State of the Art

Targeting Sarcoplasmic Reticulum Calcium Handling Proteins as Therapy for Cardiac Disease

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Sarcoplasmic reticulum (SR) calcium (Ca^{2+}) uptake and its subsequent release regulate excitation-contraction coupling of the heart. In cardiac cells (Figure 1), the SR serves as a reservoir from which Ca^{2+} is released into the cytosol via the ryanodine receptor and its associated proteins, initiating contraction of the heart.[1] Sequestration of Ca^{2+} from the cytosol into the SR lumen, and thus relaxation of the heart, is mediated by the SR Ca ATP-ase (SERCA) and its regulatory protein, phospholamban (PLN).[2] Several kinases and phosphatases regulate excitation-contraction coupling in the heart by altering the phosphorylation state of key Ca^{2+} handling proteins. Disturbed excitation-contraction coupling, in particular altered intracellular Ca^{2+} cycling, may underlie contractile dysfunction in human and animal models of heart failure.[3-6]

Heart failure is a major public health problem that affects nearly five million Americans, necessitates one million costly hospitalizations each year, and remains the leading cause of mortality and morbidity in developed nations.[7,8] Several lines of evidence report that much of the contractile deficit in human heart failure is due to diminished peak systolic Ca^{2+} transients, increased diastolic intracellular Ca^{2+} concentrations, and prolonged diastolic decay of myocyte Ca^{2+} transients (Figure 1B). A central factor limiting systolic Ca^{2+} transient amplitude is decreased SR Ca^{2+} content. Several studies reported a diminished SR Ca^{2+} content in heart failure,[9] and the fraction of SR Ca^{2+} released upon contraction is strongly influenced by the SR Ca^{2+} load.[10-14] Currently, there are two debated causes of SR Ca^{2+} depletion: (1) decreased SERCA Ca^{2+} uptake and/or an increased activity of the Na^{+}-Ca^{2+} exchanger; and (2) increased SR Ca^{2+} leak from the ryanodine receptor. There is an ongoing controversy over the contribution of each of these factors to unloading of the SR. However the relative contributions of each may vary among heart failure models and disease stages,[9,15] and it is likely that the molecular basis of heart failure may involve a combination of depressed SR Ca^{2+} uptake and enhanced SR Ca^{2+} leak.[16]

Indeed, elucidating the mechanism(s) responsible for impaired Ca^{2+} cycling in the failing heart is quite challenging, as there are many proteins involved. Alterations in expression levels, function, localization, and/or regulation of any one or a combination of these proteins may disturb intracellular Ca^{2+} homeostasis, leading to the development of heart failure. In this review, we will highlight the critical role of SR Ca^{2+} cycling proteins, SERCA, PLN, ryanodine receptor, calsequestrin, triadin, junctin, and the histidine-rich calcium binding protein (HRC), in the heart. We will also summarize studies of heart failure in human and animal models that have indicated that decreased SR Ca^{2+} uptake, alterations in SR Ca^{2+} storage, and abnormal SR Ca^{2+} re-

