Heart Failure Reviews

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Abnormalities of calcium metabolism and myocardial contractility depression in the failing heart

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Abstract Heart failure (HF) is characterized by molecular and cellular defects which jointly contribute to decreased cardiac pump function. During the development of the initial cardiac damage which leads to HF, adaptive responses activate physiological countermeasures to overcome depressed cardiac function and to maintain blood supply to vital organs in demand of nutrients. However, during the chronic course of most HF syndromes, these compensatory mechanisms are sustained beyond months and contribute to progressive maladaptive remodeling of the heart which is associated with a worse outcome. Of pathophysiological significance are mechanisms which directly control cardiac contractile function including ion- and receptor-mediated intracellular signaling pathways. Importantly, signaling cascades of stress adaptation such as intracellular calcium (Ca^{2+}) and 3′-5′-cyclic adenosine monophosphate (cAMP) become dysregulated in HF directly contributing to adverse cardiac remodeling and depression of systolic and diastolic function. Here, we provide an update about Ca^{2+} and cAMP dependent signaling changes in HF, how these changes affect cardiac function, and novel therapeutic strategies which directly address the signaling defects.

Keywords Heart failure - Arrhythmia - Calcium - Sodium - Inotropy - Catecholamine - Beta receptor - Ryanodine receptor - Phosphodiesterase - Therapy

Abbreviations *AAV* Adeno-associated virus - *AP* Action potential - *β-AR* β-Adrenergic receptor - Ca^{2+} Free ionized calcium - $[Ca^{2+}]_i$ Intracellular Ca^{2+} concentration - *CaMKII* Ca^{2+} calmodulin dependent protein kinase II - *CICR* Ca^{2+} induced Ca^{2+} release - *cAMP* 3′-5′-Cyclic adenosine monophosphate - $Ca_V1.2$ L-type Ca^{2+} channels - *DAD* Delayed after depolarization - *EC coupling* Excitation-contraction coupling - *HF* Heart failure - I_{Ca} Ca^{2+} influx current - I_{TI} Ca^{2+} activated transient inward current - *I-1* Phosphatase inhibitor 1 - *MI* Myocardial infarction - *NCX* Na^+/Ca^{2+} exchanger - *PKA* cAMP-dependent protein kinase A - *PLN* Phospholamban - *PP1* Phosphatase 1 - *SERCA2a* Sarco-endoplasmic reticulum Ca^{2+} pump - *SR* Sarcoplasmic reticulum Ca^{2+} storage organelle - *RyR2* Cardiac ryanodine receptor

Introduction

Congestive heart failure (HF) is a condition in which heart function is insufficient to supply the organs with enough blood nutrients. The consequence is a progressive cascade of changes that lead to fatigue, shortness of breath and, ultimately, death. Much progress has been made in understanding the molecular and cellular processes that contribute to HF. Despite these insights and modern treatment options, chronic HF remains a major cause of illness and death and generally has a poor prognosis. Because HF is more common with increasing age, the number of affected individuals is rising rapidly with the ageing population. Thus, new treatments directed at critical disease mechanisms are needed to halt and reverse the devastating consequences of this disease.

Here, we focus on chronic HF and our understanding of molecular disease mechanisms related to Ca^{2+} metabolism. Many insights into HF stem from the elucidation of intracellular signaling pathways that mediate cardiac performance as well as contribute to cardiac dysregulation under disease conditions. The clinically critical transition occurs when the heart can no longer provide adequate blood flow and/or pressure to meet the body's demands. Consequently, physiological countermeasures include the stimulation of neurohormonal outflow and the activation of intracellular signaling cascades. These acute responses may initially offset reduced cardiac performance. However, sustained stimulation of the heart by cellular stress signaling cascades ultimately increases the likelihood of organ failure and contributes to a worse prognosis.

Cardiac remodeling and changes in intracellular signaling

HF is characterized by chronic activation of neuro-hormonal pathways representing a compensatory response to overcome depressed cardiac function. However, over weeks to months, a chronic hyperadrenergic state ensues with elevated plasma catecholamine levels, which contribute to maladaptive cardiac chamber remodeling, progressive deterioration of pump function, and deadly arrhythmias. Due to the chronic hyperadrenergic state, desensitization of β-adrenoreceptors (β-ARs) and reduced global intracellular cAMP synthesis occur in the failing heart [1–3]. However, stimulation of β-ARs and other signaling pathways is maintained and continues to affect the remodeled cells and proteins.

A broad range of molecular pathways are involved in the development of HF, and there is likely to be substantial overlap between these pathways. Typically, cell-surface receptors are activated by ligands leading to the activation of stress-response protein kinases and phosphatases such as cAMP-dependent protein kinase A (PKA), protein kinase C (PKC), protein kinase D (PKD), mitogen activated kinases (MAPKs), Ca^{2+} calmodulin-dependent kinase II (CaMKII), and calcineurin. Chronic activation of these stress pathways and their cellular effectors including transcription factors, which target multiple genes, result in changes in cellular structure, function, and overall regulation of the heart, collectively referred to as cardiac remodeling. Additionally, an imbalance between cell survival and cell death pathways results in a low rate of cardiomyocyte apoptosis which may contribute to cell loss in HF [4, 5]. A different form of cell death, necrosis, may contribute to HF, for example through mitochondrial damage mediated by increased cytosolic Ca^{2+} concentrations in cardiomyocytes.

