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Altered sarcoplasmic reticulum calcium cycling—targets for heart failure therapy

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Abstract

Cardiac myocyte function is dependent on the synchronized movements of Ca^{2+} into and out of the cell, as well as between the cytosol and sarcoplasmic reticulum. These movements determine cardiac rhythm and regulate excitation–contraction coupling. Ca^{2+} cycling is mediated by a number of critical Ca^{2+}-handling proteins and transporters, such as L-type Ca^{2+} channels (LTCCs) and sodium/calcium exchangers in the sarcolemma, and sarcoplasmic/endoplasmic reticulum calcium ATPase 2a (SERCA2a), ryanodine receptors, and cardiac phospholamban in the sarcoplasmic reticulum. The entry of Ca^{2+} into the cytosol through LTCCs activates the release of Ca^{2+} from the sarcoplasmic reticulum through ryanodine receptor channels and initiates myocyte contraction, whereas SERCA2a and cardiac phospholamban have a key role in sarcoplasmic reticulum Ca^{2+} sequesteration and myocyte relaxation. Excitation–contraction coupling is regulated by phosphorylation of Ca^{2+}-handling proteins. Abnormalities in sarcoplasmic reticulum Ca^{2+} cycling are hallmarks of heart failure and contribute to the pathophysiology and progression of this disease. Correcting impaired intracellular Ca^{2+} cycling is a promising new approach for the treatment of heart failure. Novel therapeutic strategies that enhance myocyte Ca^{2+} homeostasis could prevent and reverse adverse cardiac remodeling and improve clinical outcomes in patients with heart failure.

Introduction

The incidence of congestive heart failure is increasing worldwide, despite important advances in pharmacological and device therapies.[1–3] Therefore, novel treatment strategies are urgently needed. An improved understanding of the molecular mechanisms involved in the pathogenesis of heart failure has led to the identification of new therapeutic targets, such as the Ca^{2+}-handling proteins in the sarcoplasmic reticulum.

Ca^{2+} has critical functions as a second messenger in a number of signaling pathways in all cell types.[4,5] Unlike other second messengers, Ca^{2+} is stored intracellularly. The membranes of the endoplasmic reticulum (and the sarcoplasmic reticulum in muscle cells), act as a major reservoir for intracellular Ca^{2+}. Cytosolic Ca^{2+} is maintained at basal levels by the actions of Ca^{2+} channels, ATPase pumps, exchangers, transporters, and Ca^{2+}-binding proteins. In the heart, Ca^{2+} regulates electrical signals that determine cardiac rhythm and

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excitation–contraction coupling, which converts the electrical stimulus to muscle contraction. Ca^{2+} might also regulate cardiac remodeling and apoptotic and necrotic cell death by activating enzymes and transcriptional gene regulation (Box 1).

Box 1

The role of Ca^{2+} in cardiac myocytes

Cytosolic levels of Ca^{2+} are regulated by various Ca^{2+} channels, ATPase pumps, Ca^{2+}-binding proteins, and Ca^{2+} transporters, which determine when and to what extent Ca^{2+} influx and efflux occur.

Signal transduction

- Ca^{2+} is a critical second messenger in G-protein-coupled pathways that induce gene transcription
- These pathways affect cardiac cell function, by activating Ca^{2+}-regulated enzymes, and might also regulate the cardiac cell population by inducing hypertrophy, necrosis, or apoptosis

Heart rhythm

- Ca^{2+} regulates electrical signals that determine the cardiac rhythm via ion currents and exchangers
- β-adrenergic stimulation results in increased Ca^{2+} entry into the cell, which ultimately leads to increased rates of myofilament contraction and relaxation
- An increase in external Ca^{2+} concentration induces shorting of phase 2 of the cardiac action potential, and consequently reduces the action potential duration
- After a delay, K^+ channels reopen, allowing diffusion of K^+ out of the cell, which causes repolarization to the resting state and reduces the duration of the action potential
- Excitation–contraction coupling converts the electrical stimulus provided by the arrival of an action potential to a mechanical response, muscular contraction
- Ca^{2+} release from the sarcoplasmic reticulum into the cytosol is required for muscle contraction: cytosolic Ca^{2+} levels activate the myofilaments and modulate their contractile properties

Mitochondrial function

- Cytosolic Ca^{2+} levels influence energy production and respiration, as ATP produced by the mitochondria is required for both muscle contraction and relaxation

Rapid changes in levels of intracellular Ca^{2+} are crucial for heart muscle contraction (systole) and relaxation (diastole). The contractile function of individual cardiac myocytes is controlled by excitation–contraction coupling (Figure 1). The arrival of an action potential depolarizes the sarcolemma and enables a small amount of extracellular Ca^{2+} to diffuse into the cell through voltage-dependent L-type calcium channels (LTCC). This Ca^{2+} influx triggers Ca^{2+} release from the sarcoplasmic reticulum through ryanodine receptor (RyR) channels. Intracellular Ca^{2+} levels then rapidly increase and Ca^{2+} binds to troponin C, resulting in muscular contraction. During cardiac relaxation, Ca^{2+} is removed from the cytosol by two main mechanisms: extrusion through the sarcolemma and reuptake into the sarcoplasmic reticulum. Extrusion occurs through sodium/calcium exchangers (NCXs) and

reuptake is mediated mainly via activation of the cardiac isoform of sarcoplasmic/endoplasmic reticulum calcium ATPase 2, SERCA2a.

Excitation–contraction coupling is modulated by other important second messengers, such as cyclic AMP (cAMP). This molecule is upregulated via activation of adenylate cyclase in response to β-adrenergic stimulation coupled with stimulatory G-protein activation. cAMP activates phosphatases and a series of protein kinases, including cAMP-dependent protein kinase (PKA),[6,7] which in turn activate β-adrenergic receptor (β-AR) signaling pathways.[8–10] PKA phosphorylates several targets that have important roles in excitation–contraction coupling. For example, phosphorylation of LTCC leads to increases in both Ca^{2+} current and force generation;[11,12] phosphorylation of the SERCA2a inhibitor, cardiac phospholamban, reduces SERCA2a inhibition, which results in an increase in Ca^{2+} resequestration into the sarcoplasmic reticulum;[13–15] and phosphorylation of troponin I reduces the sensitivity of myofilaments to Ca^{2+}, leading to enhanced Ca^{2+} resequestration and diastolic relaxation.[16–18] β-adrenergic stimulation, therefore, increases the rates of both contraction (positive inotropy) and relaxation (positive lusitropy) in cardiac myocytes.[19] The PKA-induced increase in Ca^{2+} current and amelioration of SERCA2a inhibition are major factors that enhance cardiac contractility.[20]

Defects in every stage of excitation–contraction coupling lead to diastolic and systolic abnormalities, as well as an increased propensity for ventricular arrhythmias, all of which have all been reported in patients with heart failure. These defects are the result of altered expression or function of proteins that are required for the maintenance of Ca^{2+} homeostasis. In this Review, we explain the association between abnormalities in Ca^{2+} handling in the sarcoplasmic reticulum and heart failure, and discuss the therapeutic potential of targeting key proteins involved in this process. β-ARs and adenylate cyclase are possible targets for the treatment of heart failure that have been reviewed elsewhere;[21,22] as such, they are mentioned only briefly in this Review.

Impaired Ca^{2+} cycling in heart failure

The primary cause of heart failure is diastolic dysfunction resulting from impairments in myocardial relaxation, ventricular filling, or both. Diastolic dysfunction is also associated with systolic dysfunction. At the cellular level, the causes of impaired cardiac myocyte relaxation include cytosolic Ca^{2+} overload, myofilament structural changes or dysfunction, and neurohormonal activation.

In many patients with heart failure, excitation– contraction coupling is defective. A number of abnormalities of receptors, pumps, and regulatory proteins that are involved in Ca^{2+} cycling have been shown in these patients. These abnormalities lead to a prolongation of the cytosolic Ca^{2+} transient time and, in patients with end-stage heart failure, an increase in end-diastolic Ca^{2+} concentration.[23] 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 Ca^{2+} loading via NCX. However, upregulation of NCX function, which might also occur in heart failure, could partially compensate for SERCA2a downregulation with respect to diastolic function, but would not affect systolic function.

