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REVIEW

Myocardial calcium signaling in physiology and disease

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Abstract: It is now well-established that calcium (Ca^{2+}) is a critical regulator of myocardial function and that abnormalities in cardiomyocyte intracellular Ca^{2+} dynamics contribute to pathophysiologic changes observed in several cardiac diseases, including cardiac hypertrophy, chronic heart failure, and ventricular tachyarrhythmias. Although Ca^{2+} plays a key role in maintaining cardiac excitation–contraction coupling, it is increasingly apparent that changes in myocardial Ca^{2+} also contribute to the regulation of normal and pathological signal transduction that controls myocyte growth, hypertrophic signaling, mitochondrial energetics, and transcriptional gene expression. Interestingly, experimental evidence suggests that these multifarious Ca^{2+}-dependent responses are spatially and temporally mediated by distinct cellular Ca^{2+} pools (ie, microdomains), which are generated by diverse channels and molecular signals with widely differing timescales of activation. These concepts are discussed in this review, as well as the emerging role of microRNAs in cardiac remodeling and myocardial Ca^{2+} dynamics.

Keywords: calcium signaling, calcium microdomains, hypertrophy, heart failure, arrhythmia, microRNAs

Introduction

Calcium (Ca^{2+}) and Ca^{2+}-dependent signaling are vital for the proper functioning of a healthy heart. Abnormalities in the regulation of Ca^{2+} homeostasis are associated with myocardial pathologies such as hypertrophy, heart failure, and ventricular arrhythmias.[1–3] Intracellular Ca^{2+} concentrations are therefore tightly regulated by a number of Ca^{2+}-handling enzymes, channels, and transporters located in the plasma membrane and in Ca^{2+} storage organelles, which work in concert to fine-tune a temporally and spatially precise Ca^{2+} signal during each cardiomyocyte and relaxation cycle. In addition to the well-defined Ca^{2+} transporters, new regulators have recently been identified and demonstrated to be essential for the homeostatic control of Ca^{2+} levels. Physiological maintenance of a proper cardiomyocyte Ca^{2+} signaling system not only is vital for cardiac cell contraction but also is crucial for controlling long-term myocyte responses such as metabolism, transcription, and growth. Emerging studies reveal that these Ca^{2+}-dependent responses are temporally mediated by distinct Ca^{2+} pools (ie, microdomains), which are generated by diverse channels with widely differing timescales of activation.[4–6] In this review, we discuss how myocardial Ca^{2+} signaling is essential for proper and coordinated cardiomyocyte function, as aberrant Ca^{2+}-dependent responses are linked to debilitating human diseases such as pathological cardiac hypertrophy and heart failure. We also highlight the emerging role of a number

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of microRNAs and proteins, both old and new, in the dynamic control of the myocardial Ca^{2+} signaling system.

Myocardial Ca^{2+} cycling in physiology

Ca^{2+}-induced Ca^{2+} release

Excitation–contraction (EC) coupling is a well-described, fundamental principle by which a myocyte's ionic (excitation) properties tightly coordinate its mechanical (contraction) function. In the mammalian heart, a number of ion channels and transporters ensure that calcium release and uptake in myocytes is carefully regulated during the process of contraction, as excessive quantities of calcium can lead to deleterious pathological consequences.[7] The sarcoplasmic reticulum (SR) plays an important role in orchestrating the movement of calcium during each contraction and relaxation.

Excitation leads to the opening of voltage-gated L-type Ca^{2+} channels, allowing the entry of a small amount of Ca^{2+} into the cell.[7] Through a coupling mechanism between the L-type Ca^{2+} channel (LTCC) and the SR Ca^{2+} release channel (ryanodine receptor 2 [RyR2]), a larger amount of Ca^{2+} is released through a process termed Ca^{2+}-induced Ca^{2+} release (CICR), activating the myofilaments and leading to contraction.[7] This process increases intracellular Ca^{2+} concentration from approximately 10 nM during diastole to about 1 μM during systole. The relatively increased Ca^{2+} concentration in the t-tubular SR junctions inactivates the LTCC by a Ca^{2+}-dependent inactivation and terminates there by Ca^{2+} influx to avoid Ca^{2+} overload and arrhythmias.[7,8] During relaxation, Ca^{2+} is reaccumulated back into the SR by the SR Ca^{2+} adenosine triphosphatase (ATPase) pump (SERCA2a) and extruded extracellularly primarily by the sarcolemmal Na^{+}/Ca^{2+} exchanger-1 (NCX1). The plasma membrane Ca^{2+}-ATPase pump and the mitochondrial uniporter may also contribute to this process, albeit minimally.[7] The contribution of each of these mechanisms for lowering cytosolic Ca^{2+} varies by species and is mostly determined by the abundance and activity of SERCA2a and sarcolemmal NCX1, by intracellular concentrations of Na^{+} and Ca^{2+}, and by the membrane potential.[7,9] In humans, ~75% of the Ca^{2+} is removed by SERCA2a and ~25% by the NCX1.[10,11] The Ca^{2+} pumping activity of SERCA2a is influenced by phospholamban (PLN)[12] and sarcolipin.[13] In the unphosphorylated state, PLN inhibits SERCA2a activity, whereas phosphorylation of phospholamban by cyclic adenosine monophosphate (cAMP)-dependent protein kinase and by Ca^{2+}-calmodulin-dependent protein kinase (CaMKII) reverses this inhibition.[12] CaMKII can also directly phosphorylate SERCA2a and enhances its activity.[14,15] Sarcolipin, in contrast, inhibits SERCA2a through direct binding and through stabilization of SERCA2a-PLN interaction in the absence of PLN phosphorylation and through the inhibition of PLN phosphorylation.[13] Disease-induced malfunction of several of these key EC coupling proteins and the subsequent alterations in intracellular Ca^{2+} homeostasis results in mechanical and electrical dysfunction at the molecular and the cellular levels and in the entire organ.