HF causes changes in intracellular calcium signals

HF is a syndrome which results from different insults, typically following myocardial infarction (MI), viral myocarditis, toxic cardiomyopathy, or other less frequent causes such as genetic defects. However, following chronic remodeling of the heart, cardiac phenotypes occurring from different causes show important similarities, including depressed intracellular Ca^{2+} metabolism.

Ca^{2+} is the central regulator of excitation-contraction (EC) coupling, which controls muscle contraction during each heartbeat. EC coupling is activated by an incoming action potential wavefront and the subsequent opening of voltage-dependent L-type Ca^{2+} channels ($Ca_V1.2$). A relatively small Ca^{2+} influx current (I_{Ca}) triggers a quantitatively larger intracellular Ca^{2+} release from sarcoplasmic reticulum (SR)

Ca^{2+} stores through ryanodine receptor (RyR2) Ca^{2+} release channels by the process of Ca^{2+} induced Ca^{2+} release (CICR) (Fig. 1a). CICR is followed by re-uptake of Ca^{2+} into the SR by Ca^{2+} pumps (SERCA2) and removal of Ca^{2+} from the cytosol by the Na^{+}/Ca^{2+} exchanger (NCX) (Fig. 1a). The activity of SERCA2 is regulated by phospholamban (PLN). Unphosphorylated PLN inhibits SERCA2 activity, and phosphorylation by PKA and/or CaMKII increases SR Ca^{2+} uptake. Similarly, RyR2 phosphorylation by PKA and/or CaMKII increases SR Ca^{2+} release [6, 7]. Within the approximately 12 nm wide compartment between the plasma membrane and the terminal SR membrane (junctional SR) where SR Ca^{2+} release occurs, CICR is organized within Ca^{2+} release units representing functional microdomains between T-tubuli and the terminal SR (junctional subspace). Strikingly, all components essential for CICR, including the SR Ca^{2+} release microdomain, the Ca^{2+} storage organelles, and the Ca^{2+} transport proteins themselves (Table 1), become significantly altered during remodeling of the failing heart [8].

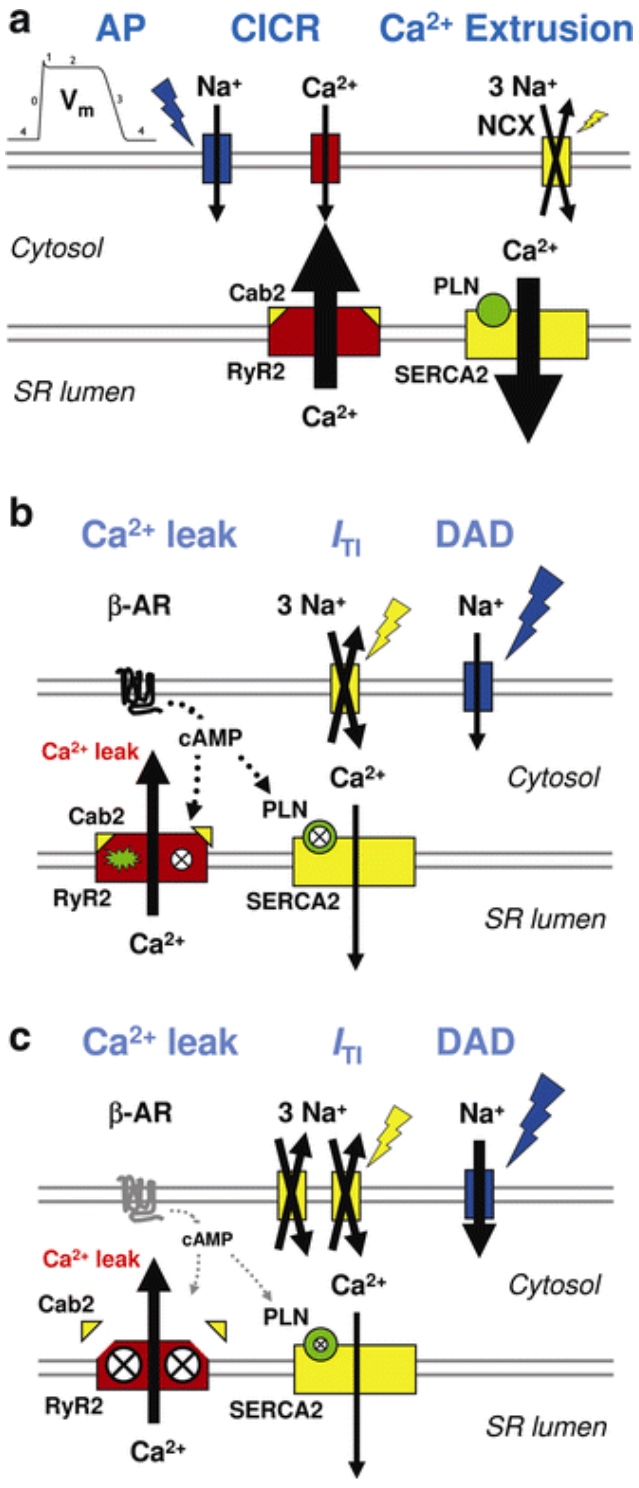

Fig. 1 **a** Physiology of excitation-contraction (EC) coupling. An incoming action potential (AP) rapidly depolarizes the