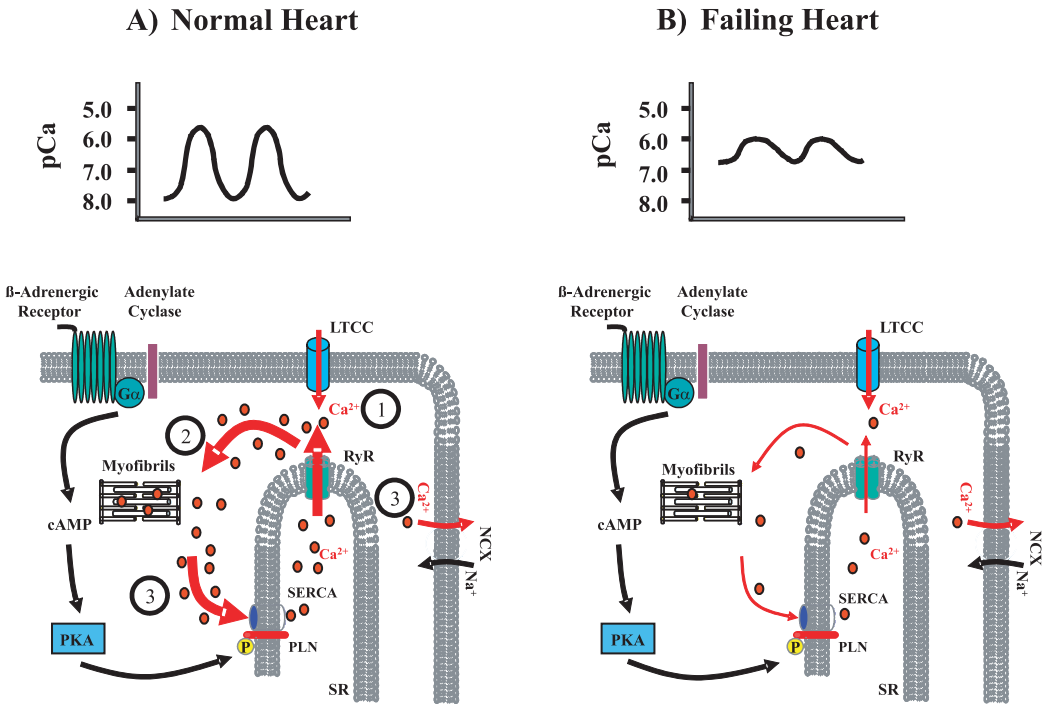

Figure 1. Schematic representation of sarcoplasmic reticulum SR Ca^{2+} cycling in normal and failing cardiomyocytes. A) Upon depolarization of the myocyte in the normal heart, Ca^{2+} enters through L-type Ca^{2+} channels (LTCC) (1), triggering a greater efflux of Ca^{2+} through the ryanodine receptors (RyR). Intracellular calcium binds to the myofibril contractile proteins, resulting in contraction of the heart (2). Subsequently, reuptake of Ca^{2+} by the SR Ca ATP-ase pump (SERCA) and/or extrusion by the sodium/calcium exchanger (NCX) initiates relaxation of the heart (3). Phospholamban (PLN) binds to SERCA in its dephosphorylated state and regulates SERCA activity. B) Myocytes in the failing heart are characterized by diminished Ca^{2+} transient amplitudes, increased diastolic Ca^{2+}, and prolonged decay of Ca^{2+} (upper panel), associated with abnormalities in excitation-contraction coupling and impaired function of the failing heart. (See text for other abbreviations).

lease contribute to cardiomyopathies, heart failure, and arrhythmias. In addition, we will review research that suggests that correcting abnormalities and imbalances of these Ca^{2+} cycling proteins, thus restoring SR Ca^{2+} cycling, may be a cardioprotective strategy in the failing heart. Finally, we will discuss naturally occurring mutations that have been identified in many SR Ca^{2+} handling proteins and associated with cardiac disease, supporting the critical role of SR Ca^{2+} cycling in the progression of cardiac disease.

Sarcoplasmic reticulum calcium uptake proteins as targets for heart failure

Sequestration of Ca^{2+} from the cytosol into the SR lumen, and thus relaxation of the heart, is mediated by SERCA and its regulatory protein PLN. Many studies in SERCA transgenic[17-19] and knockout[20,21] mice, as well as adenoviral-mediated SERCA gene transfer studies,[22,23] have suggested that there is a direct correlation between SERCA levels and modulation of cardiac contractility, supporting the notion that SERCA function is one of the fundamental determinants of cardiac contractility. PLN is a reversible inhibitor of SERCA. Phosphorylation of PLN results in an overall increase in the affinity of SERCA for Ca^{2+}, increasing SR Ca^{2+} uptake, while dephosphorylation of PLN by an SR-associated phosphatase restores the inhibitory effects of PLN on SERCA.[2] The role of PLN as a critical regulator of basal SR Ca^{2+} handling and contractility in the heart, due to PLN's ability to modulate SERCA Ca^{2+} uptake, SR Ca^{2+} load, and ultimately myocyte Ca^{2+} cycling, has been elucidated in gene knockout,[24,25] overexpression,[26] and superinhibitory mutant PLN studies.[27-30]

Since PLN and SERCA are major regulators of SR Ca^{2+} uptake and alterations in the levels or activity of either protein could contribute to decreased SR Ca^{2+} load, impaired myocyte Ca^{2+} cycling, and depressed contractile parameters in the failing heart, the expression levels and functional activity of these critical SR

