Mechanisms of Altered Ca^{2+} Handling in Heart Failure

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Abstract: **Ca^{2+} plays a crucial role in connecting membrane excitability with contraction in myocardium. The hallmark features of heart failure are mechanical dysfunction and arrhythmias; defective intracellular Ca^{2+} homeostasis is a central cause of contractile dysfunction and arrhythmias in failing myocardium. Defective Ca^{2+} homeostasis in heart failure can result from pathological alteration in the expression and activity of an increasingly understood collection of Ca^{2+} homeostatic and structural proteins, ion channels, and enzymes. This review focuses on the molecular mechanisms of defective Ca^{2+} cycling in heart failure and considers how fundamental understanding of these pathways may translate into novel and innovative therapies. (*Circ Res*. 2013;113:690-708.)**

Key Words: calcium ■ CaMKII ■ excitation-contraction coupling ■ heart failure ■ mitochondria

Among the many causes of myocardial injury that can lead to congestive heart failure (CHF), myocardial infarction (MI) is the most common in the developed world.[1] The hallmark features of heart failure include reduced contractile function manifested as blunted, slowed, dysynchronous contraction and impaired relaxation. The physiological positive force–frequency relationship and increased myocardial contractile response to increased preload are compromised in heart failure.[2] The failing heart attempts to compensate for injury by various mechanisms, such as myocardial hypertrophy, increasing filling pressure, and enhanced neurohumoral signals, which together drive a feed-forward pathophysiological spiral leading to adverse ventricular remodeling and electric instability.[3] Each of these maladaptive events is associated with loss of myocardial Ca^{2+} homeostasis.

Ca^{2+} Homeostasis and Mechanisms Underlying Excitation-Contraction Coupling

Ca^{2+} plays a crucial role in coupling cell membrane excitation and contraction, so-called excitation-contraction coupling

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Nonstandard Abbreviations and Acronyms

AAV	adeno-associated virus
ANT	adenosine nucleotide translocator
ATP	adenosine triphosphate
β-AR	beta-adrenergic receptor
CaMKII	Ca^{2+}-dependent and calmodulin-dependent protein kinase II
CHF	congestive heart failure
DAD	delayed afterdepolarization
DMD	Duchnne muscular dystrophy
EAD	early afterdepolariazation
ECC	excitation-contraction coupling
HRC	histidine-rich Ca^{2+} binding protein
I_K	voltage-gated K current
I_{KATP}	ATP-sensitive K^+ current
I_{Na}	inward Na^+ current
I_{NCX}	NCX current
I_{to}	transient outward current in the heart
K_{ATP}	cardiac ATP-sensitive K^+
LTCC	L-type calcium channel
MCU	mitochondrial Ca^{2+} uniporter
mPTP	mitochondrial permeability transition pore
NADH/NADPH	nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate hydrogen
NCX	Na^+/Ca^{2+} exchanger
NFAT	nuclear factor of activated T cells
PKA	protein kinase A
PKC	protein kinase C
RyR2	ryanodine receptor 2
SERCA2a	sarcoplasmic-endoplasmic reticulum Ca^{2+} ATPase
SR	sarcoplasmic reticulum

(ECC) (Figure 1). Cardiac contraction depends on a transient increase in the cytosolic Ca^{2+} concentration ($[Ca]^{2+}_i$) to activate cross-bridge formation between myofilament proteins that ultimately elicits pressure development in the cardiac chambers and provides energy for ejection of blood. Cardiomyocytes are packed with myofibrils enveloped in a network of Ca^{2+}-storing sarcoplasmic reticulum (SR)[4] and mitochondria.[5] ECC in ventricular myocytes is built around dyads, specialized membrane ultrastructures formed by the terminal cisternae of the SR and invaginations of the cell membrane called transverse tubules. Voltage-gated ion channels, exchangers, and Na^+/K^+ ATPase pump proteins are enriched on the transverse tubular membranes and colocalize with the intracellular ryanodine receptor (RyR2) Ca^{2+}-release channels, which are clustered on the SR membrane. ECC is initiated when the cell membrane action potential invades the myocyte along its transverse tubules. The flow of inward current depolarizes the cell membrane and rapidly (in 1–2 ms) opens voltage-gated Na^+ channels (mostly $Na_V1.5$) that are responsible for a large inward Na^+ current (I_{Na}). I_{Na} rapidly inactivates (1–2 ms) and $Na_V1.5$ channels remain inactive until the action potential is complete and the cell membrane returns to a negative resting potential (≈−90 mV). The inward I_{Na} depolarizes the cell membrane, reaching a cell membrane potential that is permissive for opening voltage-gated Ca^{2+} channels (mostly $Ca_V1.2$ in ventricular myocardium). Inward Ca^{2+} current (I_{Ca}) triggers opening of RyR2 channels by a Ca^{2+}-induced Ca^{2+} release process,[6] resulting in coordinated release of SR Ca^{2+} that contributes the major portion of myofilament-activating Ca^{2+}. The I_{Ca} contributes to the long action potential plateau (200–400 ms) characteristic of ventricular myocytes in humans.[7] The Ca^{2+} released from the SR diffuses over a short distance to engage the adjacent myofibrils, binding to troponin C of the troponin–tropomyosin complex on the actin filaments in sarcomeres, which moves tropomyosin away from the binding sites, facilitating formation of cross-bridges between actin and myosin to enable myocardial contraction. I_{Ca} inactivates by voltage-dependent and $[Ca]^{2+}_i$-dependent mechanisms[8] at the same time that voltage-gated K^+ channels open to allow an outward current that orchestrates action potential repolarization, establishing conditions required for relaxation.

Cardiac relaxation depends on a decrease in $[Ca]^{2+}_I$ that is permissive for unbinding of myofilament cross-bridges. Sequestration of cytoplasmic Ca^{2+} occurs mainly through active Ca^{2+} uptake by the SR, through the sarcoplasmic-endoplasmic reticulum Ca^{2+} ATPase (SERCA2a),[9] and to a lesser extent by extrusion to the extracellular space by the Na^+/Ca^{2+} exchanger (NCX),[10] the sarcolemmal Ca^{2+} ATPase,[11] and mitochondria.[12] The binding of Ca^{2+} rapidly activates NCX, which facilitates Ca^{2+} efflux into the extracellular milieu using the energy from the cell membrane Na^+ gradient established by the Na^+/K^+ ATPase. NCX generates a current because it exchanges 3 Na^+ for 1 Ca^{2+}, a net positive charge. Depending on the electrochemical gradient, NCX current may be inward (forward mode), extruding cytoplasmic Ca^{2+} to the extracellular space, or outward (reverse mode), importing extracellular Ca^{2+} to the cytoplasm. Thus, Ca^{2+} cycling between the extracellular space, cytosol, and SR allows rapid contraction and relaxation of the heart.

Defective ECC and Alterations of Ca^{2+}-Handling Proteins in Heart Failure

Consistently, cardiomyocytes from the failing heart show defective ECC characterized by decreased $[Ca]^{2+}_i$ transients, enhanced diastolic SR Ca^{2+} "leak," and diminished SR Ca^{2+} sequestration, events that contribute to impaired contractility and relaxation.[13] These abnormalities are attributable to alterations of a collection of key Ca^{2+}-handling proteins.

Impaired SR Ca^{2+} Release Contributes to Systolic Heart Failure

$Ca_V1.2/Na_V1.5$

Voltage-dependent opening of L-type calcium channels (LTCCs) enables cellular Ca^{2+} entry that triggers Ca^{2+}-induced Ca^{2+} release from the SR by promoting RyR2 opening, leading to myofilament cross-bridge formation and mechanical force development. The cardiac action potential plateau in ventricular myocytes is optimized for grading $Ca_V1.2$ openings to initiate Ca^{2+}-induced Ca^{2+} release and ECC. Similar to all known voltage-gated ion channels, $Ca_V1.2$ consists of a pore-forming α-subunit, auxiliary subunits, and connections to various cytoskeletal proteins.[14,15] Protein kinase A (PKA), protein kinase C (PKC), and the multifunctional Ca^{2+}-dependent and

