Altered intracellular Ca^{2+} handling in heart failure

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Structural and functional alterations in the Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} cycling abnormalities and the progression of HF. In many cases, altered Ca^{2+} cycling precedes the observed depression of mechanical performance; consequently, an amelioration of the disorder of Ca^{2+} cycling has potential as a new and intriguing therapeutic strategy against HF (5). In this review, we focus on the role of Ca^{2+} regulatory proteins in the pathogenesis of HF and on the possibility of developing a new therapeutic strategy against HF using Ca^{2+} regulatory proteins as the target.

Intracellular Ca^{2+} handling in normal cardiomyocytes

In the normal heart, intracellular Ca^{2+} movements critically regulate subsequent mechanical contractions. In cardiac excitation-contraction (E-C) coupling (Figure 1), a small amount of Ca^{2+} first enters through the L-type Ca^{2+} channel (LTCC) during membrane depolarization. This Ca^{2+} influx triggers a large-scale Ca^{2+} release through the Ca^{2+} release channel of the sarcoplasmic reticulum (SR), referred to as the ryanodine receptor (RyR). The released Ca^{2+} 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 Ca^{2+} from troponin C, followed by its reuptake into the SR through phospholamban-regulated (PLN-regulated) Ca^{2+}-ATPase (SERCA2a) and subsequent trans-sarcolemmal Ca^{2+} removal through the Na^+/Ca^{2+} exchanger (NCX) operating in its forward mode (7–9). The whole process of Ca^{2+} movement is characterized by a transient increase in intracellular [Ca^{2+}] from 100 nM to about 1 μM (8). For termination of Ca^{2+} release, RyR adaptation (10), RyR inactivation (11), and SR Ca^{2+} depletion may play important roles by acting in a synergistic manner.

Triggers for Ca^{2+} 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 Ca^{2+} current) needed to cause Ca^{2+} 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 Ca^{2+} (20–22); and/or (d) an abnormality in the channel-gating property of RyR (23–26). Not only the amount of Ca^{2+} released for a given Ca^{2+} release trigger but also the rate of Ca^{2+} 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 Ca^{2+} transient (27), a faster elevation of the cytosolic Ca^{2+} concentration might accelerate crossbridge attachment, resulting in faster and/or higher tension development. In contrast, the dyssynchronous Ca^{2+} release seen in HF might lead to a slower rate of rise in the Ca^{2+} transient (28, 29), probably leading to a dyssynchronous binding of Ca^{2+} 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 Ca^{2+} content is reportedly decreased (20–22) although the fraction of Ca^{2+} released to Ca^{2+} sequestered during Ca^{2+} 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 Ca^{2+} channel; MH, malignant hyperthermia; MyBP-Cmut, myosin-binding protein C-mutant mouse; NCX, Na^+/Ca^{2+}exchanger; PKA, protein kinase A; PLN, phospholamban; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; RyR, ryanodine receptor; SERCA2a, Ca^{2+}-ATPase; SR, sarcoplasmic reticulum.

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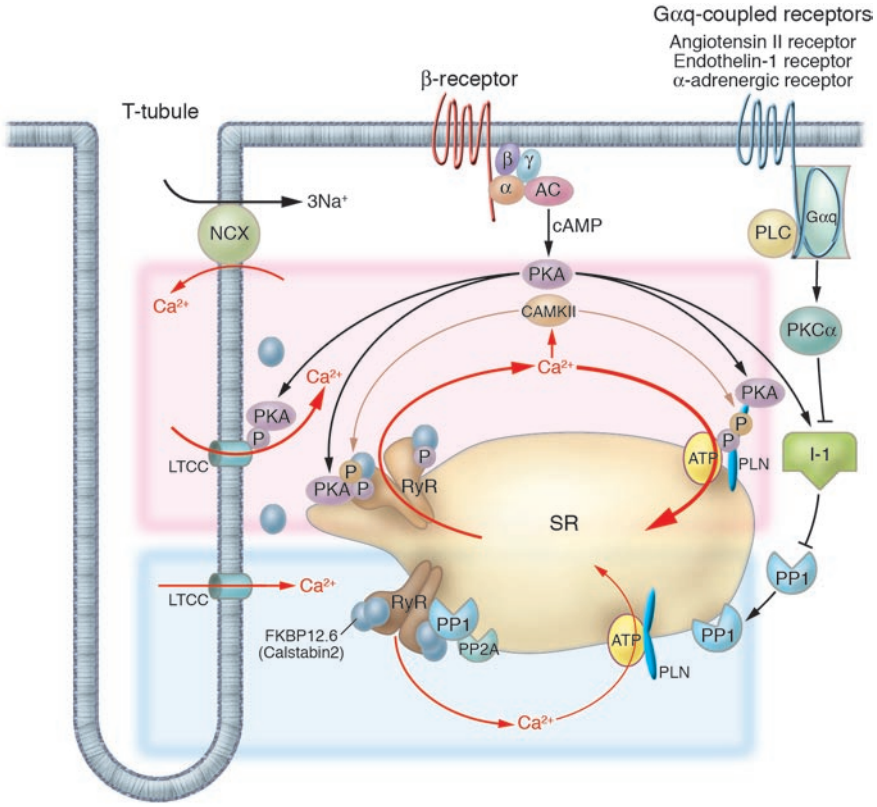

