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J Clin Invest. 2005;115(3):556-564. <https://doi.org/10.1172/JCI24159>.

Review Series

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Altered intracellular Ca²⁺ handling in heart failure

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Structural and functional alterations in the Ca²⁺ regulatory proteins present in the sarcoplasmic reticulum have recently been shown to be strongly involved in the pathogenesis of heart failure. Chronic activation of the sympathetic nervous system or of the renin-angiotensin system induces abnormalities in both the function and structure of these proteins. We review here the considerable body of evidence that has accumulated to support the notion that such abnormalities contribute to a defectiveness of contractile performance and hence to the progression of heart failure.

Introduction

Heart failure (HF) is characterized by a complex disorder that leads to a disturbance of the normal pumping of blood to the peripheral organs to meet the metabolic demands of the body. In a heart that has suffered myocardial damage, regardless of the initial cause of the damage (hypertension, myocardial ischemia, cardiomyopathy, etc.), HF eventually occurs if such damage persists for a prolonged period (1, 2). In the initial stages, compensation for the myocardial damage and maintenance of hemodynamics can occur via activation of both the sympathetic nervous system and the renin-angiotensin system, resulting in LV dilatation and/or hypertrophy. However, if the depressed cardiac function persists, with a parallel activation of neurohumoral factors, the myocardial damage becomes progressive and irreversible, and the heart can no longer meet the metabolic demand of the body, resulting in the phenotype of HF (1, 2).

A growing body of evidence has accumulated concerning the altered intracellular Ca²⁺ cycling that plays a key role in the development of HF (3–5). Recent advances in the field of molecular biology have shed light on the close relationship between Ca²⁺ cycling abnormalities and the progression of HF. In many cases, altered Ca²⁺ cycling precedes the observed depression of mechanical performance; consequently, an amelioration of the disorder of Ca²⁺ cycling has potential as a new and intriguing therapeutic strategy against HF (5). In this review, we focus on the role of Ca²⁺ regulatory proteins in the pathogenesis of HF and on the possibility of developing a new therapeutic strategy against HF using Ca²⁺ regulatory proteins as the target.

Intracellular Ca²⁺ handling in normal cardiomyocytes

In the normal heart, intracellular Ca²⁺ movements critically regulate subsequent mechanical contractions. In cardiac excitation-contraction (E-C) coupling (Figure 1), a small amount of Ca²⁺ first enters through the L-type Ca²⁺ channel (LTCC) during membrane depolarization. This Ca²⁺ influx triggers a large-scale Ca²⁺ release through the Ca²⁺ release channel of the sarcoplasmic reticulum (SR),

referred to as the ryanodine receptor (RyR). The released Ca²⁺ then binds to the troponin C within the myofilaments, which induces activation of the myofilaments and a consequent muscle contraction (6–8). Relaxation is initiated by dissociation of Ca²⁺ from troponin C, followed by its reuptake into the SR through phospholamban-regulated (PLN-regulated) Ca²⁺-ATPase (SERCA2a) and subsequent trans-sarcolemmal Ca²⁺ removal through the Na⁺/Ca²⁺ exchanger (NCX) operating in its forward mode (7–9). The whole process of Ca²⁺ movement is characterized by a transient increase in intracellular [Ca²⁺] from 100 nM to about 1 μM (8). For termination of Ca²⁺ release, RyR adaptation (10), RyR inactivation (11), and SR Ca²⁺ depletion may play important roles by acting in a synergistic manner.

Triggers for Ca²⁺ release and defective E-C coupling in HF

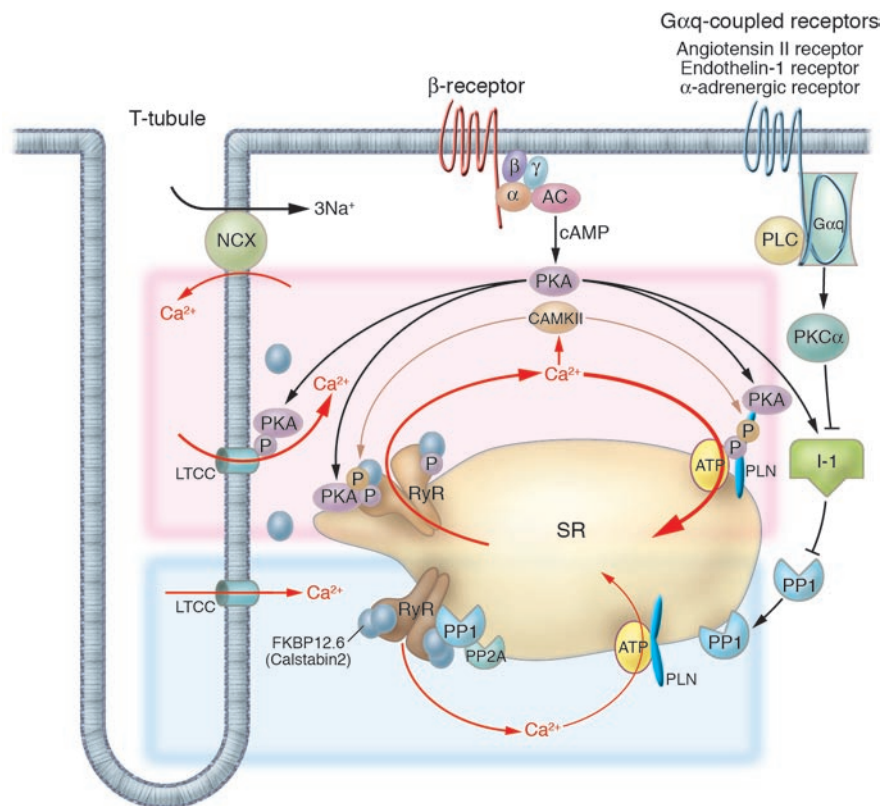
In most types of HF, the density of LTCC seems to be either unaltered or reduced (12). However, there is evidence that the function of LTCC may be altered in human HF. For instance, Shröder et al. (13) demonstrated increases in both the availability and open probability of LTCC isolated from failing human hearts, possibly due to a defect in dephosphorylation. Moreover, Chen et al. (14) recently reported that the density of LTCC was reduced in human HF although the current was maintained due to an increase in the phosphorylation level. The efficiency of the trigger (the size of the inward Ca²⁺ current) needed to cause Ca²⁺ release from the SR has been termed E-C coupling gain (15). In many cases of HF, the E-C coupling gain seems to be reduced by several factors: (a) a functional defect in LTCC (16–18); (b) an increase in the space between LTCC and RyR (19); (c) a decrease in SR Ca²⁺ (20–22); and/or (d) an abnormality in the channel-gating property of RyR (23–26). Not only the amount of Ca²⁺ released for a given Ca²⁺ release trigger but also the rate of Ca²⁺ release may be important for the contractility of the myofilaments. Since crossbridge cycling is considered to occur very rapidly from the beginning of the rising phase of the Ca²⁺ transient (27), a faster elevation of the cytosolic Ca²⁺ concentration might accelerate crossbridge attachment, resulting in faster and/or higher tension development. In contrast, the dyssynchronous Ca²⁺ release seen in HF might lead to a slower rate of rise in the Ca²⁺ transient (28, 29), probably leading to a dyssynchronous binding of Ca²⁺ to troponin C, and thereby to a slower velocity of contraction (30). However, since there is no direct evidence to support this notion, it remains to be determined whether the reduced velocity of contraction in HF really is caused by an altered gain or efficiency of E-C coupling.

In HF, the SR Ca²⁺ content is reportedly decreased (20–22) although the fraction of Ca²⁺ released to Ca²⁺ sequestered during Ca²⁺ uptake seems to be increased (25). Both an upregulation of NCX and a

Nonstandard abbreviations used: ARVD/C2, arrhythmogenic right ventricular cardiomyopathy type 2; CAMKII, calmodulin-dependent kinase II; CCD, central core disease; CPVT, catecholaminergic polymorphic ventricular tachycardia; DAD, delayed after-depolarization; E-C, excitation-contraction; FKBP, FK506-binding protein; HF, heart failure; I-1, inhibitor-1; LTCC, L-type Ca²⁺ channel; MH, malignant hyperthermia; MyBP-Cmut, myosin-binding protein C-mutant mouse; NCX, Na⁺/Ca²⁺ exchanger; PKA, protein kinase A; PLN, phospholamban; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; RyR, ryanodine receptor; SERCA2a, Ca²⁺-ATPase; SR, sarcoplasmic reticulum.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* 115:556–564 (2005).
doi:10.1172/JCI200524159.