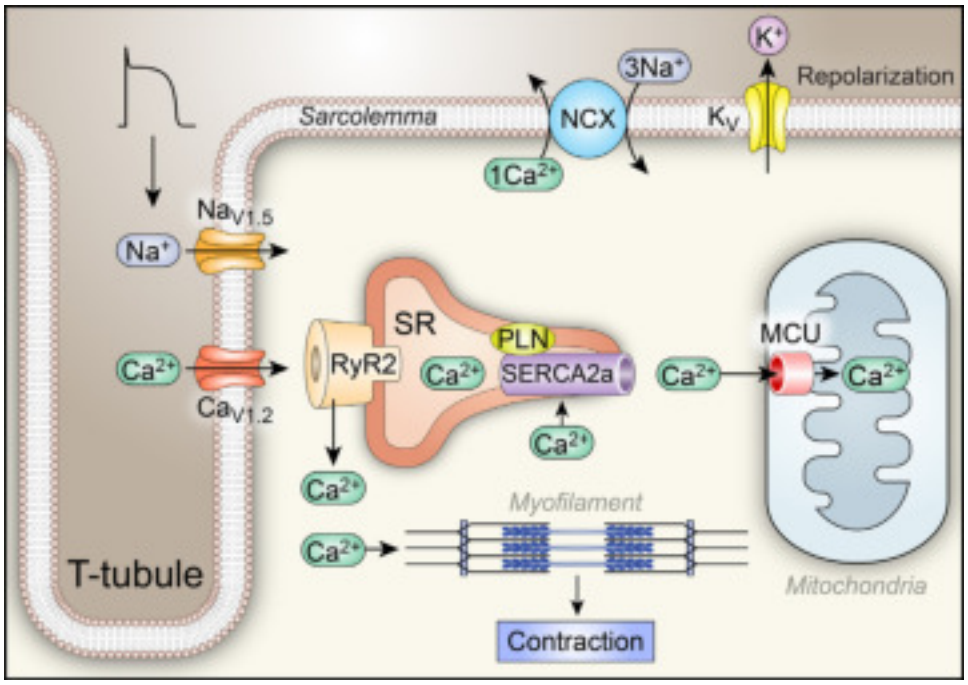

Figure 1. Ca^{2+} homeostasis and excitation-contraction coupling (ECC). The ECC process is initiated when an action potential (AP) excites the myocyte cell membrane (sarcolemma) along its transverse tubules. This depolarization rapidly opens voltage-gated Na^+ channels (mostly $Na_V1.5$) that further depolarize the cell membrane, allowing opening of voltage-gated Ca^{2+} channels (mostly $Ca_V1.2$). Inward Ca^{2+} current triggers opening of ryanodine receptor 2 (RyR2) channels by a Ca^{2+}-induced Ca^{2+} release process, resulting in coordinated release of sarcoplasmic reticulum (SR) Ca^{2+} that contributes the major portion of the myofilament-activating increase in $[Ca]^{2+}_i$. The Ca^{2+} released from the SR binds to troponin C of the troponin–tropomyosin complex on the actin filaments in sarcomeres, facilitating formation of cross-bridges between actin and myosin and myocardial contraction. Voltage-gated K^+ channels open to allow an outward current that favors AP repolarization, establishing conditions required for relaxation. Relaxation occurs when Ca^{2+} is taken back up into the SR through the action of the SR Ca^{2+} adenosine triphosphatase SERCA2a and is extruded from the cell by the sarcolemmal Na^+ and Ca^{2+} exchanger (NCX). SERCA2a is constrained by phospholamban (PLN) under resting conditions.

calmodulin-dependent protein kinase II (CaMKII) are serine-threonine kinases that catalyze ATP-dependent phosphorylation of $Ca_V1.2$ proteins[15,16] (Figure 2). CaMKII[16] and PKA[17] increase the frequency of prolonged $Ca_V1.2$ openings, whereas the functional significance of PKC actions at $Ca_V1.2$ are less clear.[15] These prolonged and frequent $Ca_V1.2$ channel openings are attributable to mode 2 $Ca_V1.2$ gating, a biophysical response shared with β-adrenergic receptor (β-AR) agonists, CaMKII, and the dihydropyridine agonist BayK 8644.[16–18] Phosphorylation by CaMKII or by PKA, the principal kinase activated by β-AR agonists, collaborates with cell membrane potential to enhance the probability of $Ca_V1.2$ opening. Mode 2 gating appears to underlie I_{Ca} facilitation, a dynamic pattern of increasing peak I_{Ca} and slowed I_{Ca} inactivation.[19] Mode 2 gating and I_{Ca} facilitation are proarrhythmic, in part, by favoring early afterdepolariazations (EADs).[16,20,21]

Elevated $[Na^+]_i$ and altered NA^+ channel properties is present in failing myocardium from humans.[22–25] Changes in $[Na^+]_i$ may have a large impact on $[Ca]^{2+}$i homeostasis.[26] Small increases in $[Na^+]_i$ may increase Ca^{2+} influx via reverse-mode NCX during systole and limit Ca^{2+} extrusion via forward-mode NCX during diastole, leading to increased subsarcolemmal $[Ca]^{2+}_i$.[27,28] Therefore, increased $[Na^+]_I$ levels lead to Ca^{2+} overload, contributing to arrhythmias and impaired diastolic function.[22] The major pathway for Na^+ influx in cardiomyocytes is through voltage-gated Na^+ channels, primarily $Na_V1.5$, which open and close rapidly (1–10 ms) to trigger the upstroke of action potential depolarization in working myocardium. CaMKII associates with and phosphorylates the $Na_V1.5$ α-subunit at a "hot spot" in the cytoplasmic I–II linker domain, an event that promotes a noninactivating, long-lasting component of I_{Na} (I_{NaL}) and arrhythmia-triggering EADs and delayed afterdepolarizations (DADs).[29,30] CaMKII inhibition reverses the increase of I_{NaL} in heart failure,[31] suggesting that $Na_V1.5$ is an important target for the antiarrhythmic effect of CaMKII inhibition.[32] $[Na^+]_i$ is also maintained by the Na^+/K^+ ATPase pump. It was reported that in failing human hearts, the tissue concentration of the Na^+/K^+ ATPase pumps are reduced.[33] Whether the functional capacity of the Na^+/K^+ ATPase pump in heart failure is altered remains inconclusive because some studies show unaltered maximum transport rate and affinity for Na^+ in a rabbit heart failure model,[34] whereas the Na^+/K^+ ATPase pump was reduced in a rat heart failure model.[35]

Reduced SR Ca^{2+} Release and Increased RyR2 Opening Probability

RyR, the largest ion channel protein (560 kDa), exists as a homotetramer (≈2.2 MDa). The predominant isoform expressed in cardiac muscle is RyR2.[36] RyR2 works as a multiprotein Ca^{2+}-release unit in which the RyR2 Ca^{2+} channel is composed of 4 membrane-spanning subunits[37] coupled to various regulatory proteins. Calsequestrin, triadin 1, and junctin bind to RyR2 at the luminal SR membrane face, where they transmit

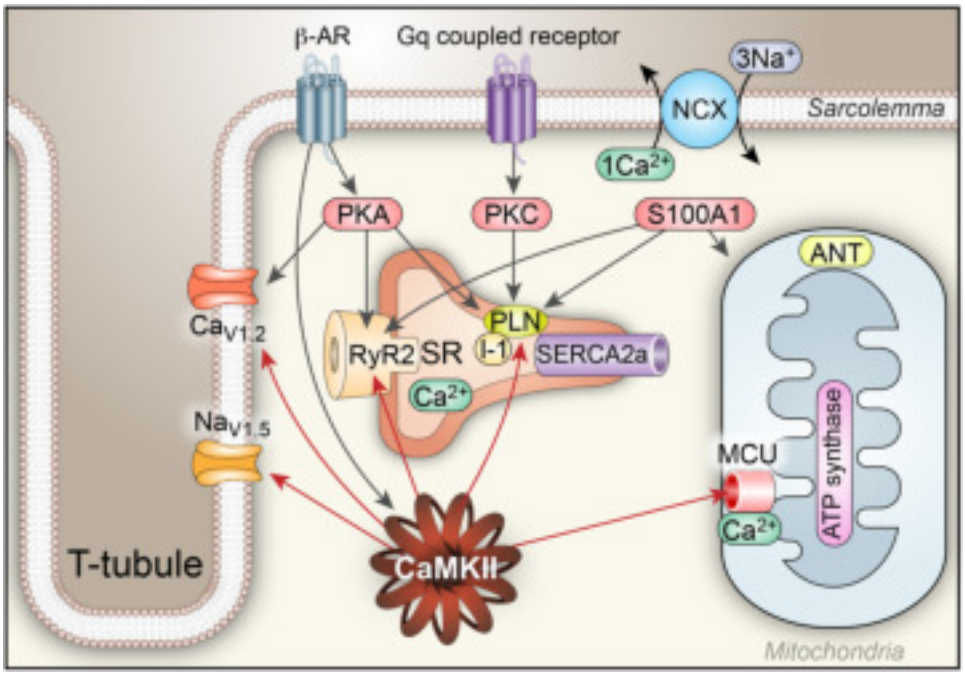

Figure 2. Regulation of $[Ca]^{2+}_i$ homeostasis by Ca^{2+}-binding proteins and kinases. Regulation of Ca^{2+} homeostasis involves a multitude of Ca^{2+}-binding proteins and enzymes, including Ca^{2+}-dependent and calmodulin-dependent protein kinase II (CaMKII), protein kinase C (PKC), protein kinase A (PKA), and S100A1. CaMKII catalyzes phosphorylation of voltage-gated Ca^{2+} channels (mostly $Ca_V1.2$ in ventricle) to increase Ca^{2+} entry, catalyzes ryanodine receptor (RyR2) phosphorylation to increase Ca^{2+} release, catalyzes phosphorylation of voltage-gated Na^+ channels (mostly $Na_V1.5$ in ventricle) to increase subsarcolemmal $[Na^+]_i$, which decreases the driving force for Ca^{2+} extrusion by the Na^+/Ca^{2+} exchanger (NCX), and catalyzes PLN phosphorylation to reduce the inhibitory activity of PLN on SERCA2a. In general, the increased phosphorylation of these proteins by CaMKII increases Ca^{2+} influx and storage by the sarcoplasmic reticulum (SR), which leads to increased systolic $[Ca]^{2+}_i$ and increased rate and magnitude of force (pressure) generation and improved lusitropy. PKA is activated by β-AR agonists and catalyzes phosphorylation of the same Ca^{2+} regulatory proteins modified by CaMKII, but at different amino acids. Classical PKC isoforms are activated downstream to a variety of G-protein-coupled receptors and are activated by increased $[Ca]^{2+}_i$, leading to decreased activity of SERCA2 by phosphorylating inhibitor 1 (I-1), resulting in PLN dephosphorylation, reducing SR Ca^{2+} load and Ca^{2+} release, causing reduced contractility. S100A1 interacts with the SERCA2a/PLN complex in a Ca^{2+}-dependent manner to augment SR Ca^{2+} uptake and increase SR Ca^{2+} content. S100A1 also directly regulates RyR2 function, stimulates ATP synthase activity, and promotes the adenosine nucleotide translocator (ANT) function to increase ATP synthesis and mitochondrial ATP efflux in cardiomyocytes.