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 β_1-AR density, resulting in an increased β_2-AR: β_1-AR ratio and uncoupling of β-ARs from G-protein complexes.[24,25] This desensitization is mediated by elevated G-protein receptor kinase activity.[6,26] 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.[27,28]

Increases in diastolic Ca^{2+} level can activate Ca^{2+}-sensitive signaling pathways in cardiac myocytes. Pathological cardiac hypertrophy is driven by a number of Ca^{2+}-sensitive signaling pathways, including the calcineurin–NFAT[29] and CaMKII–histone deacetylase[30] pathways. The sarcoplasmic reticulum Ca^{2+} sensor, stromal interaction molecule 1 (STIM1), might also activate prohypertrophic, calcium-sensitive signaling pathways.[31,32]

In heart failure, intracellular Ca^{2+} overload can result from excessive Ca^{2+} entry into the cytosol, either from the sarcoplasmic reticulum or from outside the cell. Alternatively, a decrease in Ca^{2+} efflux, or inadequate Ca^{2+} reuptake by the sarcoplasmic reticulum during diastole can result in intracellular Ca^{2+} overload in this condition (Figure 2).

Excessive Ca^{2+} entry into the cytosol

In cardiac myocytes, Ca^{2+} influx occurs almost entirely through voltage-activated LTCCs. These multisubunit complexes consist of a pore-forming α1C subunit, and the auxiliary β, α2δ, and γ subunits,[33,34] which traffic the α1C subunit to the sarcolemma and modulate the voltage dependence of channel gating.[35] Ca^{2+} influx through LTCCs triggers sarcoplasmic reticulum Ca^{2+} release, which determines the rate and magnitude of myocyte contraction via a process called Ca^{2+}-induced Ca^{2+} release. LTCC-mediated Ca^{2+} influx is also associated with cardiac hypertrophic signaling pathways; therefore, abnormalities in LTCC activity and expression can cause cardiac dysfunction. In mice, disruption of cardiac LTCC subunit genes caused defects in cardiac function and vascular remodeling that resulted in embryonic death.[36,37] Consistent with a dominant role of LTCC in Ca^{2+} transport, pharmacological blockade of LTCC effectively inhibited cardiac remodeling after pressure overload[38] and prevented cardiomyopathy in murine models.[39] Most animal models of heart failure have shown either no change or a slight decrease in the whole-cell density of LTCC current in the myocardium.[40] A murine study showed that a decrease in LTCC current caused a compensatory increase in Ca^{2+} leakage from the sarcoplasmic reticulum via ryanodine receptor 2 (RyR2), resulting in pathological cardiac hypertrophy and heart failure through calcineurin–NFAT signaling.[41] A reduction in expression of LTCC and Ca^{2+} current has not been definitively established in human heart failure. However, the single-channel activity of LTCC was significantly increased in human failing ventricular myocytes compared with nonfailing control myo-cytes.[42] The basal level of LTCC phosphorylation was also increased in failing myocardium, suggesting that LTCC phosphorylation might contribute to the blunted inotropic effects of adrenergic stimulation in the failing human heart.[42,43] Clinical trials have shown either no effect, or a detrimental effect, of LTCC-blocking agents on the survival of patients with heart failure.[44] Whether LTCC-inhibitors could be used to treat heart failure is, therefore, unclear.

Several groups of researchers have proposed that store-operated Ca^{2+} entry (SOCE, also known as capacitative Ca^{2+} entry)—a mechanism through which calcium-release-activated calcium (CRAC) channels in the plasma membrane sense depletion of intracellular Ca^{2+} and open to allow influx of Ca^{2+} from the extracellular reservoir—may be enhanced in pathological remodeling.[45] SOCE is the major Ca^{2+} entry mechanism in nonexcitable cells that lack voltage-gated Ca^{2+} channels, such as mast cells and T cells.[46] The process has an important role in signal transduction that controls Ca^{2+} homeostasis, cell differentiation, and secretion.[46] SOCE compensates for the loss of Ca^{2+} that is transported out of the cell by sarcolemmal Ca^{2+} ATPase and the NCX, and ensures proper Ca^{2+} refilling of the endoplasmic reticulum.[47] The pore-forming subunit, CRAC channel protein 1 (also known as protein orai-1), is a well-characterized component of CRAC channels in plasma membranes.[48]

The sarcoplasmic reticulum Ca^{2+} sensor STIM1 has a role in the detection of Ca^{2+} depletion and the activation of CRAC channels.[49–51] STIM1 is predominantly located in the endoplasmic or sarcoplasmic reticulum membrane. Functional EF-hand domains in the endoplasmic or sarcoplasmic reticulum luminal region of the molecule bind Ca^{2+} and detect Ca^{2+} depletion.[52,53] Dissociation of Ca^{2+} from the EF-hand domains leads to rapid oligomerization of STIM1,[52,54] relocalization of the molecule to the junction between the plasma membrane and the endoplasmic or sarcoplasmic reticulum, and activation of CRAC channels in the plasma membrane.[53,55] STIM1 directly interacts with CRAC channel protein 1, the pore-forming subunit of the CRAC channels, results in the assembly of an active tetrameric complex that opens the Ca^{2+} channel and allows Ca^{2+} influx into the cytosol.[56] Ca^{2+} influx activates calcineurin–NFAT signaling and induces transcriptional activation of immune response and prohypertrophic genes.[57,58]

SOCE has been identified in excitable cells, including skeletal muscle fibers[59–61] and smooth muscle cells.[62] In smooth and skeletal muscle cells, SOCE might be mediated by the comparatively nonselective, canonical transient receptor potential (TRPC) channels.[63,64] STIM1 directly interacts with TRPC channels, but whether STIM1-mediated gating of these channels is direct or indirect is unclear. The role of CRAC channel protein 1 in SOCE in excitable cells is also not fully understood. However, there is evidence for a role of STIM1 in SOCE-mediated cardiac pathology. In neonatal cardiac myocytes, STIM1 was required for *in vitro* hypertrophic responses induced by autocrine/paracrine growth factors and neurotransmitters (such as endothelin-1, phenyl-ephrine, and angiotensin II).[31,65] In adult rat cardiac myocytes, STIM1 overexpression increased SOCE current and activated pathological hypertrophic responses.[32] By contrast, depletion of cardiac STIM1 prevented cardio-myocyte hypertrophy and decreased NFAT activation in rats.[32] However, whether STIM1 modulation has a role in the development of heart failure remains unclear.

Reduced Ca^{2+} efflux from the cytosol

As well as acting as a Ca^{2+} reservoir, the sarcoplasmic reticulum actively maintains cytosolic Ca^{2+} concentrations during contraction–relaxation of cardiac myocytes. In humans and higher mammals, sarcoplasmic reticulum Ca^{2+} uptake is mainly regulated by SERCA2a. This Ca^{2+} pump has an important role in restoring sarcoplasmic reticulum Ca^{2+} levels in diastole and terminating Ca^{2+}- dependent force activation via Ca^{2+} 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.[66–68] An increase in end-diastolic cytosolic Ca^{2+} levels and prolongation of Ca^{2+} transient during diastole were shown in cardiac myocytes isolated from patients with end-stage heart failure.[69,70] Such abnormalities are associated with the development of enhanced diastolic tension.

In mammals, three different genes (*ATP2A1*, *ATP2A2*, and *ATP2A3*) encode SERCA protein. The phylo-genetically oldest isoform, SERCA2, is the predominant variant and three different splice transcripts have been reported: SERCA2a, SERCA2b, and SERCA2c. These transcripts differ at their C-terminus and have distinct tissue distributions. SERCA2a, which has a four-aminoacid tail and 10 transmembrane domains, is selectively expressed in the heart and in slow-twitch muscle. By contrast, SERCA2b is ubiquitously expressed and has a 49-amino-acid tail. This C-terminal extension, which has highly hydrophobic properties, might form an additional endoplasmic or sarcoplasmic reticulum transmembrane domain,[71] which could explain why SERCA2b has a higher affinity for Ca^{2+} than SERCA2a.[72] Both SERCA2a and SERCA2b bind cardiac phospholamban and they do not differ in their sensitivity to the SERCA inhibitor thapsigargin.[73] The third variant, SERCA2c, has a 61-base-pair intronic sequence between exons 20 and 21 of SERCA2a, and

a short tail of six amino acids. SERCA2c has been detected in human monocytes and fetal hearts[74] and is also expressed in skeletal muscle and cardiomyocytes.[75]

No shift in the relative amounts of the SERCA2 iso-forms has been detected in failing human hearts.[76] However, in animal models of hypertrophic[77–79] and ischemic[80,81] heart failure, a decrease in the rate of sarcoplasmic reticulum Ca^{2+} uptake as well as low levels of SERCA2a have been shown. Furthermore, a reduction in SERCA2a activity with a resulting decrease in sarcoplasmic reticulum Ca^{2+} uptake is a consistent feature of failing cardiac myocytes in humans. Data from several studies on explanted failing human hearts (obtained during transplantation procedures) suggest that sarcoplasmic reticulum Ca^{2+} transport function is impaired in end-stage human heart failure. Decreases in the level of SERCA2a mRNA, protein, or activity correlate strongly with decreased myocardial function and impaired contractile force–frequency relationships.[68,82,83]