Although Ca^{2+} regulation of EC coupling is fundamental for cardiac physiology, Ca^{2+} is also essential for the regulation of an array of Ca^{2+}-dependent regulatory processes associated with cardiac remodeling. There is emerging evidence that the induction of this Ca^{2+}-dependent remodeling is not affected by the rapid elevation in the contractile Ca^{2+} stimulus but is mediated by a more sustained Ca^{2+} signal. Several reports have now described the existence of spatially restricted and distinct Ca^{2+} pools in cardiomyocytes distinctly contractility and Ca^{2+}-dependent signaling pathways. How the cardiac myocyte distinguishes between contractile Ca^{2+} and signaling Ca^{2+} has been a matter of debate, and few hypotheses have been put forward to explain this conundrum.[4,6,16] However, a picture is now emerging that emphasizes how spatially restricted Ca^{2+} signals in local Ca^{2+} nanodomains, microdomains, and macrodomains can selectively recruit certain transcription factors and not others, or can activate certain metabolic molecules and not others, through activation of diverse Ca^{2+}-permeable ion channels with widely differing timescales of activation. A classic example of the concept of Ca^{2+} microdomains is represented by the maintenance of a very close approximation of LTCC and RyR2 in the dyads between t-tubular sarcolemma and the junctional SR that promotes contraction.[17,18] A similar microdomain also exists between mitochondria and the SR that facilitates an efficient and rapid mitochondrial Ca^{2+} uptake to meet the bioenergetics demand of the beating myocyte.[19,20] Other examples of Ca^{2+} microdomains involve local Ca^{2+} pools provided and regulated by (1) LTCC,[21,22] T-type calcium channel,[23] and plasma membrane Ca^{2+}-ATPase isoform 4[5,24–26] in nonjunctional sarcolemmal caveolae; (2) transient receptor potential channel proteins (TRPCs);[27,28] and (3) inositol 1,4,5-triphosphate receptor (IP_3R) localized to the nuclear membrane,[29,30] which are all believed to be implicated in mediating diverse Ca^{2+} responsive signal transduction in hypertrophy and heart failure.

Ca^{2+}-induced Ca^{2+} entry

Although calcium signaling is a combination of a highly organized Ca^{2+} release from intracellular Ca^{2+} stores, SR,

and Ca^{2+} entry across the plasma membrane, extracellular Ca^{2+} influx is necessary to maintain cytosolic Ca^{2+} levels. In nonexcitable cells, the mechanism that controls Ca^{2+} entry is triggered by intracellular Ca^{2+} store depletion, which activates plasma membrane Ca^{2+} channels and elicits a prolonged increase in intracellular Ca^{2+} in a process now termed store-operated Ca^{2+} entry (SOCE). This mechanism of Ca^{2+} influx plays a critical role in replenishing Ca^{2+} stores and providing sustained and precise local Ca^{2+} signals that are critical in determining a spectrum of downstream cellular responses. Stromal interacting molecule (STIM1), a recently identified single-span endoplasmic reticulum (ER) membrane molecule, has a unique and essential role in mediating Ca^{2+} entry through SOCE.[31,32]

STIM1 contains four low-Ca^{2+}-affinity luminal EF hands that are sensitive to ER Ca^{2+} concentration.[33] When ER Ca^{2+} is high, Ca^{2+} binds to STIM1 EF hands and inactivates it. However, when ER Ca^{2+} is decreased, Ca^{2+} dissociates from the EF hands and causes conformational changes that result in the activation of STIM1. Thus, STIM1 functions as a sensor of Ca^{2+} levels in the lumen of the ER and activator of the SOCE channel in the plasma membrane.[34,35] STIM1, through its Ca^{2+}-binding EF hands, can detect small changes in ER Ca^{2+} levels and responds by rapidly oligomerizing and translocating into specialized regions close to the ER-plasma membrane junctions, where it interacts with and activates plasma membrane Orai channels.[36–38] Interaction of STIM1 with Orai Ca^{2+} channels then triggers entry of Ca^{2+} and replenishment of intracellular Ca^{2+} homeostasis and activation of Ca^{2+}-dependent signaling pathways.[39,40] As Ca^{2+} levels increase in the ER lumen or at ER-plasma membrane junctions (microdomains), STIM1-Orai coupling is turned off and STIM1-induced Orai channel activation is terminated, leading to STIM1 protein de-oligomerization.[41–44]