information about SR Ca^{2+} content to RyR2.[38] It is known that congenital mutations in RyR2, calsequestrin, and triadin can cause increased SR Ca^{2+} leak, disorganized diastolic Ca^{2+} release, arrhythmias, and sudden death.[39,40]

Under physiological conditions, RyR2 opening probability is increased by the cytoplasmic Ca^{2+} trigger from I_{Ca}.[41] RyR2 activity is also regulated by multiple factors, including PKA, CaMKII, protein phosphatases 1 and 2A, calmodulin, and FKBP12.6, which are associated with the cytoplasmic face of RyR2. Marks et al[42] demonstrated that PKA phosphorylates RyR2, which enables the "fight-or-flight" response by increasing RyR2 opening probability and $[Ca]^{2+}_i$.[43] They also showed that hyperphosphorylation of RyR2 by PKA (at serine 2808) caused an FKBP12.6–RyR2 dissociation and increased RyR2 opening probability and SR Ca^{2+} leak in human[42,44] and animal models of CHF.[45–48] In addition, their results also suggest that improved cardiac function by β-AR antagonist drugs in the failing human heart is associated with restoration of FKBP12.6 levels and repair of RyR2 channel leak.[44] However, other groups reported conflicting results that phosphorylation at a single site including serine 2809 does not alter RyR2 function[49] and that phosphorylation at the S2808 site does not mediate β-AR agonist-induced cardiac response[50,51] or dysfunction after MI.[52] These highly controversial results[53] indicate that alternative mechanisms also may be important for RyR2 dysfunction in heart failure.

CaMKII is activated by β-AR agonist stimulation[54] and increased reactive oxygen species (ROS)[55] and can phosphorylate RyR2 at least 2 sites, serine 2809 and serine 2814 (S2814),[56,57] although the 2814 site appears to be preferred.[57] CaMKII-dependent RyR2 phosphorylation increases diastolic SR Ca^{2+} release.[58] Mice genetically lacking S2814A have an impaired force–frequency relationship[59] and are resistant to MI-induced heart failure and arrhythmias.[60,61] It also was shown that oxidative stress generated in the failing heart could directly alter RyR2 function by posttranslational modification, causing its increased sensitivity to activation by luminal Ca^{2+}.[62] A growing body of evidence suggests that reduced Ca^{2+} release in failing cardiomyocytes is a result of increased and improperly regulated activity of multiple Ca^{2+}-handling proteins, including $Ca_V1.2$, $Na_V1.5$, and RyR2, all of which appear to be targets of CaMKII.

Impaired Ca^{2+} Sequestration During Diastole

To achieve relaxation, cytosolic Ca^{2+} must be sequestered, mainly to the SR by SERCA2a.[9] Diastolic $[Ca]^{2+}_i$ is increased

in human heart failure, a condition that is likely related, at least in part, to defects in cytosolic Ca^{2+} removal.[63] Taken together with loss of physiological SR Ca^{2+} release, elevated diastolic $[Ca]^{2+}_i$ results in reduced contractile force, impaired relaxation, and abnormal force–frequency relationship in human heart failure. The sarcomere is the primary functional unit of cardiac muscle that is responsible for contraction and force generation. Failing myocardium is marked by spontaneous diastolic SR Ca^{2+} release, leading to spontaneous and highly variable diastolic sarcomere contractions, which significantly reduces contractile force[64,65] and contributes to the loss of inotropic effects in CHF.[65]

SR Ca^{2+} uptake is impaired in the failing human heart,[66,67] an outcome that is attributable to several mechanisms. First, there is reduced expression and activity of SERCA2a in the failing human heart.[68,69] However, in some human failing hearts, SERCA2a expression or activity is normal.[70,71] Overexpression of SERCA2a can restore the Ca^{2+} handling and the contractile function in animal models[72] and in human heart failure,[73,74] suggesting that repairing SERCA2a expression may be a viable therapy for CHF. Defects in SR Ca^{2+} release may be attributable to loss of normal "gain" of ECC, a condition in which a given I_{Ca} trigger elicits a lesser amount of SR Ca^{2+} release.[75] Comparisons of ECC gain require experimental conditions that control for SR Ca^{2+} content. Nevertheless, failing human cardiomyocytes may have preserved fractional SR Ca release[13] despite reduced SR Ca^{2+} pump activity, SR Ca^{2+} content, and systolic $[Ca]^{2+}_i$ transients, suggesting that defects in ECC gain are not an obligate aspect of failing myocardiocytes.

Second, reduced SR Ca^{2+} uptake could be attributable to increased inhibitory activity of PLN.[76,77] PLN inhibits SERCA2a in its dephosphorylated form, whereas in its phosphorylated form (by PKA at serine-16 and CaMKII at threonine-17)[78] PLN assembles into a pentamer that lacks SERCA2a inhibitory activity.

Multiple studies suggest that phosphorylation of PLN is decreased in the failing human heart, accounting for increased inhibition of SERCA2a.[77,79] For example, phosphorylation of PLN at threonine 17 is decreased in ventricular myocardium because of increased dephosphorylation by protein phosphatase 2B, also called calcineurin, despite increased activity of CaMKII in failing myocardium.[80] PLN phosphorylation at serine 16 is decreased because of increased activity of type 1 protein phosphatase in the failing human heart.[77] Several mutations in the human PLN gene (such as R9L, R9H, and L39stop)[81] have been identified that provide important insights into PLN regulation of SERCA2a. Two mutations (R9C and R14del) result in enhanced inhibition of SERCA2 by PLN, partly because of decreased PKA-mediated phosphorylation.[82,83] The phenotypes of R9C or R14del carriers include dilated cardiomyopathy and premature death.[82,83]

Another human mutation causing loss of function of PLN (Leu39stop) and uninhibited SERCA2a activity also results in dilated cardiomyopathy and premature death.[84] Genetic manipulation of PLN in mouse models yielded similar and contrasting results compared with human mutations. PLN knockout mice showed enhanced cardiac contractile function with increased affinity of SERCA2a for Ca^{2+}, consistent with the concept that PLN downregulates myocardial contractility by suppressing SERCA activity.[85] PLN knockout prevented heart failure in a mouse model of dilated cardiomyopathy caused by deficiency of the muscle-specific LIM protein.[86,87] Gene therapy with antisense against PLN improved contractile and diastolic function in isolated failing human cardiomyocytes.[88] However, PLN knockout in mice with severe cardiomyopathy attributable to transgenic overexpression of CaMKII improved SR Ca^{2+} content and myocardial contraction, but nevertheless increased mortality, mitochondrial Ca^{2+}, and myocardial cell death.[89] Taken together, these studies of mice and humans suggest that SERCA2a/PLN activity needs to be maintained within certain boundaries to support physiological function and prevent cardiomyopathy.

Another emerging regulator of SERCA activity is the Histidine-rich Ca^{2+}-binding protein (HRC), a low-affinity and high-capacity Ca^{2+}-binding protein located in the SR lumen.[90] HRC also affects RyR function through its binding to triadin, and it was suggested that HRC may mediate a cross-talk between SR Ca^{2+} uptake and release. A human HRC variant (S96A) with substitution of Ala in position 96 is associated with life-threatening ventricular arrhythmias in dilated cardiomyopathy patients, accompanied by a reduced $[Ca]^{2+}_i$ transient and a prolonged decay time.[91] Transgenic overexpression of HRC in the heart decreases SR Ca^{2+} uptake rates, suggesting that HRC inhibits SERCA2a and intracellular Ca^{2+} cycling and promotes progression to heart failure.[92] These studies suggest an important role of HRC in maintaining Ca^{2+} homeostasis in the SR.

The relative contribution of NCX to cytoplasmic Ca^{2+} sequestration is increased in failing myocardium, probably because of the decreased SR Ca^{2+} uptake.[93] Expression of NCX in human CHF has been reported to increase[10] or to be unchanged.[94] Because subsarcolemmal $[Na^+]_i$ is increased in failing ventricular myocytes, NCX current (I_{NCX}) shifts from inward to outward,[95] which contributes to prolonged cytoplasmic $[Ca]^{2+}_i$ transients, Ca^{2+} overload, and diastolic dysfunction.[22,95,96] Thus, enhanced I_{NCX} may be adaptive to defects in SERCA2a/PLN in CHF while also contributing to subsarcolemmal $[Na^+]_i$ and $[Ca]^{2+}_i$ overload in CHF.

