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Refilling Intracellular Calcium Stores

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Abstract

Within the cardiac cell, the movements of calcium ions are tightly regulated by a number of regulatory proteins including pumps, and channels. The sarcoplasmic reticulum (SR) is in large part responsible for orchestrating these movements for the normal functioning of the cardiomyocyte. Alterations of SR regulatory proteins in failing hearts leads to abnormal Ca^{2+} homeostasis and consequently to a deficient contractile state. This review focuses on the roles of SR Ca^{2+} regulators in disease states and novel strategies for therapeutic targeting of these pathways.

Keywords

Calcium; Heart failure; Sarcoplasmic Reticulum; SERCA2a

Congestive heart failure (CHF) is a clinical syndrome in which the heart is unable to pump enough blood to meet the metabolic demands of various organs in the body. CHF is a leading cause of death and morbidity in modern societies and its incidence continues to grow especially as the population ages (1). Recent treatments for CHF, focused on blocking neurohormonal pathways, increase survival, however they do not completely prevent the progress of HF. End-stage CHF has a poor prognosis, and therapeutic choices are still limited. In this context, many investigators are exploring molecular targets that can improve excitation–contraction (E–C) coupling which is deficient in CHF (2,3).

The endoplasmic reticulum (ER) plays an essential role as a signal-transducing organelle and in cardiac muscle there is a specialized ER sub-domain known as the sarcoplasmic reticulum (SR) (4). The cardiac SR is an intracellular network that surrounds the contractile apparatus. It serves not only as a calcium ion (Ca^{2+}) pool for Ca^{2+} release but it also maintains cytosolic Ca^{2+} concentrations during contraction and relaxation (5). One of the key abnormalities in HF is abnormal SR Ca^{2+} cycling which is the result of a number of processes including Ca^{2+} leak from the SR via cardiac ryanodine receptor (RyR2), and depressed SR Ca^{2+} re-uptake (6). Impaired SR Ca^{2+} re-uptake mainly caused by low expression of cardiac Sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA2a) pump, and also by an increased activity of endogenous inhibitor of SERCA2a, phospholamban (PLN).

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In this review, we aim to highlight recent insights obtained about the role and mechanisms of SR Ca^{2+} cycling and discuss the capability of SR Ca^{2+} handling proteins as a therapeutic target in heart failure.

SR Ca^{2+} cycling

Ca^{2+} is a highly versatile intracellular signaling molecule that regulates many different cellular processes, from fertilization to cell death. At any moment in time, the level of intracellular Ca^{2+} is tightly regulated by a balance between the release of Ca^{2+} into the cytosol and the removal of Ca^{2+} by the combined action of buffers, pumps and channels. Each cell type expresses a unique set of components from the Ca^{2+} -regulatory components to create Ca^{2+} -signalling systems with different spatial and temporal properties (7).

Figure 1 presents the mechanisms of SR Ca^{2+} cycling in the heart. During excitation-contraction (EC) coupling, Ca^{2+} entry through the L-type Ca^{2+} channels (LTCC) triggers Ca^{2+} release from the SR through RyR2. The combination of Ca^{2+} influx and release raises free intracellular Ca^{2+} concentration thereby allowing Ca^{2+} to associate to the myofilament proteins, troponin C resulting in sarcomere shortening and muscle contraction. Subsequent muscle relaxation is initiated by RyR2 closure accompanied by Ca^{2+} dissociation from the troponin complex. Ca^{2+} reuptake into the SR Ca^{2+} stores is catalyzed by SERCA2a and partially extruded to the external medium through the action of plasma-membrane Ca^{2+} ATPase (PMCA). In humans, SERCA2a removes 70% of Ca^{2+} and the rest is removed from cardiomyocyte by the activities $\text{Na}^{2+}/\text{Ca}^{2+}$ exchanger (NCX, 28%) and PMCA (2%) (5). Several protein kinases and phosphatases are also associated with SR Ca^{2+} cycling such as protein kinase A (PKA), Ca^{2+} /calmodulin kinase (CaMKII), and protein phosphatase type 1 (PP1).