Figure 1

Intracellular Ca^{2+} 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 Ca^{2+} through the LTCC and the subsequent large-scale Ca^{2+} release from the SR through the RyR. During diastole, cytosolic Ca^{2+} is taken up into the SR by the PLN-regulated SERCA2a pump. β receptor–mediated PKA stimulation regulates this Ca^{2+} cycling by phosphorylating LTCC, RyR, and PLN. In normal hearts, sympathetic stimulation activates β_1-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 Ca^{2+} transient and enhanced cellular contractility (pink zone signal). PP1 and PP2A regulate the dephosphorylation process of these Ca^{2+} 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 Ca^{2+} uptake. The increased Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} content observed in HF (12). The depressed SR Ca^{2+} load would reduce the E-C coupling gain, leading to contractile dysfunction as described above. In the normal contractile state, the greater SR Ca^{2+} content leads to a large fraction of the SR Ca^{2+} being released for a given Ca^{2+} trigger (7). This may be attributable to a stimulatory effect of the high intraluminal $[Ca^{2+}]$ ($[Ca^{2+}]$SR) on the channel open probability of RyRs (7, 31). Since the SR Ca^{2+} content is reduced in HF, the threshold SR content for a fractional Ca^{2+} release may be reduced, leading to a susceptibility to aberrant Ca^{2+} release (or spontaneous Ca^{2+} leak) at lower cytosolic $[Ca^{2+}]$. RyRs are coupled to proteins at the luminal SR surface (triadin, junctin, and calsequestrin) (32). Since these proteins buffer luminal Ca^{2+} and modulate the Ca^{2+} release process (32), structural and functional alterations in these proteins may be causally involved in the development of defective intraluminal $[Ca^{2+}]$ regulation seen in HF. A hypersensitivity of RyR2 channel opening to cytosolic $[Ca^{2+}]$ may contribute to the presence of a spontaneous Ca^{2+} leak at much lower levels of cytosolic $[Ca^{2+}]$ in HF than in the normal SR (i.e., approximately 100 nM during diastole). This spontaneous Ca^{2+} leak may lead to a delayed after-depolarization (DAD), which can trigger arrhythmia (33).

Altered function of SR Ca^{2+} regulatory proteins in HF

Defective FKBP12.6-mediated stabilization of RyR as a cause of HF. RyR is a Ca^{2+} release channel existing as a huge homotetramer transversing 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 Ca^{2+} 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 $[Ca^{2+}]$, owing to a partial loss of FKBP12.6 from RyR2, thus causing (in HF) a diastolic Ca^{2+} leak at a concentration of $[Ca^{2+}]$ (approximately 100 nM) at which no significant Ca^{2+} release is induced in normal hearts (39). This diastolic Ca^{2+} leak depresses the SR Ca^{2+}

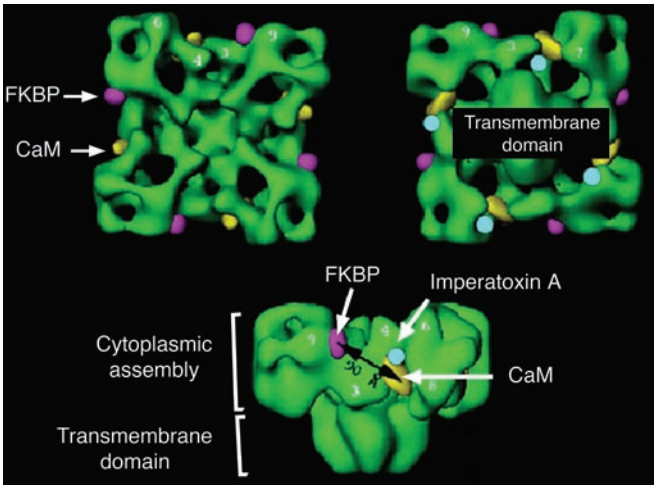

Figure 2
Three-dimensional structure of the skeletal muscle RyR, with some key sites of protein interactions. FKBP, FK506-binding protein; CaM, calmodulin. Image reprinted with permission from the *Journal of Biological Chemistry* (115).