Adenosine Triphosphate, Mitochondrial Ca^{2+} Uptake, and Retention

Adenosine triphosphate (ATP) is the predominant form of readily available energy in myocardium.[97] The Ca^{2+} concentration gradient between the extracellular and intracellular environments is massive, with approximately 10 000-fold higher extracellular than bulk cytoplasmic (≈100 nmol/L)[98] $[Ca]^{2+}_i$. Maintaining Ca^{2+} homeostasis constitutes a major ATP cost for cardiomyocytes. SERCA2a and the Na^+-K^+ ATPase are among the largest energy-consuming proteins.[99] A proper equilibrium between Ca^{2+} cycling and ATP production must be maintained to ensure proper intracellular Ca^{2+} handling and a physiological range of myocardial performance.[100,101] Mathematical modeling[102,103] and experiments in excised myocardial cell membrane patches using the ATP-sensitive K^+ current (I_{KATP}) as a readout for subsarcolemmal ATP[102,103] support a view that ATP availability can be rate-limiting under stress conditions because of high local ATP consumption and compartmentalization. Thus, it is plausible that subcellular domains of ATP deficiency contribute to myocardial dysfunction in CHF.

CHF is associated with abnormal energy metabolism, including decreased energy production and impaired energy utilization,[104–106] which appear to adversely affect $[Ca]^{2+}_i$ homeostasis.[100,106] Reduced ATP/ADP ratio, attributable to mitochondrial dysfunction, caused impaired function of SERCA2a in animal models of CHF.[107] However, Ca^{2+} transport regulates ATP production in mitochondria.[108,109] Some validated clinical therapies for CHF improve myocardial energetics and normalize $[Ca]^{2+}_i$ homeostasis. For example, β-AR antagonists were designed by Sir James Black, in part, to reduce myocardial O_2 consumption with a goal of preventing MI.[110] β-Blockers, which decrease energy consumption, have been shown to normalize the contractile function and Ca^{2+} handling in failing human hearts.[111,112] Left ventricular assist devices, which decrease the workload of the heart, improve Ca^{2+} handling in CHF patients.[14,113] Restoration of mitochondrial Ca^{2+} homeostasis by unloading mitochondrial Ca^{2+} restored cardiac energetics, including ATP synthesis.[114] Thus, CHF appears to be a condition that arises, at least in part, by interrelated defects in $[Ca]^{2+}_i$ homeostasis and metabolism, and successful CHF therapies often restore physiological $[Ca]^{2+}_i$ homeostasis and metabolism.

Mitochondrial Ca^{2+} Regulates Cell Metabolism and Cell Death

Mitochondria comprise approximately 20% to 30%[115] of cardiac mass, where they are essential for providing ATP to meet the heightened energy demand for cardiac function. Ca^{2+} appears to be a critical second messenger for communicating cellular energy demands to mitochondria for the purpose of matching ATP production by oxidative phosphorylation with metabolic requirements.[109] Oxidative phosphorylation is a Ca^{2+}-regulated process because Ca^{2+} increases the activity of key tricarboxylic acid dehydrogenases involved in producing reducing equivalents (NADH/NADPH) for electron transport.[116] Metabolic regulation by mitochondrial Ca^{2+} uptake, however, is not limited to the effects on dehydrogenases. The aspartate/glutamate exchangers located at the inner mitochondrial membrane have Ca^{2+}-binding domains, which support increased ATP production in response to local and temporal Ca^{2+} signals.[117,118] Furthermore, the close physical association between mitochondria, SR, and plasma membrane Ca^{2+} channels ensures prompt Ca^{2+} transfer to the mitochondrial matrix, which stimulates oxidative phosphorylation in response to activation of ATP-consuming processes in the cytosol.[119,120]

Compared with the SR, mitochondria have a lower affinity but a higher capacity for taking-up Ca^{2+}. Mitochondria may constitute an important buffer for cytoplasmic Ca^{2+},[119,121] but excessive accumulation of mitochondrial Ca^{2+} causes mitochondrial damage and myocardial death[122] (Figure 3). Excessive mitochondrial $[Ca]^{2+}$ ($[Ca]^{2+}_m$) and ROS[123] trigger mitochondrial permeability transition pore (mPTP) opening and subsequent dissipation of inner mitochondrial membrane potential ($\Delta\psi m$) and release of apoptotic mediators such as cytochrome C,[124] leading to cell death.[125,126] The mPTP appears to be an important but incompletely understood target for CaMKII.[127] Our group recently reported that cardiomyocytes from mice with transgenic expression of a mitochondrial-targeted CaMKII inhibitory protein[128] were able to sustain higher mitochondrial Ca^{2+} entry before mPTP opening and were resistant to programmed cell death from ischemia/reperfusion-related, catecholamine-related, and MI-related injury, suggesting that CaMKII promotes mPTP opening and myocardial death[129](Figure 3).

Mitochondria are considered a key source for pathological increases in ROS, mainly as a result of electron transport chain uncoupling at the level of complexes I and III.[123,130] Oxidative stress could damage mitochondrial DNA and proteins by forming oxidative adducts, leading to mitochondrial dysfunction, impairing myocardial energetics in heart failure. However, in heart failure, impaired mitochondrial bioenergetic function with decreased electron transport systems could cause increased oxidative stress.[131,132] Thus, mitochondrial dysfunction and ROS are tightly linked elements of an interdependent feed-forward circuit that promotes the pathogenesis of heart failure.

Mitochondrial Ca^{2+} Uniporter

The mitochondrial Ca^{2+} uniporter (MCU) is a Ca^{2+}-selective channel residing in the inner mitochondrial membrane and the major mitochondrial Ca^{2+} entry pathway.[133–135] MCU can be located in close proximity to the SR[136] and thus is exposed to high $[Ca]^{2+}$ (≈20–50 μmol/L).[137] Although the existence of the MCU was established more than 50 years ago,[138] it was not until recently that the molecular identity of MCU was discovered. MCU consists of 2 predicted membrane-spanning domains with a linker/pore loop to form a functional channel.[134,135] Overexpression of MCU increases cell death in response to challenge by proapoptotic stimuli,[135] whereas suppressing MCU with Ru360, a pharmacological antagonist related to ruthenium red, protects against ischemia-reperfusion injury.[139] We recently found that MCU is a phosphorylation substrate for CaMKII and that CaMKII-mediated increases in MCU current (I_{MCU}) required serines 57 and 92 when MCU was expressed heterologously, whereas mitochondrial-targeted CaMKII inhibition reduced I_{MCU} in myocardium.[129] The role of CaMKII signaling to MCU in heart failure is uncertain at this time, but mitochondrial CaMKII inhibition is protective against myocardial death in response to ischemia-reperfusion injury, MI, and toxic doses of isoproterenol,[129] suggesting protective effects of mitochondrial CaMKII inhibition may be mediated, at least in part, by reducing I_{MCU}.

The MICU1 is a MCU binding partner that has a single membrane-spanning domain and 2 Ca^{2+}-binding EF-hand domains.[134,139] Some recent data suggest that MICU1 is essential for setting the Ca^{2+} dependence of I_{MCU}[135,140] and preserving normal $[Ca]^{2+}_m$ by acting as a gatekeeper for Ca^{2+} uptake and preventing mitochondrial Ca^{2+} overload and excessive oxidative stress.[141] In addition, MCU regulator-1 also was recently shown to be required for MCU-dependent mitochondrial Ca^{2+} uptake and maintenance of normal cellular bioenergetics.[142] Thus, MCU appears to be a Ca^{2+}-regulated and CaMKII-regulated ion channel associated with various accessory protein subunits.

Few studies have investigated whether or how mitochondrial Ca^{2+} uptake, transport, and homeostasis are altered in heart failure. Limited indirect evidence suggests that mitochondrial Ca^{2+} uptake is reduced in failing cardiac myocytes

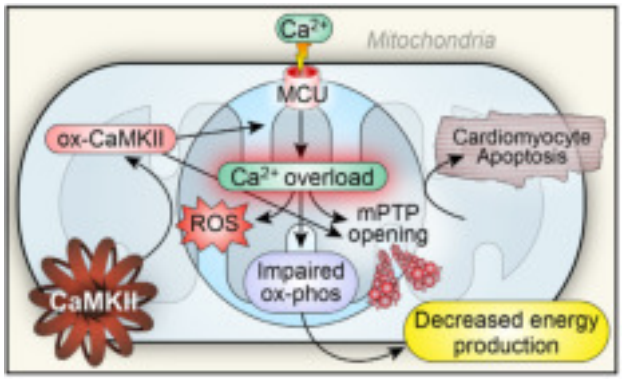

Figure 3. A scenario for mitochondrial Ca^{2+} overload, impaired metabolism, and cell death in heart failure. The mitochondrial Ca^{2+} uniporter[132] is a Ca^{2+}-selective channel residing in the inner mitochondrial membrane. Mitochondrial Ca^{2+} uniporter (MCU) is a phosphorylation substrate for Ca^{2+}-dependent and calmodulin-dependent protein kinase II (CaMKII). Mitochondrial CaMKII inhibition reduces MCU current, increases mitochondrial Ca^{2+} retention capacity, and is protective against myocardial death in response to ischemia-reperfusion injury, myocardial infarction (MI), and toxic doses of isoproterenol. Excessive mitochondrial Ca^{2+} and reactive oxygen species (ROS) trigger mitochondrial permeability transition pore (mPTP) opening, leading to cell death. Mitochondria Ca^{2+} overload also promotes ROS generation, which could oxidize CaMKII (ox-CaMKII) and cause sustained activation of CaMKII. The ox-CaMKII could enhance MCU activity and further increase mitochondrial Ca^{2+} overload, promoting mPTP opening and impairing energy metabolism in heart failure. At the same time, myocardial energy deficiency could adversely affect $[Ca]^{2+}_i$ homeostasis.

