Basic Concepts in Heart Failure

Ca\textsuperscript{2+} Cycling and New Therapeutic Approaches for Heart Failure

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Heart failure (HF) is a major health problem in Western countries. Despite significant progress in pharmacological and device-based treatment, the disease burden imposed continues to increase, particularly as the population ages. HF incidence approaches 10 per 1000 after age 65 years.\textsuperscript{1} Congestive HF is the final consequence of diverse cardiovascular disorders, including atherosclerosis, cardiomyopathy, and hypertension. Described as a complex pathophysiological syndrome that involves interactions of the circulatory, neurohormonal, and renal systems, HF is first a disease of the myocardium, although it soon induces defects in other systems.

Current treatments for HF, focused on blocking neurohormonal pathways, improve survival, but they do not halt the progression of HF. Late-stage HF has a poor prognosis, and therapeutic options are limited. Faced with these challenges, researchers are exploring novel therapeutic options.

Chronic HF is associated with increased sympathetic outflow, which may be compensatory early on, but long-term neurohormonal activation induces significant damage to the heart; in addition, it results in multiple alterations in the \beta-adrenergic receptor (\beta-AR) signaling cascade, including receptor downregulation, upregulation of receptor kinases, and increased inhibitory G-protein function.\textsuperscript{2}

The amplitude and velocity of Ca\textsuperscript{2+} cycling are regulated by a dynamic balance of phosphorylation and dephosphorylation through kinases and phosphatases. Activation of \beta-ARs stimulates cAMP production and results in protein kinase A (PKA) phosphorylation of key regulators of excitation-contraction coupling, such as L-type Ca\textsuperscript{2+} channels, phospholamban, troponin I, ryanodine receptors (RyR), myosin-binding protein C, and protein phosphatase inhibitor-1 (I-1; Figure), which leads to increased amplitude and velocity of Ca\textsuperscript{2+} cycling and increased contractility on a beat-to-beat basis.\textsuperscript{3} Protein phosphatases PP1 and PP2A counterbalance phosphorylation of these proteins. There is clear evidence that alterations in sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} cycling are a component of the impaired contractile performance of the failing heart.\textsuperscript{4,5} The abnormal intracellular Ca\textsuperscript{2+} handling includes SR Ca\textsuperscript{2+} leak through the RyR,\textsuperscript{6} decreased SR Ca\textsuperscript{2+} uptake,\textsuperscript{7} and decreased SR content. Impaired SR Ca\textsuperscript{2+} uptake results from lower expression of the SR Ca\textsuperscript{2+} pump, SERCA2a (Sarcoplasmic reticulum Ca\textsuperscript{2+} ATPase),\textsuperscript{7-9} and from an increased inhibitory function of phospholamban.\textsuperscript{10} Furthermore, Ca\textsuperscript{2+}, via the activation of various kinases and phosphatases, acts on Ca\textsuperscript{2+}-dependent transcription pathways.

Alterations in SR Ca\textsuperscript{2+} Cycling and Its Upstream Regulators in HF

Alterations of the \beta-Adrenergic Cascade

Chronic stimulation of \beta-ARs by elevated plasma catecholamines and the subsequent stimulation of the cAMP/PKA-dependent signaling pathway represents a central factor in the pathogenesis of HF.\textsuperscript{2} Three \beta-AR subtypes have been described in mammalian hearts, \beta\textsubscript{1}-, \beta\textsubscript{2}-, and \beta\textsubscript{3}-AR, with the \beta\textsubscript{1}- and \beta\textsubscript{2}-AR subtypes dominating functionally. Both are expressed in cardiac myocytes, couple primarily to the stimulatory G protein (G\textsubscript{s}), and mediate the formation of cAMP. In addition, coupling of the \beta\textsubscript{2}-AR to the inhibitory G protein (G\textsubscript{i}) has been described in several animal species\textsuperscript{11,12} and in failing human cardiomyocytes. Effects of G\textsubscript{i} coupling were evident in failing human heart from the rescue of \beta-AR sensitivity after pertussis toxin treatment of isolated myocytes.\textsuperscript{13} It is also true that \beta\textsubscript{2}-AR/G\textsubscript{i} coupling is stronger in human failing hearts than in most other species, and in myocytes, the effect is at least equal to that through \beta\textsubscript{1}-AR/G\textsubscript{i}.\textsuperscript{14,15} This is also demonstrated in atria, in which little \beta\textsubscript{2}-AR/G\textsubscript{i} coupling is seen,\textsuperscript{16} but it is not clear whether it is also true for nonfailing human ventricle. cAMP leads to activation of PKA, which phosphorylates key regulators of contraction and relaxation, as well as regulators of gene transcription, in cardiac myocytes. In addition, cAMP activates cyclic nucleotide–gated channels and the guanine nucleotide exchange factor Epac1 and can induce adult cardiac myocyte hypertrophy in a PKA-independent manner.\textsuperscript{17} A switch to G\textsubscript{i} coupling, medi-
ated by a PKA-dependent phosphorylation of the β2-AR itself, is thought to protect against apoptosis by a number of mechanisms.18,19,20