**Figure 1**

Intracellular Ca²⁺ cycling and associated signaling pathway in cardiomyocytes. On a beat-by-beat basis, a calcium transient is evoked by the initial influx of a small amount of Ca²⁺ through the LTCC and the subsequent large-scale Ca²⁺ release from the SR through the RyR. During diastole, cytosolic Ca²⁺ is taken up into the SR by the PLN-regulated SERCA2a pump. β receptor-mediated PKA stimulation regulates this Ca²⁺ cycling by phosphorylating LTCC, RyR, and PLN. In normal hearts, sympathetic stimulation activates β₁-adrenergic receptor, which in turn stimulates the production of cAMP by adenylyl cyclase and thereby activates PKA. PKA phosphorylates PLN and RyR, both of which contribute to an increased intracellular Ca²⁺ transient and enhanced cellular contractility (pink zone signal). PP1 and PP2A regulate the dephosphorylation process of these Ca²⁺ regulatory proteins (RyR, PLN, LTCC) (blue zone signaling). Activation of the Gαq-coupled receptors (angiotensin II receptor, endothelin 1 receptor, or α-adrenergic receptor) activates PLC, which in turn activates PKC-α. The PKC-α phosphorylates I-1, augmenting the activity of PP1 and causing hypophosphorylation of PLB. The PLB hypophosphorylation inhibits SERCA2a activity, thereby decreasing SR Ca²⁺ uptake. The increased Ca²⁺ level in the cytosol activates CAMKII, which affects the functions of RyR and PLN. Activation or deactivation of these molecules at a node in the signaling cascade affects beat-by-beat Ca²⁺ cycling, and such maneuvers have recently been highlighted as potential new therapeutic strategies against HF. α, G protein subunit α; β, G protein subunit β; γ, G protein subunit γ; AC, adenylyl cyclase; PLC, phospholipase C.

reduction in SERCA2a activity may be responsible for the reduced SR Ca²⁺ content observed in HF (12). The depressed SR Ca²⁺ load would reduce the E-C coupling gain, leading to contractile dysfunction as described above. In the normal contractile state, the greater SR Ca²⁺ content leads to a large fraction of the SR Ca²⁺ being released for a given Ca²⁺ trigger (7). This may be attributable to a stimulatory effect of the high intraluminal [Ca²⁺] ([Ca²⁺]_{SR}) on the channel open probability of RyRs (7, 31). Since the SR Ca²⁺ content is reduced in HF, the threshold SR content for a fractional Ca²⁺ release may be reduced, leading to a susceptibility to aberrant Ca²⁺ release (or spontaneous Ca²⁺ leak) at lower cytosolic [Ca²⁺]. RyRs are coupled to proteins at the luminal SR surface (triadin, junctin, and calsequestrin) (32).

Since these proteins buffer luminal Ca²⁺ and modulate the Ca²⁺ release process (32), structural and functional alterations in these proteins may be causally involved in the development of defective intraluminal [Ca²⁺] regulation seen in HF. A hypersensitivity of RyR2 channel opening to cytosolic [Ca²⁺] may contribute to the presence of a spontaneous Ca²⁺ leak at much lower levels of cytosolic [Ca²⁺] in HF than in the normal SR (i.e., approximately 100 nM during diastole). This spontaneous Ca²⁺ leak may lead to a delayed after-depolarization (DAD), which can trigger arrhythmia (33).

Altered function of SR Ca²⁺ regulatory proteins in HF

Defective FKBP12.6-mediated stabilization of RyR as a cause of HF. RyR is a Ca²⁺ release channel existing as a huge homotetramer transverse the SR membrane (34) (Figure 2). Three mammalian isoforms of RyR have been identified. Of these, RyR1 is found in skeletal muscle while RyR2 is predominantly expressed in cardiac muscle (35, 36). RyR3 is ubiquitously expressed at low levels and has functional properties that differ from those of both RyR1 and RyR2 (37).

Each monomer contains approximately 5,000 amino acids and has a molecular weight of 565 kDa. RyR is also a scaffolding protein to which numerous key regulatory proteins are bound, thus forming the junctional complex (38–40). It associates with FK506-binding protein (FKBP), calmodulin, protein kinase A (PKA), protein phosphatase 1 (PP1), and protein phosphatase 2A (PP2A). The RyRs are closely associated with LTCC, and this spatial association of the 2 channels forms a key functional unit in cardiac E-C coupling (32).

One of these accessory proteins, FKBP12.6, plays an important role in the stabilization of the channel (in other words, in the maintenance of the closed state of the channel). FKBP12.6 binds to RyR2 with a stoichiometric ratio of 1 FKBP12.6 to 1 RyR2 monomer,

or 4 FKBP12.6s to 1 tetramer (41). Marx et al. (39) reported that in human HF, and in an experimental model of HF, PKA-mediated hyperphosphorylation of RyR2 occurs, and this in turn dissociates FKBP12.6 from RyR2, leading to a diastolic Ca²⁺ leak through RyR2. Long-term hyperphosphorylation of RyR2 can be maintained through a reduction in the protein abundance of PP1 and PP2A, both of which are tightly coupled to RyR2 (39). In a lipid-bilayer experiment, single-channel activity was found to be hypersensitized to [Ca²⁺], owing to a partial loss of FKBP12.6 from RyR2, thus causing (in HF) a diastolic Ca²⁺ leak at a concentration of [Ca²⁺] (approximately 100 nM) at which no significant Ca²⁺ release is induced in normal hearts (39). This diastolic Ca²⁺ leak depresses the SR Ca²⁺

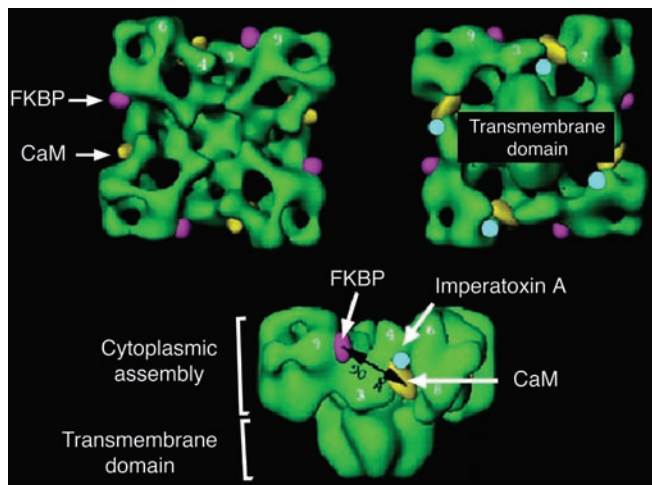


Figure 2

Three-dimensional structure of the skeletal muscle RyR, with some key sites of protein interactions. FKBP, FK506-binding protein; CaM, calmodulin. Image reprinted with permission from the *Journal of Biological Chemistry* (115).

load and serves as a substrate for DAD, which can trigger cardiac arrhythmia and lead to sudden death (42–44). The dissociation of FKBP12.6 from RyR2 also functionally uncouples multiple RyR2s and disturbs both the simultaneous opening of RyR2s during systole and their simultaneous closing during diastole (26, 39). In vivo, Shannon et al. (45) did indeed find a diastolic Ca^{2+} leak in a rabbit model of myocardial infarction. Earlier, we found that in a canine model of pacing-induced HF, PKA-hyperphosphorylation of RyR2 occurred in association with a conformational change in RyR2 and a subsequent prominent Ca^{2+} leak through RyR2 (23), although Jiang et al. later obtained conflicting results using the same model (46).