because there is reduced open probability of Ca^{2+} conductance pathways in mitoplasts isolated from failing myocardium and decreased $\Delta\psi m$,[143] the electric driving force for mitochondrial Ca^{2+} uptake.[106] There is an emerging view that defective cytosolic Na^+ and Ca^{2+} homeostasis affects mitochondrial Ca^{2+} transport in heart failure. Mitochondrial Ca^{2+} efflux is mainly enabled by the mitochondrial Na^+/Ca^{2+} exchanger.[144] Elevated $[Na^+]_i$ stimulates mitochondrial Na^+/Ca^{2+} exchanger and mitochondrial Ca^{2+} efflux and reduces steady-state $[Ca]^{2+}_m$.[144] Thus, mitochondria are a critical interface between Ca^{2+} metabolism and are key determinants of myocardial survival in response to clinically relevant forms of pathological stress. A growing body of evidence suggests that mitochondria play a central role in heart failure.

Transverse Tubules

Transverse tubules are deep invaginations of the ventricular myocyte cell membrane (sarcolemma), where voltage-gated Ca^{2+} channels are richly expressed and tightly coupled with SR RyR2, forming dyads to enable Ca^{2+}-induced Ca^{2+} release. There is emerging evidence that normal transverse tubular ultrastructure is disrupted in heart failure.[145,146] Transverse tubules can become spatially dispersed, leaving RyRs "orphaned" from their dyadic association with $Ca_V1.2$,[147] which impairs Ca^{2+}-induced Ca^{2+} release. In addition, Ca^{2+} transients in these regions will depend on Ca^{2+} diffusion and propagated Ca^{2+} release, thus contributing to dysynchronous Ca^{2+} sparks, inefficient ECC, and a propensity toward arrhythmias. Recent studies suggest that junctophilin-2 may play a crucial role in maintenance of normal transverse tubular ultrastructure[145,148] and association of $Ca_V1.2$ with RyR2,[148,149] whereas targeted suppression of microRNA, which inhibits junctophilin, prevents disruption of T-tubule structure and transition to heart failure from hypertrophy.[150] β-AR antagonists[151] and sildenafil[152] can defend against transverse tubular disruption in animal models of heart failure. Thus, improved understanding of the interface between membrane and regulatory cytoskeletal proteins may lead to new therapeutic targets to preserve cellular architecture that is required for physiological Ca^{2+} homeostasis.

Myofilament and Cytoskeletal Proteins

Abnormal Ca^{2+} homeostasis and myofilament function impair cardiac contractile function and trigger ventricular arrhythmias in heart failure.[153] Ankyrins are adapter proteins that attach membrane proteins to the spectrin-actin–based membrane skeleton and thus are intimately involved in ion channel and transporter signaling complexes in the cardiovascular system.[154] Ankyrin dysfunction has been linked with abnormal ion channel and transporter membrane organization and human arrhythmias.[155,156] Genetic defects in ankyrins cause altered Na^+ and Ca^{2+} transport and enhanced RyR2 openings, contributing to loss of $[Ca]^{2+}_i$ homeostasis,[157] activation of CaMKII, and arrhythmias.[158] It was recently reported that ankyrin B plays a cardioprotective role against ischemia-induced cardiac dysfunction and ankyrin-B levels are decreased in human heart failure.[159]

Titin is a large myofilament protein that spans half of the sarcomere and functions as a molecular spring that provides passive stiffness to cardiac myocytes.[160] Titin isoform composition and phosphorylation regulate myocardial diastolic function.[160] Titin expression was reported to be increased in pressure-overload hypertrophy but was decreased in decompensated CHF,[161,162] suggesting that titin could contribute to the loss of compliance and decreased contractile function featured in heart failure. Titin knockout mice demonstrated reduced SR Ca^{2+} uptake accompanied by reduced levels of PLN and SERCA2a, and these mice had development of cardiac hypertrophy and heart failure.[163] CaMKII phosphorylates titin and modulates passive force generation in normal and failing myocardium.[164] Deranged CaMKII-dependent titin phosphorylation occurs in heart failure and contributes to altered diastolic stress.[164] These findings suggest that titin is a participant in Ca^{2+}-related defects in heart failure, and suggest that titin could emerge as a target for future heart failure therapies.

Dystrophin is a cytoplasmic protein and a crucial part of the dystroglycan complex, which consists of tightly associated transmembrane and cytoskeletal proteins that serve to connect the cytoskeleton to the extracellular matrix.[165] Mutation of the dystrophin gene and absence of dystrophin cause Duchnne muscular dystrophy (DMD), a fatal X-linked disease,[166] which results in a skeletal as well as a dilated cardiomyopathy. Cardiac involvement including heart failure accounts for 20-30% of the mortality in DMD patients.[167] An MDX mouse, which is a model of DMD and lacks the protein dystrophin, has decreased levels of SR luminal Ca^{2+}-binding proteins,[168] decreased SERCA2a expression,[169] and an increase in resting $[Ca]^{2+}_i$.[170] Patients with DMD are at increased risk for fatal cardiac arrhythmias.[167,171] MDX mice were shown to have "leaky" RyR2 because of S-nitrosylation of the channel and

FKBP 12.6 depletion.[172] Suppressing the RyR2-mediated diastolic SR Ca^{2+} leak by inhibiting FKBP 12.6 depletion prevented any fatal sudden cardiac arrhythmias in DMD mice, suggesting that leaky RyR2 triggers ventricular arrhythmia in DMD.[172] Recent studies show that CaMKII inhibition or interbreeding into a genetic background with a knock-in RyR2 S2814A mutation that is resistant to CaMKII prevents arrhythmogenic Ca^{2+} waves and ventricular tachycardia in MDX mice,[173] suggesting that CaMKII phosphorylation at S2814A of RyR2 contributes to the arrhythmia in MDX mice and possibly in DMD patients. Combined, these studies suggest that myofilament and cytoskeletal proteins are intimately involved in Ca^{2+} homeostasis and contribute to pathogenesis of heart failure and arrhythmias.

Alterations in Regulatory Mechanisms in Heart Failure

Ca^{2+} and Calmodulin-Dependent Protein Kinase II

CaMKII is a multifunctional serine-threonine protein kinase that is abundant in nerve and muscle. There are 4 different CaMKII encoding genes, with each encoding a distinct CaMKII isoform (α, β, γ, δ). CaMKIIδ appears to be the main isoform expressed in the heart, but CaMKIIγ is also present.[174] Whether these 2 main isoforms have selective roles in cardiac pathophysiology is unclear at this point, because there are few studies investigating the role of CaMKIIγ. Transaortic banding induced increased expression of both CaMKIIδ and CaMKIIγ isoforms[175] and conditional double-knockout of CaMKIIδ and CaMKIIγ caused decreased phosphorylation of target proteins.[164] A recent study suggests that CaMKIIγ is enriched in mitochondria.[176] CaMKII connects intracellular Ca^{2+} signaling to ECC and regulates both SR Ca^{2+} uptake and release (Figure 2). CaMKII acts on multiple Ca^{2+} homeostatic proteins involved in ECC,[32] including voltage-gated Ca^{2+} channels,[16] RyR2,[177] and PLN.[178] In general, CaMKII-mediated phosphorylation of Ca^{2+} homeostatic proteins enhances their activity and promotes performance of physiological events such as ECC and fight-or-flight mechanical and heart rate responses.