cell membrane potential (V_m) in phase 0 through opening of voltage-dependent Na^+ channels ($Na_V1.5$). Subsequently, EC coupling is initiated through the opening of voltage-dependent L-type Ca^{2+} channels ($Ca_V1.2$) and the plasma membrane Ca^{2+} influx current (I_{Ca}). I_{Ca} activates ryanodine receptors (RyR2s) and intracellular Ca^{2+} release from sarcoplasmic reticulum (SR) stores, also known as Ca^{2+} induced Ca^{2+} release (CICR). CICR is followed by extrusion of Ca^{2+} from the cytosol into the SR by Ca^{2+} pumps (SERCA2) and to the extracellular compartment by the Na^+/Ca^{2+} exchanger (NCX) operating in its forward mode (3 Na^+ in for each Ca^{2+} out), which creates an electrogenic inward current. SR Ca^{2+} leak is inhibited by the calstabin2 (Cab2) subunit which stabilizes the RyR2 closed state. The SERCA2 pump rate is inhibited by the phospholamban (PLN) subunit in its dephosphorylated state. Ca^{2+} release and uptake occur cyclically during each heart beat and represent 60–90% of Ca^{2+} signaling during EC coupling depending on the species studied. **b** EC coupling abnormalities in CPVT. RyR2 missense mutations significantly increase the propensity for intracellular Ca^{2+} leak in resting cardiomyocytes (during diastole) with filled SR Ca^{2+} stores. Stimulation of β-adrenergic receptors (β-ARs) during stress adaptation results in RyR2 and PLN phosphorylation by PKA (indicated by) which increases SR Ca^{2+} release and uptake, respectively. However, RyR2 mutations (as indicated by *green star*) in the PKA phosphorylated Ca^{2+} release channel lead to partial calstabin2 depletion, a significant gain-of-function defect of RyR2, and intracellular Ca^{2+} leak. RyR2 Ca^{2+} leak activates depolarizing transient inward currents (I_{TI}) supposedly through abnormal forward mode NCX activity. If I_{TI} currents reach a critical threshold of membrane potential instability in phase 4 of the cardiac AP, Na^+ channels are activated leading to delayed after depolarizations (DADs) which underly triggered activity. **c** EC coupling abnormalities in HF. HF is a chronic hyperadrenergic state which results in downregulation of β-AR signaling and reduced intracellular cAMP synthesis. However, maintained hyperadrenergic stimulation of β-ARs during HF results in chronic RyR2 PKA *hyper*phosphorylation (indicated by large), depletion of the stabilizing calstabin2 subunits as well as other components of the channel complex including phosphodiesterase 4D3 (PDE4D3). PDE4D3 depletion causes a chronically reduced cAMP hydrolysis in the channel complex and contributes to RyR2 PKA hyperphosphorylation induced intracellular Ca^{2+} leak. On the other hand, PLN is chronically PKA hypophosphorylated (indicated by small) creating constitutively inhibited state of SERCA2 and reduced SR Ca^{2+} uptake. Additionally, NCX expression is significantly increased leading to abnormally increased Ca^{2+} extrusion to the extracellular side and depletion of intracellular Ca^{2+} stores. Despite Ca^{2+} store depletion, DADs and triggered activity are frequent in HF possibly due to increased SR Ca^{2+} leak and proarrhythmogenic inward NCX and late $I_{Na,L}$ currents

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Table 1 Major calcium handling abnormalities in the failing heart

Gene	Physiological function	Pathophysiology in HF	Genetic syndromes
NCX1, SLC8A1	Na^+/Ca^{2+} exchange	I_{NCX} gain-of-function	n/a
	Forward mode	Contractile dysfunction	
	Reverse mode	Pro-arrhythmogenic	
RyR2	Ryanodine receptor isoform 2	Intracellular Ca^{2+} leak	CPVT (ARVC2 ?)
	Intracellular Ca^{2+} release	Contractile dysfunction	Missense mutations
			HF, SSS, AVND
	Contractile activation in systole	Pro-arrhythmogenic	Deletion mutation
ATP2A2	SERCA2a pump	Loss-of-function	Darier disease
	Intracellular SR Ca^{2+} uptake	Depressed SR Ca^{2+} uptake	
	Muscle relaxation in diastole	Depressed contraction and relaxation	
PLN	Phospholamban	Gain-of-function	Dilated cardiomyopathy, early onset
	Constitutive inhibition of SERCA2a pump function	Increase in ratio of PLN:SERCA2a results in decreased SERCA2a function	Missense and deletion mutations

Gene	Physiological function	Pathophysiology in HF	Genetic syndromes
	PKA and/or CaMKII phosphorylation of PNL increases SR Ca^{2+} uptake and contractile function	PLN hypophosphorylation is associated with decreased SR Ca^{2+} uptake and contractile dysfunction	

ARVC2 arrhythmogenic right ventricular cardiomyopathy type 2, *AVND* atrioventricular node dysfunction, *CPVT* catecholaminergic polymorphic ventricular tachycardia, *SSS* sick sinus node, *n/a* not available

Milestones toward imaging of intracellular calcium metabolism

The study of intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, in heart function requires to monitor the dynamics of $[Ca^{2+}]_i$ in living cells. However, the free $[Ca^{2+}]_i$ is heterogeneous even in resting cells and ranges from nanomolar to micromolar concentrations in the cytoplasm and SR Ca^{2+} stores, respectively [9]. During electrical cell activation or stimulation by hormone receptors, the resultant changes in the cytoplasmic and organellar Ca^{2+} concentrations occur as spatially and temporally defined patterns within cellular microdomains [10]. Thus, understanding of changes in Ca^{2+} signaling during HF has been largely dependent on methods to monitor Ca^{2+} concentrations in living cells.