Ca^{2+} handling proteins have been investigated in human heart failure. Several studies reported SERCA mRNA and protein expression levels to be significantly decreased in the failing human heart.[31,32] Interestingly, SERCA protein levels were significantly reduced in failing but not in compensated hypertrophied human myocyardium, although mRNA levels of SERCA were reduced in both hypertrophied and failing hearts, suggesting that a decrease in SERCA protein correlates with the development of myocardial failure.[33] In addition, several studies indicated decreased SERCA activity in the failing human myocardium.[31,32] Hasenfuss et al[34] demonstrated a significant correlation between SERCA protein level, SR Ca^{2+} uptake, and myocardial function in failing hearts. A decrease in PLN mRNA has been consistently observed in failing human myocardium; however, no alterations in PLN protein levels have been demonstrated in failing hearts to date.[35,36] Interestingly, hypophosphorylation of PLN was reported in human failing hearts,[37] and this may be due to increased protein phosphatase (PP1) activity in heart failure.[38] Given that SERCA protein levels were found to be decreased by a greater proportion than protein levels of PLN, and that PLN was shown to be hypophosphorylated in the failing myocardium, inhibition of SERCA is predicted to be more pronounced in the human failing myocardium compared to the non-failing myocardium, providing an explanation for the observed decrease in SERCA activity in the human failing heart.[39] Indeed, decreased SERCA activity, due to decreased SERCA protein levels and/or increased inhibition of SERCA activity by PLN, may be responsible for impaired removal of cytosolic Ca^{2+}, decreased SR Ca^{2+} load, and impaired SR Ca^{2+} release, which are characteristic of the failing heart.

Since it has been suggested that the PLN/SERCA ratio and the degree of PLN inhibition of SERCA are important determinants of depressed SR function and altered Ca^{2+} cycling in the failing human myocardium, SERCA and PLN are attractive therapeutic targets for heart failure. SERCA overexpression or PLN inhibition were remarkably successful in improving myocardial function in a variety of experimental heart failure models,[19,40-49] as well as in human heart failure.[50,51] However, PLN ablation did not rescue all forms of cardiomyopathy,[52-54] indicating that augmenting SR calcium cycling may not benefit all forms of heart failure, especially if impaired intracellular calcium cycling is not associated with the observed phenotype.

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. Although sequence analysis revealed some nucleotide changes in the SERCA2a genomic sequence, none of these genetic variations resulted in amino acid alterations, indicating that SERCA2a is highly conserved and the gene is tightly regulated, which may be essential for controlling intracellular Ca^{2+} homeostasis and excitation-contraction coupling. Three naturally occurring mutations were discovered in PLN; two were linked to dilated cardiomyopathy[55,56] and one was linked to hypertrophic cardiomyopathy.[57] These findings indicated that PLN may be a candidate gene responsible for cardiomyopathy since: a) long-term effects of the specific inhibition of PLN phosphorylation are deleterious and trigger dilated cardiomyopathy;[55] b) the absence of PLN in humans results in lethal heart failure;[56] and c) increased PLN promoter activity triggers hypertrophic cardiomyopathy.[57] Clearly, elucidating more specific roles that PLN plays in the development of cardiomyopathy may aid in the development and improvement of drugs for the treatment of cardiac disease and might ultimately lead to the generation of novel genetic therapy for human heart failure.

In summary, PLN is a critical regulator of basal SR Ca^{2+} handling and contractility in the heart, due to PLN's ability to modulate SERCA Ca^{2+} uptake, SR Ca^{2+} load, and, ultimately, myocyte Ca^{2+} 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 Ca^{2+} homeostasis and depressed contractility, but may be critical mechanisms underlying the heart failure phenotype. Thus, increasing the reuptake of Ca^{2+} 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.

Sarcoplasmic reticulum calcium storage/release proteins as targets for heart failure

While SERCA and PLN regulate Ca^{2+} uptake and relaxation of the heart, SR Ca^{2+} sequestration and the subsequent Ca^{2+} release are facilitated by a massive macromolecular ryanodine receptor complex, strategically localized at the site where a key step in excitation-contraction coupling occurs. Strong evidence suggests that: (a) four SR junctional proteins, the ryanodine receptor, triadin, junctin, and calsequestrin, associate into a stable quaternary complex at the cardiac SR junction-