CaMKII consists of stacked hexamers and each monomer consists of an N-terminus catalytic domain and a C-terminus association domain that flank a core regulatory domain.[179] The "hypervariable" region located between the association and regulatory domains is likely responsible for tuning the Ca^{2+} sensitivity of CaMKII activation.[179] CaMKII is activated when $[Ca]^{2+}_i$ binds to calmodulin (CaM), causing conformational changes that release the catalytic domain from the negative regulation by the autoinhibitory region of the regulatory domain.[179]

Under diastolic, resting $[Ca^{+}]_i$ in the presence of low ROS, CaMKII is enzymatically inactive because of the binding of catalytic domain to an autoinhibitory region. Sustained activation of CaMKII by binding to calcified calmodulin (Ca^{2+}/CaM) leads to threonine 287 autophosphorylation (the numbering varies slightly between isoforms), CaM trapping, and CaMKII activation that is autonomous from Ca^{2+}/CaM (Figure 4).[180] Ca^{2+}/CaM autonomous (constitutively active) CaMKII is also generated by oxidation of paired regulatory domain methionines (281/282).[55] In this setting, oxidized CaMKII resets its Ca^{2+} sensitivity so that lower levels of intracellular Ca^{2+} are required for initial activation.[181] Thus, both threonine 287 autophosphoryation and methionine 281/282 oxidation can convert CaMKII into a constitutively active enzyme. The constitutively active forms of CaMKII appear to be particularly effective at driving myocardial disease phenotypes.[21,182–184] Thus, CaMKII is a highly regulated

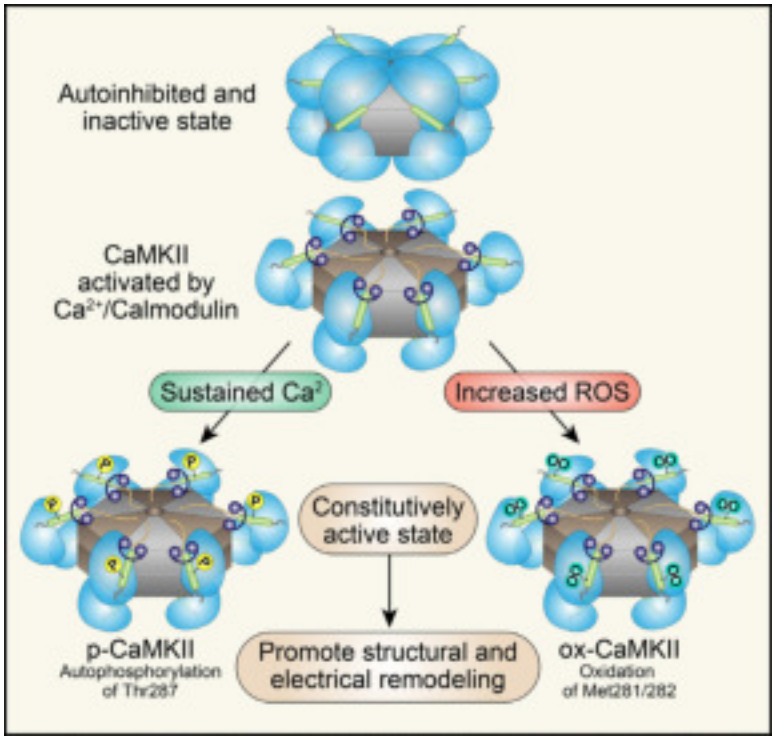

Figure 4. Structure and activation of Ca^{2+}-dependent and calmodulin-dependent protein kinase II (CaMKII). CaMKII consists of stacked hexamers and each monomer consists of an N-terminus catalytic domain and a C-terminus association domain that flank a core regulatory domain. CaMKII is activated when $[Ca]^{2+}_i$ binds to calmodulin, causing CaMKII to assume an active, extended conformation. Sustained binding to calcified calmodulin (Ca^{2+}/CaM) leads to threonine 287 autophosphorylation and sustained CaMKII activation. Oxidation of paired regulatory domain methionines (281/282) also causes sustained activation of CaMKII as oxidized CaMKII resets its Ca^{2+} sensitivity so that lower levels of intracellular Ca^{2+} are required for initial activation. Thus, both threonine 287 autophosphoryation and methionine 281/282 oxidation can convert CaMKII into a constitutively active enzyme to drive myocardial disease phenotypes.

signal, but under pathological stress CaMKII undergoes post-translational modifications that convert it into a Ca^{2+}/CaM-autonomous enzyme with the potential to promote heart failure and arrhythmias.

Chronic and excessive neurohormonal activation contributing to the progression of CHF cause increased $[Ca]^{2+}_i$ and ROS,[185,186] causing sustained activation of CaMKII. Increased myocardial CaMKII activity and expression have been found in various animal models[187,188] and in patients with heart failure.[189] Mice with myocardial transgenic CaMKII overexpression have development of heart failure and premature sudden death.[190] CaMKII activation by β-AR stimulation causes fetal gene induction, pathological hypertrophy,[54,191] myocardial apoptosis,[192] arrhythmia,[193] and worsening heart failure after MI.[55] Angiotensin II activates CaMKII by methionine oxidation and promotes cardiomyocyte death,[55,181] which contributes to sinus node dysfunction,[183] a frequent counterpart to heart failure. Aldosterone activates CaMKII by methionine oxidation and CaMKII activation by aldosterone leads to increased death after MI by increasing the propensity to myocardial rupture.[182] Intriguingly, excessive oxidized CaMKII activates a myocyte enhancer factor-2 transcriptional signaling pathway to increase myocardial expression of matrix metalloproteinase-9 that contributes to myocardial matrix instability and sudden death attributable to postmyocardial infarction cardiac rupture.[182]

We recently found that hyperglycemia also leads to increased methionine 281/282 oxidized CaMKII in diabetic patients and in mice, and increased oxidized CaMKII is a necessary signal for diabetes-associated excess mortality in a mouse model of MI.[184] We found that ROS was increased in cardiac myocytes exposed to hyperglycemia and that mitochondrial-targeted antioxidant therapy or a knock-in mutation of CaMKIIδ to prevent oxidative activation (M281/281V) were both effective at preventing excess diabetes-attributable mortality after MI.[184] Importantly, CaMKII inhibitors significantly improved the force–frequency relationship in failing human cardiomyocytes.[194] CaMKIIδ$^{-/-}$ knockout mice are resistant to myocardial hypertrophy and pressure overload–induced heart failure,[195,196] and mice with transgenic myocardial CaMKII inhibition are resistant to heart failure from MI.[54] Taken together, this evidence indicates that CaMKII plays an important role in connecting upstream signals, such as neurohumoral activation, hyperglycemia, ischemic injury and infarction with defective Ca^{2+} signaling, and downstream pathological outcomes important for CHF.

Protein Kinase A

PKA is the principal upstream kinase activated by β-AR agonists. There are multiple β-AR subtypes, including β_1-AR, β_2-AR, and β_3-AR.[197,198] β-ARs belong to the large family of G-protein-coupled receptors with 7 transmembrane domains[199] and contain phosphorylation sites[200] that serve as targets for protein kinases, including PKA and PKC.[201] The binding of circulating adrenergic amine agonists to β-ARs activates adenylate cyclase and simulates cAMP production to release the catalytically active subunit of PKA.

PKA, in turn, catalyzes phosphorylation of multiple Ca^{2+}-regulatory proteins, including PLN, L-type Ca^{2+} channels, and RYR2. Under physiological conditions, activation of the β-AR signaling pathway through PKA stimulates Ca^{2+} influx and increases SR Ca^{2+} uptake and storage by the SR, leading to increased systolic $[Ca]^{2+}_i$ transients and thus increased contractile function and lusitropy.[4] However, in the failing heart, chronically elevated adrenergic agonist activity leads to down-regulation of β_1-AR signaling with decreased β_1-AR density[202,203] and uncoupling of β_2-AR from downstream effector molecules, including Ca^{2+}-regulatory target proteins such as PLN,[204] leading to inefficient ECC and decreased contractile function. These changes impair the ability of the failing heart to increase contractility to meet hemodynamic demands.

Widely established benefits of β-AR antagonist drugs in treating heart failure[44] strongly support that altered β-AR signaling is maladaptive and promotes heart failure progression. However, the mechanisms of therapeutic benefit for β-AR antagonist drugs are likely to be diverse. β-AR antagonists preserve transverse tubular ultrastructure,[151] reverse RyR2 hyperphosphorylation,[44,204] and decrease SR Ca^{2+} leak,[44,205] leading to increased contractility in heart failure. In addition, β-AR agonist stimulation causes apoptosis via activation of a mitochondrial death pathway,[206] whereas β-AR antagonists such as carvedilol can protect mitochondria from oxidative stress–induced mPTP opening.[207,208]

PKA-dependent β-AR signaling desensitizes after sustained β_1-AR agonist stimulation.[209] In contrast, CaMKII signaling in ECC is persistent and may be necessary to sustain positive inotropic actions of prolonged catecholamine signaling.[210] Epac is a guanine nucleotide exchange protein that directly binds to and is activated by cAMP in parallel to the classical PKA signaling pathway. Epac was shown to mediate β-AR–induced cardiomyocyte hypertrophy[210,211] and arrhythmias,[212] to modulate cardiac nuclear Ca^{2+} signaling by increasing nuclear Ca^{2+} through phospholipase C, inositol trisphosphate, and CaMKII, and to activate the transcription factor MEF2.[213] A recent study demonstrated that Epac may mediate cardioprotection from cell death induced by β-AR activation.[214] Thus, β-AR stimulation activates multiple signaling pathways, including cAMP/PKA, cAMP/Epac, and the CaMKII pathway. In our view, it is not yet clear how much of the therapeutic benefit of β-AR antagonist drugs is attributable to reduced PKA activity or what portion is attributable to reduction in the activity of other downstream signals, such as CaMKII.