There are several lines of evidence that the different coupling of β1-AR and β2-AR has consequences for cardiac function (for review, see Lohse et al): (1) Although β1-AR stimulation induces cardiomyocyte growth, β2-AR stimulation does not. (2) Transgenic mice with heart-specific overexpression of β1-AR develop progressive cardiac hypertrophy and dysfunction, whereas β2-AR transgenic mice display a long-standing augmentation of cardiac function; cardiac disease occurs only at high levels of β2-AR overexpression.23,24 (3) Isolated cardiac myocytes undergo apoptosis on β1-AR selective stimulation, whereas β2-AR stimulation may protect against β1-AR-induced apoptosis.25–27 Interestingly, β1-AR overexpression causes early dysfunction of Ca cycling,28 and the resultant cardiac damage can be markedly reduced by different strategies to improve Ca cycling, for example, phospholamban knockout29 or Na/H-exchanger inhibition.30 Numerous studies have demonstrated a reduction in β1-AR correlated with the severity of HF, whereas β2-AR levels remained unchanged in most studies. In addition, the remaining β-ARs are desensitized in the failing heart owing to increased G-protein–coupled receptor kinase activity.35 Inhibition of upregulated G-protein–coupled receptor kinase activity has been proposed as a therapeutic strategy for HF.33 An upregulation of Goi and a downregulation of Gβγ, together with a downregulation of the adenylyl cyclase type V and VI are also observed repeatedly in HF.34,35 Beta2-AR/Gi coupling may be clinically relevant because it has been suggested that some β-blockers can cause the β2-AR to activate Gi-dependent pathways directly and that the use of β1-AR blockers in combination with a β2-AR agonist would be a more appropriate treatment for HF.37 This is a strategy that was used in a study that showed recovery of patients in whom a left ventricular assist device had been implanted to the point that the device could be explanted.38

HF is accompanied by increased global phosphatase activity, as well as SR-associated type 1 (PP-1) phosphatase.39 Overexpression of PP-1 catalytic subunit in the mouse heart, to similar levels as those observed in human failing hearts, caused depressed function and dilated cardiomyopathy.40 This PP-1 activity is regulated by the endogenous I-1 inhibitor. I-1 becomes active on its phosphorylation at Thr35 by PKA, which allows for amplification of the β-adrenergic responses in the heart. I-1 is also phosphorylated at Ser67 and/or Thr75 by protein kinase C, and this results in enhanced phosphatase activity, depressed function, and attenuation of the stimulatory effects of PKA signaling.41,42 In addition, inhibitor-2, another endogenous PP-1 inhibitor, has also been shown to increase cardiac contractility and Ca2+ cycling in a transgenic model,43 and inhibition of PP-1 by inhibitor-2 gene delivery ameliorates HF progression in a model of genetic cardiomyopathy.44

Recently, compartmentalization of different components of the cAMP signaling pathway has raised great interest. Beta-ARs, G proteins, adenylyl cyclases, PKA anchoring proteins, phosphatases, and phosphodiesterases have been reported to be localized in distinct cellular subcompartments and cause subcellular compartmentalization of cAMP.45,46 In adult cardiomyocytes, β1-AR stimulation induced a widespread cAMP signal, whereas β2-AR stimulation induced a localized cAMP signal.47 Compartmentalization of cAMP could be due to localized sites of synthesis by adenylyl cyclases, degradation by phosphodiesterases, or cAMP buffering by binding proteins.48 Loss of spatial cAMP localization has been seen after phosphodiesterase inhibition with a PKA-based sensor45,46 but not with a monomolecular cAMP sensor.47 Compartmentalization, together with alterations in expression and activity of phosphodiesterases, contributes to altered cAMP signaling in HF.49,50 In addition, in cardiac myocytes, as demonstrated in vascular smooth muscle cells, cAMP can be extruded from the cell by members of the multidrug-resistance–associated proteins such as MRP4.51 The importance of cAMP extrusion in HF remains to be demonstrated. Finally, the topography of cardiac myocytes is altered in HF with the loss of T tubules, and this could also affect the localization of β-ARs and the β-adrenergic signaling cascade.