Attempts to reproduce the altered channel-gating property seen in HF have not been successful in intact myocytes. Independent groups have reported that phosphorylation at serine2808 or serine2809 does not cause FKBP12.6 dissociation from RyR2 and that the constitutive phosphorylation of serine2808 or serine2809 by mutations (S2808D or S2809D) fails to disrupt the FKBP12.6-RyR2 interaction (47, 48). To explain these apparently contradictory findings, Wehrens et al. (49) recently provided data suggesting that overexpression of FKBP12.6 outside the physiological range (47, 48) overwhelms the shift in FKBP12.6-binding affinity induced by PKA phosphorylation, allowing FKBP12.6 to bind to PKA-phosphorylated RyR2. Regarding other findings that seem to conflict with the PKA-hyperphosphorylation theory of HF (39), Li et al. (50) found that PKA phosphorylation of RyR did not increase calcium sparks in permeabilized myocytes. However, this study was performed under conditions in which cytosolic Ca^{2+} is clamped at 50 or 10 nM, which is lower than diastolic Ca^{2+} concentrations. It appears that this may match the physiology of E-C coupling because increased Ca^{2+} release under resting diastolic conditions would cause a serious problem. Valdivia et al. (51) showed that PKA phosphorylation caused an initial transient RyR2 opening in response to a jump in $[\text{Ca}^{2+}]_i$, followed by a rapid deactivation of channel gating. Eisner et al. (52) showed that the abrupt increase in RyR2 opening induced by caffeine in intact cells has only transient effects on the amplitude of the Ca^{2+}

transient (due to autoregulation). That is to say, the additional Ca^{2+} released by enhanced RyR2 opening will be rapidly removed by NCX during the subsequent beat, thereby reducing the SR Ca^{2+} available for the next beat. In the steady state, the reduced SR Ca^{2+} content offsets the effects of increased RyR2 opening with the result that Ca^{2+} transients are almost unchanged. These studies may provide a mechanism for transiently increasing systolic SR Ca^{2+} release in a physiological manner to increase cardiac contractility. An important problem in HF is that RyR2s are chronically PKA hyperphosphorylated with a partial loss of FKBP12.6 and that as a result the channels become leaky. These leaky RyR2 channels may reduce the SR Ca^{2+} load and in turn lead to the reduced contractility of cardiac muscle in failing hearts.

Mutations within RyR as a cause of defective channel opening. More than 40 RyR1 mutations have been found in patients with malignant hyperthermia (MH) or central core disease (CCD) (33). It has been shown that such RyR1 mutations in MH and CCD produce an abnormal mode of channel gating that alters the Ca^{2+} inactivation process and makes the channel hyper- and hyposensitive to activating and inactivating ligands, respectively (53). The mutation sites cluster into 3 major regions (N-terminal, central, and C-terminal). To date, more than 30 mutations have been found in the analogous RyR2 regions in patients with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD/C2) or catecholaminergic polymorphic ventricular tachycardia (CPVT) (54–63) (Table 1). This suggests that these 3 regions represent domains that are critical for the regulation of both RyR1 and RyR2 and that these domains are also involved in the pathogenesis of RyR-linked skeletal and cardiac muscle diseases. Interestingly, 1 of the cardiomyopathy (ARVD/C2) mutations in the N-terminal domain of RyR2, Arg176Gln, corresponds exactly to the Arg163Cys human MH mutation of RyR1. Likewise, some RyR2 mutations either match exactly or are located very close to some of the mutations found in RyR1.

The above findings strongly suggest that each mutation site is crucial for the maintenance of the normal channel-gating property. Marks and colleagues recently investigated the pathogenic role of RyR2 mutations by evaluating channel activity in recombinant RyR2 containing the same single-point mutation as that seen in CPVT patients (64). They found that FKBP12.6-deficient mice and CPVT-associated RyR2 mutants exhibited a significantly increased open probability of the channel only during exercise or in the PKA-phosphorylated state, that these RyR2 mutants displayed a reduced affinity of FKBP12.6 for RyR2, and that a constitutively active recombinant FKBP12.6 (FKBP12.6-D37S, a mutant form of FKBP12.6 with serine residue 37 substituted for aspartic acid) that can bind to PKA-phosphorylated RyR2 reversed the hyperactivity of channel gating seen in PKA-phosphorylated mutated RyR2 (64). These findings are compatible with the clinical finding that CPVT patients do not exhibit arrhythmia at rest but may suffer lethal arrhythmia during exercise (65).

Recently, Jiang et al. (66) reported that the mutant RyR2 linked to CPVT and sudden death increased the sensitivity of single RyR2 channels to activation by luminal Ca^{2+} and enhanced the basal level of $[\text{^3H}]$ ryanodine binding, even without PKA-phosphorylation. The discrepancies between these reports might be partly explained by FKBP12.6 being absent from the RyR2 mutant studied by Jiang et al. (66) but present in the RyR2 mutant studied by Wehrens et al. (64). It remains to be determined whether the resting channel-gating property of the FKBP12.6-depleted, mutant RyR2 linked to



Table 1
RyR2 mutations found in patients with arrhythmogenic right ventricular cardiomyopathy and CPVT

Amino acid	Disease	Domain	References
R176Q	ARVD/C2	N-terminal	(56)
R414L	CPVT	N-terminal	(62)
I419F	CPVT	N-terminal	(62)
R420W	ARVD/C2	N-terminal	(61, 57)
L433P	ARVD/C2	N-terminal	(56)
S2246L	CPVT	Central	(54, 59, 61)
V2306I	CPVT	Central	(58)
R2311D	CPVT	Central	(59)
P2328S	CPVT	Central	(55)
N2386I	ARVD/C2	Central	(56)
A2387P	CPVT	Central	(60)
Y2392C	ARVD/C2	Central	(57)
A2403T	CPVT	Central	(62)
R2474S	CPVT	Central	(54)
T2504M	ARVD/C2	Central	(56)
L2534V	CPVT	Central	(63)
L3778F	CPVT	C-terminal	(59)
G3946S	CPVT	C-terminal	(59)
N4097S	CPVT	C-terminal	(61)
N4104K	CPVT	C-terminal	(54)
E4146K	CPVT	C-terminal	(61)
T4158P	CPVT	C-terminal	(61)
Q4201R	CPVT	C-terminal	(55)
R4497C	CPVT	C-terminal	(54, 61)
F4499C	CPVT	C-terminal	(62)
N4504I	CPVT	C-terminal	(60)
A4510T	CPVT	C-terminal	(62)
A4608P	CPVT	C-terminal	(60)
V4653F	CPVT	C-terminal	(55)
G4671R	CPVT	C-terminal	(62)
V4771I	CPVT	C-terminal	(59)
I4848V	CPVT	C-terminal	(62)
A4860G	CPVT	C-terminal	(59)
I4867M	CPVT	C-terminal	(59)
V4880A	CPVT	C-terminal	(60)
N4895D	CPVT	C-terminal	(59)
P4902L	CPVT	C-terminal	(58)
E4950K	CPVT	C-terminal	(59)
R4959Q	CPVT	C-terminal	(58)

The reported mutations cluster in 3 regions homologous to 3 MH/CCD regions (that is, N-terminal, central, and C-terminal regions).

CPVT and sudden cardiac death can be altered even without PKA phosphorylation and how PKA phosphorylation affects the channel activity in the FKBP12.6-depleted, mutant RyR2.

Domain-domain interaction: a key mechanism for stabilization of RyR. On the grounds that mutations in either the N-terminal or the central domain produce abnormal modes of RyR channel gating, generally referred to as hyperactivation and hypersensitization effects (33, 53), Ikemoto et al. (67) proposed an intriguing hypothesis. In this hypothesis, the 2 domains (N-terminal and central domain) interact with each other to act as a regulatory switch for channel-gating activity, with a tight zipping of the interacting domains serving to stabilize the channel. A mutation in either domain weakens the interdomain interaction, thus increasing the tendency toward unzipping, which causes activation and leakiness of the Ca²⁺ channel (67). For instance, 1 of the domain peptides, DP4, which cor-

responds to the Leu2442-Pro2477 region of the central domain, has been found to enhance [³H]ryanodine binding and to induce Ca²⁺ release from the SR, thereby inducing contraction, in skinned muscle fibers at an inhibitory Mg²⁺ concentration (68). DP4 is also known to increase the frequency of Ca²⁺ sparks in saponin-permeabilized fibers (69) and to increase the open probability of single channels (69). In addition, a cardiac domain peptide corresponding to the Gly2459-Pro2494 region of RyR2 (DPc10) has been shown to produce significant activation of the RyR2 Ca²⁺ channel at low Ca²⁺ concentrations in a way similar to that described for DP4 (70). An Arg-to-Ser mutation in the peptide that mimics the Arg2474-to-Ser2474 human CPVT mutation completely abolished both the hyperactivation and the hypersensitization effects seen with DPc10 (70). In light of these data, it might be anticipated that a mutation in the Gly2460-Pro2495 domain of RyR2 would not only make the Ca²⁺ channel leaky but also increase its sensitivity to various pharmacological agonists, leading to diastolic Ca²⁺ overload, as widely seen in failing hearts. Since the binding region of FKBP12.6 to RyR2, which seems to reside in residues 2361–2496 according to Marx et al. (39), is included in this sequence in DPc10 (residues 2460–2495), there may be a close mechanistic relationship between PKA-mediated FKBP12.6 dissociation and domain-domain interaction.