Protein Kinase C

PKC is a family of serine-threonine protein kinases that are present in a wide variety of tissues, including myocardium. PKCα is the most abundantly expressed isoform of the myocardial PKC family. Receptors for activated C kinase are isoform-selective anchoring proteins for PKCs.[215] Receptors for activated C kinase are important for determining the subcellular localization of PKC isoenzymes.[215] PKCα plays an important role in regulating myocardial contractility. For example, mice with PKCα deletion demonstrate an increase in $[Ca]^{2+}_i$ transients and contractility, whereas overexpression of PKCα diminishes contractility.[216] PKCα knockout mice are protected from pressure overload–induced heart failure and from dilated

cardiomyopathy induced by deleting the gene-encoding muscle LIM protein (*Csrp3*), and are protected from cardiomyopathy associated with overexpression of type 1 protein phosphatase.[216] One experimentally validated pathway for PKCα action to decrease $[Ca]^{2+}_i$ transients is that PKCα suppresses SERCA2a activity by phosphorylating inhibitor-1, resulting in increased type 1 protein phosphatase activity and dephosphorylation of PLN.[216] Decreased SERCA2a activity thus reduces SR Ca^{2+} load, leading to reduced Ca^{2+} release during systole, hence reducing contractility. Other PKC isoforms (δ) and (E) may play a significant role in promoting hypertrophy.[217,218] Taken together, these results from animal models support a potential role for PKC in promoting heart failure progression.

S100A1

S100A1 belongs to the S100 protein family, a group of EF-hand–containing Ca^{2+}-binding proteins. S100A1 shows highest expression in human cardiac muscle and is preferentially expressed in the left ventricle. S100A1 has a molecular weight of 10.4 kDa and contains 2 functional EF-hand Ca^{2+}-binding motifs. On Ca^{2+} binding, S100A1 undergoes a conformational change to expose a hydrophobic pocket for binding to target proteins.[219] The Ca^{2+} binding affinity of S100A1 is tightly regulated by posttranslational modifications, including S-nitrosylation and S-glutathionylation of a cysteine residue in the C-terminal region.[220–222] Either modification enhances Ca^{2+} affinity by several orders of magnitude, which augments the ability of S100A1 to sense Ca^{2+} oscillations over a wide dynamic range.[220–222] S100A1 has emerged as a key regulator of Ca^{2+} cycling and cardiac contractile function.[220,223] S100A1 enhances SR Ca^{2+} uptake and increases SR Ca^{2+} content.[109,223] S100A1 also directly regulates RyR2 function.[223,224] More recently, S100A1 was found to reside in mitochondria, where it stimulates ATP synthase (complex V) activity and promotes the adenosine nucleotide translocator function to increase ATP synthesis and mitochondrial ATP efflux in cardiomyocytes.[109,225]

S100A1 knockout mice had impaired contractility and showed enhanced proarrhythmogenic susceptibility to acute β-AR agonist stimulation and pressure overload induced by chronic transaortic constriction.[226,227] There was impaired SR Ca^{2+} uptake, increased SR Ca^{2+} leakage, and a reduced SR Ca^{2+} load in heart tissues from the S100A1 knockout mice.[228,229] The S100A1 knockout mice also demonstrated excessive mortality and accelerated CHF after MI, as well as increased post-MI cardiac remodeling.[228,229] In contrast, mice with myocardial S100A1 overexpression showed enhanced contractile responses to β-AR stimulation, improved $[Ca]^{2+}_i$ homeostasis, improved survival, and preserved left ventricular function after MI.[229] In human heart samples with dilated and ischemic cardiomyopathy, S100A1 mRNA and protein expression were found to be downregulated.[230,231] Decreased S100A1 expression levels also were shown in experimental HF animal models and correlated with the severity of heart failure and mortality.[229,232] These results suggest that S100A1 plays an important role in regulating Ca^{2+} cycling and contractile function, whereas loss of S100A1 may contribute to heart failure in the setting of pathological stress.

Calcineurin

Calcineurin, also known as protein phosphatase 2B, is a Ca^{2+}/CaM-activated serine-threonine phosphatase and the first Ca^{2+}-dependent signaling molecule explicitly linked to myocardial hypertrophy and heart failure.[233,234] Calcineurin signaling stimulates cardiac hypertrophy[235,236] and remodeling through activation of the nuclear factor of activated T-cell (NFAT) transcription factor. On calcineurin-mediated dephosphorylation, NFAT translocates to the nucleus and activates cardiac transcription.[237] The calcineurin–NFAT signaling pathway in myocardium appears to be activated only when there are pathological increases in $[Ca]^{2+}_I$, whereas it is not activated during physiological hypertrophy induced by exercise or pregnancy,[238] suggesting that calcineurin signaling is tightly coupled with pathological defects in Ca^{2+} homeostasis.

There is increased calcineurin activity or expression in animal models[235] and in patients with myocardial hypertrophy and heart failure.[232,239,240] Overexpression of calcineurin causes myocardial hypertrophy, heart failure, and premature death.[234,238] Calcineurin inhibition by cyclosporin prevented hypertrophy in mice genetically predisposed to development of hypertrophic cardiomyopathy and in a rat model of pressure overload–induced hypertrophy.[244] Calcineurin Aβ-knockout mice, with an 80% decrease in calcineurin enzymatic activity in the heart, show decreased hypertrophic responses induced by pressure overload or agonist infusion, including angiotensin II and isoproterenol.[241] Intriguingly, CaMKII expression and activity were increased in calcineurin transgenic mice.[193] CaMKII inhibition improved contractile function, reduced arrhythmias, and decreased mortality in mice with myocardial transgenic overexpression of a constitutively active form of calcineurin without substantially reducing calcineurin-evoked myocardial hypertrophy.[193,238] We interpret these findings to suggest that myocardial dysfunction and high mortality in calcineurin transgenic mice are, at least in part, attributable to downstream activation of CaMKII and independent of myocardial hypertrophy. The interactions between calcineurin and CaMKII are complex, as highlighted by the finding that CaMKII catalyzed phosphorylation of calcineurin prevents full activation of calcineurin by inhibiting Ca^{2+}/CaM binding. Thus, CaMKII may act as an antihypertrophic agent in the context of the calcineurin/NFAT pathway.[243] Overall, these findings support a view that calcineurin is an important regulator of cardiac hypertrophy and heart failure but leave open the question of which downstream events are critical for the cardiomyopathic actions of calcineurin.

Arrhythmias as a Common Cause of Death in Heart Failure

Heart failure, especially in patients with left ventricular ejection fractions less than 30%, is associated with a high rate of arrhythmia-induced sudden death.[244] Various factors appear to enhance the probability of arrhythmias, including defective $[Ca]^{2+}_i$ homeostasis. Many ion channels respond to loss of normal $[Ca]^{2+}_i$ homeostasis by contributing to cell membrane hyperexcitability. However, as exemplified by the Cardiac Arrhythmia Suppression Trial (CAST)[245] and Survival with Oral d-Sotalol (SWORD),[246] ion channel antagonist therapies

are not effective in preventing sudden death in patients at high risk. In contrast, neurohumoral antagonist drugs that serve as mainstay therapeutics for heart failure, such as β-AR,[247] angiotensin II,[248] and mineralocorticoid receptor antagonists,[249] are effective in reducing sudden death. These findings suggest that signals that modulate ionic currents are better therapeutic targets than ion channels.

Electric Remodeling

Proarrhythmic electric remodeling is a term used to describe multiple changes in ionic currents that collectively lead to action potential and QT interval prolongation and favor arrhythmias in failing ventricular myocardium. Prolongation of the action potential plateau, in particular, contributes to a proarrhythmic substrate for noninactivating components of $Na_V1.5$ current[30,250] and $Ca_V1.2$ channels in a high-activity gating mode.[16] A comprehensive review of electric remodeling in heart failure is beyond the scope of this review but has been published elsewhere.[251] Voltage-gated K currents (I_K) are the major driving force for myocardial membrane repolarization,[252] and failing myocardium is consistently reported to show reduced repolarizing I_K that contributes to proarrhythmic action potential and QT interval prolongation.[253] Interestingly, excessive CaMKII activity also contributes to reduced I_K in failing myocardium by phosphorylation of the pore-forming α-subunit of the voltage-dependent K^+ channel 4.3 at Ser^{550}, which encodes a class of rapidly inactivating I_K, including the transient outward current in the heart.[254]

Cardiac ATP-sensitive K^+ (K_{ATP}) channels are metabolic sensors activated in response to various forms of cardiac stress, including ischemia and neurohormonal activation, leading to membrane hyperpolarization, decreased action potential duration, and contractility.[255] Hence, K_{ATP} channels play an important role in improving cellular energy efficiency and stress resistance. Association of K_{ATP} with Ankyrin B via the C-terminus of Kir6.2, the pore-forming unit, was shown to be important for K_{ATP} channel trafficking and membrane metabolic regulation.[256] One recent study suggests that CaMKII couples the surface expression of cardiac K_{ATP} channels with Ca^{2+} signaling to regulate energy efficiency and stress resistance, because Ca^{2+}-dependent activation of CaMKII results in phosphorylation of Kir6.2, the pore-forming subunit, and promotes internalization of K_{ATP} channels.[257] CaMKII also affects trafficking of a variety of voltage-gated K^+ currents, with the net effect of reducing repolarizing K^+ current and prolonging the action potential.[258] These findings suggest that $[Ca]^{2+}_i$ may feed-back to control multiple ionic currents through activation of CaMKII and that excessive CaMKII activity in CHF contributes to the proarrhythmic substrate and the enhanced risk for sudden death in structural heart disease by altering ion channel function and membrane expression.