**Figure.** Simplified representation of the main Ca2+ handling mechanisms deregulated in HF. Depicted in gray are systems that are either downregulated or inhibited, and depicted in red are systems that are upregulated or activated in HF. GPCR indicates G-protein–coupled receptors; GRKs, G-protein receptor kinases; AC, adenylyl cyclase; PDE4D3, phosphodiesterase type 4D3; Icα, Ca2+ influx through L-type Ca2+ channels; HCN, hyperpolarization-activated cyclic nucleotide–gated channels; NCX, sodium-calcium exchanger; CSQ, calsequestrin; PLN, phospholamban; SAP, stress-activated protein kinase; JNK, c-Jun NH2-terminal kinase; CaN, calcineurin; NFAT, nuclear factor of activated T cells; CaMK, Ca2+/calmodulin kinase; HDAC, histone deacetylase; and MEF2, myocyte-enhancing factor 2. Red dots indicate phosphorylation; red arrows, phosphorylation; blue arrows, transport; and black arrows, activation.

**Alterations in SR Ca2+ Cycling**

Defective intracellular Ca2+ homeostasis has been reported consistently in HF.53 Prolonged decay of the Ca2+ transient and increased diastolic [Ca2+]i contribute to slowed relaxation.54,55 Several defects in intracellular Ca2+ homeostasis...
have been reported, including depressed Ca\(^{2+}\) uptake, decreased storage, and release of the SR associated with diastolic SR Ca\(^{2+}\) leak. The Figure presents the main mechanisms of SR Ca\(^{2+}\) cycling shown to be altered in HF, together with alterations in the upstream regulatory mechanisms and downstream consequences.

**SR Ca\(^{2+}\) Uptake**

A decrease in Sarco/endoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA) activity was described in the 1970s,\(^\text{56}\) and this was associated with a decrease in SERCA2a expression in the early 1990s.\(^\text{7–9}\) The decrease in the SERCA2a level is related to the intensity and duration of cardiac overload. Reduced SERCA2a activity and SR Ca\(^{2+}\) uptake may lead to an increase in diastolic Ca\(^{2+}\), an abnormally long time course of Ca\(^{2+}\) transient, and a decrease in SR Ca\(^{2+}\) release. Results from transgenic mice reveal a primordial role for SERCA2a dysfunction in induction of signaling pathways that lead to cardiac hypertrophy and failure. Indeed, deletion of the SERCA2 gene (ATP2A2) is lethal, but heterozygous mice are viable and develop cardiac hypertrophy.\(^\text{55}\) Increasing the cardiac load by aortic banding results in accelerated HF progression in SERCA2\(^{-/-}\) mice compared with wild-type controls.\(^\text{58}\) However, in humans, there is no evidence for cardiac hypertrophy or dysfunction in patients who have a mutation of the ATP2A2 gene that leads to haploinsufficiency (Diarer disease); upregulation of the normal allele is a possible explanation for the absence of cardiac hypertrophy.\(^\text{59}\)

Phospholamban is a 52-amino-acid protein that controls the affinity of SERCA2 for Ca\(^{2+}\). In its unphosphorylated form, phospholamban inhibits SERCA2 Ca\(^{2+}\) affinity, and phosphorylation by PKA and Ca\(^{2+}\)/calmodulin kinase (CaMKII) relieves this inhibition and results in enhanced contractility. As indicated above, the levels of SERCA2 decrease significantly in end-stage cardiomyopathy, whereas most studies reported unchanged protein levels of phospholamban. This leads to an increase in the ratio of phospholamban to SERCA2, diminished Ca\(^{2+}\) affinity of SERCA2, and depressed Ca\(^{2+}\) cycling. Furthermore, in failing hearts, the phosphorylation levels of phospholamban at Ser16 and Thr17 are decreased,\(^\text{60,61}\) which indicates an increased inhibitory function of phospholamban. Consistent with these observations, human mutations in phospholamban, which lead to a superinhibitory form or to abnormal interaction with PKA, have been linked to hypertrophic or dilated cardiomyopathy.\(^\text{52,63}\) In addition, 2 single-nucleotide transitions at −49 bp\(^\text{64}\) or −77 bp\(^\text{65}\) upstream of the transcription start site in the phospholamban promoter region, were identified in humans with dilated cardiomyopathy and hypertrophic cardiomyopathy, respectively. These 2 mutations were associated with increased activities of the phospholamban gene promoter in in vitro assays.