NCX. In intact hearts, trans-sarcolemmal Ca²⁺ removal occurs through the NCX, acting in its forward mode (8). In hypertrophied or failing hearts, cytosolic [Na⁺] has been shown to be elevated (71–73), leading to activation of the reverse mode of NCX, which causes a Ca²⁺ influx (74). Although this Ca²⁺ influx via NCX is not as efficient for triggering Ca²⁺ release as the inward Ca²⁺ current through LTCC (75), it may contribute to an increase in SR Ca²⁺ content that is favorable for an increase in contractility. In failing hearts, the increased Ca²⁺ influx via the reverse mode of NCX may not be completely taken up during diastole by SERCA2a, owing to its decreased activity, resulting in the increase in diastolic [Ca²⁺] that leads to impairment of relaxation (74). As heart rate increases through an activation in sympathetic tone, cytosolic [Na⁺] is elevated, and the subsequent rise in Ca²⁺ influx via NCX contributes to a frequency-dependent increase in contractility (positive staircase) (72). In failing hearts, this response is blunted, and the diastolic [Ca²⁺] is elevated due to the combined effect of a decreased SR Ca²⁺ uptake and an additional Ca²⁺ influx via NCX in its reverse mode (74). Moreover, the increased Ca²⁺ influx that occurs during the later phase of the action potential causes a tail Ca²⁺ transient that induces DAD and triggers arrhythmia (76). The abundance of NCX protein is reportedly increased in both experimental and human failing hearts although some studies showed an unchanged level (12). This may possibly be explained by differences in the stage and/or severity of the HF.

SERCA2a and PLN. Many studies have demonstrated a reduced expression of SERCA2a protein in failing hearts, although some studies have shown an unaltered expression (12). Consistently, previous studies have indicated that SR Ca²⁺ uptake (or SERCA2a activity) is reduced in the failing animal or human myocardium (25, 72, 77). A decrease in the PLN mRNA level has been consistently observed in failing hearts. However, some studies report that the PLN protein level was unchanged (12), resulting in the protein expression of SERCA2a relative to PLN being diminished (3, 4). This ratio (i.e., the protein expression of SERCA2a relative to PLN) indicates the extent of Ca²⁺ pump inhibition, and hence the basal level of SERCA2a activity is at a lower level in failing hearts than in normal hearts (3, 4). Regarding the phosphorylation

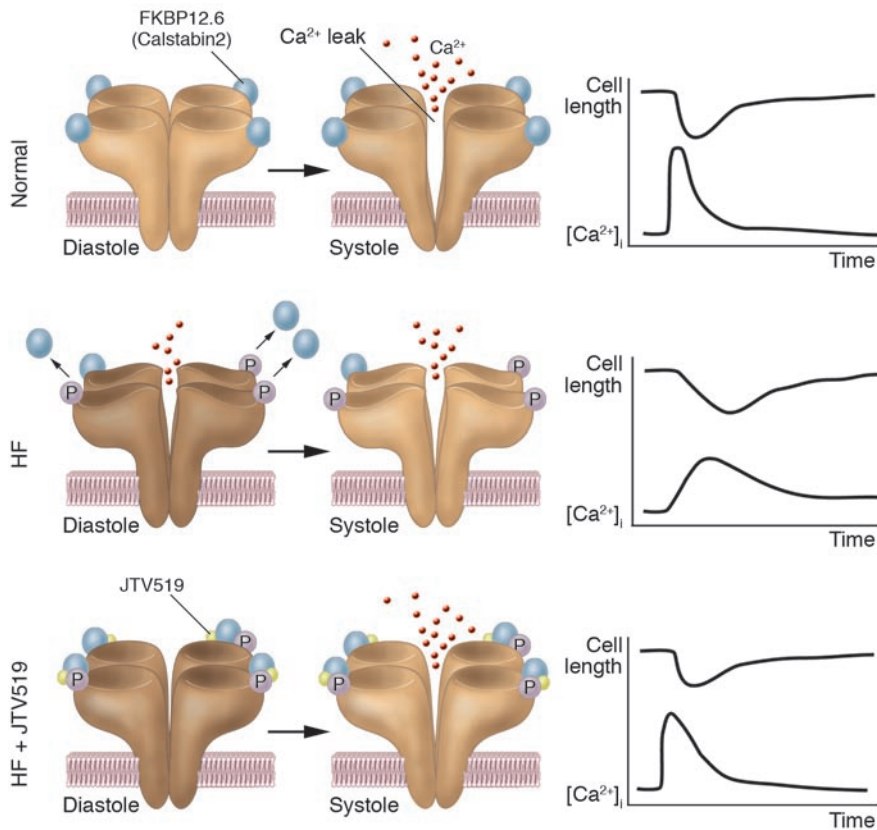


Figure 3 Therapeutic strategy involving FKBP12.6-mediated stabilization of RyR. A small influx of Ca^{2+} through the LTCC leads to the release of a large amount of Ca^{2+} from the SR through RyR in the normal heart. In HF, however, PKA-mediated hyperphosphorylation of RyR2 occurs, and this in turn dissociates FKBP12.6 from RyR2, leading to a diastolic Ca^{2+} leak through RyR2. This results in the Ca^{2+} transient being diminished (due to the reduced SR Ca^{2+} content and dyssynchronous Ca^{2+} release). Administration of a new compound, the 1,4-benzothiazepine derivative JTV519, normalizes this abnormal channel gating by restoring the conformational state of RyR and by rebinding FKBP12.6 to the channel complex. Thereby, JTV519 normalizes Ca^{2+} cycling and contractile function in failing cardiac myocytes and hence provides chronic suppression of progressive left ventricular dysfunction in HF. P, PKA phosphorylation at serine 2809; $[\text{Ca}^{2+}]_i$, intracellular $[\text{Ca}^{2+}]$.

of PLN, the level of serine 16 phosphorylation has variously been reported to be reduced (78–80) or unaltered (81, 82) in HF whereas the level of threonine 17 phosphorylation has consistently been reported to be decreased (82, 83). Threonine 17 phosphorylation is affected by the decreased calmodulin-dependent kinase II (CAMKII) activity in HF whereas serine 16 phosphorylation is mainly affected by PKA activity. The level of CAMKII activity also affects the ser-38 residue, which is the calcium-binding domain of SERCA2a, thereby regulating calcium uptake (84). The altered phosphorylation state of PLN may be responsible for the reduced SR calcium-uptake activity seen in HF. Type-1 PP1, which makes up a major protein of the serine/threonine protein phosphatases present in the cardiac myocyte, may also play an important role in regulating PLN phosphorylation since PP1 has been shown to be hyperactivated concurrently with a reduced level of serine 16 phosphorylation in PLN in several models of HF (85–87) and since overexpression of PP1 catalytic subunit α in the mouse heart was shown to lead to marked left ventricular dilation and premature death due to severe HF (88). Decreased threonine 35 phosphorylation in inhibitor-1 (I-1), an endogenous inhibitor of PP1, is further associated with increased PP1 activity in the failing heart (88).

A recent report by Braz et al. (89) demonstrated that PKC regulation of PP1 activity is critical. In HF, not only is the β -adrenergic system stimulated, but the receptor-operated signalings triggered by angiotensin II, endothelin, and the α -adrenergic system are chronically activated, contributing to depressed contractility and to a progression of both remodeling and apoptosis (1, 2). The common key enzyme in the downstream events in these receptor-operated systems is PKC. It has been demonstrated that PKC- α , which is the predominant PKC isoenzyme expressed in

the heart (90), plays a key role in regulating cardiac contractility and Ca^{2+} handling in myocytes (89). PKC- α directly phosphorylates serine 67 in I-1, thereby augmenting the activity of PP1 and causing hypophosphorylation of PLN (89). This finding may in part answer the question of why SERCA2a activity is found to be reduced in HF, apart from the more obvious possibility of a reduced abundance of SERCA2a protein.