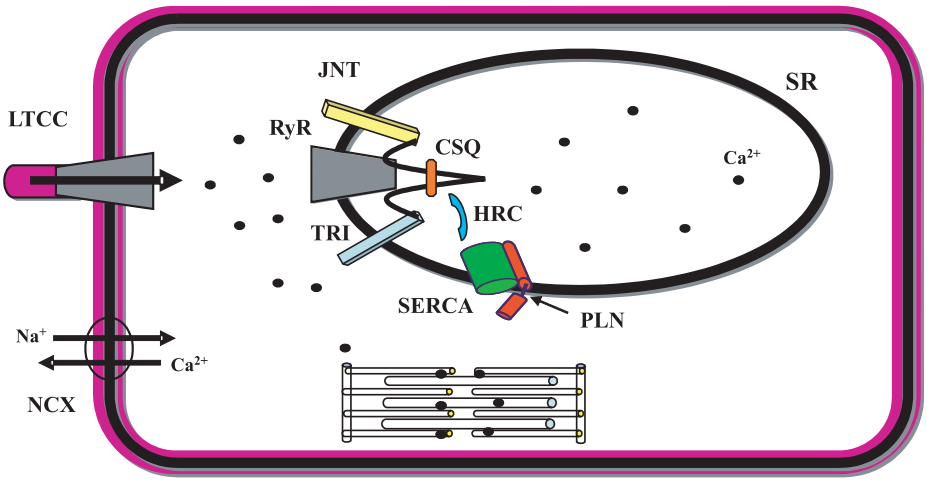

Figure 2. Schematic representation of the quaternary complex regulating SR Ca^{2+} storage and release. Ryanodine receptor (RyR) forms a stable quaternary complex at the cardiac SR junctional membrane with triadin (TRI), junctin (JNT), and calsequestrin (CSQ). The interactions between these proteins are Ca^{2+}-sensitive, and this complex is necessary for proper SR Ca^{2+} storage and release. A recently discovered histidine-rich calcium binding protein (HRC) also plays an important role in SR Ca^{2+} cycling. (See text for other abbreviations).

al membrane (Figure 2); (b) the interactions between these proteins are Ca^{2+}-sensitive; and (c) this complex regulates SR Ca^{2+} storage and release.[58,59] Since depletion of the SR Ca^{2+} load will impair the ability of the SR to release Ca^{2+} during systole to initiate contraction,[60] expression levels and activity of the critical proteins involved in SR Ca^{2+} storage and release have been investigated in human and experimental models of heart failure. Indeed, alterations in the levels of the ryanodine receptor, triadin, junctin, and calsequestrin may affect Ca^{2+} storage in the SR, the amount of Ca^{2+} which is available to be released from the SR, and the amount of SR Ca^{2+} leak during diastole, thus contributing to the pathology of the failing heart.

Ryanodine Receptor

Ryanodine receptor channels are part of massive macromolecular complexes which play a critical role in excitation-contraction coupling in the heart.[61,62] Alterations in levels of the ryanodine receptor and accessory proteins that regulate the activity of the ryanodine receptor affect cardiac Ca^{2+} homeostasis. Mice lacking the ryanodine receptor died at embryonic day 10 with morphological abnormalities in the heart tube, indicating that the ryanodine receptor is required for cellular Ca^{2+} homeostasis to maintain the developing SR.[63] Marks and colleagues[64,65] demonstrated that FKBP12.6 affects the gating and stabilizes the function of the ryanodine receptor channel, while FKBP12.6 ablation in mice resulted in dysregulation of Ca^{2+} release, cardiac hypertrophy,[66] severe dilated cardiomyopathy with ventricular septal defects,[67] and exercise-induced cardiac ventricular arrhythmias, which triggered sudden cardiac death.[65] Thus, altered ryanodine receptor activity may contribute to the pathology of the failing heart.

Since increased SR Ca^{2+} leak through the ryanodine receptor has been suggested as a significant component of altered excitation-contraction coupling in heart failure, several research laboratories have investigated ryanodine receptor levels and activity in human heart failure. There have been reports of decreased mRNA expression levels of the ryanodine receptor in human ischemic and dilated cardiomyopathy.[68] One study revealed decreased ryanodine binding,[68] other studies revealed no significant differences in binding,[69,70] while another study revealed increased ryanodine binding in failing human hearts.[71] At the protein level, there were no alterations in the ryanodine receptor.[31] Furthermore, the activity of the ryanodine receptor, measured under voltage-clamp conditions, showed normal properties in failing human hearts.[70,72] Hasenfuss et al[32] reported no differences in mRNA or protein expression levels of FKBP12.6 in failing and non-failing human myocardium from ischemic or dilated cardiomyopathy. One group of researchers reported hyperphosphorylation of the ryanodine receptor in human heart failure,[73] while another group found no differences between the phosphorylation state of the ryanodine receptor in failing and non-failing human myocardium.[70]