Recently, the antiapoptotic HS-1-associated protein X-1 (HAX-1) was identified as a phospholamban-binding partner.\(^\text{66}\) HAX-1 was primarily targeted to mitochondria but redistributed and colocalized with phospholamban at the endoplasmic reticulum on cotransfection with phospholamban in HEK 293 cells. Interestingly, the presence of phospholamban enhanced the antiapoptotic and protective effects of HAX-1 against hypoxia/reoxygenation-induced cell death. Thus, the discovery of the phospholamban/HAX-1 interaction reveals a new regulatory mechanism of cardiac function and may constitute an important interface between Ca\(^{2+}\) homeostasis and cell survival.

**SR Ca\(^{2+}\) Load and Storage**

Major emphasis has been placed on correcting either the depressed SR Ca\(^{2+}\) uptake or impaired SR Ca\(^{2+}\) release to restore cellular Ca\(^{2+}\) homeostasis and contractile performance in HF, but current evidence indicates that SR Ca\(^{2+}\) load and storage also play an important role in regulation of overall cardiac function. Ca\(^{2+}\) load is regulated by calsequestrin.

Calsequestrin is a high-capacity Ca\(^{2+}\) binding protein in the SR lumen. Alterations in calsequestrin levels are associated with cardiac remodeling\(^\text{67}\) and ventricular arrhythmias.\(^\text{68,69}\) Interestingly, mutations in CASQ2 have been shown to cause an autosomal-recessive form of catecholaminergic polymorphic ventricular tachycardia (CPVT), whereas CPVT and arrhythmogenic right ventricular dysplasia have been associated with autosomal-dominant mutations in the SR Ca\(^{2+}\) release channel/ryanodine receptor (RyR2) gene.\(^\text{69,70}\)

Calsequestrin is likely linked to RyR via junctin and triadin, forming a quaternary RyR-triadin-junctin-calsequestrin ensemble that is believed to modulate RyR Ca\(^{2+}\) release.\(^\text{71}\) Alterations in the levels of junctin and triadin, which may anchor RyR2 to calsequestrin, have been shown to impair SR Ca\(^{2+}\) release. Importantly, junctin levels were significantly downregulated and virtually undetectable in human failing hearts,\(^\text{72}\) which may constitute an additional contributor to susceptibility toward arrhythmias. In mice, junctin ablation was associated with enhanced SR Ca\(^{2+}\) leak, which induced fatal arrhythmias through activation of delayed afterdepolarizations under stress.\(^\text{73}\) The delayed afterdepolarizations appeared to be linked to junctin deficiency rather than the accompanying upregulation of the Na\(^+\)-Ca\(^{2+}\) exchanger protein and activity.\(^\text{74}\) These findings support a prominent role of junctin in the regulation of RyR-mediated SR Ca\(^{2+}\) release and both basal and β-adrenergically stimulated cardiac function.

Besides calsequestrin, the histidine-rich Ca\(^{2+}\) binding protein (HRC) is another low-affinity, high-capacity Ca\(^{2+}\) binding protein. HRC is an SR luminal Ca\(^{2+}\) binding protein that is associated with SERCA2 and triadin.\(^\text{75}\) Importantly, a genetic variant in the HRC gene, Ser96Ala, is associated with ventricular arrhythmias in patients with idiopathic dilated cardiomyopathy.\(^\text{76}\) In addition, HRC is significantly downregulated in HF patients and in animal models of HF.\(^\text{77}\)