New treatment for HF by modulation of Ca^{2+} regulatory proteins

Stabilization of RyR. The RyR2 has been shown to be hyperphosphorylated by PKA in both human and experimental HF (23, 39, 91–94), although admittedly Jiang et al. did not observe PKA hyperphosphorylation of RyR2 in a canine model of HF (46). Many large clinical trials have shown that treatment with a β blocker restores cardiac function and reduces the rate of mortality in patients with HF (2, 95). Several researchers have reported recently that in experimental and human HF, β blockers reversed PKA-mediated hyperphosphorylation of RyR2, restored the stoichiometry of the RyR2 macromolecular complex, restored normal single-channel function, and inhibited the Ca^{2+} leak (91–93). These findings may provide a molecular basis for the common clinical observation that the use of β receptor blockers improves the prognosis of patients with HF. In a canine model of HF, we found that the angiotensin II–receptor blocker valsartan, which has been used in the treatment of HF in the clinical setting, also normalizes the Ca^{2+} regulatory process through a β blocker-like action (94). By acting on the presynaptic angiotensin II receptor, valsartan inhibited norepinephrine release and stimulated norepinephrine uptake back into the synaptic pool, with the result that adrenergic signals were

**Table 2**Effect of in vivo molecular intervention upregulating SR Ca²⁺ uptake in animal models of HF

Model	Evaluated phenotype	Target gene	Rescue effect	References
SERCA2a				
Aortic-banded rat	HF	SERCA2a GT	Yes	(97)
Aortic-banded rat	Metabolism and survival	SERCA2a GT	Yes	(98)
Aortic-banded mouse	LV dysfunction	SERCA2a Tg	Yes	(100)
Diabetic cardiomyopathy rat	LV dysfunction	SERCA2a Tg	Yes	(99)
Aortic-banded mouse	LV hypertrophy and HF	SERCA2a Tg	Yes	(101)
Aortic-banded rat	HF	SERCA2a Tg	Yes	(102)
Aortic-banded rat	Arrhythmia	SERCA2a GT	Yes	(103)
PLN				
MLPKO mouse	DCM and HF	PLN KO	Yes	(106)
Tropomodulin mutant Tg mouse	DCM and HF	PLN KO	No	(111)
Calsequestrin Tg mouse	LV hypertrophy and HF	PLN KO	Yes	(107)
MHC (R403Q) mutant Tg mouse	LV hypertrophy and HF	PLN KO	Yes	(108)
BI014.6 hamster	DCM and HF	PLNS16E GT	Yes	(109)
Gaq Tg mouse	LV hypertrophy and HF	PLN KO	No	(112)
MyBP-C(Mut) Tg mouse	HCM and HF	PLN KO	No	(112)
Postinfarction rat	LV remodeling and HF	PLNS16E GT	Yes	(110)
PP1/PKC-α				
MLPKO mouse	DCM and HF	PKC- α KO	Yes	(89)

GT, gene transfer; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy.

not overtransmitted into the cell. This would lead to a reduction in the PKA-hyperphosphorylation of RyR2 and to an inhibition of the Ca²⁺ leak in the failing heart (94).

Since a conformational change in RyR2 precedes the Ca²⁺ leak (23), an amelioration of this conformational change could be a new therapeutic strategy against HF (Figure 3). Using a canine model of HF, we recently found that chronic administration of a new compound, the 1,4-benzothiazepine derivative JTV519, improved contractility and prevented the development of LV remodeling and HF, presumably by stabilization of RyR2 (80). In JTV519-untreated hearts, RyR2 was PKA-hyperphosphorylated with a dissociation of FKBP12.6 whereas the reverse of these states was true of JTV519-treated hearts, in which channel phosphorylation returned toward the levels seen in the normal heart (80). Using FKBP12.6^{+/-} mice, Wehrens et al. (49) demonstrated that JTV519 increased the affinity of FKBP12.6 for RyR2, which stabilized the closed state of RyR2 and prevented the Ca²⁺ leak that triggers arrhythmias. In their study, FKBP12.6^{-/-} mice showed an increase in RyR2 open probability, ventricular tachycardia, and sudden cardiac death upon either exercise or PKA-phosphorylation. JTV519 did not prevent arrhythmias in FKBP12.6^{-/-} mice, indicating that the presence of FKBP12.6 in the heart is required for the therapeutic effects of JTV519 to be expressed (49), although it needs to be determined whether the same is true in FKBP12.6-depleted (by PKA-phosphorylation or FK506) RyR2. Lehnart et al. (96) found that recombinant RyR2 channels containing the missense mutations seen in CPVT patients (RyR2-P2328S, RyR2-Q4201R, and RyR2-V4653F) showed defective channel-gating properties (that is, an increase in open probability and resistance to Mg²⁺-induced inhibition after PKA phosphorylation) and that JTV519 normalized this abnormal channel gating via a rebinding of FKBP12.6 to the channel complex. Collectively, the above data suggest that stabilization of RyR2 may represent a new molecular target for the

treatment or prevention of exercise-induced arrhythmias and sudden death in patients with CPVT mutations and HF.

Overexpression of SERCA2a and PLN inhibition. One intriguing therapeutic approach to the treatment of HF may be to restore the depressed Ca²⁺ uptake activity that is generally considered to play a key pathogenic role in the development of this condition. The enhanced contractility associated with SERCA2a overexpression has been reported to be protective against both HF and cardiac hypertrophy (97–102). For example, in a pressure-overload rat model of HF, adenovirus-mediated overexpression of SERCA2a has been found to rescue depressed contractility and survival without adverse effects on energy metabolism (97, 98) and cardiac arrhythmia (103). The enhanced contractility induced by SERCA2a overexpression is due to an enhanced SR Ca²⁺ content and the resulting increase in Ca²⁺ efflux during systole (104, 105). An inhibition of PLN and the subsequent increase in SERCA2a activity appear to be promising strategies for the treatment of HF. Indeed, PLN gene ablation has been shown to prevent ventricular dysfunction, fibrosis, and development of HF both in dilated cardiomyopathy and hypertrophic cardiomyopathy (106–108). An in vivo gene transfer of a dominant-negative PLN mutant (which leads to greater SERCA2a activity) rescued HF both in a cardiomyopathic hamster model (109) and in rats with myocardial infarction (110). In contrast to these studies showing the functional benefit of enhanced SERCA2a activity in failing myocardium, other studies showed that PLN ablation failed to rescue the cardiomyopathic phenotype in several models of cardiomyopathy (i.e., tropomodulin-overexpressing transgenic [TOT] mouse [ref. 111], Gaq-transgenic mouse, and myosin-binding protein C-mutant mouse [MyBP-Cmut] [ref. 112]). These data suggest that the ablation or inhibition of PLN may not always be effective for the treatment of HF (Table 2). Independent groups have reported that human PLN mutations lead to dilated cardiomyopathy with Arg9Cys (113) and Leu39stop mutations (114). The PLN with Arg9Cys mutation was



found to interact abnormally with PKA and lack the ability to be phosphorylated at Ser16, thereby dominantly inhibiting SERCA2a function (113). These data support the notion that PLN inhibition is a promising therapeutic approach for human HF. On the other hand, the fact that PLN with Leu39stop mutation lacks transmembrane peptide and thereby disables PLN retention in the SR (114) raises the possibility that innate absence of PLN in the SR may cause long-term adverse effects in the human heart. However, the lod score for the linkage between Leu39stop mutation and HF was low, and moreover, the physiological significance of Leu39stop mutation was not evaluated in the animal model. Further investigation is still needed to determine whether either overexpression of SERCA2a or PLN inhibition can be a new therapeutic strategy against human HF. It is also of great interest that, as recently demonstrated, overexpression of I-1 (88) or ablation of PKC- α (89) leads to increased myocyte contractility in the human failing myocyte and muscle LIM protein (MLP)-deficient cardiomyopathy, respectively, presumably by inhibiting the increased PP1 activity seen in the failing heart. Further assessment will be needed to determine whether PP1 inhibition might be beneficial in the long-term setting of HF.