SERCA2a activity is tightly regulated by the small inhibitory peptide cardiac phospholamban. This protein binds to SERCA2a and induces a conformational change that reduces the Ca^{2+}-binding affinity of SERCA2a and the rate of Ca^{2+} uptake by the sarcoplasmic reticulum.[84,85] In most failing human hearts, cardiac phospholamban protein expression is unaltered,[86,87] but a decrease in the level of cardiac phospholamban mRNA has been reported in patients with dilated or ischemic cardio-myopathies.[88,89] Interestingly, the decrease in levels of SERCA2a in failing myocardium is greater than the decrease in levels of cardiac phospholamban;[90] therefore, the ratio of cardiac phospholamban to SERCA2a increases, resulting in increased inhibition of SERCA2a. A reduction in phosphorylation of cardiac phospholamban can also increase SERCA2a inhibition in failing myocardium.[91] In animal models of heart failure, phosphorylation of cardiac phospholamban was reduced at either Ser16,[92] Thr17,[93,94] or both.[95] Similarly, reduced phosphorylation of cardiac phospholamban at Ser16, but no change in phosphorylation at Thr17, was shown in samples from failing human hearts.[92,96] The reduction in phosphorylation at Ser16 correlated with a decrease in the sensitivity of SERCA2a to high Ca^{2+} levels in human failing myocardium.[92,96] Cardiac phospholamban is dephosphorylated by protein phosphatase 1 (PP1)[97] and, as we will discuss in more detail later in this Review, increased PP1 activity has been shown in animal models of heart failure.[98] These data suggest that the level of phospholamban phosphorylation might depend on the severity of heart failure, and could be affected by β-AR signaling pathways through alterations in the activation of protein kinase A (PKA).

Mutations in the *PLN* gene, which encodes cardiac phospholamban, are associated with human heart failure. In patients with familial dilated cardiomyopathy, mutations in the coding region of the *PLN* gene have been identified.[97] *PLN* mutations, such as an arginine to cysteine substitution in codon 9 (Arg9Cys) and an arginine deletion in codon 14 (Arg14del), might enhance cardiac phospholamban-mediated inhibition of SERCA2a activity.[99–101] Transgenic mice that expressed the Arg9Cys mutant developed terminal heart failure that resulted in premature death.[100] In addition, two more mutations at Arg9 (Arg–Leu and Arg–His substitutions) were identified in a cohort of patients with heart failure.[99] A mutation in the coding region of the *PLN* gene that resulted in a termination codon (Leu39stop) was identified in two families with hereditary heart failure.[102] Patients who were homozygous for this mutation, which prevented phospholamban expression, developed dilated cardiomyopathy and heart failure.[102]

NCX has a major role in the extrusion of Ca^{2+} through the sarcolemma into the extracellular space and contributes to Ca^{2+} removal during mammalian cardiac myocyte relaxation.[103] Some studies have shown increased NCX expression in failing human hearts.[104,105] In human heart failure, NCX upregulation might compensate, at least in part, for any decline in

SERCA2a activity because an increase in NCX activity might accelerate the removal of Ca^{2+} from failing cardiac myocytes during relaxation. However, another study showed no change in NCX expression in myocardium from terminally failing human hearts.[106] A study in which the level of NCX mRNA in myocardium from failing human hearts was analyzed showed no correlation between NCX mRNA expression and the severity of disease.[107] NCX mRNA was upregulated only in end-stage heart failure.[107] However, NCX mRNA levels do not always indicate the level of NCX protein expression or activity.[108,109] Although increased NCX activity in heart failure might initially be adaptive, excessive or sustained NCX activation could contribute to a further decrease in the Ca^{2+} content of the sarcoplasmic reticulum as a result of increased cytosolic Ca^{2+} removal. This decrease could lead to diminished systolic Ca^{2+} transients, and reduced contractile function. Some studies have suggested that NCX is involved in Ca^{2+} influx, which might occur via a reverse mode in the presence of high levels of intra-cellular Na^{+} and positive membrane potentials.[110,111] In the forward mode, NCX transports Na^{+} into the cell and reduces diastolic Ca^{2+} levels by extruding Ca^{2+}. However, during the action potential and/or when intracellular Na^{+} concentration is increased, NCX can function in a reverse mode and transport Ca^{2+} into the cell. This Ca^{2+} influx might promote sarcoplasmic Ca^{2+} loading and, together with the LTCC, modulate the release of Ca^{2+} from the sarcoplasmic reticulum, resulting in an increase in intracellular Ca^{2+} concentration.

Reduced sarcoplasmic reticulum Ca^{2+} content

A reduction in Ca^{2+} uptake by the sarcoplasmic reticulum reduces the amount of stored Ca^{2+} available for release into the cytosol. In addition, decreased Ca^{2+} storage reduces excitation–contraction coupling and contributes to dysfunction of systolic contractility.[112]

The most abundant acidic sarcoplasmic reticulum protein is calsequestrin (CASQ). In humans, two isoforms of CASQ have been identified; CASQ-1 is found in skeletal muscle, and CASQ-2 in cardiac muscle.[113] CASQ-2 is primarily responsible for the Ca^{2+} storage capacity of the myocardial sarcoplasmic reticulum. The protein has a high capacity and a moderate affinity for Ca^{2+} and acts as a Ca^{2+} buffer that decreases the concentration of free luminal Ca^{2+} to facilitate further SERCA2a-mediated Ca^{2+} uptake into the sarcoplasmic reticulum.[113] The large Ca^{2+} buffering range of CASQ-2 facilitates the large storage capacity of the sarcoplasmic reticulum as well as the rapid release of Ca^{2+} into the cytosol.[113] In addition, CASQ-2 has a role in regulating the open probability of RyR2 channels and, therefore, the amount of Ca^{2+} release from the sarcoplasmic reticulum.[114,115] The levels of CASQ-2 mRNA and protein do not change substantially in failing human hearts. However, CASQ-2 is associated with certain types of arrhythmia, such as catecholaminergic polymorphic ventricular tachycardia (CPVT), which is characterized by stress-induced or exercise-induced ventricular tachycardias that lead to sudden cardiac death at young ages (9 ± 4 years).[116] In mice, CASQ-2 knockout caused an autosomal-recessive form of CPVT, although Ca^{2+} transients in cardiac myocytes were unaltered.[117] Mutations in *CASQ2*, the gene that encodes CASQ-2, also cause CPVT.[118] In humans, a missense mutation in a highly conserved region of *CASQ2* that resulted in an amino acid change from aspartic acid to histidine at codon 307 (Asp307His) caused autosomal-recessive CPVT.[118] The Asp307His mutation also caused RyR2 dysfunction and a CVPT phenotype in a mouse model.[119]

Cardiac CASQ-2 is glycosylated and then trimmed by a series of mannosidases located within distinct intra-cellular compartments.[120] This glycosylation prevents premature polymerization of CASQ-2, and ensures transport of the protein to the sarcoplasmic reticulum. Interestingly, defective glycosylation of CASQ-2 was identified in canine ventricles following rapid-pacing-induced heart failure.[120] The levels of glycan forms of CASQ-2 that were characteristic of a junctional sarcoplasmic reticulum location were

reduced by 80%, and the level of the mannose 8,9 CASQ-2 glycoform, characteristic of a rough endoplasmic reticulum location, was increased twofold in failing canine hearts compared with healthy controls.[120] Data from CASQ-2 trafficking studies in adult rats also suggested that abnormal glycosylation might result in retention of CASQ-2 in the rough endoplasmic reticulum of cardiac myocytes.[121,122] These findings suggest that defective post-translational processing of CASQ-2 might lead to impaired CASQ-2 cellular compartmentalization during heart failure.

Triadin and junctin are transmembrane sarcoplasmic reticulum proteins that form a complex with CASQ-2 and RyR2. This complex, which anchors CASQ-2 to the luminal membrane in close proximity to the Ca^{2+}-release channel, might have an important role in the regulation of Ca^{2+} release from the sarcoplasmic reticulum. Triadin and junctin might be involved in luminal Ca^{2+} sensing by mediating the interaction between CASQ-2 and RyR2,[123] and might be required for the normal operation of Ca^{2+} release. The levels of triadin and junctin are reduced in failing human myocardium.[124] However, whether this reduction causes heart failure, or if the downregulation is an adaptive response that improves Ca^{2+} handling in failing cardiac myocytes, is not clear. Overexpression of triadin in adult rat ventricular myocytes increased RyR2 channel activity and arrhythmia.[125] Downregulation of junctin in failing human hearts might reduce sarcoplasmic Ca^{2+} leak in response to chronic β-adrenergic stimulation and improve cardiac function.[126] Cardiac-specific overexpression of β_1-AR in transgenic mice resulted in a decrease in junctin protein levels and progression of hypertrophy.[126,127] Abnormalities in sarcoplasmic reticulum protein trafficking and assembly of the Ca^{2+}-release complex, which result in impaired Ca^{2+} release from the sarcoplasmic reticulum, might contribute to heart failure.

