Histidine-rich calcium binding protein; I-1: Inhibitor-1; LTCC: L-type Ca2+ channels; LVEF: Left ventricular ejection fraction; NCX: Na+/ Ca2+ exchanger; PKA: Protein kinase A; PKC: Protein kinase C; PLB: Phospholamban; PP1: Protein phosphatase 1; RyR2: Ryanodine receptor-2; SERCA2a: Sarcoplasmic reticulum ATP-ase; SGLT-2I: Sodium-glucose cotransporter-2 inhibitors; SOCE: Store-operated Ca2+ entry; SR: Sarcoplasmic reticulum.

COMPETING INTERESTS

The authors declared that they do not have any conflict of interest.

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AUTHORS' CONTRIBUTION

All authors were involved in the design, write up and preparing the manuscript to be submitted. All authors have read and agreed the manuscript.

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