Conclusions and perspectives

Recent progress in molecular cardiology makes it possible to envision a new therapeutic approach to HF, targeting key molecules involved in intracellular Ca²⁺ handling (such as RyR, SERCA2a, PLN, and others). Controlling these molecular functions has been found to be beneficial in certain experimental conditions. However, not all investigators are agreed that such therapies can usefully be extended to all types of failing hearts. Depending on the experimental conditions or on the model of HF, both positive and

negative results (i.e., benefit or no benefit with the above therapies) have been obtained. With regard to this, it is important to notice whether the animal models accurately reflect the human disorder or the underlying human biology. Because of the heterogeneous nature of human HF examined in various studies (i.e., different stages and etiologies), caution should be exercised when trying to decide whether this approach might be generally applicable to the treatment of HF. In this regard, further investigation is clearly needed. Moreover, in contrast to many experimental situations, in which treatments are administered before HF develops, human HF has to be cured after it has developed. At present, little information is available to indicate whether manipulations targeting Ca²⁺ regulatory proteins are effective after HF has developed as well as before. Nevertheless, new forms of therapy targeting Ca²⁺ regulatory proteins should open up a new chapter in the potential treatment of HF.

Acknowledgments

This work was supported by grants-in-aid for scientific research from The Ministry of Education in Japan (16590689, 16209026, 14370228, and 15590754 to M. Yano, Y. Ikeda, and M. Matsuzaki) and by grants-in-aid from the Takeda Science Foundation (to M. Yano and Y. Ikeda).

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1. Francis, G.S. 2001. Pathophysiology of chronic heart failure. *Am. J. Med.* **110**:37S-46S.
2. Braunwald, E., and Bristow, M.R. 2000. Congestive heart failure: fifty years of progress. *Circulation*. **102**:IV14-IV23.
3. Hasenfuss, G., and Pieske, B. 2002. Calcium cycling in congestive heart failure. *J. Mol. Cell. Cardiol.* **34**:951-969.
4. Meyer, M., et al. 1995. Alterations of sarcoplasmic reticulum proteins in failing human dilated cardiomyopathy. *Circulation*. **92**:778-784.
5. Houser, S.R., and Margulies, K.B. 2003. Is depressed myocyte contractility centrally involved in heart failure? *Circ. Res.* **92**:350-358.
6. Fabiato, A. 1983. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *Am. J. Physiol.* **245**:C1-C14.
7. Bers, D.M. 2002. Cardiac excitation-contraction coupling. *Nature*. **415**:198-205.
8. Bers, D.M. 2001. *Excitation-contraction coupling and cardiac contractile force*. Kluwer Academic Publishers. Dordrecht, The Netherlands. 427 pp.
9. Bassani, J.W., Bassani, R.A., and Bers, D.M. 1994. Relaxation in rabbit and rat cardiac cells: species-dependent differences in cellular mechanisms. *J. Physiol.* **476**:279-293.
10. Fill, M., et al. 2000. Ryanodine receptor adaptation. *J. Gen. Physiol.* **116**:873-882.
11. Sitsapesan, R., and Williams, A.J. 2000. Do inactivation mechanisms rather than adaptation hold the key to understanding ryanodine receptor channel gating? *J. Gen. Physiol.* **116**:867-872.
12. Hasenfuss, G. 1998. Alterations of calcium-regulatory proteins in heart failure. *Cardiovasc. Res.* **37**:279-289.
13. Schröder, F., et al. 1998. Increased availability and open probability of single L-type calcium channels from failing compared with nonfailing human ventricle. *Circulation*. **98**:969-976.
14. Chen, X., et al. 2002. L-type Ca²⁺ channel density and regulation are altered in failing human ventricular myocytes and recover after support with mechanical assist devices. *Circ. Res.* **91**:517-524.
15. Wier, W.G., and Balke, C.W. 1999. Ca²⁺ release mechanisms, Ca²⁺ sparks, and local control of excitation-contraction coupling in normal heart muscle. *Circ. Res.* **85**:770-776.
16. Piot, C., et al. 1996. High frequency-induced upregulation of human cardiac calcium currents. *Circulation*. **93**:120-128.
17. Barrere-Lemaire, S., Piot, C., Leclercq, F., Nargeot, J., and Richard, S. 2000. Facilitation of L-type calcium currents by diastolic depolarization in cardiac cells: impairment in heart failure. *Cardiovasc. Res.* **47**:336-349.
18. He, J., et al. 2001. Reduction in density of transverse tubules and L-type Ca²⁺ channels in canine tachycardia-induced heart failure. *Cardiovasc. Res.* **49**:298-307.
19. Gomez, A.M., et al. 1997. Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure. *Science*. **276**:800-806.
20. O'Rourke, B., et al. 1999. Mechanisms of altered excitation-contraction coupling in canine tachycardia-induced heart failure, I: experimental studies. *Circ. Res.* **84**:562-570.
21. Lindner, M., Erdmer, E., and Beuckelmann, D.J. 1998. Calcium content of the sarcoplasmic reticulum in isolated ventricular myocytes from patients with terminal heart failure. *J. Mol. Cell. Cardiol.* **30**:743-749.
22. Hobai, I.A., and O'Rourke, B. 2001. Decreased sarcoplasmic reticulum calcium content is responsible for defective excitation-contraction coupling in canine heart failure. *Circulation*. **103**:1577-1584.
23. Yano, M., et al. 2000. Altered stoichiometry of FKBP12.6 versus ryanodine receptor as a cause of abnormal Ca²⁺ leak through ryanodine receptor in heart failure. *Circulation*. **102**:2131-2136.
24. Ono, K., et al. 2000. Altered interaction of FKBP12.6 with ryanodine receptor as a cause of abnormal Ca²⁺ release in heart failure. *Cardiovasc. Res.* **48**:323-331.
25. Yamamoto, T., et al. 1999. Abnormal Ca²⁺ release from cardiac sarcoplasmic reticulum in tachycardia-induced heart failure. *Cardiovasc. Res.* **44**:146-155.
26. Marx, S.O., et al. 2001. Coupled gating between cardiac calcium release channels (ryanodine receptors). *Circ. Res.* **88**:1151-1158.
27. Blinks, J.R., and Hannon, J.D. 1996. Evaluation of changes in myofibrillar Ca²⁺ sensitivity in intact cardiac cells. In *Molecular physiology and pharmacology of cardiac ion channels and transporters*. M. Morad, S. Ebashi, W. Trautwein, and K. Kurachi, editors. Kluwer Academic. Dordrecht, The Netherlands/London, United Kingdom. 513-529.
28. Litwin, S.E., Zhang, D., and Bridge, J.H. 2000. Dys-synchronous Ca²⁺ sparks in myocytes from infarcted hearts. *Circ. Res.* **87**:1040-1047.
29. Sah, R., Ramirez, R.J., and Backx, P.H. 2002. Modulation of Ca²⁺ release in cardiac myocytes by changes in repolarization rate: role of phase-1 action potential repolarization in excitation-contraction coupling. *Circ. Res.* **90**:165-173.
30. Sjaastad, I., Wasserstrom, J.A., and Sejersted, O.M. 2003. Heart failure — a challenge to our current concepts of excitation-contraction coupling. *J. Physiol.* **546**:33-47.
31. Shannon, T.R., and Bers, D.M. 2004. Integrated Ca²⁺ management in cardiac myocytes. *Ann. N. Y. Acad. Sci.* **1015**:28-38.
32. Bers, D.M. 2004. Macromolecular complexes regulating cardiac ryanodine receptor function. *J. Mol. Cell. Cardiol.* **37**:417-429.