Sarcoplasmic reticulum histidine-rich calcium-binding protein (HRC) interacts with triadin and SERCA2a, and might have a role in both Ca^{2+} release and uptake.[127] In adult rat cardiac myocytes, overexpression of HRC increased the Ca^{2+} storage capacity of the sarcoplasmic reticulum but decreased sarcoplasmic reticulum Ca^{2+} release and the Ca^{2+} transient duration, resulting in a reduction in contractility.[128] HRC expression is significantly reduced in failing human and animal hearts.[128] This reduction might be a compensatory mechanism that increases Ca^{2+} release from the sarcoplasmic reticulum in an attempt to improve cardiac function during heart failure.[128] A *HRC* gene polymorphism, which results in the amino acid change Ser96Ala, was associated with ventricular arrhythmias in patients with idiopathic dilated cardiomyopathy.[129]

S100 calcium binding protein A1 (S100A1), a member of the S100 family of proteins that contain EF-hand Ca^{2+}-binding motifs, is located in the sarcoplasmic reticulum, sarcomere, and mitochondria, and is highly expressed in cardiac myocytes.[130] S100A1 stabilizes RyR2 in diastole and augments Ca^{2+} release during systole. S100A1 also increases SERCA2a activity during the relaxation phase.[131] Myocardial levels of S100A1 are decreased in patients with heart failure as a result of cardio-myopathy.[132] Gain-of-function and loss-of-function experiments have been used to characterize the *in vivo* and *ex vivo* positive inotropic effects of S100A1.[133–135] Cardiac-restricted overexpression of S100A1 in transgenic mice had chronic cardiac inotropic effects that were independent of β-adrenergic signaling,[133] and an increase in S100A1 protein levels in isolated adult and neonatal murine cardiac myocytes enhanced their systolic and diastolic performance through modulation of cellular Ca^{2+} handling.[133,134] By contrast, S100A1-deficient mice showed severely impaired inotropic and lusitropic responses to β-adrenergic stimulation and acute or chronic hemodynamic stress.[135] These impairments were at least partly caused by a reduction in cardiac Ca^{2+} sensitivity.[135] A decrease in S100A1 protein levels might, therefore, contribute to Ca^{2+} dysregulation and impaired contractile function in human heart failure. Interestingly, myocardial contractility is also regulated by energy metabolism and S100A1 interacts with

mitochondrial ATP synthase, resulting in an increase in the activity of the enzyme and increased production of ATP.[136] These data suggest that S100A1 might also have a role in cardiac metabolism. However, further investigation of the potential mechanism of S100A1-mediated metabolism in cardiac myocytes is required.

Sarcoplasmic reticulum Ca^{2+} leakage

Ca^{2+} channels within the sarcoplasmic reticulum are nonselective and have high-conductance cation transport properties, which facilitate rapid, localized Ca^{2+} release. Abnormalities in the structure and function of the sarcoplasmic reticulum Ca^{2+} channels have been associated with cardiac arrhythmia and human heart failure.[137,138]

Release of Ca^{2+} from the sarcoplasmic reticulum into the cytosol, which is required for contractile activation of the heart, is mediated by RyR2.[139] The RyR2 tetramer is a massive macromolecular signaling complex that binds kinases and phosphatases as well as adaptor and targeting proteins.[140] Most of these regulatory proteins, including calmodulin, CaMKII, FKBP 12.6, PKA, PP1, and calcineurin, bind to the cytosolic region of RyR2, whereas triadin and junctin bind to the luminal sarcoplasmic region.[123,141–147] Several studies have shown increased sarcoplasmic reticulum Ca^{2+} leak during diastole as a result of RyR2 dysfunction in heart failure.[148,149] This diastolic Ca^{2+} leak depletes Ca^{2+} stores and presumably impairs the contractility of cardiac myocytes.

Expression of RyR2 is unchanged in failing human hearts.[150,151] However, several studies showed an increased level of PKA-mediated phosphorylation of RyR2 in human heart failure.[152,153] Chronic hyperactivity of β-AR signaling might lead to hyperactivation of PKA and subsequent hyperphosphorylation of RyR2 in failing hearts. However, the effect of RyR2 hyperphosphorylation on channel activation is not clear. Some studies showed that PKA-mediated phosphorylation of RyR2 activated the channel,[152,153] whereas other studies showed that the open channel probability of RyR2 was slightly decreased in response to phosphorylation.[154,155] Alternatively, PKA-mediated phosphorylation might destabilize RyR2 channels by inducing dissociation of the accessory protein FKBP12.6.[153] PKA levels are unchanged in patients with heart failure, but levels of phosphatases in the RyR2 protein complex, such as PP1 and protein phosphatase 2A (PP2A), are reduced. These changes resulted in a reduced rate of dephosphorylation of RyR2 (at Ser2,809 in rabbit and Ser2,808 in humans and mice) and dissociation of FKBP12.6 from the RyR2 complex.[153,156] FKBP12.6 depletion increases the Ca^{2+} sensitivity of RyR2 and results in leakage of Ca^{2+} from the sarcoplasmic reticulum during diastole. Abnormal interactions between FKBP12.6 and RyR2 have been shown in left-sided heart failure.[157,158] Impaired binding of FKBP12.6 to RyR2 might also result in diastolic Ca^{2+} leakage from the sarcoplasmic reticulum, which could potentially initiate ventricular arrhythmias. However, further work is required to elucidate the mechanisms by which PKA modulates RyR2 channel activation in intact cells and to clarify the effect of FKBP12.6 binding on single-channel RyR2 function.

Several missense mutations in cardiac RyR2 have been identified in patients with CPVT.[159] A pathogenic role of the *RyR2* mutation Arg4497Cys was shown in a conditional knock-in mouse model.[160] The arrhythmia morphology, arrhythmia severity, and incomplete responses to β-blockers, of Arg4497Cys mutant mice were similar to that of patients with CPVT.

Altered Ca^{2+}-activated signaling

Abnormal Ca^{2+} cycling can affect multiple signaling pathways in the heart and result in hypertrophic growth and cardiac remodeling, which frequently progress to heart failure.

Changes in the morphological and functional properties of the myocardium ultimately require reprogramming of cardiac myocyte gene expression.[161]

Calcineurin (also known as Ca^{2+}/calmodulin dependent protein phosphatase 2B) is a serine/threonine phosphatase that has a key role in coupling Ca^{2+} signaling to cellular responses, through interaction with NFAT.[162] Increases in intracellular Ca^{2+} levels activate calcineurin phosphatase activity. The activated protein binds and dephosphorylates NFAT, which translocates from the cytosol to the nucleus and induces gene expression.[163,164] In the heart, NFAT interacts directly with the cardiac-restricted zinc finger transcription factor GATA4, which regulates expression of hypertrophic genes, including β-myosin heavy chain, atrial natriuretic factor, and B-type natriuretic peptide.[165,166] In mice, constitutive activation of calcineurin resulted in progressive decompensation, dilatation, and the development of heart failure.[167,168] This progression recapitulated the development of hypertrophic cardiomyopathy in human heart failure.[167,168] However, the effect of pharmacological inhibition of calcineurin (using cyclosporine A) on hypertrophy in rat and mouse models is unclear. Two studies showed that treatment with cyclosporine A prevented or attenuated cardiac hypertrophy in aortic-banded mice,[169,170] but there was no evidence that the drug inhibited cardiac hypertrophy in other studies that used the same experimental model.[171,172] High-dose cyclosporine A treatment might result in severe adverse effects that could mask the therapeutic effects of the drug.[168] A complete understanding of the role of the calcineurin–NFAT pathway in the progression of hypertrophy is required to facilitate the development of antihypertrophic drugs.