Ca^{2+} indicators form selective and reversible complexes with free Ca^{2+} ions. The physicochemical differences of the free and bound indicator allow for the fluorescence detection of changes in $\Delta[Ca^{2+}]_i$ usually by the absorbance and/or emission of light [11]. Thus, the concentration of free Ca^{2+} is measured indirectly by monitoring the amount of the free versus the Ca^{2+} complexed indicator. The chemiluminescent photoprotein aequorin purified from jellyfish can be microinjected into tissues or cells and applied as a Ca^{2+} indicator [12]. Moreover, recombinant aequorin can be targeted to specific subcellular compartments through signal sequences [13]. However, recombinant aequorin emits a relatively small signal upon Ca^{2+} binding which compromises the measurement of Ca^{2+} concentrations in smaller cells at the cost of low time resolution [14]. Tsien and colleagues synthesized the first, rationally designed, fluorescent Ca^{2+} indicator for intracellular use based on the Ca^{2+}-chelator EGTA [15]. The widespread and successful use of fluorescent polycarboxylate dyes started with the introduction of lipophilic acetoxymethyl (AM) ester derivatives allowing efficient and stable indicator loading of living cells without potentially damaging pipette injection techniques [16].

Global intracellular calcium abnormalities in heart failure

Pioneering studies used injection of the aequorin Ca^{2+} sensor into physiologically contracting muscle preparations from explanted human hearts. Aequorin loaded muscles from failing hearts showed a reduced capacity to restore nanomolar resting Ca^{2+} levels during diastole in conjunction with depressed muscle contraction and relaxation [17]. Different from healthy heart muscle and pronounced at faster rates, the aequorin injected failing heart muscle showed a reduced amplitude of the intracellular Ca^{2+} transient ($\Delta[Ca^{2+}]_i$) together with diminished force production [18]. Additionally, myothermal measurements in isolated muscle strip preparations from failing and non-failing human hearts showed that the thermal equivalent of total Ca^{2+} cycling is reduced significantly in HF [19]. The rate of heat production was significantly reduced indicating reduced SR Ca^{2+} uptake [19].

Consistent with multicellular muscle preparations, dissociated single cardiomyocytes from failing human hearts displayed a prolonged relaxation, depressed systolic contraction, and elevated diastolic tension [20]. Contractile dysfunction of failing cardiomyocytes occurred in conjunction with abnormal $[Ca^{2+}]_i$ metabolism including reduced SR Ca^{2+} release, elevated resting $[Ca^{2+}]_i$, and a reduced rate of Ca^{2+} removal [21, 22]. For late stage HF it is now accepted that cardiomyocytes and/or muscle preparations from explanted patient or animal hearts exhibit a reduced $\Delta[Ca^{2+}]_i$ amplitude and a slowed decay of the global intracellular Ca^{2+} transient [17, 23–25]. Thus, depressed contractility in HF appears to be associated with cellular signaling abnormalities at the level of the global intracellular Ca^{2+} transient.

Notably, the combination of an abnormal intracellular Ca^{2+} transient together with depressed contractile function all occur from quite different forms of cardiac insults. Such phenotypic changes have been documented in dilated human cardiomyopathy [23, 26], hypertension induced hypertrophy HF in salt-sensitive rats [24], rats with myocardial infarction [27], mice with muscle LIM protein knockout [28], mice with replication-restricted full-length Coxsackievirus B3 overexpression and myocarditis [29], and mice overexpressing either the catalytic protein kinase A (PKA)-Cα subunit [30] or the cytosolic CaMKIIδ_C splice variant [31]. Thus, different HF models appear to agree that qualitatively similar changes of the intracellular Ca^{2+} transient and subsequent alterations of EC coupling and contractile dysfunction occur.

Local intracellular calcium abnormalities in heart failure

While cardiac contraction and relaxation cycles are controlled by a cell-wide (global) signaling event, the Ca^{2+} transient, intracellular Ca^{2+} release occurs within local (subcellular) Ca^{2+} release units as evidenced by elementary Ca^{2+} release events measured with bright fluorescent polycarboxylate dyes [8, 32]. How do local, elementary Ca^{2+} release events, known as Ca^{2+} sparks, modulate the intracellular Ca^{2+} transient? At a diastolic $[Ca^{2+}]_i$ of approximately 100 nM, Ca^{2+} sparks occur at a very low rate (100 s^{-1} $cell^{-1}$). When the AP occurs, Ca^{2+} influx causes a large increase in cytosolic $[Ca^{2+}]_i$ within the tight junctional subspace (Fig. 1a). A raise of junctional $[Ca^{2+}]_i$ above nanomolar concentrations increases the spark rate 10^3 to 10^6 fold. Therefore, the I_{Ca} influx current becomes amplified by a dramatic increase in Ca^{2+} spark rate during CICR. This implies that modulation of the Ca^{2+} spark rate may control the Ca^{2+} transient amplitude and cardiac force development (inotropy). Indeed, imaging experiments of subcellular Ca^{2+} signaling have provided evidence that β-adrenergic stimulation increases local Ca^{2+} release possibly due to RyR2 phosphorylation [33].