33. Brini, M. 2004. Ryanodine receptor defects in muscle genetic diseases. *Biochem. Biophys. Res. Commun.* **322**:1245–1255.
34. Wagenknecht, T., et al. 1989. Three-dimensional architecture of the calcium channel/foot structure of sarcoplasmic reticulum. *Nature.* **338**:167–170.
35. Marks, A.R., et al. 1989. Molecular cloning and characterization of the ryanodine receptor/junctional channel complex cDNA from skeletal muscle sarcoplasmic reticulum. *Proc. Natl. Acad. Sci. U. S. A.* **86**:8683–8687.
36. Otsu, K., et al. 1990. Molecular cloning of cDNA encoding the Ca²⁺ release channel (ryanodine receptor) of rabbit cardiac muscle sarcoplasmic reticulum. *J. Biol. Chem.* **265**:13472–13483.
37. Takeshima, H., et al. 1995. Ca²⁺-induced Ca²⁺ release in myocytes from dyspedic mice lacking the type-1 ryanodine receptor. *EMBO J.* **14**:2999–3006.
38. Brillantes, A.B., et al. 1994. Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell.* **77**:513–523.
39. Marx, S.O., et al. 2000. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell.* **101**:365–376.
40. Marks, A.R., Marx, S.O., and Reiken, S. 2002. Regulation of ryanodine receptors via macromolecular complexes: a novel role for leucine/isoleucine zipers. *Trends Cardiovasc. Med.* **12**:166–170.
41. Marks, A.R. 2002. Ryanodine receptors, FKBP12, and heart failure. *Front. Biosci.* **7**:d970–d977.
42. Marks, A.R., Reiken, S., and Marx, S.O. 2002. Progression of heart failure: is protein kinase a hyperphosphorylation of the ryanodine receptor a contributing factor? *Circulation.* **105**:272–275.
43. Lehnart, S.E., Wehrens, X.H., and Marks, A.R. 2004. Calstabin deficiency, ryanodine receptors, and sudden cardiac death. *Biochem. Biophys. Res. Commun.* **322**:1267–1279.
44. Wehrens, X.H., and Marks, A.R. 2003. Altered function and regulation of cardiac ryanodine receptors in cardiac disease. *Trends Biochem. Sci.* **28**:671–678.
45. Shannon, T.R., Pogwizd, S.M., and Bers, D.M. 2003. Elevated sarcoplasmic reticulum Ca²⁺ leak in intact ventricular myocytes from rabbits in heart failure. *Circ. Res.* **93**:592–594.
46. Jiang, M.T., et al. 2002. Abnormal Ca²⁺ release, but normal ryanodine receptors, in canine and human heart failure. *Circ. Res.* **91**:1015–1022.
47. Strange, M., Xu, L., Balshaw, D., Yamaguchi, N., and Meissner, G. 2003. Characterization of recombinant skeletal muscle (Ser-2843) and cardiac muscle (Ser-2809) ryanodine receptor phosphorylation mutants. *J. Biol. Chem.* **278**:51693–51702.
48. Xiao, B., Sutherland, C., Walsh, M.P., and Chen, S.R. 2004. Protein kinase A phosphorylation at serine-2808 of the cardiac Ca²⁺-release channel (ryanodine receptor) does not dissociate 12.6-kDa FK506-binding protein (FKBP12.6). *Circ. Res.* **94**:487–495.
49. Wehrens, X.H., et al. 2004. Protection from cardiac arrhythmia through ryanodine receptor-stabilizing protein calstabin2. *Science.* **304**:292–296.
50. Li, Y., Kranias, E.G., Mignery, G.A., and Bers, D.M. 2002. Protein kinase A phosphorylation of the ryanodine receptor does not affect calcium sparks in mouse ventricular myocytes. *Circ. Res.* **90**:309–316.
51. Valdivia, H.H., Kaplan, J.H., Ellis-Davies, G.C., and Lederer, W.J. 1995. Rapid adaptation of cardiac ryanodine receptors: modulation by Mg²⁺ and phosphorylation. *Science.* **267**:1997–2000.
52. Eisner, D.A., Choi, H.S., Diaz, M.E., O'Neill, S.C., and Trafford, A.W. 2000. Integrative analysis of calcium cycling in cardiac muscle. *Circ. Res.* **87**:1087–1094.
53. McCarthy, T.V., Quane, K.A., and Lynch, P.J. 2000. Ryanodine receptor mutations in malignant hyperthermia and central core disease. *Hum. Mutat.* **15**:410–417.
54. Priori, S.G., et al. 2001. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation.* **103**:196–200.
55. Laitinen, P.J., et al. 2001. Mutations of the cardiac ryanodine receptor (RyR2) gene in familial polymorphic ventricular tachycardia. *Circulation.* **103**:485–490.
56. Tiso, N., et al. 2001. Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). *Hum. Mol. Genet.* **10**:189–194.
57. Baucce, B., et al. 2002. Screening for ryanodine receptor type 2 mutations in families with effort-induced polymorphic ventricular arrhythmias and sudden death: early diagnosis of asymptomatic carriers. *J. Am. Coll. Cardiol.* **40**:341–349.
58. Laitinen, P.J., Swan, H., and Kontula, K. 2003. Molecular genetics of exercise-induced polymorphic ventricular tachycardia: identification of three novel cardiac ryanodine receptor mutations and two common calsequestrin 2 amino-acid polymorphisms. *Eur. J. Hum. Genet.* **11**:888–891.
59. Priori, S.G., et al. 2002. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. *Circulation.* **106**:69–74.
60. Bagattin, A., et al. 2004. Denaturing HPLC-based approach for detecting RyR2 mutations involved in malignant arrhythmias. *Clin. Chem.* **50**:1148–1155.
61. Tester, D.J., Spoon, D.B., Valdivia, H.H., Makielski, J.C., and Ackerman, M.J. 2004. Targeted mutational analysis of the RyR2-encoded cardiac ryanodine receptor in sudden unexplained death: a molecular autopsy of 49 medical examiner/coroner's cases. *Mayo Clin. Proc.* **79**:1380–1384.
62. Choi, G., et al. 2004. Spectrum and frequency of cardiac channel defects in swimming-triggered arrhythmia syndromes. *Circulation.* **110**:2119–2124.
63. Hasdemir, C., Priori, S.G., Overholt, E., and Lazzara, R. 2004. Catecholaminergic polymorphic ventricular tachycardia, recurrent syncope, and implantable loop recorder. *J. Cardiovasc. Electrophysiol.* **15**:729.
64. Wehrens, X.H., et al. 2003. FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. *Cell.* **113**:829–840.
65. Marks, A.R., Priori, S., Memmi, M., Kontula, K., and Laitinen, P.J. 2002. Involvement of the cardiac ryanodine receptor/calcium release channel in catecholaminergic polymorphic ventricular tachycardia. *J. Cell. Physiol.* **190**:1–6.
66. Jiang, D., et al. 2004. RyR2 mutations linked to ventricular tachycardia and sudden death reduce the threshold for store-overload-induced Ca²⁺ release (SOICR). *Proc. Natl. Acad. Sci. U. S. A.* **101**:13062–13067.
67. Ikemoto, N., and Yamamoto, T. 2002. Regulation of calcium release by interdomain interaction within ryanodine receptors. *Front. Biosci.* **7**:d671–d683.
68. Lamb, G.D., Posterino, G.S., Yamamoto, T., and Ikemoto, N. 2001. Effects of a domain peptide of the ryanodine receptor on Ca²⁺ release in skinned skeletal muscle fibers. *Am. J. Physiol. Cell Physiol.* **281**:C207–C214.
69. Shtifman, A., et al. 2002. Interdomain interactions within ryanodine receptors regulate Ca²⁺ spark frequency in skeletal muscle. *J. Gen. Physiol.* **119**:15–32.
70. Yamamoto, T., and Ikemoto, N. 2002. Peptide probe study of the critical regulatory domain of the cardiac ryanodine receptor. *Biochem. Biophys. Res. Commun.* **291**:1102–1108.
71. Gray, R.P., McIntyre, H., Sheridan, D.S., and Fry, C.H. 2001. Intracellular sodium and contractile function in hypertrophied human and guinea-pig myocardium. *Pflugers Arch.* **442**:117–123.
72. Pieske, B., et al. 1995. Alterations in intracellular calcium handling associated with the inverse force-frequency relation in human dilated cardiomyopathy. *Circulation.* **92**:1169–1178.
73. Pieske, B., Maier, L.S., Bers, D.M., and Hasenfuss, G. 1999. Ca²⁺ handling and sarcoplasmic reticulum Ca²⁺ content in isolated failing and nonfailing human myocardium. *Circ. Res.* **85**:38–46.
74. Pieske, B., and Houser, S.R. 2003. [Na⁺]_i handling in the failing human heart. *Cardiovasc. Res.* **57**:874–886.
75. Sipido, K.R., Maes, M., and Van de Werf, F. 1997. Low efficiency of Ca²⁺ entry through the Na⁺/Ca²⁺ exchanger as trigger for Ca²⁺ release from the sarcoplasmic reticulum. A comparison between L-type Ca²⁺ current and reverse-mode Na⁺/Ca²⁺ exchange. *Circ. Res.* **81**:1034–1044.
76. Pieske, B., et al. 2002. Rate dependence of [Na⁺]_i and contractility in nonfailing and failing human myocardium. *Circulation.* **106**:447–453.
77. Limas, C.J., et al. 1987. Calcium uptake by cardiac sarcoplasmic reticulum in human dilated cardiomyopathy. *Cardiovasc. Res.* **21**:601–605.
78. Schwinger, R.H., et al. 1999. Reduced Ca²⁺-sensitivity of SERCA 2a in failing human myocardium due to reduced serin-16 phospholamban phosphorylation. *J. Mol. Cell. Cardiol.* **31**:479–491.
79. Sande, J.B., et al. 2002. Reduced level of serine(16) phosphorylated phospholamban in the failing rat myocardium: a major contributor to reduced SERCA2 activity. *Cardiovasc. Res.* **53**:382–391.
80. Yano, M., et al. 2003. FKBP12.6-mediated stabilization of calcium-release channel (ryanodine receptor) as a novel therapeutic strategy against heart failure. *Circulation.* **107**:477–484.
81. Bohm, M., Reiger, B., Schwinger, R.H., and Erdmann, E. 1994. cAMP concentrations, cAMP dependent protein kinase activity, and phospholamban in non-failing and failing myocardium. *Cardiovasc. Res.* **28**:1713–1719.
82. Neticadan, T., Temsah, R.M., Kawabata, K., and Dhalla, N.S. 2000. Sarcoplasmic reticulum Ca²⁺/Calmodulin-dependent protein kinase is altered in heart failure. *Circ. Res.* **86**:596–605.
83. Mishra, S., Sabbah, H.N., Jain, J.C., and Gupta, R.C. 2003. Reduced Ca²⁺-calmodulin-dependent protein kinase activity and expression in LV myocardium of dogs with heart failure. *Am. J. Physiol. Heart Circ. Physiol.* **284**:H876–H883.
84. Frank, K.F., Bolck, B., Erdmann, E., and Schwinger, R.H. 2003. Sarcoplasmic reticulum Ca²⁺-ATPase modulates cardiac contraction and relaxation. *Cardiovasc. Res.* **57**:20–27.
85. Neumann, J., et al. 1997. Increased expression of cardiac phosphatases in patients with end-stage heart failure. *J. Mol. Cell. Cardiol.* **29**:265–272.
86. Huang, B., Wang, S., Qin, D., Boutjdir, M., and El-Sherif, N. 1999. Diminished basal phosphorylation level of phospholamban in the postinfarction remodeled rat ventricle: role of beta-adrenergic pathway, G(i) protein, phosphodiesterase, and phosphatases. *Circ. Res.* **85**:848–855.
87. Gupta, R.C., et al. 2003. Cardiac SR-coupled PP1 activity and expression are increased and inhibitor 1 protein expression is decreased in failing hearts. *Am. J. Physiol. Heart Circ. Physiol.* **285**:H2373–H2381.
88. Carr, A.N., et al. 2002. Type 1 phosphatase, a negative regulator of cardiac function. *Mol. Cell. Biol.* **22**:4124–4135.
89. Braz, J.C., et al. 2004. PKC-alpha regulates cardiac contractility and propensity toward heart failure. *Nat. Med.* **10**:248–254.
90. Pass, J.M., et al. 2001. Enhanced PKC beta II translocation and PKC beta II-RACK1 interactions in PKC epsilon-induced heart failure: a role for RACK1. *Am. J. Physiol. Heart Circ. Physiol.* **281**:H2500–H2510.
91. Reiken, S., et al. 2003. Beta-blockers restore calcium release channel function and improve cardiac muscle performance in human heart failure. *Circulation.* **107**:2459–2466.