Whether cardiomyocytes possess a similar functional SOCE mechanism is not clear, as Ca^{2+} entry through SOCE requires depletion of SR/ER Ca^{2+} store content, a condition that is hardly achieved in vivo in physiological conditions. However, SOCE has emerged as a potential mechanism regulating Ca^{2+} transients in cardiomyocytes, and a number of recent studies have provided evidence that SOCE is, for instance, operational in neonatal cardiomyocytes.[45–48] Hulot et al have reported that STIM1 is abundant in neonatal cardiomyocytes but marginally expressed in adult cardiomyocytes.[48] Interestingly, these studies also reported that STIM1 manipulation did not have any influence on cardiac EC coupling. These observations were further confirmed by Luo et al, who have also demonstrated that SOCE is nearly absent in adult cardiomyocytes but is highly active in neonatal myocytes or adult cardiomyocytes under pathological cardiac remodeling.[49] This latter study also demonstrated that STIM1 expression levels correlate with the magnitude of SOCE activation and the subsequent increase in Ca^{2+} entry.[49]

Although the SOCE process has been initially reported to mainly result from the coupling of STIM1 with Orai Ca^{2+} channels,[47,50,51] STIM1 is now known to interact with and activate several TRPCs (transient receptor potential channels), which have long been linked to SOCE.[52,53] Recent evidence suggests, for example, that TRPC1, TRPC3, and TRPC6 are implicated in cardiac hypertrophic responses.[54–58] However, even though STIM1appears to function as a master regulator of SOCE protein complexes, many questions pertaining to its role in cardiomyocytes invite future answers. For instance, what are the coupling partners of STIM1 in the heart? Do Orai and TRP channels act synergistically, or redundantly, and do they have different timescales of activation? Do they activate a common Ca^{2+} pool, and/or do they elicit different Ca^{2+}-dependent signaling pathways? What is the role of STIM2, Orai1, Orai2 and Orai3, and other TRP channels in the heart?

Emerging results from a number of studies show that adult cardiomyocytes may not need SOCE per se to regulate SR Ca^{2+} loading or contractile Ca^{2+} pools, but this process has a functional significance in fetal and neonatal cardiomyocytes and during cardiac growth and remodeling.[7] Moreover, studies have reported that STIM1 interacts with and controls the function of Cav1.2 voltage-operated Ca^{2+} channel (LTCC) in smooth muscle and neuron.[59,60] STIM1 interacts with the C-terminal end of the LTCC, inhibiting its function and decreasing its surface expression, thus leading to its inactivation.[60] Interestingly, this action of STIM1 on LTCC is thought to favor Ca^{2+} entry via the Orai channel.[60] Given the importance of LTCC in Ca^{2+} signaling in cardiac hypertrophy, it is not known whether STIM1 has a similar effect on LTCC in cardiomyocytes, an observation that remains to be determined.

Ca^{2+} signaling in hypertrophy and heart failure

Intracellular Ca^{2+} handling in hypertrophy and heart failure

The remarkable low cytosolic Ca^{2+} gradient is tightly maintained, as noted earlier, by the concerted activity of several highly conserved families of Ca^{2+} handling pumps, channels, and transporters that regulate a balance between Ca^{2+} entry, extrusion, and storage.[61] Although initial modifications of

Ca^{2+} handling are beneficial, several lines of evidence now suggest that Ca^{2+} dysregulation contributes to pathological hypertrophy and heart failure.

Heart failure is characterized by a number of abnormalities at the cellular level in the various steps of EC coupling. The major abnormality in Ca^{2+} cycling that occurs in heart failure is the observation that SR Ca^{2+} stores are significantly reduced. When these stores are depleted, SR Ca^{2+} release is curtailed in terms of both its amplitude and duration, and as a result, reduced contraction force is generated. The likely cause of this deficiency (depressed SR load) is the differential changes in gene expression and activity of key Ca^{2+} regulatory proteins. Ca^{2+} transients recorded from failing human myocardial cells or trabeculae reveal a significantly prolonged Ca^{2+} transient with an elevated end-diastolic intracellular Ca^{2+}. A decrease in SERCA2a activity and Ca^{2+} uptake have been shown to be responsible for abnormal Ca^{2+} homeostasis in both experimental and human failing hearts.[62,63] Associated with a defective Ca^{2+} uptake, there is a decrease in the relative ratio of SERCA2a/PLN in these failing hearts. With a decrease in SERCA2a expression and an increase in PLN expression, the SERCA2a/PLN ratio is significantly decreased, leading to a slower relaxation.