The β -adrenergic receptors (β -AR), which belong to the family of G-protein coupled receptors, transmit exogenous signals that activate effector proteins within cardiomyocytes. Once a specific β -AR is activated, signal transduction is initiated by the activation of oligomeric G proteins. For instance, G_1 -ARs activate G_s proteins through increased cyclic AMP (cAMP) level via adenylyl cyclases. G_s signaling, acts as the stimulator, initiates the activation of the (PKA) and cAMP, which modulate the activity of key proteins for cardiac function, including LTCC, RyR2, PLN, troponin I, hyperpolarization-activated cyclic-nucleotide-gated channels, and myosin-binding proteins. In contrast, G_i signaling, acts as an inhibitor of the system, decreases cAMP levels, activates mitogen-activated protein kinases (MAPKs), and modulates receptor signaling and the activation of transcriptional factors. Moreover, decreased cardiac β -AR density, β -AR de-sensitization, and activated G protein-coupled receptor kinases (GRK) are important feature of chronic HF patients (8). These abnormalities are implicated in the decreased responsiveness of β -AR in HF cardiac myocytes.

SR Ca^{2+} handling proteins in HF

Defective intracellular Ca^{2+} homeostasis through alternations of Ca^{2+} handling proteins has been investigated in both experimental and human HF (table 1). A decrease in lusitropy in HF is characterized by a prolonged decay of Ca^{2+} transient and increased diastolic intracellular Ca^{2+} (9). These defects have been linked to at least three factors: (i) decreased Ca^{2+} re-uptake into the SR, (ii) decreased Ca^{2+} storage of the SR, and (iii) Ca^{2+} leak from the SR. Recently several proteins are reported as major regulators.

SR Ca²⁺ load

SR Ca²⁺ uptake is mainly regulated by SERCA2a Ca²⁺ pump. SERCA2a activity inhibited via PLN phosphorylation by reducing its affinity for Ca²⁺. In mammals, three different genes (*ATP2A1-3*) each encode at least two different SERCA protein isoforms. SERCA2 is the predominant variant of all SERCA isoforms and phylogenetically the oldest. Three different splice transcripts are reported, SERCA2a, SERCA2b, and SERCA2c, which only differ at the C-terminus. SERCA2a is mainly expressed in the heart, on the other hand, low amounts of SERCA2b (10) and possibly even lower expressions of SERCA2c have also been detected. Functional differences between the isoforms SERCA2a and SERCA2b were demonstrated from studies of transgenic animals. Long-term expression of SERCA2a in transgenic animals improves contractility without harmful effects, but substitute of SERCA2a to SERCA2b isoform causing cardiac dysfunction and hypertrophy (11,12). A number of studies collectively have shown that significantly reduced SERCA2a expression and activity in failing myocardium. Several studies in human hearts, primarily explanted human hearts with advanced heart failure, suggest that SR Ca²⁺ transport function is altered in end-stage human heart failure. A decrease in the level of SERCA2a pump (mRNA, protein, or activity) has been strongly correlated with decreased myocardial function and impaired force-frequency response (13,14). Impaired re-uptake of Ca²⁺ into the SR, reflected by a reduced SERCA2a protein level, would at least partially explain the lower SR Ca²⁺ concentration and intracellular Ca²⁺ transient.

As an endogenous regulator of SERCA2a, PLN controls the affinity of SERCA2a for Ca²⁺. With an unchanged PLN level in HF, the PLN/SERCA2a ratio increases, diminishing Ca²⁺ affinity of SERCA2a. Moreover, decreased PLN phosphorylation by PKA and CaMKII at Ser16 and Thr17, respectively, which relieves SERCA2a inhibition is a further complicating factor that would contribute to the impaired cardiac contractility of the failing heart (15,16). Dephosphorylation of PLN is also controlled by protein phosphatase 1 (PP1) activity which is increased in HF. However, PP1 is itself inhibited by its endogenous modulator, inhibitor-1 (I-1) which can be activated by PKA and inhibited by PKC (17).