An important advance why changes in the intracellular Ca^{2+} transient occur in HF come from combined voltage-clamp and Ca^{2+} spark imaging experiments to assess if changes in EC coupling are responsible for depressed cardiac function. A rat model of hypertension induced HF with preserved Ca^{2+} influx current (I_{Ca}) density showed that the ability of any given I_{Ca} to activate SR Ca^{2+} sparks ($\Delta[Ca^{2+}]_i$) was significantly decreased [24]. Thus, HF is accompanied by a decrease in the gain of EC coupling ($\Delta[Ca^{2+}]_i/I_{Ca}$) which has been confirmed in rats with myocardial infarction [27], dogs with pacing-induced HF [34], mice with viral myocarditis [29], and muscle LIM protein knockout mice [35]. Additionally, structural changes of the T-tubules, SR storage organelles, and/or the architecture of the junctional microdomain cleft are all likely contributors to defective EC coupling by altering the geometry of the Ca^{2+} release unit potentially resulting in orphaned RyR2 release channels and/or abnormal CICR [36, 37].

Abnormal calcium release triggers fatal arrhythmias

Sudden unexpected death accounts for up to 50% of all deaths in HF patients and is most often due to

ventricular tachyarrhythmias [38]. Both reentry and focal mechanisms have been documented in patients with ischemic cardiomyopathy [39]. At the cellular level, arrhythmias have been associated with Ca^{2+} induced electrical abnormalities including SR Ca^{2+} overload leading to intracellular Ca^{2+} waves and Ca^{2+} activated transient inward current (I_{TI}) [40–42]. In digitalis treated cells, I_{TI} was shown to initiate delayed after depolarizations (DADs) [43]. Association of SR Ca^{2+} leak with activation of a depolarizing Na^{+}/Ca^{2+} exchange current is a likely mechanism of arrhythmogenic I_{TI} in HF [44–46].

Genetic linkage and translational studies have significantly advanced our understanding about specific Ca^{2+} dependent arrhythmia mechanisms (Table 1). For instance, RyR2 missense mutations may cause stress-induced syncope and sudden death in a syndrome called Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) [47, 48]. Consistent with a β-AR and cAMP mediated stress mechanism, PKA phosphorylated RyR2 channels containing CPVT mutations showed a significant gain-of-function defect and a Ca^{2+} leak mediated arrhythmia trigger mechanism [49, 50]. Indeed, cardiomyocytes from knockin mice with the RyR2-R2474S mutation identified earlier in CPVT patients [47] showed Ca^{2+} leak mediated I_{TI} currents and DADs during stimulation with catecholamines (Fig. 1b) [51]. While we have to anticipate additional mechanisms of Ca^{2+} leak in HF, mechanistic linkage of CPVT mutant RyR2 dependent Ca^{2+} leak to arrhythmia initiation [51] provides a specific mechanism of Ca^{2+} dependent arrhythmia initiation which can be targeted and tested by a novel class of RyR2 stabilizing compounds [51] (Table 2).

Table 2 Clinical and novel drug rationales to treat HF

Target	Drug	Advantage	Disadvantage
NCX transporter			
Forward mode	SEA0400	Reduced infarct size	OTA
Reverse mode	SEA0400, KB-R7943	n/a	Negative inotropic?
RyR2 channel			
Phosphorylation	β-Blockers	Survival, progression	Indirect mechanism
	ACE inhibitors	Survival, progression	Indirect mechanism
Pore block	Tetracaine	n/a	OTA; n/a
Stabilization	JTV519	Specificity	OTA
	S107	Specificity	n/a
SERCA2 pump			
Stimulation	Gingerol	Specificity	Toxicity; n/a
Overexpression	AAV-SERCA2a	Specificity	Gene therapy

OTA off-target activity, *n/a* not available

Storage organelle and calcium transport dysfunction

Ca^{2+} is the central intracellular messenger which links an incoming action potential to myofilament activation and cardiac contraction. Apart from EC coupling, $[Ca^{2+}]_i$ is subject to physiological stress adaptation and a higher systolic $\Delta[Ca^{2+}]_i$ increases force development and cardiac output (inotropy) [33]. Following the realization that SR Ca^{2+} uptake might be depressed in failing cardiomyocytes, the intracellular storage organelles and SR Ca^{2+} content became a focus of intense research interest.

HF has been associated with decreased function of different Ca^{2+} transport mechanisms (Table 1) including the sarcoendoplasmic reticulum (SR) Ca^{2+} ATPase (SERCA2) [52, 53] and with increased function of the Na^{+}/Ca^{2+} exchanger (NCX) [54]. The ratio of PLN:SERCA2 is a critical determinant of cardiac Ca^{2+}

homeostasis, and increases in this ratio have been suggested to contribute to increased diastolic Ca^{2+} levels and cardiac dysfunction [53, 55, 56]. On the other hand, due to the changes in the relative expression and function of NCX *and* SERCA2, a net shift toward increased Ca^{2+} extrusion to the extracellular space and a net decrease in SR Ca^{2+} uptake occurs (Fig. 1c). Therefore, intracellular Ca^{2+} stores may develop a relatively Ca^{2+} depleted state which has been confirmed experimentally in cardiomyocytes from dogs and humans with HF [26, 34]. Decreased SR Ca^{2+} load may result in a decreased amplitude and slower upstroke of the intracellular Ca^{2+} transient. Since $\Delta[Ca^{2+}]_i$ is an important mediator of cardiac force development, a decreased $\Delta[Ca^{2+}]_i$ due to decreased SR load may result in decreased force development. However, SR Ca^{2+} store depletion in HF does not necessarily prevent an increase in Ca^{2+} spark frequency [31] likely due to chronically increased RyR2 phosphorylation [57] and consistent with an increased propensity for Ca^{2+} induced arrhythmias in HF.