Although alterations in the protein expression levels of the ryanodine receptor or FKBP12.6 have not been reported in human heart failure, it is possible that disturbed interactions between the two proteins may exist in the failing heart. In various diverse animal models of heart failure, the degree of PKA phosphorylation of the ryanodine receptor correlated with the degree of cardiac dysfunction in these animal models, suggesting

that PKA hyperphosphorylation of the ryanodine receptor is likely to be a general feature of heart failure.[74] PKA hyperphosphorylation 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 increased opening of the channel and disturbed functional coupling of ryanodine receptors.[64,73] Furthermore, destabilization and reduced functional coupling of ryanodine receptor channels, as a result of a disturbed interaction with FKBP12.6, may result in defective closure of ryanodine receptor channels, which could be associated with diastolic SR Ca^{2+} leak, depletion of SR Ca^{2+} load, and reduced excitation-contraction coupling.[64,75] It is important to note that another laboratory reported no differences in the PKA-dependent phosphorylation of the ryanodine receptor, the association of FKBP12.6 with the ryanodine receptor, nor the activity of the ryanodine receptor channels at diastolic Ca^{2+} levels in failing human and canine myocardium, compared to non-failing myocardium.[70] Nevertheless, independently of the concept of ryanodine receptor hyperphosphorylation in heart failure, there is substantial evidence that a leak of Ca^{2+} from the SR not only decreases SR Ca^{2+} content and the subsequent release of systolic Ca^{2+} but may also trigger arrhythmias.[65,75-78]

In light of the evidence that increased SR Ca^{2+} leak is detrimental and that disrupted interactions between FKBP12.6 and the ryanodine receptor result in SR Ca^{2+} leak, FKBP12.6 may be a therapeutic target to stabilize the ryanodine receptor. Indeed, overexpression of FKBP12.6 in isolated rabbit cardiomyocytes reduced spontaneous SR Ca^{2+} leak, increased SR Ca^{2+} content, and promoted SR Ca^{2+} release during activation.[79] Furthermore, the use of the agent JTV519, which restored FKBP12.6-mediated stabilization of the ryanodine receptor, not only prevented SR Ca^{2+} leak through the ryanodine receptor, but also improved ventricular function and prevented the development of heart failure in a canine model,[77] as well as preventing fatal ventricular arrhythmias in a FKBP12.6 heterozygous knockout mouse model.[78] Thus, reducing SR Ca^{2+} leak may prove to be a therapeutic strategy in restoring SR Ca^{2+} cycling of the failing heart, as well as in preventing arrhythmias.

Naturally occurring heritable mutations in the ryanodine receptor are associated with stress-induced sudden cardiac death in humans.[80-84] These studies provide a link between altered ryanodine receptor channel function and exercise-induced ventricular arrhythmias and suggest that defective ryanodine receptor channel function, due to depletion of FKBP12.6, may be a molecular mechanism underlying arrhythmias. Although the mechanisms for the mutations remain to be elucidated, the greater amount of Ca^{2+} released from the mutant hearts may induce an elevated diastolic cytoplasmic Ca^{2+}, resulting in diastolic afterdepolarizations that can initiate fatal ventricular tachyarrhythmias.[65,85-87] Indeed, experimental data suggest that abnormal Ca^{2+} release by mutant ryanodine receptors is associated with the development of cardiac disease; thus, targeting deficient ryanodine receptor activity may be a therapeutic strategy to treat the common disorder of ventricular arrhythmias.

In summary, the ryanodine receptor is part of a macromolecular complex and proper formation of a stable Ca^{2+} release complex at the cardiac SR junctional membrane is required for appropriate regulation of Ca^{2+} release from the SR. Thus, increasing regulation of the ryanodine receptor and decreasing spontaneous SR leak may be a promising therapeutic strategy for heart failure. Indeed, normalizing intracellular Ca^{2+} handling through regulation of expression and function of the ryanodine receptor may prove to be beneficial in cardiac disease. However, much more intensive research must be performed to further investigate this possibility.