SR Ca²⁺ store

During a regular Ca²⁺ transient only 40~60% of the total SR Ca²⁺ is released thereby leaving a sizable Ca²⁺ reserve within the SR. This implies that a number of mechanisms are activated to terminate the Ca²⁺ release process before the SR Ca²⁺ store is emptied.

Calsequestrin (CASQ), high-capacity Ca²⁺ binding protein in the SR lumen, have been shown to play a major role as SR Ca²⁺ storage which was bound to approximately 50~75% of Ca²⁺ in the SR in cardiomyocytes. Changes in calsequestrin levels are associated with cardiac remodeling and ventricular arrhythmias in rodent models (18).

Another SR luminal protein, histidine rich Ca²⁺ binding protein (HRC) has been reported to be an important SR Ca²⁺ storage protein, which binds to SERCA2a and triadin. Decreased HRC levels were detected in both HF patients and animal model of HF (19).

Two anchoring proteins, junctin and triadin, are transmembrane proteins that appear to associate with each other, RyR2 and CASQ. They stabilize the structure at the junctional SR membrane and regulate both SR Ca²⁺ storage and release (20). The TG animal results reveal a critical role for junctin and triadin in cardiac EC coupling (21,22). However, their expression levels in human HF have not been clearly assessed.

SR Ca²⁺ release

RyR2 channels are part of a massive macromolecular signaling complex, which play a crucial role in excitation-contraction coupling in the heart. During diastolic phase, the resting state of the cardiac cycle, RyR2 must lock tightly otherwise Ca²⁺ will leak uncontrollably into the cytosol from the SR. Thus, opening and closing of the tetrameric channel requires strict regulation by several inhibitory and activating accessory proteins. Recently, the phosphorylation state of RyR2 has been considered as the major trigger factor. In HF, PKA-mediated hyperphosphorylation of RyR2 at S2809 has been suggested to induce diastolic release of Ca²⁺ through dissociation of the channel-stabilizing protein, FKBP12.6 (23). More recently, other groups have shown that PKA-mediated hyperphosphorylation of RyR-S2809 plays no role in SR Ca leak (24). Currently, CAMKII phosphorylation of RYR2 has emerged as an important modulator of the channel function in diseased states (25).

Therapeutic targets for HF

Restoring SR Ca²⁺ transport has been the focus of sustained investigations in the past few years to rescue HF. Among the major SR proteins that have been investigated, altering SERCA2a and/or PLN levels or activity to restore disturbed Ca²⁺ uptake into the SR and preventing SR Ca²⁺ leak have been proposed as a promising therapeutic strategies for HF.

SERCA2a restoration

Transgenic animal models have been developed to define the beneficial role of the SERCA pump in Ca²⁺ homeostasis and cardiac physiology. Transgenic (TG) mice overexpressing SERCA2a by 1.5-fold resulted in SR Ca²⁺ transport and enhanced rates of cardiac contractility (26,27). No cardiac pathology has been observed in these animals, suggesting that SERCA2a overexpression (albeit at low levels) can be tolerated by the heart. On the other hand, disruption of the SERCA2 allele is lethal, with the homozygous null (SERCA2^{-/-}) mice die early in development. Heterozygous (SERCA2^{+/-}) mice showed 65% of SERCA2 protein levels compared with wild type hearts. Although no cardiac pathology was exhibited, when stressed by pressure overload, they developed heart failure much more quickly than WT control (28). In addition, these mice show impaired intracellular Ca²⁺ homeostasis and decreased rates of cardiac contractile function (28,29). Thus, these studies employing TG and gene knockout animal models showed that SERCA pump level is a critical determinant of SR Ca²⁺ uptake function and changes in its level can modify baseline contractility.