Using transgenic, and gene transfer approaches in isolated rat cardiac myocytes and failing human myocytes, increasing levels of PLN relative to SERCA2a significantly altered intracellular Ca^{2+} handling by prolonging the relaxation phase of the Ca^{2+} transient, decreasing Ca^{2+} release, and increasing resting calcium.[10–12,61] Overexpression of SERCA2a for instance in neonatal rat cardiomyocytes largely "rescued" the phenotype created by decreasing the SERCA2a/PLN ratio.[64] More important, in human cardiomyocytes isolated from the left ventricle of patients with end-stage heart failure, gene transfer of SERCA2a resulted in an increase in both protein expression and pump activity and induced a faster contraction velocity and enhanced relaxation velocity, thereby restoring these parameters to levels observed in nonfailing hearts.[65] In an animal model of pressure-overload hypertrophy in transition to failure, in which SERCA2a protein levels and activity are decreased and severe contractile dysfunction is present, overexpression of SERCA2a by gene transfer in vivo restored both systolic and diastolic function to normal levels.[62,63] These studies provide strong evidence that overexpression of SERCA2a to rescue disturbed Ca^{2+} cycling and myocardial function of the failing heart is indeed possible, validating the feasibility of cardiac gene transfer in patients with heart failure.[66,67] The recent successful and safe completion of a Phase II trial targeting the cardiac SERCA2a has the potential to open a new era for gene therapy for heart failure.[67–69] In contrast to SERCA2a, most studies from hypertrophied and failing hearts have shown an increase in both NCX1 messenger (m)RNA and protein,[70,71] suggesting that enhanced NCX1 function compensates for defective SR removal of Ca^{2+} from the cytoplasm in the failing heart, but at the cost of further depleting the SR-releasable pool of Ca^{2+} and further increasing the probability of arrhythmogenicity. However, the exact role of NCX1 in disease and whether it participates as a compensatory or maladaptive mechanism remains controversial.

It was demonstrated that RyR2 phosphorylation is highly increased in heart failure and that this "hyperphosphorylation" increases the RyR2 gating activity and causes a persistent leak of Ca^{2+} from the SR, further impairing the SR Ca^{2+} load.[72] Usually, the RyR is closed during diastole to allow for SERCA2a Ca^{2+} uptake. Ca^{2+} release through RyR2 is regulated in part by the interaction of RyR2 with calstabin2 (also known as FKBP12.6). Protein kinase A (PKA)-induced hyperphosphorylation of RyR2 in failing hearts causes calstabin2 to dissociate from the RyR2 channel complex and destabilize the closed state of the channel, resulting in increased spontaneous diastolic RyR2 activity, elevated diastolic SR Ca^{2+} leak, reduced SR Ca^{2+} load, and decreased Ca^{2+} transients.[1,73,74] Other suggested mechanisms for increased RyR2 open probability include oxidative modification of the RyR and increased RyR phosphorylation by CaMKII.[75] Furthermore, the gating of RyR2 is also controlled by a score of other Ca^{2+} regulatory proteins including triadin/junction/calsequestrin complex, sorcin, calmodulin, and the protein phosphatases PP1 and PP2A.[76,77] Diastolic leak of SR Ca^{2+} has been proposed as the mechanism responsible for delayed after-polarization, ventricular arrhythmia trigger, and sudden death in heart failure.[78] Although substantial data have accumulated in support of the RyR hyperphosphorylation hypothesis, many reports have questioned it and, using a similar canine model of heart failure (in addition to human tissue), have found that the Ca^{2+} sensitivity of the RyR opening was unaffected.[78,79] Furthermore, the association of calstabin2 with RyR2 has been reported to be insensitive to the degree of PKA phosphorylation.[80] Although impaired RyR function is likely to be involved in the abnormal Ca^{2+} handling commonly observed in heart failure, the exact alterations in RyR function in heart failure have yet to be defined.

Another mechanism for aberrant Ca^{2+} signaling in heart failure is the function of inositol 1,4,5-triphosphate gated Ca^{2+} release channels (IP_3R) located in the SR and nuclear envelope. Although the physiological role of IP_3R

in cardiomyocytes has been a matter of debate, recent evidence supports a prominent role[81] in EC coupling and SR Ca^{2+} release: their location near the RyR2 within the dyadic functional microdomains suggests that they can trigger CICR and enhance EC coupling.[82–84] The expression of IP_3R, which is typically many-fold less abundant than RyR in normal cardiomyocytes,[29,84] increases significantly in both human and animal models of hypertrophy and heart failure, particularly their expression in the dyadic junction, suggesting that these channels may be associated with pathological signaling.[85,86] Studies in neonatal rat ventricular myocytes suggest that activation of IP_3R may be linked to α1 adrenergic receptor (α1AR)-induced Ca^{2+} spark rate and global Ca^{2+} oscillations, as well as catecholamine-induced cardiomyocyte hypertrophic growth.[87] Heart-specific genetic manipulations of IP_3R signaling demonstrate that IP_3R expression enhances cardiac hypertrophy remodeling in vivo in response to agonist stimulation.[30] Increased IP_3-induced Ca^{2+} release in the perinuclear region can directly activate Ca^{2+}-dependent transcription factors that permit expression of hypertrophic genes.[83,85] However, enhanced SR Ca^{2+} release through IP_3R has the potential to trigger arrhythmogenic events.[85]

New players in cardiomyocyte Ca^{2+} dynamics and cardiomyopathy

New findings have illustrated the regulatory roles of novel candidates in myocyte Ca^{2+} cycling and cardiac pathophysiology. Only a few examples of relevance to the topic of this review are addressed here.