CaMKII can also be activated in response to increased levels of intracellular Ca^{2+}.[173] The protein has a role in multiple cellular processes that are important in the transition from early structural heart disease to heart failure and sudden death,[173] including regulation of hypertrophic growth (mediated by class II histone deacetylase), MEF2 transcriptional activity, and inflammatory responses via activation of NFκB.[173] Consistent with the role of CaMKII in the regulation of hypertrophic gene expression, CaMKII expression and activity correlated with the progression of heart failure in patients with structural heart disease post-myocardial infarction.[174,175] Overexpression of CaMKIIδ in the hearts of transgenic mice induced a hypertrophic phenotype that rapidly transitioned to dilated cardiomyopathy and abnormal intracellular Ca^{2+} homeostasis,[176] resembling the remodeled myocardium in human heart failure. By contrast, CaMKII inhibition in animal models, by either genetic or pharmacological approaches, prevented phenotypic changes that are associated with heart failure, such as arrhythmias, hypertrophy, and myocardial dysfunction.[177–179]

Importantly, CaMKII might have a role in the regulation of myocyte Ca^{2+} homeostasis.[180] The protein can phosphorylate and, therefore, modulate the function of key Ca^{2+}-handling proteins, such as cardiac phospholamban, RyR2, and LTCC.[173] CaMKII-dependent phosphorylation of cardiac phospholamban at Thr17 can lead to an increase SERCA2a activity and thereby accelerate sarcoplasmic reticulum Ca^{2+} transport.[181] Cardiac phospholamban is phosphorylated at Ser16 by PKA and at Thr17 by CaMKII, but the relative importance of these dual phosphorylation sites in the regulation of cardiac contractility is difficult to assess. A number of studies showed that phosphorylation at Ser16 was required to allow phosphorylation at Thr17 and so maximize the contractile effects of β-adrenergic stimulation.[182–184] However, Thr17 phosphorylation that was independent of Ser16 phosphorylation has also been reported.[185,186] In mice, targeted inhibition of CaMKII activity using an inhibitory peptide resulted in a substantial decrease in phosphorylation of phospholamban at Thr17, but had no effect on phosphorylation at Ser16.[186] The mice developed dilated heart failure.[186]

CaMKII can phosphorylate RyR2 at Ser2,808 (or Ser2,809, depending on species)—which is also a PKA phosphorylation site—and at other sites, including Ser2,815. This phosphorylation increases the activity of the RyR2 channels.[187] Endogenouse CaMKII increased the amount of Ca^{2+} release from the sarcoplasmic reticulum for a given sarcoplasmic reticulum Ca^{2+} content in intact cardiomyocytes.[188] Consistent with this finding, transgenic mice overexpressing CaMKIIδc (the major cardiac isoform) had an increased Ca^{2+} spark frequency (indicative of diastolic spontaneous SR Ca^{2+} release) and low diastolic Ca^{2+} content compared with wild-type mice.[189] Overexpression of CaMKIIδc causes heart failure.[189]

CaMKII also associates with LTCC complexes and increases the open probability of the channels, resulting in an increase in Ca^{2+} current and augmentation of cellular Ca^{2+} signaling via a process called facilitation.[190] CaMKII-dependent facilitation of LTCCs requires Thr498 phosphorylation in the β2a subunit to enhance regulatory phosphorylation.[191] A role of CaMKII in the development of early after-depolarization and arrhythmias through LTCC activation has been shown in mouse models.[190,192] Increases in the speed of sarcoplasmic reticulum Ca^{2+} uptake and release as a result of CaMKII-mediated phosphorylation of Ca^{2+}-handling proteins might provide more time for diastolic filling of the ventricles at higher heart rates.

Potential therapeutic strategies

Improvements in knowledge of Ca^{2+} dynamics in health and disease have led to increased understanding of the therapeutic potential of targeting Ca^{2+}-handling proteins. A number of pharmacological and gene therapy strategies aimed at restoring the function of the sarcoplasmic reticulum—either by increasing Ca^{2+} uptake or preventing Ca^{2+} leakage—have been investigated (Table 1).

Restoring Ca^{2+} uptake

Modulation of SERCA2a activity is critical for improving Ca^{2+} uptake by the sarcoplasmic reticulum. SERCA2a overexpression leads to an improvement in cardiac function in many animal models of heart failure. Transgenic rats with increased SERCA2a expression showed enhanced contractility and better survival after pressure overload than wild-type rats.[193] Similarly, initial data indicated that SERCA2a restoration by gene transfer increased contractility in isolated failing human cardiac myocytes.[194] These data, which have been validated in independent animal studies,[195,196] support the hypothesis that *in vivo* gene delivery of SERCA2a could rescue the heart failure phenotype. Furthermore, SERCA2a overexpression in failing rat hearts increased the phosphocreatine:ATP ratio[197] and decreased the oxygen cost of left ventricular contraction.[198] These findings suggest that restoration of sarcoplasmic reticulum Ca^{2+} transport could normalize energy metabolism and utilization in failing hearts.

In many small-animal studies, adenoviral delivery of SERCA2a has been used to restore SERCA2a expression in failing hearts.[199,200] However, expression of adenovirally delivered transgenes is short-lived and the adenoviral vector induces an inflammatory response.[201] To avoid these problems, new vectors, such as various recombinant adeno-associated virus (AAV) serotypes, are now being used. These vectors are nonpathogenic, nonreplicative, and nonintegrating.[202]

In pigs with volume-overload heart failure, an increase in sarcoplasmic reticulum Ca^{2+} uptake via AAV-mediated SERCA2a gene transfer was beneficial for maintenance of cardiac function.[196] In this study, the viral vector containing SERCA2a was administered by intracoronary artery infusion, and ventricular remodeling and improvements in cardiac

parameters (including ejection fraction and peak left ventricular pressure rate) were observed 2 months after SERCA2a gene transfer. Improvements in left ventricular function have also been reported in sheep with rapid-pacing-induced heart failure.[203]

Other animal studies have shown an association between SERCA2a overexpression and reduced frequency of arrhythmias.[195,204–206] However, in one study involving a rat model in which SERCA2a was constitutively overexpressed, investigators found a delay in myocardial failure after myocardial infarction in rats at a cost of increased acute arrhythmias.[207] Notably, though, this latter study did not involve gene transfer of SERCA2a and the overexpression of SERCA2a was not evident at the protein level. One of the early concerns with SERCA2a overexpression was that, with additional SERCA2a pumps, the SR would trigger calcium release and cause arrhythmias, especially if the RyR2 are leaky.[208] However, experimental models have shown that SERCA2a overexpression decreased ventricular arrhythmias in both small[204] and large[195] animal models of ischemia reperfusion. More-recent studies have shown that overexpression of SERCA2a suppresses electrical alter-nans,[205] interrupting an important pathway leading to cardiac fibrillation, and decrease SR Ca^{2+} leak in a model of advanced heart failure.[206] In a rat model of heart failure induced by myocardial infarction, SERCA2a gene therapy stabilized sarcoplasmic reticulum Ca^{2+} load, reduced RyR2 phosphorylation, and decreased Ca^{2+} leakage from the sarcoplasmic reticulum.[206] These data suggest that SERCA2a has positive inotropic and lusitropic effects, and SERCA2a gene therapy could have antiarrhythmic effects in patients with heart failure.

CUPID (Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease), the first clinical trial of AAV1 delivery of SERCA2a in patients with advanced heart failure, began in 2007. The main objective was to investigate the safety and biological effects of restoring SERCA2a activity in patients with advanced heart failure. Phase I was an open-label, sequential-dose-escalation study in nine patients with advanced heart failure (NYHA class III or IV).[209] AAV1–SERCA2a was administered by single coronary artery infusion at doses ranging from 1.4×10^{11}–3.0×10^{12} DNAse-resistant particles (DRP). There were no unexpected safety concerns and several patients showed improvements in a number of relevant parameters, including heart failure symptoms and cardiac function, at 6-month follow-up. The phase I trial, therefore, demonstrated safety and efficacy of the therapy despite the small number of enrolled patients.

Phase II of the CUPID trial was a double-blind, placebo-controlled, randomized study of 39 patients with advanced heart failure.[210] In this study, the participants received either placebo or one of three doses of AAV1–SERCA2: 6×10^{11} DRP (low-dose), 3×10^{12} DRP (mid-dose) or 1×10^{13} DRP (high-dose). Each dose group contained eight patients. At 6 months of follow-up, the patients treated with high-dose AAV1–SERCA2a showed improvement or stabilization of heart failure symptoms (assessed using the NYHA classification and Minnesota Living With Heart Failure Questionnaire), functional status (assessed using a 6 min walk test and measurement of maximal oxygen consumption), and hemodynamic performance (left ventricular ejection fraction and left ventricular end-systolic volume), as well as a decrease in levels of the heart failure biomarker N-terminal B-type natriuretic propeptide. At 12 months follow-up, a significant decrease in cardiovascular events (hospitalizations related to heart failure, episodes of worsening of heart failure, death, or the requirement for left ventricular assist devices or cardiac transplant) was evident in the patients who received high-dose AAV1–SERCA2a compared with those who received placebo (HR = 0.12, $P = 0.003$). Moreover, during the 12-month study, the estimated average duration of cardiovascular hospitalizations was considerably shorter for patients in the high-dose AAV1–SERCA2a group than in the placebo group (0.4 days versus 4.5 days). The primary end point success criteria were defined as achieving efficacy in either the

group-level analysis, with improvement in at least two of the four efficacy domains (symptomatic, functional, biomarker, and left ventricular function or remodeling), the individual-level analysis, or Kaplan–Meier analysis of the outcome end point (time to death, or requirement for ventricular assist device or transplantation). These analyses showed that treatment with high-dose AAV1–SERCA2a was better than placebo. Statistical significance for all three criteria was defined as $P<0.2$, rather than $P<0.05$, because additional requirements were imposed on the study outcomes to control for the probability of obtaining positive study results by chance alone.