Targeting of SERCA2a by pharmacological means has not yielded any specific agents. This has led to the consideration of gene therapy as a method to specifically enhance SERCA2a activity. Even though a number of vectors were used to deliver SERCA2a gene to the heart, recombinant adeno-associated vectors have proven to have the essential features for long term expression. Recombinant AAV based vectors are non-pathogenic, devoid of viral coding sequences, and mediate long-term gene expression in the absence of an immune or inflammatory response (30). Moreover, there is a serotype for specific tissue to target. In pre-clinical HF models including rodents, porcine, and sheep, AAV-mediated SERCA2a overexpression was well tolerated and significantly improves cardiac contractility, cardiac metabolism (as measured by the ratio of creatine phosphate to ATP ratio), and energy utilization (as measured by restoration of the O₂ consumption to E_{max} relationship to normal levels) even when the underlying pathophysiology or insult (eg, mitral valve rupture or pacing induced heart failure) was not removed (31–33). Especially, the restoration of the energetics is an important issue because increased myocyte contractility is associated with more energy requirement. These findings showed that beneficial remodelling following

SRECA2a overexpression occurred within the cardiomyocytes restoring the metabolic machinery to normal.

A first-in-human, phase 1 trial (CUPID) was recently conducted restoring the levels of this key enzyme in HF patients by gene transfer of the SERCA2a cDNA via a recombinant AAV vector (AAV1.SERCA2a). The results of the phase 1 study demonstrated an acceptable safety profile, and improvements at 6 months across symptomatic, functional, biomarker and LV function/remodeling parameters (34,35). More recently a phase 2 trial was completed of 39 patients with advanced HF who were randomized to receive intracoronary adeno-associated virus 1 (AAV1) mediated SERCA2a gene delivery (in one of 3 doses (low dose – 6×10^{11} DNase Resistant Particles (DRP), middle dose – 3×10^{12} DRP and high dose – 1×10^{13} DRP) versus placebo. At six months, the AAV1.SERCA2a treated patients, demonstrated improvement or stabilization in NYHA class, MLWHFQ, 6MWT, VO2 max, NT-proBNP levels, and LV end-systolic volumes. Significant increases in time to adjudicated CV events, and a decreased frequency of CV events per patient were observed on AAV1.SERCA2a. No increases in adverse events, disease-related events, laboratory abnormalities or arrhythmias were observed in AAV1.SERCA2a treated patients compared to placebo. Even though larger studies are needed to establish AAV.SERCA2a as a treatment modality for advanced HF, the CUPID trial demonstrated that SERCA2a is a critical target in the pathogenesis of HF.

Phospholamban inhibition

Many studies using genetically manipulated animal models have established PLN as an important factor of both contractility and β -agonist responses in the heart (36). Gene transfer of dominant-negative mutants of PLN such as K3E/R14E, V49A improved SR function and myocyte contractility in an animal model of HF. Adenoviral-mediated PLN ablation restored contractile function in human failing cardiomyocyte. These findings support the idea that targeting PLN can enhance cardiac contractility in HF.

More recently, to attenuate inhibitory effect of PLN on SERCA2a, AAV9-mediated expression of inhibitory RNA PLN or activation of PP1 led to dephosphorylation of PLN have been used to reduce PLN levels or activity (37).