Calsequestrin

Calsequestrin, a high-capacity calcium binding protein located in the lumen of the SR, is important in SR Ca^{2+} storage and release. To elucidate the physiological significance of cardiac calsequestrin in excitation-contraction coupling, the levels of calsequestrin were altered in transgenic mice and isolated rat ventricular myocytes. Results in transgenic mice (10-20 fold calsequestrin overexpression) revealed that exceptionally heavy Ca^{2+} buffering by calsequestrin strongly depresses fractional SR Ca^{2+} release, triggering a cascade of molecular events which activate a program of cardiac hypertrophy that may transition to heart failure.[88-92] In contrast to the results in calsequestrin overexpressing mouse models, more moderate levels of adenoviral-induced overexpression (2-4 fold) of calsequestrin in adult ventricular myocytes resulted in increased SR Ca^{2+} load, enhanced Ca^{2+} transient and Ca^{2+} spark amplitude, and prolonged time of SR Ca^{2+} release.[93] Furthermore, 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 Ca^{2+} load and spontaneous

SR Ca^{2+} release.[93,94] Terentyev et al[93] demonstrated that calsequestrin is a key determinant of the amount of Ca^{2+} released by the ryanodine receptor channel through stabilization of local luminal Ca^{2+} in the vicinity of ryanodine receptor channels and modulation of the Ca^{2+}-dependent closure of the channels. In addition, Gyorke et al[59] recently demonstrated that calsequestrin is a luminal Ca^{2+} sensor that inhibits ryanodine receptor channel activity at low luminal Ca^{2+}, 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 Ca^{2+} storage and release may be more complex than originally speculated.

An impaired ability of the SR to sequester and subsequently release Ca^{2+} may contribute to the pathology of the failing heart; thus, many studies investigated the expression level of calsequestrin, a regulator of SR Ca^{2+} storage and release, in failing hearts. In multiple studies, levels of calsequestrin mRNA and protein were unaltered in human failing myocardium, compared to non-failing myocardium,[35,95-98] suggesting that the capacity of the SR to bind Ca^{2+} is not changed in the failing human myocardium.[31] Although total calsequestrin levels in heart failure were not altered, Kiarash et al[99] reported defective glycosylation of calsequestrin in a canine model of heart failure, suggesting that altered calsequestrin processing and protein or membrane trafficking may be defective in heart failure.

Interestingly, mutations in calsequestrin are associated with cardiac disease in human patients. Specifically, missense mutations in the calsequestrin gene are associated with catecholamine-induced polymorphic ventricular tachycardia (CPVT).[100,101] Data suggest that the underlying cause of CPVT might be related to abnormal Ca^{2+} release caused by reduced calsequestrin levels (due to nonsense mutations), as spontaneous Ca^{2+} release can induce inward currents and oscillations in the membrane potential, resulting in triggered arrhythmias.[93] Furthermore, point mutations resulting in loss of normal calsequestrin function may affect calsequestrin's ability to regulate Ca^{2+} release through the ryanodine receptor following excitation and result in the disease phenotype.[102] Given calsequestrin's recently proposed role in regulating ryanodine receptor Ca^{2+} release through stabilization of local luminal Ca^{2+} and modulation of the Ca^{2+}-dependent closure of the channels,[93] the abnormal regulation of the ryanodine receptor in the presence of reduced calsequestrin levels (or mutant calsequestrin) may explain the increased incidence of ventricular arrhythmias associated with mutations of calsequestrin.[93,94] Thus, pathological links have been established between specific, clinically-relevant mutations in the calsequestrin 2 gene and the arrhythmogenic behavior underlying CPVT. These studies provide new clues for further elucidation of the structural-functional relationships within the junctional Ca^{2+} signaling complex and for further development of therapeutic strategies to prevent or treat arrhythmias.

Thus, calsequestrin plays an integral role in excitation-contraction coupling, in combination with the ryanodine receptor, triadin, and junctin. Although calsequestrin protein levels are not altered in the failing myocardium, naturally occurring mutations in the calsequestrin gene must be further investigated to determine whether calsequestrin mutations may contribute to impaired Ca^{2+} handling in the failing heart. In addition, efforts to target these mutations and repair Ca^{2+} cycling may prove to be beneficial in the context of cardiac disease.

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 Ca^{2+} storage and release.[58,59] The data from transgenic mouse models with cardiac-specific overexpression of triadin[103,104] or junctin[105-107] suggest that junctin and triadin act not only as structural anchoring proteins between calsequestrin and the ryanodine receptor, but also as important regulators of Ca^{2+} handling and contractile properties in the heart during excitation-contraction coupling.