Two clinical trials of AAV1–SERCA2a therapy in patients with heart failure are ongoing. A randomized, double-blind study of 16 patients who have received a left ventricular assist device for an accepted clinical indication is being carried out in the UK to evaluate the safety and feasibility of AAV1–SERCA2a therapy, and a Phase 2 trial in patients with severe heart failure is being conducted in France to evaluate structural changes in response to the therapy.

Reducing SERCA2a inhibition by targeting cardiac phospholamban is an alternative strategy for increasing SERCA2a activity. Cardiac phospholamban modulation using antisense RNA,[211] a dominant-negative phospholamban mutant,[212] or cardiac-phospholamban-targeted antibodies can increase SERCA2a activity.[213] However, pharmacological approaches to cardiac phospholamban modulation have not been successful because of non-specificity of the pharmacological agents.[91] An *in vitro* study showed that siRNA-mediated inhibition was not sufficient for modulation of cardiac phospholamban in cardiac myocytes, perhaps owing to poor stability of the RNA transcript.[214] However, myocardial phospholamban expression silencing, using an AAV9 vector encoding a short hairpin RNA, resulted in heart failure rescue, increased SERCA2a activity, and improved left ventricular function in aortic-banded rats.[215] This result suggests that AAV-mediated RNA-interference therapies might be beneficial for patients with cardiac hypertrophy and heart failure.

The serine/threonine phosphatases PP1 and PP2 have a role in regulation of cardiac contractility.[216] In particular, the negative regulator of Ca^{2+} cycling PP1, which localizes to the sarcoplasmic reticulum, has been implicated in the regulation of the β-AR response.[217] Dephosphorylation of phospholamban restores inhibition of SERCA, and inhibition of Ca^{2+} uptake into the sarcoplasmic reticulum[218] and an increase in PP1 activity[219,220] was shown in failing human hearts. PP1 is regulated by endogenous protein phosphatase 1 regulatory subunit 1A (IPP-1; also known as protein phosphatase inhibitor 1).[221] IPP-1 is activated by PKA-mediated phosphorylation at Thr35 and specifically inhibits PP1 activity. The protein could, therefore, act an amplifier of β-adrenergic responses in the heart.[222,223] In heart failure, IPP-1 expression is decreased and the protein is dephosphorylated and inactive.[224] The decrease in the level of active IPP-1 results in a reduction in PP1 inhibition, activation of cardiac phospholamban, and inhibition of SERCA2a activity.[221] PP1 inhibitors could, therefore, potentially be used to increase SERCA2a activity in patients with heart failure. In rats with heart failure, this approach enhanced contractility under basal conditions and after β-adrenergic stimulation.[225] In another study, inducible overexpression of IPP-1c in mouse hearts increased cardiac phospholamban phosphorylation at Ser16, but had no effect on phosphorylation of RyR2 at Ser2,808, troponin I at Ser23 or Ser24, or myosin binding protein C at Ser282.[226] These data confirm that IPP-1c specifically inhibits cardiac phospholamban. In addition, IPP-1c expression improved postischemic cardiac performance[226] and had cardioprotective effects (namely, attenuation of the endoplasmic reticulum stress response) in adult transgenic mice.[226] IPP-1c overexpression enhanced cardiac contractility in young mice, but resulted in contractile dysfunction and ventricular dilatation in response to β-adrenergic stimulation in aged mice.[227] By contrast, IPP-1c-mediated, PP1-specific inhibition increased Ca^{2+} uptake into the sarcoplasmic reticulum

without direct modification of sarcoplasmic reticulum Ca^{2+} release in rat cardiac myocytes.[228] These data suggest that IPP-1c could be an interesting new target for gene therapy in heart failure.

The post-translational modification of small ubiquitin- related modifier 1 (SUMO-1) could be targeted to increase SERCA2a activity. SUMO-1 is a unique polypeptide that covalently conjugates to target proteins. This binding, termed SUMOylation, regulates many aspects of protein function, such as subcellular localization, protein–protein interactions, and transcriptional activity.[229] A number of target proteins that are regulated by SUMOylation have been implicated in human disease.[230] For example, a decrease in lamin A SUMOylation could be a causative factor in familial dilated cardiomyopathy; two different mutations in lamin A have been identified in patients with this disease.[231,232] These mutations, which cause the amino-acid substitutions Glu203Gly and Glu203Lys, are likely to lead to defective lamin A SUMOylation as well as alterations in nuclear morphology and the subcellular localization of lamin A.[232] We have shown that the activity and stability of SERCA2a in cardiac myocytes is modulated by SUMO-1, and that cardiac expression of SUMO-1 is significantly reduced in humans and animals with heart failure.[233] Cardiac-specific overexpression of SUMO-1 in transgenic mice restored SERCA2a function, improved hemodynamic performance, and significantly increased survival following thoracic aortic constriction.[233] Consistent with these findings, a long-term cardiac benefit of SUMO-1 over-expression (using AAV9-mediated *SUMO1* gene delivery) has been shown in mouse models of heart failure. Transgene-mediated SUMO-1 overexpression rescued pressure-overload-induced cardiac dysfunction concomitantly with increased SERCA2a function and improved left ventricular dilatation, functional deterioration, and hemodynamic performance 2 months after gene transfer (4 months after thoracic aortic constriction).[233] SUMO-1 overexpression also significantly reduced mortality among the mice with heart failure ($P<0.05$).[233] Depletion of cardiac SUMO-1 using AAV9-mediated delivery of a small hairpin RNA in normal mice resulted in a decline in SERCA2a function and expression, and a dramatic increase in mortality.[233] Importantly, these beneficial effects of SUMO-1 overexpression did not occur when SERCA2a was downregulated,[233] suggesting a SERCA2a-dependent role of SUMO-1 in cardiac function. Further studies are required to define the molecular mechanism of SERCA2a regulation by SUMOylation and to investigate the association between low SUMO-1 expression and heart failure.

Preventing Ca^{2+} leak

Hyperphosphorylation of RyR2 by PKA or CaMKII destabilizes the channel and might cause channel dysfunction.[234,235] Two strategies for enhancing RyR2 stabilization—increasing FKBP12.6 binding and reducing hyperphosphorylation—have been investigated. Several animal studies have been carried out to investigate the effects of FKBP12.6 overexpression in failing hearts. In one study, adenovirus-mediated FKBP12.6 over-expression reduced Ca^{2+} leakage from the sarcoplasmic reticulum as well as Ca^{2+} spark frequency and amplitude in isolated rabbit cardiac myocytes.[236] In another study, cardiac-specific overexpression of FKBP12.6 in mice led to significantly improved cardiac function after myocardial infarction.[237] Improved cardiac function was also shown in transgenic mice that overexpressed a mutant form of FKBP12.6 with a high binding affinity for hyper-phosphorylated RyR2 *in vitro*.[237] These results support the hypothesis that increased binding of FKBP12.6 to RyR2 might improve cardiac function in heart failure.[237]

Pharmacological strategies aimed at reducing Ca^{2+} leakage through RyR2 have also been investigated. These strategies involve the use of agents that interact with RyR2 and inhibit Ca^{2+} release from the sarcoplasmic reticulum, either by altering the gating of the RyR2 channel or controlling ion translocation. Unfortunately, most of the classic RyR2-targeting drugs, such as dantrolene and flecainide, have unacceptable adverse effects or lack long-

term efficacy.[238,239] JTV-519 (also known as K201) is a promising new drug with cardioprotective effects.[240] The drug is a 1,4-benzothiazepine derivative that has structural similarities to diltiazem, a voltage-dependent LTCC blocker used in the treatment of hypertension and some types of arrhythmias.[241] JTV-519 has cardioprotective and antiarrhythmic properties.[242,243] The agent improved the contractility of paced hearts and prevented development of heart failure in dogs, probably by preventing dissociation of FKBP12.6 from RyR2 and, therefore, improving defective channel gating.[244] JTV-519 treatment also increased FKBP12.6 binding to hyperphosphorylated RyR2 and improved cardiac function in dogs with pacing-induced heart failure.[244] In guinea pigs[245] and rats,[246] JTV-519 prevented myocardial injury caused by ischemia, and provided more-effective myocardial protection than Ca^{2+}-channel blockers and [247] In one study, the presence of FKBP12.6 β_1-AR blockers. was not required for JTV-519 function and the drug did not prevent delayed after-depolarizations and ventricular arrhythmias in a mouse model of CPVT.[248] The suggestion that FKBP12.6 might be necessary for RyR2 conformational stabilization during the closed state is still controversial. However, JTV-519 has clear beneficial effects in heart failure and most studies have shown that the drug improves arrhythmia induced by defective RyR2 protein.