RyR2 stabilization

FKBP12.6 affects the gating and stabilizes the function of the RyR2, while FKBP12.6 ablation in mice resulted in deregulation of Ca^{2+} release, cardiac hypertrophy, severe dilated cardiomyopathy with ventricular septal defects, and exercise-induced cardiac ventricular arrhythmias, which triggered sudden cardiac death. Mutant RyR2 in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT) have shown reduced affinity for FKBP12.6 and have similar defective single-channel properties as RyR2 from FKBP12.6^{-/-}. In addition, the sustained activation of the sympathetic nervous system in HF apparently results in PKA-induced hyperphosphorylation of RyR2, leading to FKBP12.6 dissociation and diastolic leakage of Ca^{2+} from the SR. The benzodiazepine derivative agent, JTV519 has been shown to improve cardiac function, by enhancing FKBP12.6-RyR2 interactions (38). More recently, a small molecule (S107) that prevents depletion of calstabin1 from the Ryanodine complex has also been shown to prevent SR Ca^{2+} leak (39,40). Thus, stabilization of RyR2 complex could be an important target for the treatment of HF.

Conclusion

SR calcium cycling is crucial to the process of excitation–contraction coupling and dysfunction of several calcium cycling proteins have been strongly associated with the pathogenesis of human heart failure. A large body of experimental findings to date have clearly demonstrated the concept that reversal of impaired calcium handling can prevent and/or partially reverse progression of heart failure. The clinical trial of gene delivery of AAV1.SERCA2a in patients with heart failure, with its positive biological signals, has clearly demonstrated that SERCA2a is an important therapeutic target. The new molecular based strategies for restoring calcium cycling may provide a new therapeutic paradigm for patients with severe heart failure.

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References

1. Lloyd-Jones D, Adams RJ, Brown TM, et al. Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation* 121:e46–e215. [PubMed: 20019324]
2. Gwathmey JK, Slawsky MT, Hajjar RJ, Briggs GM, Morgan JP. Role of intracellular calcium handling in force-interval relationships of human ventricular myocardium. *J Clin Invest* 1990;85:1599–613. [PubMed: 2332508]
3. Hasenfuss G, Pieske B. Calcium cycling in congestive heart failure. *J Mol Cell Cardiol* 2002;34:951–69. [PubMed: 12234765]
4. Pozzan T, Rizzuto R, Volpe P, Meldolesi J. Molecular and cellular physiology of intracellular calcium stores. *Physiol Rev* 1994;74:595–636. [PubMed: 8036248]
5. Bers DM. Cardiac excitation-contraction coupling. *Nature* 2002;415:198–205. [PubMed: 11805843]
6. Meyer M, Schillinger W, Pieske B, et al. Alterations of sarcoplasmic reticulum proteins in failing human dilated cardiomyopathy. *Circulation* 1995;92:778–84. [PubMed: 7641356]
7. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* 2003;4:517–29. [PubMed: 12838335]
8. Bristow MR. beta-adrenergic receptor blockade in chronic heart failure. *Circulation* 2000;101:558–69. [PubMed: 10662755]
9. Morgan JP, Erny RE, Allen PD, Grossman W, Gwathmey JK. Abnormal intracellular calcium handling, a major cause of systolic and diastolic dysfunction in ventricular myocardium from patients with heart failure. *Circulation* 1990;81:III21–32. [PubMed: 2153479]
10. Vangheluwe P, Louch WE, Ver Heyen M, Sipido K, Raeymaekers L, Wuytack F. Ca²⁺ transport ATPase isoforms SERCA2a and SERCA2b are targeted to the same sites in the murine heart. *Cell Calcium* 2003;34:457–64. [PubMed: 14572804]
11. He H, Giordano FJ, Hilal-Dandan R, et al. Overexpression of the rat sarcoplasmic reticulum Ca²⁺-ATPase gene in the heart of transgenic mice accelerates calcium transients and cardiac relaxation. *J Clin Invest* 1997;100:380–9. [PubMed: 9218515]
12. Baker DL, Hashimoto K, Grupp IL, et al. Targeted overexpression of the sarcoplasmic reticulum Ca²⁺-ATPase increases cardiac contractility in transgenic mouse hearts. *Circ Res* 1998;83:1205–14. [PubMed: 9851937]
13. Bavendiek U, Brixius K, Munch G, Zobel C, Muller-Ehmsen J, Schwinger RH. Effect of inotropic interventions on the force-frequency relation in the human heart. *Basic Res Cardiol* 1998;93 (Suppl 1):76–85. [PubMed: 9833134]
14. Arai M, Alpert NR, MacLennan DH, Barton P, Periasamy M. Alterations in sarcoplasmic reticulum gene expression in human heart failure. A possible mechanism for alterations in systolic and diastolic properties of the failing myocardium. *Circ Res* 1993;72:463–9. [PubMed: 8418995]