Although much research has suggested that triadin and junctin are critical proteins involved in SR Ca^{2+} storage and release, their expression levels in failing hearts have not been assessed. Since inherited cardiomyopathies may be associated with genes involved in SR Ca^{2+} cycling (PLN, ryanodine receptor, and calsequestrin), 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. Clearly, 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

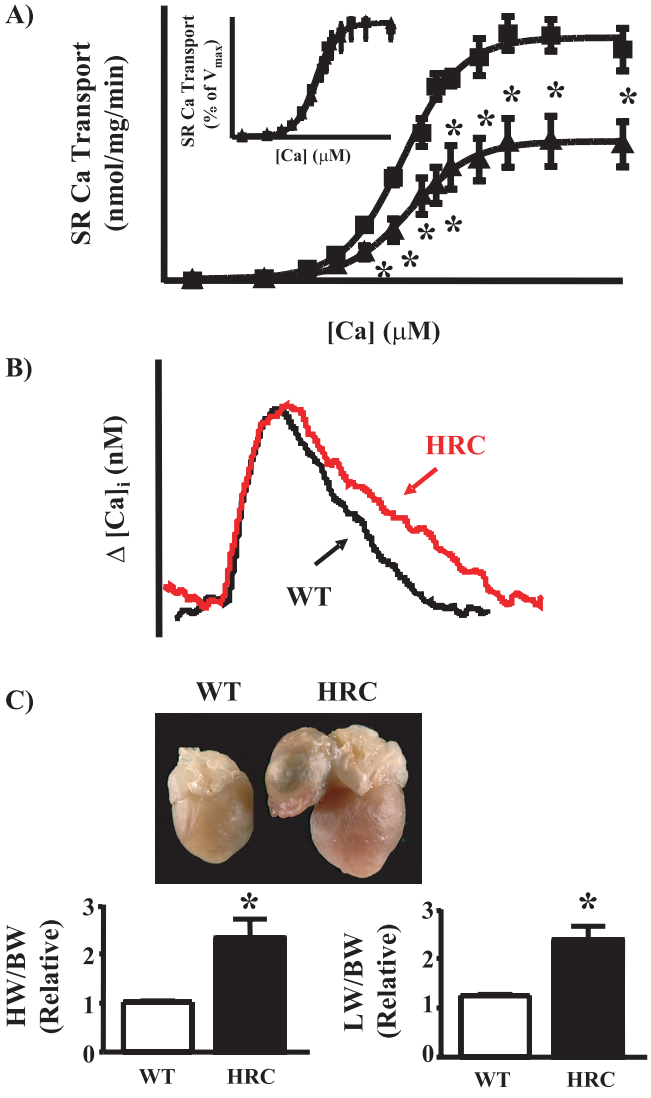

Figure 3. HRC regulation of SR Ca sequestration and cardiac function. A) SR Ca^{2+} uptake rates were significantly depressed in 3 month old HRC (▲) hearts over a wide range of [Ca^{2+}], compared to wild type (WT) (■). There was no difference in the apparent affinity of SERCA for Ca^{2+} between WT and HRC overexpressing hearts (inset). B) While the peak of twitch Ca^{2+} transients was not different, the time constant of twitch Ca^{2+} decline was significantly prolonged in 3 month old HRC overexpressing myocytes, compared to WT. C) A representative photograph of 18 month old WT and HRC overexpressing hearts. Note the dramatic enlargement of the left atrium and severe hypertrophy, which is reflected in the ~2.3-fold increased HW/BW ratio in HRC mice. HRC mice were characterized by labored breathing, pulmonary edema (increased LW/BW), and chest congestion, indicating congestive heart failure in these animals at 18 months of age. (See text for other abbreviations.)

for improving Ca^{2+} handling in the context of cardiac disease.

Histidine-rich calcium binding protein: does it play a role in SR Ca^{2+} cycling?