Rycals (CPU0213, S107, and S44121) are drugs that stabilize RyR2–FKBP complexes and inhibit Ca^{2+} leakage from the sarcoplasmic reticulum; they could, therefore, also have beneficial effects in patients with heart failure.[249] In one study, the rycal S107 reduced biochemical and histological evidence of skeletal muscle damage, improved skeletal muscle function, and increased exercise performance in wild-type mice subjected to intensive exercise and in a mouse model of Duchenne muscular dystrophy.[250] To our knowledge, cardioprotective effects of rycal drugs have not yet been shown. However, the efficacy of S44121 is currently being evaluated in patients with chronic heart failure who are at risk of ventricular arrhythmias.[251] Suppression of CaMKII hyperactivity after chronic β-AR stimulation has been suggested as a therapy for heart failure. However, whether elevated CaMKII activity is a causative factor in the development of heart failure or a consequence of the disease is not clear.[252] Pharmacological inhibition of CaMKII using KN-92 and KN-93 reduced RyR2 phosphorylation and Ca^{2+} leak from the sarcoplasmic reticulum in human myocardium.[253] However, these drugs are not specific inhibitors of CaMKII, but rather inhibit all CaMK iso-forms, as well as LTCC.[254] Peptide inhibitors of CaMKII have low potency as well as poor specificity (they also inhibit other CaMKs and PKA).[255] Cardiac-specific CaMKII inhibitors that could be used to treat patients with steady-state heart failure are not currently available.

Another potential target for heart failure therapy is the RyR2-stabilizing protein, S100A1. Reduced S100A1 expression has been implicated in cardiomyopathies,[256] and animal models have shown the potential of S100A1 gene therapy for treatment of heart failure.[257–259] For example, in rat models of heart failure, AAV6-mediated overexpression of S100A1 improved cardiac function parameters, including ejection fraction, and reversed left ventricular remodeling.[259] Adenovirus-mediated S100A1 overexpression also improved the function of failing cardiac myocytes isolated from patients with ischemic heart failure.[260] This result suggests that S100A1 could be a therapeutic target in human heart failure. The safety and efficacy of coronary venous AAV9–S100A1 delivery has been shown in a pig model of postischemic heart failure; contractile function and cardiac remodeling were significantly improved.[261] However, this system has not yet been evaluated in human heart failure.

Conclusions

Improved understanding of the mechanisms of Ca^{2+} cycling in cardiac cells has led to the development of novel therapeutic strategies for heart failure. These strategies include the targeting of proteins implicated in Ca^{2+} cycling abnormalities, such as Ca^{2+}-handling proteins in the sarcoplasmic reticulum. Targeting these proteins to enhance Ca^{2+} uptake into the sarcoplasmic reticulum or block sarcoplasmic reticulum Ca^{2+} leakage could have substantial therapeutic benefits in patients with heart failure. 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 Ca^{2+}-handling proteins will ultimately guide the future development of novel treatment modalities for patients with heart failure.

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Key points

- Ca^{2+} cycling defects in cardiac myocytes are a hallmark of heart failure
- Ca^{2+}-handling abnormalities in failing cardiac myocytes include reduced Ca^{2+} uptake, decreased sarcoplasmic reticulum Ca^{2+} sequestration, and defective Ca^{2+} release from the sarcoplasmic reticulum, resulting in cytosolic Ca^{2+} overload
- Changes in the expression and activity of Ca^{2+}-handling proteins have been described in patients with chronic heart failure
- Restoration of sarcoplasmic reticulum Ca^{2+} uptake through activation of sarcoplasmic/endoplasmic reticulum calcium ATPase 2a is a promising strategy for the treatment of patients with heart failure
- Reducing Ca^{2+} leakage from the sarcoplasmic reticulum by targeting calcium/calmodulin-dependent protein kinase type II or stabilizing ryanodine receptors is also a promising strategy for treatment of patients with heart failure

Review criteria

A search for original articles published between 1970 and 2012 and focusing on the sarcoplasmic reticulum was performed in MEDLINE and PubMed. The search terms used were “sarcoplasmic reticulum”, “calcium”, “SERCA2a”, and “ryanodine receptor”, alone and in combination. All articles identified were English-language, full-text papers. We also searched the reference lists of identified articles for further relevant papers.

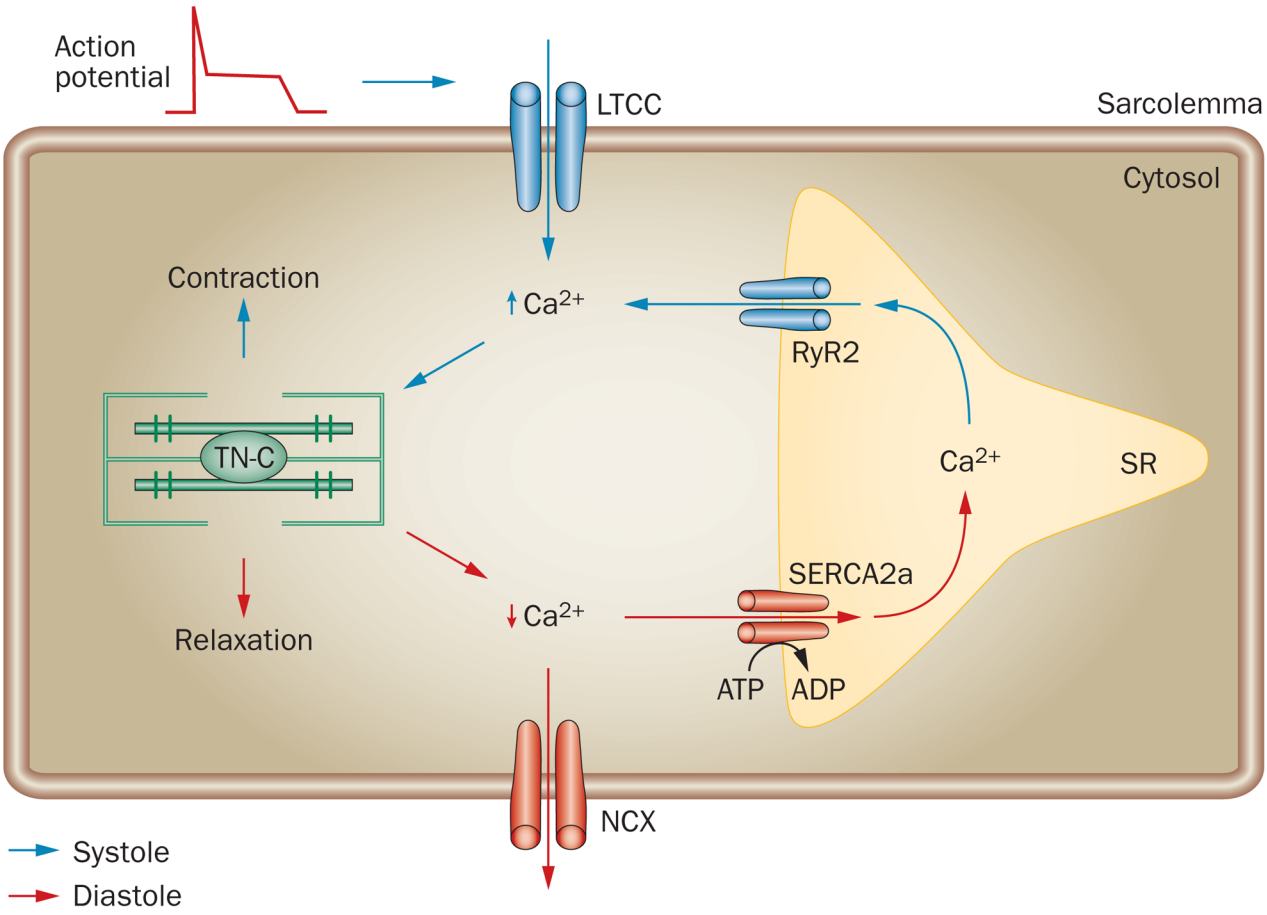

Figure 1.