15. Schwinger RH, Munch G, Bolck B, Karczewski P, Krause EG, Erdmann E. Reduced Ca(2+)-sensitivity of SERCA 2a in failing human myocardium due to reduced serin-16 phospholamban phosphorylation. *J Mol Cell Cardiol* 1999;31:479–91. [PubMed: 10198180]
16. Haghighi K, Gregory KN, Kranias EG. Sarcoplasmic reticulum Ca-ATPase-phospholamban interactions and dilated cardiomyopathy. *Biochem Biophys Res Commun* 2004;322:1214–22. [PubMed: 15336969]
17. Rodriguez P, Mitton B, Nicolaou P, Chen G, Kranias EG. Phosphorylation of human inhibitor-1 at Ser67 and/or Thr75 attenuates stimulatory effects of protein kinase A signaling in cardiac myocytes. *Am J Physiol Heart Circ Physiol* 2007;293:H762–9. [PubMed: 17416610]
18. Chopra N, Kannankeril PJ, Yang T, et al. Modest reductions of cardiac calsequestrin increase sarcoplasmic reticulum Ca2+ leak independent of luminal Ca2+ and trigger ventricular arrhythmias in mice. *Circ Res* 2007;101:617–26. [PubMed: 17656677]
19. Arvanitis DA, Vafiadaki E, Fan GC, et al. Histidine-rich Ca-binding protein interacts with sarcoplasmic reticulum Ca-ATPase. *Am J Physiol Heart Circ Physiol* 2007;293:H1581–9. [PubMed: 17526652]
20. Muller FU, Kirchhefer U, Begrow F, Reinke U, Neumann J, Schmitz W. Junctional sarcoplasmic reticulum transmembrane proteins in the heart. *Basic Res Cardiol* 2002;97 (Suppl 1):I52–5. [PubMed: 12479235]
21. Terentyev D, Cala SE, Houle TD, et al. Triadin overexpression stimulates excitation-contraction coupling and increases predisposition to cellular arrhythmia in cardiac myocytes. *Circ Res* 2005;96:651–8. [PubMed: 15731460]
22. Kirchhefer U, Neumann J, Bers DM, et al. Impaired relaxation in transgenic mice overexpressing junctin. *Cardiovasc Res* 2003;59:369–79. [PubMed: 12909320]
23. Gyorke S, Terentyev D. Modulation of ryanodine receptor by luminal calcium and accessory proteins in health and cardiac disease. *Cardiovasc Res* 2008;77:245–55. [PubMed: 18006456]
24. Guo T, Cornea RL, Huke S, et al. Kinetics of FKBP12.6 binding to ryanodine receptors in permeabilized cardiac myocytes and effects on Ca sparks. *Circ Res* 2010;106:1743–52. [PubMed: 20431056]
25. Chelu MG, Sarma S, Sood S, et al. Calmodulin kinase II-mediated sarcoplasmic reticulum Ca2+ leak promotes atrial fibrillation in mice. *J Clin Invest* 2009;119:1940–51. [PubMed: 19603549]
26. Loukianov E, Ji Y, Grupp IL, et al. Enhanced myocardial contractility and increased Ca2+ transport function in transgenic hearts expressing the fast-twitch skeletal muscle sarcoplasmic reticulum Ca2+-ATPase. *Circ Res* 1998;83:889–97. [PubMed: 9797337]
27. Mork HK, Sjaastad I, Sande JB, Periasamy M, Sejersted OM, Louch WE. Increased cardiomyocyte function and Ca2+ transients in mice during early congestive heart failure. *J Mol Cell Cardiol* 2007;43:177–86. [PubMed: 17574269]
28. Periasamy M, Reed TD, Liu LH, et al. Impaired cardiac performance in heterozygous mice with a null mutation in the sarco(endo)plasmic reticulum Ca2+-ATPase isoform 2 (SERCA2) gene. *J Biol Chem* 1999;274:2556–62. [PubMed: 9891028]
29. Schultz Jel J, Glascock BJ, Witt SA, et al. Accelerated onset of heart failure in mice during pressure overload with chronically decreased SERCA2 calcium pump activity. *Am J Physiol Heart Circ Physiol* 2004;286:H1146–53. [PubMed: 14630633]
30. Hajjar RJ, del Monte F, Matsui T, Rosenzweig A. Prospects for gene therapy for heart failure. *Circ Res* 2000;86:616–21. [PubMed: 10746995]
31. Sakata S, Lebeche D, Sakata N, et al. Restoration of mechanical and energetic function in failing aortic-banded rat hearts by gene transfer of calcium cycling proteins. *J Mol Cell Cardiol* 2007;42:852–61. [PubMed: 17300800]
32. Kawase Y, Ly HQ, Prunier F, et al. Reversal of cardiac dysfunction after long-term expression of SERCA2a by gene transfer in a pre-clinical model of heart failure. *J Am Coll Cardiol* 2008;51:1112–9. [PubMed: 18342232]
33. Byrne MJ, Power JM, Prevolos A, Mariani JA, Hajjar RJ, Kaye DM. Recirculating cardiac delivery of AAV2/1SERCA2a improves myocardial function in an experimental model of heart failure in large animals. *Gene Ther* 2008;15:1550–7. [PubMed: 18650850]