CaMKII and Arrhythmia

Heart failure is a condition of increased oxidant stress, loss of $[Ca]^{2+}_i$ homeostasis, and activation of CaMKII. CaMKII exerts proarrhythmic effects through actions at multiple protein targets that are key components of Ca^{2+} homeostasis, including CaV1.2,[16,259] NaV1.5,[31,250] and RyRs[57] (Figure 5). CaMKII increases phosphorylation of a CaV1.2 β-subunit (β_{2a}) at

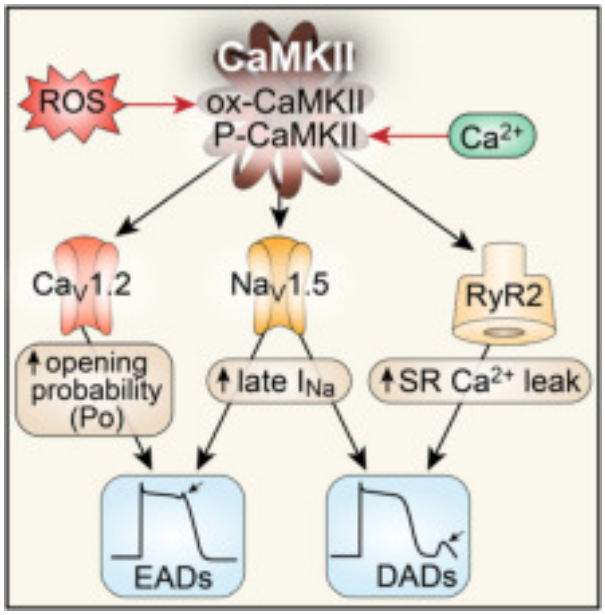

Figure 5. Ca^{2+}-dependent and calmodulin-dependent protein kinase II (CaMKII) and mechanisms of arrhythmia. Sustained activation of CaMKII by oxidative stress and elevated $[Ca]^{2+}_i$ contributes to arrhythmia in heart failure by several mechanisms. CaMKII phosphorylates L-type Ca channels ($Ca_V1.2$) to increase its open probability, causing early afterdepolarizations (EADs). Increased I_{Ca} also contributes to action potential prolongation, augmented $[Ca]^{2+}_i$, and delayed afterdepolarizations (DADs). CaMKII phosphorylates Na^+ channels ($Na_V1.5$) and enhances the long-lasting late I_{Na} (gain of function), promoting EADs and increasing subsarcolemmal $[Na^+]_i$ to favor DADs. CaMKII favors phosphorylation of ryanodine receptor (RyR2) to increase sarcoplasmic reticulum (SR) Ca^{2+} leak, which shifts Na^+/Ca^{2+} exchanger (NCX) to a forward mode, causing DADs. CaMKII contributes to arrhythmogenic structural features of injured myocardium by promoting myocyte death and collagen deposition.

Thr498,[259] leading to high-activity mode 2 gating, intracellular Ca^{2+} overload, and EADs.[16] Phosphorylation of RyR2 at Ser2814 by CaMKII increases diastolic SR Ca^{2+} leak,[57] which is proarrhythmic[260] by triggering DADs. CaMKII acts on Nav1.5, the predominant cardiac voltage-gated Na^+ channel, and increases I_{NaL},[30,31,250] which prolongs action potential and triggers early EADs.[31,250] CaMKII inhibition has been shown to prevent or suppress ventricular arrhythmias in myocardial tissues and animal models.[260,261] This evidence consistently suggests that CaMKII can promote arrhythmias and sudden death, and that CaMKII inhibition can reduce or prevent arrhythmias.

Reverse ECC

Diseased myocardium is nonuniform in ECC, with damaged and nondamaged regions as well as inhomogeneous border zone areas bridging damaged and healthy tissue. Arrhythmogenic contractile waves were observed in nonuniform failing myocardium.[262] A potential mechanism underlying this phenomenon is reverse ECC,[263] a process during which abnormal contractions of damaged regions cause regional increase of $[Ca]^{2+}_i$, leading to arrhythmogenic contractile waves. Aftercontractions appear to be initiated by the weak and damaged region during regular contractions and propagate into neighboring myocardium.[264] These contractile waves are likely attributable to mechanical

effects of damaged myocardium, such as stretching and release, and regional elevation of $[Ca]^{2+}_i$ as a result of damage.[265] When cardiac muscle is damaged, intracellular Ca^{2+} waves are initiated locally but propagate into adjacent tissues.[266] Diffusing Ca^{2+} ions activate neighboring SR, which in turn triggers further Ca^{2+} release from SR. These Ca^{2+} waves may give rise to premature contractions and trigger arrhythmias.[267] Purkinje fibers are particularly prone to proarrhythmic $[Ca]^{2+}_i$ waves and may serve as an arrhythmia focus for injured myocardium.[268] Another potential mechanism underling arrhythmogenic Ca^{2+} waves are the activation of stretch-activated channels, which are nonselective cation channels activated by mechanical stress.[269] In the MDX mouse, lack of dystrophin results in increased activity of stretch-activated channels and increased resting intracellular $[Ca]^{2+}_i$ in skeletal muscles.[270] Stretch-activated channels also have been reported in ventricular cardiomyocytes[271] and are proposed to play a role in tachycardia-induced chronic heart failure.[272] Thus, the role of Ca^{2+} in maladaptive contractions may be proarrhythmic.

Therapeutic Targets for Heart Failure

Current drug therapies for CHF are mainly designed to counteract overactivation of the sympathetic and renin angiotensin–aldosterone systems, which is known to prolong survival.[247–249] Advanced CHF associated with increased risk of fatal arrhythmias also can be managed by surgically implantable cardioverter defibrillator, cardiac resynchronization therapy, and mechanical ventricular assist devices. However, currently available pharmacological and device therapies are far from ideal because they fail to fully correct underlying molecular abnormalities involved in systolic and diastolic dysfunction as well as adverse structural and proarrhythmic electric remodeling. Given the central role of Ca^{2+} signaling in the progression of CHF, restoration of normal $[Ca]^{2+}_i$ homeostasis is a promising strategy to forestall progression and improve function of failing cardiomyocytes.

RyR2

CHF is a condition of leaky RyR2, decreased SR Ca^{2+} content, and reduced $[Ca]^{2+}_i$ transients. Leaky RyR2 can contribute to myocardial dysfunction and arrhythmias.[58,238] Overexpression of the RyR2 regulatory protein FKBP12.6 caused increased SR Ca^{2+} content and improved myocyte shortening in isolated cardiomyocytes.[238] RyR2 leak also can potentially be directly targeted by pharmacological agents shown to improve cardiac function[238] and prevent arrhythmias.[275] For example, K201, a benzothiazepine derivative and inhibitor of RyR2 was shown to stabilize RyR2s and decrease SR Ca^{2+} leak.[274] So-called Rycals, K201-congeners, have emerged as promising agents for targeting RyR2 and reducing arrhythmias and heart failure.[36] Another Rycal compound, ARM036, also a benzothiazepine derivative, is in phase II trials for heart failure and catecholaminergic polymorphic ventricular tachycardia. It is anticipated that information regarding the potential clinical benefits of pharmacological therapy aiming to modulate RyR2 function will soon become available.

Ca^{2+}-Dependent and Calmodulin-Dependent Protein Kinase II

CaMKII links Ca^{2+} homeostasis and cardiac function in myocardium under physiological conditions. Under pathological conditions such as heart failure characterized by excessive neurohormonal activation and oxidative stress, CaMKII activation is sustained, which promotes diastolic Ca^{2+} leak and arrhythmias. Animal studies consistently demonstrate that CaMKII inhibition reduces heart failure and arrhythmias, reducing or preventing sudden death. In our view, CaMKII is a highly validated target that connects to most or all aspects of defective $[Ca]^{2+}_i$ homeostasis in heart failure. However, to determine whether the experimentally observed benefits of CaMKII inhibition are applicable to human heart failure, CaMKII inhibitory drugs with drug-like properties and adequate specificity and safety will need to be developed.

Protein Kinase C

PKCα has been identified to have critical roles in the pathogenesis of heart failure. Deletion of the PKCα gene[216,275] or inhibition with drugs[133,276,277] have shown dramatic protective effects against the development of heart failure of various etiologies, including ischemia, pressure overload, or dilated cardiomyopathy induced by deleting LIM protein in animal models. However, clinical trials with PKC inhibitors or receptors for activated C kinase inhibitor peptides were largely disappointing for improving heart failure[278] or reducing myocardial injury in MI patients.[279,280] Transfer of genes encoding S100A1 and SERCA2a are discussed elsewhere in this compendium.

Conclusion

It is now clear that impaired $[Ca]^{2+}_i$ homeostasis is a key feature of heart failure that contributes to contractile dysfunction and arrhythmias. Defective Ca^{2+} homeostasis in heart failure is most often the result of altered expression and function of a group of $[Ca]^{2+}_i$-handling and structural proteins, ion channels, and enzymes. Numerous laboratories have contributed to the improved understanding of these pathways and this new knowledge has bolstered the quest to develop novel and improved therapeutics. We expect that the next several years will witness the initial results of several promising heart failure therapies designed to correct defects in myocardial $[Ca]^{2+}_i$ homeostasis.

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M.E. Anderson is a named inventor on intellectual property claiming to treat myocardial infarction by CaMKII inhibition and is a cofounder of Allosteros Therapeutics, a biotech company aiming to develop enzyme-based therapies.

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