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 [Ca^{2+}], 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 finding 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 [^{3}H]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 Ca^{2+} channel (67). For instance, 1 of the domain peptides, DP4, which corresponds to the Leu2442-Pro2477 region of the central domain, has been found to enhance [^{3}H]ryanodine binding and to induce Ca^{2+} release from the SR, thereby inducing contraction, in skinned muscle fibers at an inhibitory Mg^{2+} concentration (68). DP4 is also known to increase the frequency of Ca^{2+} 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 Ca^{2+} channel at low Ca^{2+} 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 Ca^{2+} channel leaky but also increase its sensitivity to various pharmacological agonists, leading to diastolic Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} influx (74). Although this Ca^{2+} influx via NCX is not as efficient for triggering Ca^{2+} release as the inward Ca^{2+} current through LTCC (75), it may contribute to an increase in SR Ca^{2+} content that is favorable for an increase in contractility. In failing hearts, the increased Ca^{2+} 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 [Ca^{2+}] 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 Ca^{2+} influx via NCX contributes to a frequency-dependent increase in contractility (positive staircase) (72). In failing hearts, this response is blunted, and the diastolic [Ca^{2+}] is elevated due to the combined effect of a decreased SR Ca^{2+} uptake and an additional Ca^{2+} influx via NCX in its reverse mode (74). Moreover, the increased Ca^{2+} influx that occurs during the later phase of the action potential causes a tail Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} 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

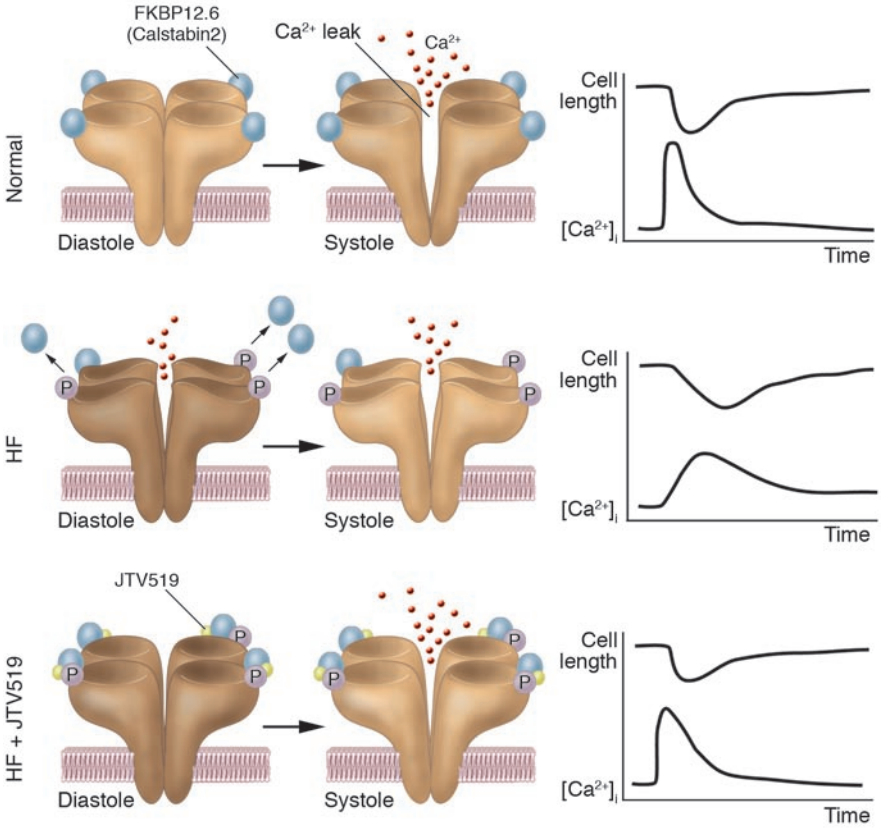

Figure 3
Therapeutic strategy involving FKBP12.6-mediated stabilization of RyR. A small influx of Ca^{2+} through the LTCC leads to the release of a large amount of Ca^{2+} from the SR through RyR in the normal heart. In HF, however, PKA-mediated hyperphosphorylation of RyR2 occurs, and this in turn 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; $[Ca^{2+}]_i$, intracellular $[Ca^{2+}]$.

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 Ca^{2+} 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)
BIO14.6 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 Ca^{2+} leak in the failing heart (94).

Since a conformational change in RyR2 precedes the Ca^{2+} 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 Ca^{2+} 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 Mg^{2+}-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 Ca^{2+} 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 Ca^{2+} content and the resulting increase in Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} regulatory proteins are effective after HF has developed as well as before. Nevertheless, new forms of therapy targeting Ca^{2+} regulatory proteins should open up a new chapter in the potential treatment of HF.

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