34. Hajjar RJ, Zsebo K, Deckelbaum L, et al. Design of a phase 1/2 trial of intracoronary administration of AAV1/SERCA2a in patients with heart failure. *J Card Fail* 2008;14:355–67. [PubMed: 18514926]
35. Jaski BE, Jessup ML, Mancini DM, et al. Calcium upregulation by percutaneous administration of gene therapy in cardiac disease (CUPID Trial), a first-inhuman phase 1/2 clinical trial. *J Card Fail* 2009;15:171–81. [PubMed: 19327618]
36. del Monte F, Harding SE, Dec GW, Gwathmey JK, Hajjar RJ. Targeting phospholamban by gene transfer in human heart failure. *Circulation* 2002;105:904–7. [PubMed: 11864915]
37. Pathak A, del Monte F, Zhao W, et al. Enhancement of cardiac function and suppression of heart failure progression by inhibition of protein phosphatase 1. *Circ Res* 2005;96:756–66. [PubMed: 15746443]
38. Lehnart SE, Terrenoire C, Reiken S, et al. Stabilization of cardiac ryanodine receptor prevents intracellular calcium leak and arrhythmias. *Proc Natl Acad Sci U S A* 2006;103:7906–10. [PubMed: 16672364]
39. Fauconnier J, Thireau J, Reiken S, et al. Leaky RyR2 trigger ventricular arrhythmias in Duchenne muscular dystrophy. *Proc Natl Acad Sci U S A* 2010;107:1559–64. [PubMed: 20080623]
40. Bellinger AM, Reiken S, Dura M, et al. Remodeling of ryanodine receptor complex causes “leaky” channels: a molecular mechanism for decreased exercise capacity. *Proc Natl Acad Sci U S A* 2008;105:2198–202. [PubMed: 18268335]
41. Prestle J, Dieterich S, Preuss M, Bieligg U, Hasenfuss G. Heterogeneous transmural gene expression of calcium-handling proteins and natriuretic peptides in the failing human heart. *Cardiovasc Res* 1999;43:323–31. [PubMed: 10536662]
42. Marx SO, Reiken S, Hisamatsu Y, et al. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* 2000;101:365–76. [PubMed: 10830164]
43. Gergs U, Berndt T, Buskase J, et al. On the role of junctin in cardiac Ca²⁺ handling, contractility, and heart failure. *Am J Physiol Heart Circ Physiol* 2007;293:H728–34. [PubMed: 17400717]