Molecular mechanisms of depressed SR calcium uptake

Decreased SERCA2 function has been associated with changes in the regulatory subunit PLN (Fig. 1c). PLN is a phosphoprotein which inhibits SERCA2 in its dephosphorylated state, while phosphorylation of PLN during β-AR stimulation relieves this inhibitory effect. The regulatory role of PLN in myocardial contractility has been established through the generation and characterization of genetically altered mouse models, which revealed a correlation between PLN expression and contractile function [58–60]. PLN phosphorylation in HF is chronically decreased (PLN *hypo*phosphorylation) which may directly contribute to depressed SR Ca^{2+} uptake [61]. Chronic PLN hypophosphorylation can be predicted to compromise cardiac stress adaptation mediated by cAMP and PKA. Overexpression of the cardiac SERCA2a isoform or the constitutively PKA phosphorylated PLN-Ser^{16} improved function in rats following aortic banding-induced HF [62] and decreased arrhythmia susceptibility following ischemia-reperfusion cardiomyopathy in pigs [63].

Phospholamban is potently inhibited by phosphatase 1 (PP1), which in turn is inhibited by phosphatase inhibitor I-1 following PKA phosphorylation and I-1 activation. Consistent with this mechanism, overexpression of an activated I-1 form in mice protects the animals from HF development [64]. Moreover, the identification of PLN mutations in patients with dilated cardiomyopathy has strengthened its critical role in cardiac function. Thus, inhibition of PLN activity and restoration of SR Ca^{2+} cycling were suggested to hold promise for treating heart failure [65].

Molecular mechanisms of intracellular calcium leak

In samples from patients and animals with HF, RyR2 is chronically PKA *hyper*phosphorylated contributing to intracellular Ca^{2+} leak and remodeling of the macromolecular channel complex [7, 66, 67]. If the hyperadrenergic state in HF leads to secondary downregulation of β-adrenergic signaling and globally reduced intracellular cAMP synthesis, which molecular mechanism maintains RyR2 PKA hyperphosphorylation and intracellular Ca^{2+} leak? During HF, phosphatases (PP1 and PP2A) are depleted from the RyR2 complex which may contribute to a reduced rate of RyR2 dephosphorylation [61, 66]. In human HF, the cAMP-specific phosphodiesterase isoform PDE4D3 was found to be decreased in the RyR2 complex paralleled by a decrease in cAMP hydrolyzing activity [57]. Thus, PKA hyperphosphorylation and calstabin2 depletion may both contribute to chronic RyR2 activation and intracellular Ca^{2+} leak (Fig. 1c). Indeed, single RyR2 channels from human failing hearts showed an increased open probability consistent with intracellular Ca^{2+} leak [66].

Using site-directed mutagenesis of RyR2, a specific Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) phosphorylation site (Ser^{2814}) distinct from the PKA phosphorylation site (Ser^{2808}) has been

identified [6]. CaMKII phosphorylation increased RyR2 Ca^{2+} sensitivity and open probability. Since CaMKII was activated by higher cardiac pacing rates, RyR2 CaMKII phosphorylation may contribute to enhanced CICR and enhanced contractility at higher heart rates. However, the rate-dependent increase in RyR2 phosphorylation by CaMKII as seen in sham-operated, healthy rat hearts was compromised in rat hearts with HF [6]. Transgenic mice overexpressing the cytosolic CaMKIIδ_C isoform develop cardiac hypertrophy, HF, and intracellular Ca^{2+} leak [68]. Acute overexpression of CaMKIIδ_C in cardiomyocytes resulted in significant SR Ca^{2+} leak without changes in contractile function [69]. Adenoviral CaMKII overexpression was not associated with calstabin2 dissociation from RyR2 [69] confirming earlier results [6]. On the other hand, PKA phosphorylation of RyR2 resulted in depletion of the stabilizing calstabin2 subunit [6], and decreased calstabin2 levels have been linked to Ca^{2+} triggered arrhythmias in the structurally normal heart [49]. Disruption of RyR2 PKA phosphorylation in Ser2808Ala knockin mice with myocardial infarct induced HF has provided evidence that PKA hyperphosphorylation induced SR Ca^{2+} leak may directly contribute to HF progression [70]. However, the significance of concomitant PKA and/or CaMKII RyR2 phosphorylation in Ca^{2+} dysregulation during HF has not been addressed conclusively.