**SR Ca\(^{2+}\) Release**

Increased diastolic [Ca\(^{2+}\)], most likely transient in nature, may result from SR Ca\(^{2+}\) leak through RyR2, which displays an increased sensitivity to Ca\(^{2+}\)-induced Ca\(^{2+}\) release and incomplete channel closure during diastole in HF.\(^\text{78}\) This leak may induce Ca\(^{2+}\) waves and lead to delayed afterdepolarizations, a major trigger for ventricular arrhythmias. Interestingly, mutations in RyR2 are present in the majority of patients with CPVT.\(^\text{79}\) Chronic sympathetic activity in HF was shown to induce PKA hyperphosphorylation of RyR2 at
S2008/2009 (depending on the species), \textsuperscript{78,80,81} which resulted in dissociation of the channel-stabilizing protein calstabin2 (FKB12.6), \textsuperscript{78,82} This PKA hyperphosphorylation has been linked to phosphodiesterase 4D3 deficiency in the RyR2 complex in failing hearts. \textsuperscript{83} The site of PKA phosphorylation (Ser 2008 versus Ser 2030) and its importance in HF are a matter of debate but have been discussed in detail elsewhere. \textsuperscript{84} RyR2 is also phosphorylated by CaMKII on Ser 2814/2815, which enhances open-channel probability\textsuperscript{85} and SR Ca\textsuperscript{2+} leak.\textsuperscript{86,87} RyR2 phosphorylation by CaMKII is induced by activation of EPAC (exchange protein directly activated by cAMP).\textsuperscript{88} Furthermore, CaMKII is involved in the apoptotic effect of chronic \(\beta\textsubscript{-}AR\) stimulation.\textsuperscript{89} Thus, the EPAC/CaMKII/RyR2 phosphorylation pathway may be the link between \(\beta\textsubscript{-}AR\) and apoptosis. A recent study using CaMKI\(\textsuperscript{b}\) ablated mice suggested that CaMKII contributes to cardiac decompensation by enhancing RyR2 phosphorylation and SR Ca\textsuperscript{2+} leak.\textsuperscript{90} Despite some controversy about RyR2 phosphorylation at its PKA versus CaMKII sites, there is a consensus that increased RyR2 sensitivity to Ca\textsuperscript{2+} and RyR2 aberrant function caused by hyperphosphorylation are key mechanisms in the pathogenesis of HF.

**SR Ca\textsuperscript{2+} Cycling Therapeutic Strategies in HF**

Ca\textsuperscript{2+} cycling defects are potential therapeutic targets for HF and Ca\textsuperscript{2+} cycling proteins, and their regulators are considered potential targets for the treatment of HF.

**Increasing SR Ca\textsuperscript{2+} Uptake**

Two approaches have been proposed to increase SERCA activity: SERCA overexpression and phospholamban inhibition.

**SERCA Overexpression**

Transgenic animals with cardiac-specific expression of either SERCA2a or SERCA1a showed improved contractility under baseline conditions and after pressure overload.\textsuperscript{91–93} The mortality rate after aortic banding in animals overexpressing SERCA2a was identical to that in the wild-type controls.\textsuperscript{92} The results regarding arrhythmias are controversial. Although Chen et al\textsuperscript{94} reported an increased frequency of arrhythmias leading to mortality after myocardial infarction in transgenic rats, dramatic and significant decreases in all indices of ventricular arrhythmias were observed after postischemic injury in SERCA1a-overexpressing hearts from transgenic mice and in rodent and porcine hearts after gene transfer of SERCA2a.\textsuperscript{93,95} An excess of SERCA2a expression may lead to increased SR Ca\textsuperscript{2+} content and SR/endoplasmic reticulum stress, but interestingly, even if high amounts of SERCA2a messenger RNA are expressed in the adult heart, the level of protein increase does not exceed 30\% to 50\%.\textsuperscript{91,96}