Figure 1. The main mechanisms of SR Ca^{2+} cycling in the heart

Solid lines indicate the Ca^{2+} cycling in the cardiomyocyte. Upon a depolarizing signal, extracellular Ca^{2+} enters the cytosol via the LTTC, which triggers induces the release of a greater amount of Ca^{2+} through the RYR2 which initiates contraction at the myofilaments. Removal of Ca^{2+} during diastole is primarily facilitated via SERCA2a and to a lesser extent by the NCX, and PMCA. Increase the cytosolic Ca^{2+} concentration activates CaMKII. CaMKII phosphorylates the LTTC, RyR2, and PLN; Long dash dot lines. Dash lines delineate β -adrenergic stimulation, AC is activated, which leads to production of cAMP and PKA activation. PKA then phosphorylates the RyR2, TnI, and PLN, which augments contractility. Also, PKA phosphorylates I-1, and then I-1 activate PP1, which cause hypophosphorylation of PLN. AC, adenylyl cyclase; β -AR, β -adrenergic receptor; CASQ, calsequestrin; CaMKII, Ca^{2+} /calmodulin-dependent protein kinase; G α S, GTP binding protein; HRC, histidin-rich Ca^{2+} binding protein; I-1, inhibitor-1; LTTC, L-type Ca^{2+} channel; NCX, $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger; PKA, protein kinase A; PLN, phospholamban; PMCA, plasma-membrane Ca^{2+} -ATPase; RyR2: ryanodine receptor 2; SERCA2a, SR Ca^{2+} -ATPase; TnI: troponin I.

Table 1

Alterations of SR Ca²⁺ cycling proteins level in failing hearts

Protein Name	Molecular Function	Outcome in HF
SERCA2a	Catalyzes the hydrolysis of ATP coupled with the translocation of Ca ²⁺ from the cytosol into the SR lumen	Reduced (14,41)
PLN	Regulate the activity of the Ca ²⁺ pump of cardiac SR	Unchanged (41)
RyR2	Communication between transverse-tubules and SR. Contraction of cardiac muscle is triggered by release of Ca ²⁺ from SR following depolarization of T- tubules	Unchanged (18)
FKBP12.6	Associates with the RyR2 in cardiac muscle SR and play a unique physiological role in excitation- contraction coupling in cardiac muscle	Unchanged/ Dissociates FKBP12.6 from RyR2 (42)
CASQ2	Acts as an internal Ca ²⁺ store in muscle. The release of Ca ²⁺ bound to CASQ2 through a Ca ²⁺ release channel triggers muscle contraction	Unchanged (41)
HRC	May play a role in the regulation of Ca ²⁺ sequestration or release in the SR of skeletal and cardiac muscle	Reduced (19)
TRDN	May be involved in anchoring CASQ to the junctional SR and allowing its functional coupling with the RyR	Reduced (43) (may compensatory mechanism)
JCN	Forms a quaternary complex with the Ca ²⁺ release channel, RyR, TRD and CASQ2	Reduced (43) (may compensatory mechanism)

SR; sarcoplasmic reticulum; SERCA2a, SR-Ca²⁺ ATPase; PLN, phospholamban; RyR2, cardiac ryanodine receptor; FKBP12.6/calstabin2, FK506 binding protein 12.6; CASQ, calsequestrin; HRC, histidine-rich Ca²⁺ binding protein; TRDN, triadin; JCN, junction