TRPC

Very recently, another family of intracellular Ca^{2+} permeable ion channels, unrelated to RyR and IP_3R, has been implicated in myocardial Ca^{2+} signaling and cardiac physiology and pathology: the TRPCs. At least five subclasses of TRPC are highly expressed in the heart.[27,88] TRPCs have been mapped to different structures in the cell, such as the plasma membrane, or assembled with caveolar complexes,[89,90] the Golgi apparatus,[91] and the SR/ER.[92] Although the TRPC channels do not appear necessary for the regulation of SR Ca^{2+} cycling and EC coupling, they clearly contribute to the dynamics of local Ca^{2+} pools and the stimulation of Ca^{2+}-dependent signaling pathways associated with cardiac hypertrophy and heart failure. Interestingly, the activity or expression of TRPC channels is upregulated in animal models of pathological hypertrophy and heart failure and in human heart failure samples[28,56,57,93] or in cultured neonatal cardiomyocytes after hypertrophic agonist stimulation.[55,94] Experimental cardiac genetic manipulations in vivo have also suggested prohypertrophic effects of TRPC channels. For instance, TRPC3 and TRPC6 transgenic overexpression in mice both impaired cardiac function and induced cardiomyopathy, most likely through activation of the calcineurin-nuclear factor of activated T-cells (NFAT) module.[54,57] Conversely, genetic deletion of TRPC1, selective inhibition of TRPC3, or inhibition of TRPC6 effectively blocked cardiac hypertrophy and attenuated calcineurin-NFAT activation in mice subject to transverse aortic constriction (TAC) surgery.[56,93,95,96] Similarly, silencing TRPC3 and TRPC6 in vitro in cultured neonatal cardiomyocytes abrogated angiotensin II–dependent hypertrophic response and calcineurin-NFAT signaling,[58] whereas activation of TRPC3 or TRPC7 promoted cardiomyocyte apoptosis that could contribute to myocardial injury.[28,97,98] Thus, TRPC channels are emerging as prohypertrophic effectors and key components in Ca^{2+} signaling pathways in maladaptive programming of cardiac growth.

CIB1

Ca^{2+} and integrin-binding protein 1 (CIB1, also called calmyrin) is a sarcolemmal protein that has been identified as a regulator of calcineurin-NFAT signaling and cardiac hypertrophy in the heart.[99] CIB1 interacts with the calcineurin regulatory subunit (calcineurin B) and regulates its activity. Calcineurin has been previously reported to interact with LTCC and activates its Ca^{2+} gating properties.[22] Given its location on the plasma membrane, CIB1 is believed to interact with LTCC and recruits calcineurin to the channel for activation. Genetic manipulations have demonstrated that deletion of CIB1 led to significant reduction in calcineurin-NFAT activation and prevented calcineurin translocation to the membrane, whereas overexpression of CIB1 produced the opposite effect.[99] This study established CIB1 as a new regulator of pathological cardiac hypertrophy and calcineurin-NFAT signaling in the heart.

Epac

Epac (exchange protein directly activated by cAMP), a cAMP target, has emerged as a new regulator of cardiac dysfunction. Two Epac isoforms have been identified: Epac1 (with one cAMP-binding domain) and Epac2 (with two cAMP-binding sites).[100] Recent evidence shows Epac is involved in the regulation of myocardial Ca^{2+} handling and cardiac hypertrophy.[101–103] Pharmacological activation of Epac1 with the cAMP analog 8-(4-chlorophenylthio)-2′-O-methyladenosine-3′,5′-cyclic mono-phosphate was shown to enhance SR Ca^{2+} release via phospholipase Cε[102,104] and

CaMKII,[103,104] suggesting that Epac1-induced SR Ca^{2+} leak can also reduce SR Ca^{2+} load and release.[105] These effects are similar to those evoked by β-adrenergic receptor (β-AR) activation. Indeed, β-AR stimulation activated Epac1 and altered SR Ca^{2+} leak by increasing CaMKII-dependent phosphorylation of RyR2.[103,104,106]

Previous studies have demonstrated that chronic activation of β-AR, as it occurs in heart failure conditions, can provoke cardiac arrhythmias as a result of abnormal diastolic SR Ca^{2+} leaks via phosphorylation of RyR2,[107,108] raising the possibility that the Epac1-induced SR Ca^{2+} leak pathway also may contribute significantly to β-AR-induced arrhythmias. Indeed, pharmacological activation of Epac1 in whole hearts increased diastolic SR Ca^{2+} leak and ventricular arrhythmogenesis.[106,109] Although there is a general agreement on the role of Epac in myocyte Ca^{2+} mishandling, its role in pathological remodeling is less certain. Epac is upregulated in experimental animal models of hypertrophy[110] and in late-stage failing human heart samples.[111] Furthermore, Epac1 overexpression induced hypertrophic response in cultured neonatal[101] and adult cardiomyocytes,[110] whereas Epac1 silencing protected against β-AR–induced hypertrophy.[110] However, recent work in Epac knockout mice has shown that Epac has no effect on cardiac function, baseline myocyte Ca^{2+} signaling, or early-stage pressure overload–induced hypertrophy,[109] but plays a more substantial role in pathological conditions such as arrhythmia and heart failure.[106,109] Furthermore, this study also demonstrated that Epac2 isoform, and not Epac1, mediates SR Ca^{2+} leak via β_1-AR, causing CaMKII-dependent RyR2 phosphorylation and SR Ca^{2+} leak and arrhythmia.[109] Epac1 is, however, believed to mediate a Ca^{2+} pool that is involved in transcriptional signaling.[112] Indeed, Epac1 can effectively activate the hypertrophic transcriptional signaling such as calcineurin-NFAT and CaMKII-histone deacetylase pathways, possibly via an IP_3-IP_3R modulation.[105, 113]