Improvements in systolic and diastolic function after SERCA2a overexpression have been demonstrated in many studies and in various conditions: In isolated cardiac myocytes from failing hearts, in healthy small animals by gene transfer and in transgenic animal models, in small-animal models of HF (gene transfer and transgenics), and by gene transfer in large-animal models.\textsuperscript{97} SERCA2a rescues depressed contractility and survival\textsuperscript{98} while at the same time improving both energy metabolism (as evidenced by restoration of the creatine phosphate–to-ATP ratio) and energy utilization (as evidenced by restoration of the O\textsubscript{2} consumption–to-E\textsubscript{max} relationship to normal levels).\textsuperscript{98,99} These results have been discussed recently in a comprehensive review.\textsuperscript{97} By lowering cytosolic Ca\textsuperscript{2+}, SERCA2a expression inhibits calcineurin activity and activation of the Nfat (nuclear factor of activated T cells) pathway.\textsuperscript{100} In addition, an increase in diastolic Ca\textsuperscript{2+} leads to activation of Ca\textsuperscript{2+}-activated proteases and kinases (Figure), which in the long term leads to apoptosis and breakdown of vital structural proteins. This may explain why SERCA2a gene transfer inhibits hypertrophic, hyperplastic, and apoptotic signaling pathways mediated by calcineurin. Another reason for the beneficial role of SERCA2a overexpression may be that it reduces the effect of oxidative stress. Indeed, high levels of oxygen-derived free radicals are generated during myocardial ischemia/reperfusion, and this damages SERCA2a,\textsuperscript{101} potentially contributing to cellular Ca\textsuperscript{2+} overload and myocardial injury. Finally, overexpression of SERCA2a increases coronary blood flow and thus improves cardiac function through a direct effect on endothelial cells.\textsuperscript{102} The contrast between the beneficial effects of restoration of SERCA2a activity and the damaging consequences of \(\beta\textsubscript{-}agonist/phosphodiesterase inhibitor inotropic agents is striking, particularly because SERCA2a stimulation (by \(\beta\textsubscript{-}\)adrenergic-dependent removal of phospholamban inhibition) is a key part of the action of these agents. It can be hypothesized that the arrhythmias and cell death produced by excess cAMP relate more to other targets, such as the RyR and L-type Ca\textsuperscript{2+} channel, which have the potential to cause cytoplasmic Ca\textsuperscript{2+} overload when stimulated excessively.

The beneficial results observed in preclinical testing of targeting Ca\textsuperscript{2+} cycling in the failing cardiac myocyte have led to the initiation of clinical trials in patients with HF to enhance SR Ca\textsuperscript{2+} uptake. Because pharmacological targeting of SERCA2a or phospholamban has not yielded agents that have high specificity for either SERCA2a or phospholamban, a gene therapy approach was begun more than 10 years ago. After a great deal of toxicology work, this led to the initiation and recent completion of a first-in-humans phase 1 clinical trial, Calcium Upregulation by Percutaneous administration of gene therapy In cardiac Disease (CUPID), of gene therapy for patients with advanced HF using an adeno-associated type 1 vector carrying SERCA2a.\textsuperscript{103} The safety profile of adeno-associated virus gene therapy in these patients along with the positive biological signals obtained from this phase 1 trial have led to the initiation of a phase 2 trial of AAV1.SERCA2a in patients in New York Heart Association class III/IV.\textsuperscript{104}

**Phospholamban Inhibition**

As outlined above, phospholamban is a critical regulator of cardiac contractility and a cause of inherited cardiomyopathy in humans. Thus, suppression of the inhibitory effect of phospholamban is a promising approach to improving cardiac function. Indeed, recombinant adenovirus-mediated gene transfer in failing human cardiac myocytes with the antisense phospholamban resulted in enhanced contractile properties, similar to cardiac myocytes infected with SERCA2.\textsuperscript{105} In
addition, overexpression of the phospholamban-dominant negative mutants K3E/R14E or V49A in cardiac myocytes improved SR function and myocyte contractility.\textsuperscript{106,107} Furthermore, phenotypic rescue of HF mouse models by genetic complementation with phospholamban ablation also provided evidence that targeting phospholamban may restore cardiac function and potentially serve as a therapeutic modality in HF.\textsuperscript{107} More recently, chronic inhibition of phospholamban with gene transfer of small interfering RNA with adenovirus type 9 vectors in a rodent model of HF resulted in improved contractility, reversal of adverse remodeling, and a decrease in hypertrophy and fibrosis.\textsuperscript{108} These findings support the notion that targeting phospholamban can enhance cardiac contractility. Furthermore, phenotypic rescue of HF mouse models by genetic complementation with phospholamban ablation also provided evidence that targeting phospholamban may restore cardiac function and potentially serve as a therapeutic modality in HF.\textsuperscript{107} Phospholamban mutations have also been identified in human patients, and the inheritance of Arg9Cys or R14del-phospholamban, which act as superinhibitors, was linked to dilated cardiomyopathy\textsuperscript{62,109}; however, another Leu39stop-phospholamban mutant that resulted in apparent phospholamban ablation\textsuperscript{62} was also associated with dilated cardiomyopathy. Thus, caution should prevail in targeting phospholamban as a therapeutic modality in human HF, although a caveat of the Leu39stop-phospholamban human studies is that the number of affected individuals is low, and the score for linkage of the mutation to the disease is low.