92. Reiken, S., et al. 2001. Beta-adrenergic receptor blockers restore cardiac calcium release channel (ryanodine receptor) structure and function in heart failure. *Circulation*. **104**:2843–2848.
93. Doi, M., et al. 2002. Propranolol prevents the development of heart failure by restoring FKBP12.6-mediated stabilization of ryanodine receptor. *Circulation*. **105**:1374–1379.
94. Okuda, S., et al. 2004. Valsartan restores sarcoplasmic reticulum function with no appreciable effect on resting cardiac function in pacing-induced heart failure. *Circulation*. **109**:911–919.
95. Bristow, M.R. 2000. Beta-adrenergic receptor blockade in chronic heart failure. *Circulation*. **101**:558–569.
96. Lehnart, S.E., et al. 2004. Sudden death in familial polymorphic ventricular tachycardia associated with calcium release channel (ryanodine receptor) leak. *Circulation*. **109**:3208–3214.
97. Miyamoto, M.I., et al. 2000. Adenoviral gene transfer of SERCA2a improves left-ventricular function in aortic-banded rats in transition to heart failure. *Proc. Natl. Acad. Sci. U. S. A.* **97**:793–798.
98. del Monte, F., et al. 2001. Improvement in survival and cardiac metabolism after gene transfer of sarcoplasmic reticulum Ca^{2+} -ATPase in a rat model of heart failure. *Circulation*. **104**:1424–1429.
99. Trost, S.U., et al. 2002. Overexpression of the sarcoplasmic reticulum Ca^{2+} -ATPase improves myocardial contractility in diabetic cardiomyopathy. *Diabetes*. **51**:1166–1171.
100. Ito, K., et al. 2001. Transgenic expression of sarcoplasmic reticulum Ca^{2+} atpase modifies the transition from hypertrophy to early heart failure. *Circ. Res.* **89**:422–429.
101. Nakayama, H., et al. 2003. Cardiac-specific overexpression of a high Ca^{2+} affinity mutant of SERCA2a attenuates in vivo pressure overload cardiac hypertrophy. *FASEB J.* **17**:61–63.
102. Muller, O.J., et al. 2003. Transgenic rat hearts overexpressing SERCA2a show improved contractility under baseline conditions and pressure overload. *Cardiovasc. Res.* **59**:380–389.
103. del Monte, F., et al. 2004. Abrogation of ventricular arrhythmias in a model of ischemia and reperfusion by targeting myocardial calcium cycling. *Proc. Natl. Acad. Sci. U. S. A.* **101**:5622–5627.
104. He, H., et al. 1997. Overexpression of the rat sarcoplasmic reticulum Ca^{2+} ATPase gene in the heart of transgenic mice accelerates calcium transients and cardiac relaxation. *J. Clin. Invest.* **100**:380–389.
105. Baker, D.L., et al. 1998. Targeted overexpression of the sarcoplasmic reticulum Ca^{2+} -ATPase increases cardiac contractility in transgenic mouse hearts. *Circ. Res.* **83**:1205–1214.
106. Minamisawa, S., et al. 1999. Chronic phospholamban-sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy. *Cell*. **99**:313–322.
107. Sato, Y., et al. 2001. Rescue of contractile parameters and myocyte hypertrophy in calsequestrin overexpressing myocardium by phospholamban ablation. *J. Biol. Chem.* **276**:9392–9399.
108. Freeman, K., et al. 2001. Alterations in cardiac adrenergic signaling and calcium cycling differentially affect the progression of cardiomyopathy. *J. Clin. Invest.* **107**:967–974.
109. Hoshijima, M., et al. 2002. Chronic suppression of heart-failure progression by a pseudophosphorylated mutant of phospholamban via in vivo cardiac rAAV gene delivery. *Nat. Med.* **8**:864–871.
110. Iwanaga, Y., et al. 2004. Chronic phospholamban inhibition prevents progressive cardiac dysfunction and pathological remodeling after infarction in rats. *J. Clin. Invest.* **113**:727–736. doi:10.1172/JCI200418716.
111. Delling, U., Sussman, M.A., and Molkenin, J.D. 2000. Re-evaluating sarcoplasmic reticulum function in heart failure. *Nat. Med.* **6**:942–943.
112. Song, Q., et al. 2003. Rescue of cardiomyocyte dysfunction by phospholamban ablation does not prevent ventricular failure in genetic hypertrophy. *J. Clin. Invest.* **111**:859–867. doi:10.1172/JCI200316738.
113. Schmitt, J.P., et al. 2003. Dilated cardiomyopathy and heart failure caused by a mutation in phospholamban. *Science*. **299**:1410–1413.
114. Haghghi, K., et al. 2003. Human phospholamban null results in lethal dilated cardiomyopathy revealing a critical difference between mouse and human. *J. Clin. Invest.* **111**:869–876. doi:10.1172/JCI200317892.
115. Wagenknecht, T., et al. 1997. Locations of calmodulin and FK506-binding protein on the three-dimensional architecture of the skeletal muscle ryanodine receptor. *J. Biol. Chem.* **272**:32463–32471.