Excitation–contraction coupling. During systole, the action potential depolarizes the sarcolemma. This depolarization enables a small amount of extracellular Ca^{2+} to enter the cytosol through the LTCCs. Ca^{2+} entry triggers the release of Ca^{2+} from the SR through RyR2 channels. The intracellular Ca^{2+} concentration increases and binding of Ca^{2+} to TN-C activates myofilaments, resulting in muscle contraction. Removal of cytosolic Ca^{2+} during diastole is primarily facilitated by SERCA2a, which returns Ca^{2+} to the SR. Some Ca^{2+} also exits the cell through NCX. The decrease in intracellular Ca^{2+} leads to dissociation of Ca^{2+} from TN-C and muscle relaxation. Abbreviations: LTCC, voltage-dependent L-type Ca^{2+} channel; NCX, Na^{+}/Ca^{2+} exchanger; RyR2, ryanodine receptor 2; SERCA2, sarcoplasmic/endoplasmic reticulum calcium ATPase 2; SR, sarcoplasmic reticulum; TN-C, troponin C.

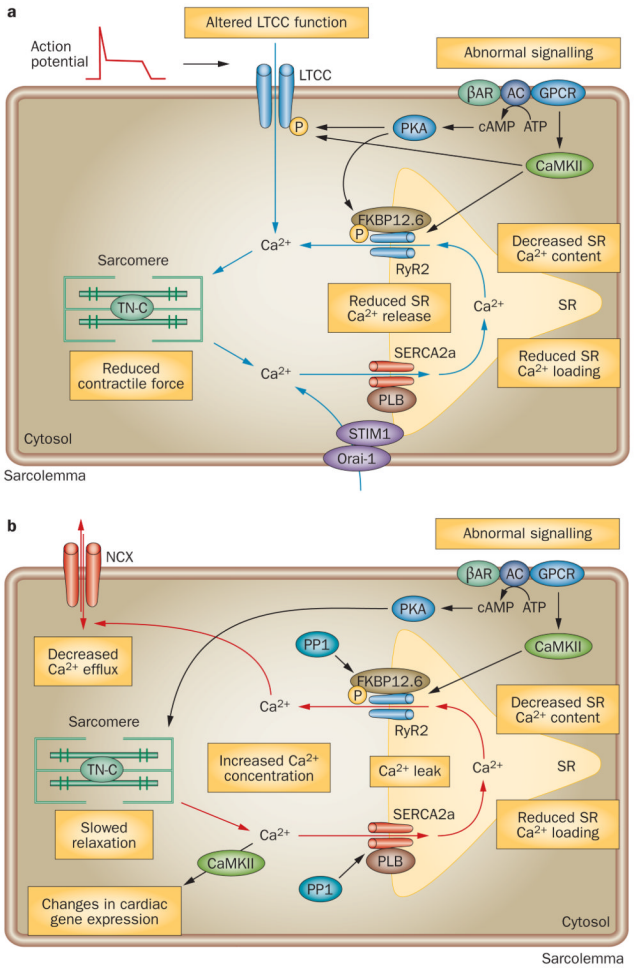

Figure 2.

Abnormal intracellular Ca^{2+} handling in failing cardiomyocytes results in reduced contractile force and prolonged relaxation. **a.** Reduced SR Ca^{2+} storage and release cause abnormal systolic cardiomyocyte function. Abnormal β-AR and GPCR signaling increase the expression of PKA and CaMKII, which hyperphosphorylate and alter the function of RyR2 and LTCC. FKBP12.6 also contributes to stabilization of the RyR2 open probability. Impaired SERCA2a function decreases SR Ca^{2+} loading, resulting in reduced SR Ca^{2+} content and release and, therefore, reduced contractility. Alterations in LTCC function and STIM1–ORAI1-mediated store-operated Ca^{2+} influx can also result in abnormal Ca^{2+} handling during systole. **b.** Reduced SR Ca^{2+} resequestration is a key abnormality in diastole. Decreased SERCA2a activity, downregulation of SERCA2a, a decreased PLB:SERCA2a ratio, or PLB hypophosphorylation cause prolonged intracellular Ca^{2+} transients, reduced SR Ca^{2+} loading, and slowed cardiomyocyte relaxation. An increase in levels of NCX might be a compensatory response to prevent Ca^{2+} overloading. While increased NCX activity would be initially adaptive, excessive or sustained NCX activation could contribute to decreased sarcoplasmic reticulum Ca^{2+} content by removing cytosolic Ca^{2+}, and reducing systolic Ca^{2+} transients and contractile function. SR Ca^{2+} leak resulting from impaired RyR2 function and increased expression of PP1 can reduce SR Ca^{2+} content and increase cytosolic Ca^{2+} concentration during diastole. On activation, the β-AR–AC–GPCR complex synthesizes cAMP, which activates PKA. Hyperactive PKA decreases the Ca^{2+} sensitivity of the myofilaments and prolongs relaxation. Intracellular Ca^{2+} overload

stimulates CaMKII, which contributes to SR diastolic Ca^{2+} leak by hyperphosphorylating RyR2 and induces the transduction of pathological Ca^{2+} signaling. Abbreviations: AC, adenylate cyclase; β-AR, β-adrenergic receptor; CaMKII, calcium and calmodulin-dependent protein kinase type II; FKBP12.6, FK506 binding protein; GPCR, G-protein-coupled receptor; LTCC, voltage-dependent L-type Ca^{2+} channel; NCX, Na^{+}/Ca^{2+} exchanger; Orai-1, calcium-release activated calcium channel protein 1; P, phosphorylation; PLB, cardiac phospholamban; PP1, protein phosphatase 1; RyR2, ryanodine receptor 2; SERCA2a, sarcoplasmic/endoplasmic reticulum calcium ATPase 2a; STIM1, stromal interaction molecule 1.

Table 1

Therapies for heart failure that target Ca^{2+}-handling proteins

Target	Strategy	Effects in heart failure	Clinical stage of development
Restoration of Ca^{2+} uptake into the sarcoplasmic reticulum			
SERCA2a	Increasing expression by AAV1-mediated gene delivery	Rescues depressed myocardial contractility and increases survival	Clinical trials: Phase I/II CUPID[209] demonstrated safety and efficacy in patients with advanced heart failure, Phase II NCT00454818[210] is ongoing
SUMO1	Increasing expression by manipulation of post-translationally modified protein	Improves SERCA2a activity and stability	Beneficial effects of AAV9-mediated *SUMO1* gene delivery shown in mouse models[233]
Phospholamban	Decreasing expression by ablation or depletion using dominant-negative molecules	Increases SERCA2a activity Reduces phospholamban-mediated SERCA2a inhibition	Beneficial in experimental animal models[211–213,215] Outcome in human heart failure is unclear
IPP-1	Overexpression of constitutively active form (IPP-1c)	Conditional amplification of β-adrenergic signaling Enhanced phospholamban phosphorylation	Beneficial effects of IPP-1 gene transfer shown in murine models of heart failure[225]
Prevention of Ca^{2+} leakage from the sarcoplasmic reticulum			
RyR2	Channel stabilization Decreased probability of open channels	Prevention of sarcoplasmic reticulum dysfunction and possible antiarrhythmic effects	Agents that stabilize RyR2, such as JTV-519 and S44121, are being tested in animal models[262,263] and in muscle preparations from patients with heart failure and arrhythmias[247]
CaMKII	Inhibition of CaMKII hyperactivity	Prevention of pathological β-AR mediated signaling Prevention of arrhythmia	Inhibition of CaMKII using inhibitory peptides or small molecules beneficial in animal models of structural heart disease[177–179]
S100A1	Overexpression	Stabilization of RyR2 in diastole and reduction in arrhythmic risk Increased SERCA2a activity	Safety and efficacy shown in preclinical large animal model[261] as well as in human cardiomyocytes[230] Additional beneficial effects shown in a rat model of myocardial infarction when S100A1 gene therapy was combined with β-AR blockers[259]

Abbreviations: AAV, adeno-associated virus; β-AR, β-adrenergic receptor; CaMKII, calcium/calmodulin-dependent protein kinase type II; IPP-1, endogenous protein phosphatase 1 regulatory subunit 1A; RyR2, ryanodine receptor 2; S100A1, S100 calcium binding protein A1; SERCA2a, sarcoplasmic and endoplasmic reticulum calcium ATPase 2a; SUMO-1, small ubiquitin-related modifier 1.