Electrical remodeling and ionic imbalance in hypertrophy and heart failure

Although changes in many ion channel currents have been reported in cardiac hypertrophy, reduction in the density of the transient outward K^+ current (I_{to}) is the most prominent ionic current change resulting in action potential duration (APD) prolongation in a process referred to as electrical remodeling.[114–116] Prolongation of the APD is consistently observed in experimental models of cardiac hypertrophy and failure. APD prolongation and downregulation of I_{to1} are also observed in compensated hypertrophy[117] and in end-stage human heart failure.[118] In the mammalian heart, I_{to1} is encoded by Kv4.2, Kv4.3, or a combination of the two.[119] Associated with the reduction in I_{to1} density, downregulation of Kv4.2 and/or Kv4.3 mRNA and protein expression levels has been observed in cardiac tissue derived from diseased hearts.[115,120,121] APD has been shown to strongly influence Ca^{2+} transient amplitude in normal[122] and hypertrophied[120] myocytes, which may help support contraction of the compromised myocardium but may also harm the myocardium by increasing the propensity to develop arrhythmias[119] and by activating hypertrophic signaling pathways.

The relationships between reductions of I_{to1} and concomitant APD prolongation and cardiac hypertrophy and heart failure have been explored using a number of techniques. Cardiac-specific ablation of I_{to1} by overexpression of a Kv4.2 channel with a single missense mutation (W362F) did not induce cardiac hypertrophy.[123] However, cardiac specific overexpression of dominant-negative Kv4.2 channel (truncated channel: Kv4.2 N) resulted in hypertrophy and cardiomyopathy along with a prolonged APD.[124] APD prolongation is associated with an enhanced propensity to develop cardiac arrhythmias,[125,126] which may contribute to the high incidence of sudden death observed in patients with heart failure.[119,127] The mechanism by which APD prolongation contributes to arrhythmogenesis might be related to the intracellular Ca^{2+} overload it generates. APD prolongation can elevate $[Ca^{2+}]_i$, and several groups have demonstrated that modulation of APD is an important determinant of calcium influx through LTCC.[128,129] We have shown that cardiac gene transfer of Kv4.3-based I_{to} can increase I_{to} density, shorten APD, decrease Ca^{2+} influx, and attenuate cardiac hypertrophy in vitro[130] and in vivo.[131] Interestingly, restoration of the otherwise downregulated expression of the potassium channel interacting protein 2 (KChIP2, a K_V4 subunit) in hypertrophied hearts, evoked similar effects.[132] Therefore, a reduction in I_{to} density and APD prolongation represent early electrical remodeling events in the diseased myocardium, pointing toward a potential role in disease initiation and progression.

Ca^{2+} signaling in cardiac arrhythmia

Pathogenesis and the role of calcium overload during cardiac arrhythmia

Ventricular arrhythmias are a major cause of death in patients with heart failure. The role of abnormal cytoplasmic Ca^{2+} regulation is thought to be a critical and perhaps common mechanism underlying cardiac dysfunction and the genesis of ventricular arrhythmias.[133] In fact, the well-described phenomenon of Ca^{2+}-mediated arrhythmias was originally

observed as a result of digitalis intoxication. By raising intracellular Na^+, cardiac glycosides reduce Ca^{2+} efflux by NCX and favor net Ca^{2+} uptake by the SR. At high doses, these agents can produce Ca^{2+} overload of the SR and result in the spontaneous release of Ca^{2+} by RyR, thereby generating a net depolarizing transient inward current mediated by NCX. Spontaneous rises in membrane potential caused by the activation of the transient inward current manifest as delayed after-depolarizations (DADs) that can be of sufficient magnitude to produce a full-blown premature action potential. A variety of complementary changes occurring as a result of left ventricular dysfunction in heart failure can reduce the threshold for the induction of DAD-mediated triggered beats, among which is the upregulation of NCX and the increased open probability of PKA-hyper-phosphorylated RyR2 channels. Catecholaminergic polymorphic ventricular tachycardia and arrhythmogenic right ventricular dysplasia, both of which are characterized by mutations in RyR2 genes, are two forms of familial cardiomyopathies-associated arrhythmias.[134] Wehrens et al demonstrated that catecholaminergic polymorphic ventricular tachycardia mutant RyR2 channels have a decreased binding affinity for calstabin2.[135] Although these mutant channels are indistinguishable from wild-type channels at rest, they exhibit exaggerated calstabin2 dissociation from RyR2 in response to PKA-mediated phosphorylation, causing more SR Ca^{2+} release and diastolic Ca^{2+} leak.[136] In addition to DADs, early after-depolarizations are another source of triggered activity in cardiomyocytes, which is secondary to abnormal Ca^{2+} cycling and results from the reactivation of LTCC during conditions of prolonged action potential repolarization.[137,138] Furthermore, abnormal Ca^{2+} cycling by the SR has also been implicated in the pathogenesis of reentrant arrhythmias by promoting action potential alternans and/or spiral wave break-ups leading to the degeneration of ventricular tachycardia and ventricular fibrillation.[137,139,140] As such, changes in Ca^{2+} handling in general, and Ca^{2+} overload in particular, are thought to form the triggers for lethal arrhythmias at the cellular level, which, under appropriate conditions, might produce sustained arrhythmias at the organ-system level.