In HF, decreased phospholamban phosphorylation may be associated with decreased kinase (PKA) activity and/or, more interestingly, increased PP-1 activity. The latter may be caused by dephosphorylation or inactivation of I-1. Thus, overexpression of the PP-1 catalytic subunit in mice demonstrated significantly decreased phosphorylation of phospholamban and closely recapitulated the phenotype of HF.\textsuperscript{109} In addition, expression of a constitutively active I-1 restored contractile properties in failing rat hearts\textsuperscript{110} and failing human cardiac myocytes under isoproterenol treatment.\textsuperscript{40} Furthermore, infection of adult and neonatal rat cardiac myocytes with an adenovirus encoding the full-length I-1 was associated with a marked increase in phospholamban phosphorylation and cardiac contractility.\textsuperscript{111} More interestingly, temporally controlled expression of active I-1 (T35D) in the adult heart elicited significant enhancement of SR Ca\textsuperscript{2+} transport and contractile function, which was associated with preferential phospholamban phosphorylation.\textsuperscript{39} On ischemia/reperfusion-induced injury, hearts with active I-1 expression exhibited attenuated infarct size and apoptosis, as well as necrosis, which resulted in improved contractile function and recovery.\textsuperscript{39} These data suggest that increased I-1 activity enhances Ca\textsuperscript{2+} cycling and improves mechanical recovery and cell survival after an ischemic insult.

**Fixing SR Ca\textsuperscript{2+} Leak**

Because diastolic SR Ca\textsuperscript{2+} leak is an important contributing factor to HF, it was of major interest to develop drugs that prevent SR Ca\textsuperscript{2+} leak by stabilizing RyR2.\textsuperscript{112} A benzodiazepine derivative (JTV519) was shown to improve cardiac function, possibly by enhancing calstabin2 (FKB12.6)-RyR2 interactions.\textsuperscript{82} JTV519 increases the affinity of calstabin2 for PKA-hyperphosphorylated RyR2, which stabilizes the closed state of RyR2 and prevents diastolic SR Ca\textsuperscript{2+} leak.\textsuperscript{113} JTV519 rescued calstabin2 binding to recombinant RyR2-harboring mutations observed in patients with CPVT\textsuperscript{114} and inhibited pacing-induced arrhythmias in calstabin2\textsuperscript{27} mice.\textsuperscript{115} JTV519 was without effect in homozygous calstabin2-deficient mice, which indicates that the presence of calstabin2 is necessary for JTV519 activity. S107 is a new member of a class of drugs called "rycals." S107 stabilizes the RyR1/2-calstabin1/2 complexes and inhibits SR Ca\textsuperscript{2+} leak, reduces biochemical and histological evidence of muscle damage, improves muscle function, and increases exercise performance in murine skeletal muscles in wild-type mice subjected to severe exercise and in a murine model of Duchenne muscular dystrophy (mdx).\textsuperscript{116,117} Knock-in mice heterozygous for the CPVT-linked RyR2-R2474S mutation exhibited ventricular arrhythmias and sudden cardiac death after exercise followed by catecholamine treatment.\textsuperscript{118} In addition, they exhibited spontaneous generalized tonic-clonic seizures, which occurred in the absence of cardiac arrhythmias. Treatment with S107 inhibited the channel leak, prevented cardiac arrhythmias, and raised the seizure threshold.\textsuperscript{118} Thus, S107 can rescue RyR dysfunction observed in the heart, skeletal muscle, and brain and could be a candidate for treatment of ventricular arrhythmias, muscle fatigue, and epilepsy.

In summary, dysregulation of Ca\textsuperscript{2+} cycling appears to play a major role in HF. Several interesting targets have been identified in recent years; however, finding therapeutic strategies to address these targets has proven difficult. This may make indirect strategies (ie, targeting proteins that interfere with the key targets) or new forms of interference such as gene therapy necessary. There can be no doubt that research on these key events in cardiac physiology not only will continue to produce a better picture of the complex etiology of HF but also will ultimately be translated into new treatment strategies.

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112


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