Altered sodium handling in heart failure

Intracellular Na^+ and Ca^{2+} concentrations are intricately coupled through the NCX current. In addition, Na^+ influx through sarcolemmal $Na_V1.5$ channels contributes to intracellular $[Na^+]_i$ homeostasis (Fig. 1a). Studies about $[Na^+]_i$ in human myocardium showed a stimulation rate dependent increase in $[Na^+]_i$ [71, 72]. For any given stimulation rate, human end-stage failing myocardium showed a shift toward higher intracellular $[Na^+]_i$ [71]. In a rabbit HF model with elevated $[Na^+]_i$, the function of the Na^+/K^+-ATPase function was found normal [73]. A potential role of altered Na^+ channel inactivation and a significantly increased late $I_{Na,L}$ in HF as a cause of intracellular $[Na^+]_i$ overload has been documented in animal models of HF and human failing myocardium [74, 75]. Slow pacing of failing cardiomyocytes with increased $[Na^+]_i$ may enhance Ca^{2+} influx from the extracellular side through reverse mode NCX contributing to increased SR Ca^{2+} load and force development. Indeed, at higher pacing rates failing cardiomyocytes with high $[Na^+]_i$ are prone to diastolic Ca^{2+} overload and contractile dysfunction. Interestingly, pharmacological inhibition of $I_{Na,L}$ was found to improve diastolic dysfunction in failing human myocardium [76]. Additionally, increased late $I_{Na,L}$ may result in Ca^{2+} induced electrical cardiomyocyte dysfunction as shown in *SCN5A*-ΔKPQ knockin mice resembling the Long-QT3 syndrome [77].

Altered force frequency relation in the failing human heart

Pathophysiological consequences of altered EC coupling have an immediate impact on cardiac stress adaptation during higher heart rates. The force–frequency relation (FFR) or staircase phenomenon of healthy human myocardium and within physiological limits describes a heart rate dependent increase in contractile force and cardiac output. However, in failing human hearts or isolated muscle preparations, the frequency-dependent potentiation of contractile force is blunted or, even worse, inversed [78]. Alteration of the FFR in the failing human heart has been accepted as a functional milestone which may partially explain the decreased exercise capacity of symptomatic HF patients.

Using aequorin Ca^{2+} measurements it has been shown that a positive FFR in normal healthy myocardium is associated with a positive $\Delta[Ca^{2+}]_i$ transient amplitude–frequency relation, and vice versa, a negative FFR in the failing myocardium is associated with an inverted $\Delta[Ca^{2+}]_i$–frequency relation [18]. Alterations of the frequency response of contractile performance have been confirmed in vivo in HF patients [79]. A

different experimental protocol which is thought to enable increased Ca^{2+} accumulation into the SR stores in diastole, uses post-rest pacing which typically produces an increase in developed force in healthy myocardium also referred to as 'post-rest potentiation'. However, alterations in post-rest potentiation that may underlie reduced stress adaptation and the FFR inversion have been associated with a blunted post-rest $\Delta[Ca^{2+}]_i$ increase of SR Ca^{2+} content (Fig. 2) [80]. While in healthy human myocardium a pronounced upregulation of SR Ca^{2+} content occurs at higher heart rates, failing human myocardium shows a blunted regulation of SR Ca^{2+} content [26]. Therefore, the chronic hyperadrenergic state and associated higher heart rates in HF patients may create an increased risk for Ca^{2+} induced cardiac dysfunction and arrhythmias. Accordingly, novel therapeutic strategies have successfully used pharmacological heart rate reduction in HF patients to improve prognostic outcome [81].