Targeting myocardial calcium cycling to prevent arrhythmia

To highlight the importance of SR Ca^{2+} in the regulation of ventricular arrhythmia, we have addressed the issue of restoring the Ca^{2+} load of the SR through SERCA2a overexpression, using genetic strategies.[141] We and others have previously demonstrated that targeted delivery of SERCA2a to the heart can significantly improve contractile function in vitro and in vivo, normalize metabolism and intracellular signaling pathways, and improve survival and mechano-energetic properties at no cost of oxygen consumption.[62,63,142,143] Whether these potential benefits of SERCA2a can be further extended to improve cardiac arrhythmias was addressed using a well-documented Ca^{2+} overload rat model of ischemia/reperfusion (IR). Cardiac IR induces ventricular arrhythmias, including ventricular premature beats, ventricular tachycardia, and ventricular fibrillation, in both experimental animal models and in man.[7] We found that SERCA2a overexpression significantly decreased ventricular arrhythmias during IR and 24 hours later, using continuous telemetry.[141] In addition, SERCA2a overexpression significantly reduced infarct size and improved wall thickening in the anterior wall, which may have contributed to the decrease in ventricular arrhythmias because the size of the infarct is associated with the incidence and frequency of arrhythmias. A decrease in diastolic Ca^{2+} and better handling of intracellular ions during the rush of reperfusion are both associated with improved survival of the cardiomyocyte. The reduced incidence and severity of threatening arrhythmias of IR in the SERCA2a-overexpressing animals also corresponded to a preservation of muscle function, as demonstrated by improved wall thickening and hemodynamic measurements. These findings were recently extended by other studies that demonstrated that SERCA2a gene transfer in animal models of heart failure significantly reduced arrhythmogenic cardiac alternans,[144] reduced SR Ca^{2+} leak through reduction of RyR phosphorylation, and attenuated ventricular arrhythmias in vivo.[66]

Although targeting SR Ca^{2+} cycling proteins can abrogate Ca^{2+}-mediated arrhythmic triggers and prevent the adverse electrophysiological remodeling associated with IR injury, other candidates have also been shown to exert beneficial antiarrhythmic effects. Protein kinase C epsilon (PKCε) activation confers cardioprotection from IR injury in various cell cultures, isolated perfused heart models, and transgenic mice.[145] Furthermore, in vivo activation of PKCε was shown to protect the ischemic myocardium from reperfusion arrhythmias, whereas its inhibition exacerbates their incidence.[146] CaMKII has been functionally implicated in the mediation of frequency-dependent acceleration of relaxation, RyR2-mediated SR Ca^{2+} leak, and Ca^{2+} current facilitation,[147] all of which lead to perturbations in intracellular and SR Ca^{2+} balances and triggered arrhythmias. In addition, CaMKII expression and activity are known to increase in patients and in many animal models of structural heart disease. Hence, its inhibition has been shown in several studies to

reduce hypertrophy, cardiac dysfunction, and importantly, ventricular arrhythmias.[148,149]

MicroRNA-mediated Ca^{2+} signals and cardiac hypertrophy

Recent studies have uncovered key roles for a family of newly discovered, small, noncoding regulatory RNA molecules, known as microRNAs (miRNAs), in the control of diverse aspects of biological processes, including cardiovascular biology.[150] In addition to their role in cardiac development,[151–153] miRNAs are also critically involved in the pathological process of adult hearts, both in humans and rodents, including cardiac hypertrophy,[154–156] heart failure,[157–159] cardiomyopathy,[159] and arrhythmogenesis.[160] Here we briefly touch on some of the miRNAs that have been implicated in Ca^{2+} signaling and EC coupling. For a more comprehensive understanding of the pivotal function of miRNA in cardiovascular pathologies, the reader is referred to more recent reviews on the subject.[81,161,162] At this time, it is estimated that there are 150 to 200 miRNAs expressed in the cardiovascular system. For example, with respect to hypertrophy, multiple miRNAs, including the muscle-specific miRNAs (miR-1, miR-133, miR-208, and miR-499) and other miRNAs (miR-195, miR-21, and miR-18b), have been identified as participating in and can independently determine the pathological process. miR-195 was upregulated during cardiac hypertrophy, and its overexpression resulted in pathological cardiac growth and heart failure in transgenic mice.[158] miR-133 was down-regulated in mouse and human models of cardiac hypertrophy. Although overexpression of miR-133 inhibited cardiac hypertrophy,[163] suppression of miR-133 induced hypertrophy.[154] In a separate study, miR-21 and miR-18b were shown to be antihypertrophic.[155] miR-208a is upregulated in human hearts after myocardial infarction,[164] and two-fold overexpression of miR-208 in mice is sufficient to promote cardiac hypertrophy and arrhythmia.[165]