Evidence suggests that the histidine-rich calcium binding protein (HRC) regulates Ca^{2+} homeostasis in the heart. There is debate over whether HRC is located on the cytoplasmic surface of the SR[108-111] or in the SR lumen.[112-115] However, the strongest evidence suggests that HRC is a luminal SR protein (Figure 2). Lee et al[116] reported that HRC is an additional component of the SR quaternary structure involved in Ca^{2+} storage and release. The deduced amino acid sequence of HRC revealed structural features which are analogous to calsequestrin, suggesting that HRC may play a similar role to calsequestrin during excitation-contraction coupling, as a Ca^{2+} storage protein.[116,117] In addition, HRC associated with triadin in a Ca^{2+} dependent manner, implicating HRC in the regulation of Ca^{2+} release from the SR.[108,116] Two studies showed that adenoviral-mediated HRC overexpression in neonatal[118] or adult[119] rat cardiac myocytes significantly enhanced the SR Ca^{2+} storage capacity, resulting in altered Ca^{2+} homeostasis. Furthermore, we recently demonstrated that HRC is an integral regulatory protein in SR Ca^{2+} sequestration and cardiac function. Cardiac-specific overexpression of HRC in transgenic mice resulted in impaired SR Ca^{2+} uptake, leading to delayed cardiomyocyte Ca^{2+} transient decay, cardiac remodeling, and left ventricular dysfunction, which progressed to heart failure (Figure 3).[120]

Decreased protein levels of HRC have been reported in human and animal models of heart failure:[119] however, the potential role of HRC in diminished intracellular Ca^{2+} transients observed in failing hearts has not been investigated. Given HRC's newly discovered role in regulating SERCA activity,[120] it is possible that downregulation of HRC may represent an initial compensatory mechanism in the failing heart to increase SR Ca^{2+} uptake, especially in the face of the already decreased SERCA activity.[31,32] However, since HRC and SERCA are downregulated to different extents, there is an apparent increase in the relative ratio of HRC/SERCA2, which may contribute to further inhibition of the compromised SR Ca^{2+} sequestration in the failing myocardium. It is also likely that HRC levels may be critical for proper SR Ca^{2+} cycling and any alteration, such as decreased levels of HRC in human and experimental animal models of heart failure,[119] or increased HRC levels in HRC overexpressing rat cardiac myocytes[119] or HRC transgenic hearts,[120] results in abnormal Ca^{2+} cycling and cardiac dysfunction.

In mice and in humans, the HRC gene has been linked to cardiac disease, suggesting that abnormal properties of the HRC protein may affect the physiological function of SR Ca^{2+} cycling, contributing to the pathology of the failing heart. Van den Broek et al[121] speculated that the HRC gene may be the candidate gene responsible for dystrophic cardiac calcification (DCC) in inbred mouse strains. Given HRC's properties and location in the SR, aberrant HRC protein production may affect cellular calcium homeostasis, resulting in increased fluctuation or average concentration of ionized calcium within the cell, contributing to the pathology of DCC. In humans, localization of the HRC gene coincides with the region to which the gene for myotonic dystrophy was assigned[122] and to the region of the polymorphic marker which links to the disease locus for cardiac conduction disease.[123] Since an impairment of calcium handling and excitation-contraction coupling may lead to myotonia in myotonic dystrophy and may contribute to the pathology of cardiac conduction disease, a critical role for HRC in human clinical diseases may correlate. Although Hofmann et al[122] identified naturally occurring mutations in the HRC gene of normal individuals, specific HRC polymorphisms have not yet been linked to cardiac disease.

In conclusion, by further elucidating the physiological role of HRC in SR Ca^{2+} cycling, investigating its expression levels in the failing heart, and exploring if naturally occurring mutations exist in HRC and correlate with cardiac disease, we will determine whether HRC may be a target for heart failure therapy. Indeed, if HRC is a critical protein involved in SR Ca^{2+} cycling, targeting HRC may be a potential therapeutic strategy for the treatment of cardiac diseases.

Conclusion

Ca^{2+} cycling in the cardiac myocyte is certainly a complex process, and further elucidation of the proteins involved will be vital if we are to gain a better understanding of the basic physiology and pathophysiology of the heart. Intense research efforts described in this review have delineated the integral role of SR Ca^{2+} cycling in the progression of cardiac disease. The findings in genetically altered mouse models and in human heart failure have elucidated the specific role that SR Ca^{2+} handling proteins play in the failing heart and have provided evidence that targeting these proteins may be therapeutic in cardiac disease. Currently, studies are being designed to link naturally occurring heritable mutations in these important Ca^{2+} cycling proteins to cardiac disease. The information generated as a result of these further studies should lead to the generation of novel genetic therapy for cardiac disease.

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