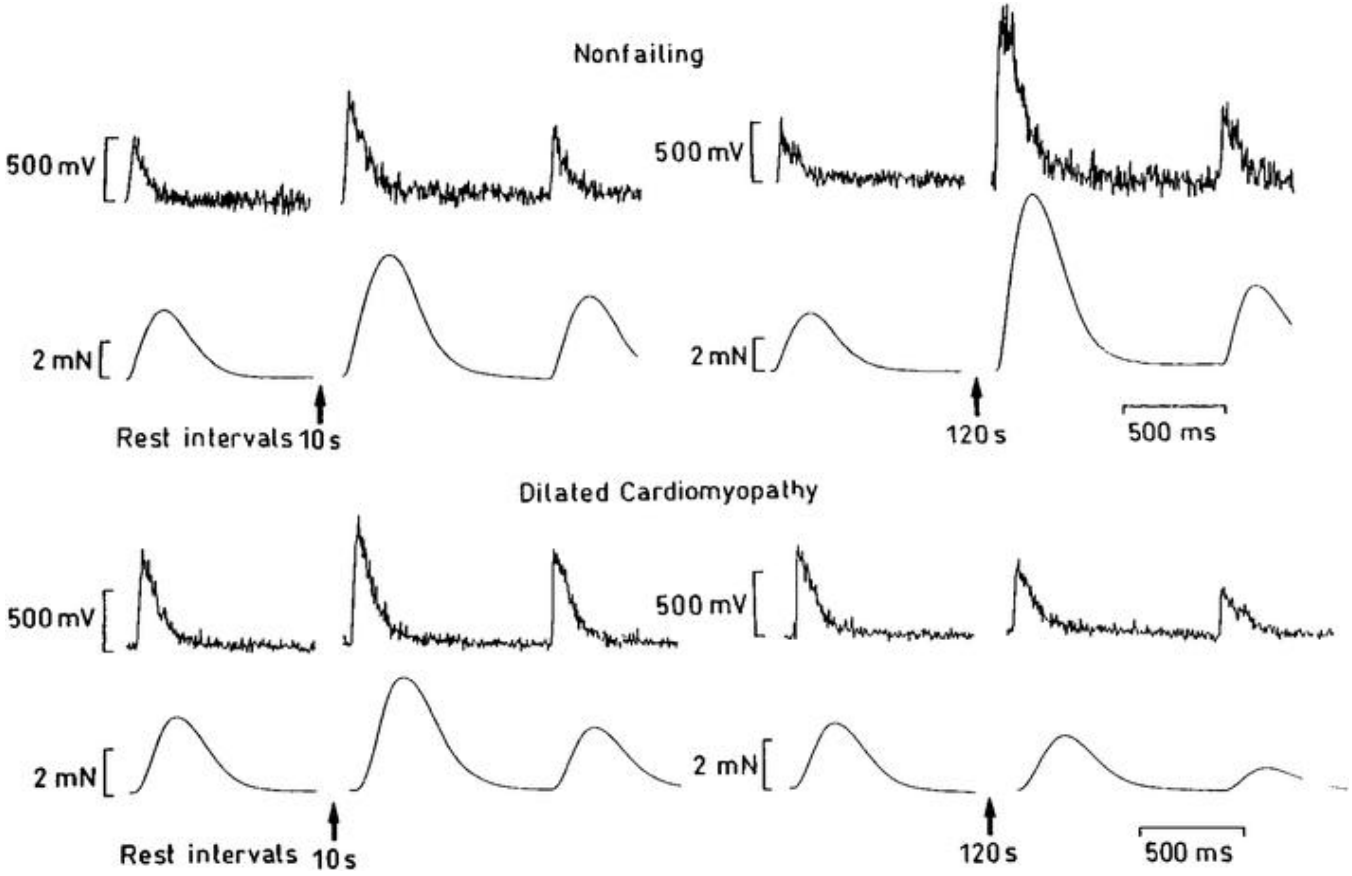

Fig. 2 Representative traces of aequorin-based Ca^{2+} signals and corresponding isometric forces from human nonfailing (*top*) and failing (*bottom*) myocardial muscle preparations. *Upper panel*: Nonfailing myocardium shows post-rest potentiation of the intracellular Ca^{2+} transient and force development which increases from 10 to 120 s rest period. *Lower panel*: Failing myocardium shows depressed post-rest intracellular Ca^{2+} transient and force development after 120 s rest. Steady-state pre-rest signals are shown on the left of each trace; first and second post-rest signals are shown afterwards; post-rest signals represent 10 s (*left*) and 120 s (*right*), dimensions as indicated. Reproduced with permission from the Journal of Clinical Investigation (Pieske et al. [80])

Therapeutic rationales to modulate calcium handling in heart failure

Several therapeutic rationales exist which aim to correct known molecular Ca^{2+} signaling abnormalities in HF as summarized in Table 2. Among these strategies, NCX blockers may have therapeutic utility if mode-selective block can be established and/or risks for disturbing intracellular Ca^{2+} metabolism can be avoided. Partial inhibition of NCX by SEA-0400 in $MLP^{-/-}$ knockout cardiomyocytes with heart failure

showed a net gain of $[Ca^{2+}]_i$ and SR load but no improvement in contractility whereas cardiomyocytes from mice with aortic-banding induced hypertrophy and HF showed improved contractile function [82]. Further development of NCX blockers for HF will depend on the critical assessment of the potential benefits of NCX reduction versus effects on $[Ca^{2+}]_i$ by refining mode dependence and/or including additional targeting strategies.

Some traditional ion channel blockers like tetracaine inhibit ion permeation through RyR2 channels, however, lack of specificity and potential side effects on contractile function indicate significant limitations toward therapeutic applicability [83, 84]. Recently, the efficacy of novel RyR2 channel stabilizing drugs of the 1,4-benzothiazepine class (JTV519 or K201) which stabilize the RyR2 closed state but do not block ion permeation has been established in animal models of heart failure where they inhibit progression of cardiac remodeling and dysfunction [57, 85, 86]. RyR2 stabilizing compounds with high specificity and cardiac activity have been developed [51] and their efficacy as potential HF treatment is under investigation. Additionally, β-blockers and angiotensin-II receptor blockers have been associated with beneficial effects on RyR2 channel dysfunction in HF through mechanisms which indirectly prevent excess post-translational modification of RyR2, e.g. by PKA hyperphosphorylation or nitrosylation [87–89].

Adeno-associated viruses (AAVs) are currently best suited for myocardial gene delivery due to minimal pathogenicity and several serotypes exhibit tropism for the heart. Following identification and comprehensive characterization of SERCA2a as a potential therapeutic target in HF, cardiac gene therapy trials using replication-deficient viral vectors (AAV-SERCA2a) have been approved by the US Food and Drug Administration and are under review with the UK regulatory authorities for clinical trials [90].

Summary and future perspectives

HF is characterized by chronic maladaptive changes and a poor prognosis. Chronic activation of intracellular signaling pathways mediates structural and functional remodeling of the failing heart. Changes in EC coupling at the level of local and global Ca^{2+} signals represent a key mechanism of contractile depression and arrhythmia propensity. It has become increasingly clear that cardiac remodeling in HF occurs within cytosolic Ca^{2+} signaling microdomains. Translational approaches about the local and global Ca^{2+} signaling mechanisms, remodeling mechanisms, and related disease processes will be of key importance to develop novel and specific therapeutic rationales. We anticipate that Ca^{2+} imaging techniques will significantly increase our understanding of the cardiac pathophysiology underlying HF and boost development of novel therapeutic strategies in the future.

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