However, despite these observations, contradictory miRNA data have been reported under comparable experimental conditions. For instance, using similar pressure-overload (TAC) mouse model of hypertrophy, miR-21 was reported to be highly upregulated 1 to 2 weeks post-TAC but decreased to a normal level after 3 to 4 weeks,[156,166] whereas other studies reported a sustained expression over time.[155,158] miR-1 was identified as being down-regulated or unchanged[156,166] in TAC models and upregulated in dilative cardiomyopathy and in patients with coronary artery disease,[157,160] as well as in cardiomyocytes on oxidative stress.[167] We have demonstrated that miR-1 is down-regulated in pressure overload–induced cardiac hypertrophy, and chronic restoration of miR-1 expression in vivo by adeno-associated-virus 9-mediated gene transfer protected against the maladaptive cardiac remodeling induced by pressure overload.[168] Clearly, differences in the published findings can be attributed to the different experimental models and protocols used, the extent of the disease progression, the developmental stage, and the times at which the measurements were made and the nature of pathological stresses applied. Recent studies have demonstrated that cardio-enriched miRNAs can be released into the circulation and could readily be detected in circulating blood. These circulating miRNAs may potentially constitute excellent candidate biomarkers for various diseases, including myocardial infarction and heart failure.[169–171]

With respect to Ca^{2+} signaling regulation, few microRNAs have been shown to directly or indirectly control Ca^{2+} cycling and regulate important targets involved in cardiomyocyte EC coupling. Manipulation of miR-1 has been shown to mediate Ca^{2+}-dependent arrhythmogenesis through modulation of ion channels function and their associated regulators. For instance, overexpression of miR-1 in rat ventricular myocytes enhanced CaMKII-dependent phosphorylation of LTCC and RyR2 by targeting protein phosphatase activity localized to the channels, leading to increased channel activity and disruption of Ca^{2+} cycling and promotion of arrhythmogenesis.[172] Interestingly, similar effects were evoked by miR-133 overexpression.[173] MiR-1 was also shown to modulate Ca^{2+} transients in cardiomyocytes by repressing sorcin expression in the heart failure model.[174] This is important, since sorcin, as a Ca^{2+} modulator, is known to interact with and regulate several ionic channels, including LTCC, RyR2, NCX1, and SERCA2a, and thus plays an important role in the regulation of EC coupling. Moreover, miR-1 was also demonstrated to target and repress NCX1, a key regulator of Ca^{2+} influx,[175,176] and annexin A5,[176] further highlighting the pivotal role for miR-1 as a regulator of cardiomyocyte Ca^{2+} homeostasis. Interestingly, miR-214 was reported to target and repress NCX1 and protects cardiomyocytes from Ca^{2+}-induced cell death during ischemia/reperfusion injury.[177] In addition, miR-133a, which is bi-cistronically clustered with miR-1, was shown to have a strong effect on Ca^{2+} signaling pathways in cardiomyocytes by targeting calcineurin,[178] NFATc4,[179] and IP_3R2.[180] The latter study also showed that IP_3-induced Ca^{2+} release significantly contributes, in a feed-forward mechanism, to the down-regulation of miR-133a during hypertrophy and accentuation of pathological remodeling.[180] In contrast, levels of miR-21 and miR-132, and miR-328 were upregulated after isoproterenol stimulation and

atrial fibrillation, respectively.[181,182] miR-328 targeted and suppressed both the α1c and β1 subunits of the LTCC, whereas miR-21 and miR-132 both targeted β1 subunits of the channel, which may explain the dramatic reduction in the L-type Ca^{2+} current and the subsequent alterations in EC coupling observed in these studies.

Conclusion

In heart physiology, Ca^{2+} is a double-edged sword for both life and death. Although it is required for proper cardiac maintenance, abnormalities in Ca^{2+} cycling and signaling are at the heart of contractile deficiency and the progression to cardiac hypertrophy and heart failure. Understanding the normal pathophysiology of myocardial Ca^{2+} signaling is important for devising adequate and appropriate strategies to correct Ca^{2+}-stimulated maladaptive remodeling. Clinical trials targeting normalization of SR Ca^{2+} uptake (SERCA2a gene therapy) and reduction of SR Ca^{2+} leak (RyR stabilizers) are currently underway in patients with heart failure. Likewise, it is expected that new advances in myocardial Ca^{2+}-dependent therapies will emerge in the near future, such as defining the sources of signaling Ca^{2+} and identifying pathological microRNAs involved in the induction of heart failure and arrhythmias.

Disclosure

The authors report no conflicts of interest in this work.

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