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The Fire From Within

The Biggest Ca²⁺ Channel Erupts and Dribbles

Mark E. Anderson

CaMKII Is a Pluripotent Signaling Molecule in Heart

The multifunctional Ca²⁺ and calmodulin (CaM)-dependent protein kinase II (CaMKII) is a serine threonine kinase that is abundant in heart where it phosphorylates Ca²⁺_i homeostatic proteins. It seems likely that CaMKII plays an important role in cardiac physiology because these target proteins significantly overlap with the more extensively studied serine threonine kinase, protein kinase A (PKA), which is a key arbiter of catecholamine responses in heart. However, the physiological functions of CaMKII remain poorly understood, whereas the potential role of CaMKII in signaling myocardial dysfunction and arrhythmias has become an area of intense focus. CaMKII activity and expression are upregulated in failing human hearts and in many animal models of structural heart disease.¹ CaMKII inhibitory drugs can prevent cardiac arrhythmias^{2,3} and suppress afterdepolarizations⁴ that are a probable proximate focal cause of arrhythmias in heart failure. CaMKII inhibition in mice reduces left ventricular dilation and prevents disordered intracellular Ca²⁺ (Ca²⁺_i) homeostasis after myocardial infarction.⁵ CaMKII overexpression in mouse heart causes severe cardiac hypertrophy, dysfunction, and sudden death that is heralded by increased SR Ca²⁺ leak⁶; these findings go a long way to making a case for CaMKII as a causative signal in heart disease and arrhythmias but do not identify critical molecular targets or test the potential role of CaMKII in a large non-rodent animal model. The work by Ai et al in this issue of *Circulation Research* makes an important contribution by demonstrating CaMKII upregulation causes increased Ca²⁺ leak from ryanodine receptor (RyR) Ca²⁺ release channels in a clinically-relevant model of structural heart disease.⁷

Ryanodine Receptors Are Central

Ca²⁺_i release controls cardiac contraction, and most of the Ca²⁺_i for contraction is released from the intracellular sarcoplasmic reticulum (SR) through ryanodine receptors (RyR). RyRs are huge proteins (565 kDa) that assemble with a fourfold symmetry to form a functional Ca²⁺ release channel. Approximately 90% of the RyR is not directly required to form the pore but instead protrudes into the cytoplasm where

it binds numerous proteins, including PKA, CaMKII, CaM, and FK12.6 (calstabin). Cardiac contraction is initiated when Ca²⁺ current (*I*_{Ca}), through sarcolemmal L-type Ca²⁺ channels (LTCC), triggers RyR opening by a Ca²⁺-induced Ca²⁺ release (CICR) mechanism. LTCCs “face off” with RyRs across a highly ordered cytoplasmic cleft that delineates a kind of Ca²⁺ furnace during each CICR-initiated heart beat (Figure). CICR has an obvious need to function reliably, so it is astounding to consider how this feed forward process is intrinsically unstable. The increased instability of CICR in heart failure is directly relevant to arrhythmias initiated by afterdepolarizations. RyRs partly rely on a collaboration of Ca²⁺-sensing proteins in the SR lumen to grade their opening probability and the amount of SR Ca²⁺ release to a given *I*_{Ca} stimulus. Thus the SR Ca²⁺ content is an important parameter for setting the inotropic state, and heart failure is generally a condition of reduced SR Ca²⁺ content and diminished myocardial contraction.

Kinases Facilitate Communication Between LTCCs and RyRs

LTCCs and RyRs form the protein machinery for initiating contraction in cardiac and skeletal muscle, but in cardiac muscle communication between these proteins occurs without a requirement for physical contact. PKA is preassociated with LTCCs and RyRs, and PKA-dependent phosphorylation increases LTCC⁸ and RyR⁹ opening. The resultant increase in Ca²⁺_i is an important reason for the positive inotropic response to catecholamines. The multifunctional Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) is activated by increased Ca²⁺_i, and so catecholamine stimulation activates CaMKII in addition to PKA.⁵ In contrast to PKA, which is tightly linked to inotropy, CaMKII inhibition does not cause a reduction in fractional shortening during acute catecholamine stimulation in mice.⁵ Prolonged catecholamine exposure does reduce contractile function by uncertain mechanisms that require CaMKII.¹⁰ CaMKII colocalizes with LTCCs¹¹ and RyRs,¹² and CaMKII can also increase LTCC¹³ and RyR¹² opening probability in cardiac myocytes. The ultrastructural environment of LTCCs and RyRs is well-suited for a Ca²⁺_i-responsive kinase to serve as a coordinating signal between LTCCs and RyRs during CICR. The recently identified role of CaMKII in heart failure suggests the possibility that excessive CaMKII activity could cause or contribute to CICR defects present in heart failure.

Heart Failure Is a Disease of Disordered Ca²⁺_i Homeostasis

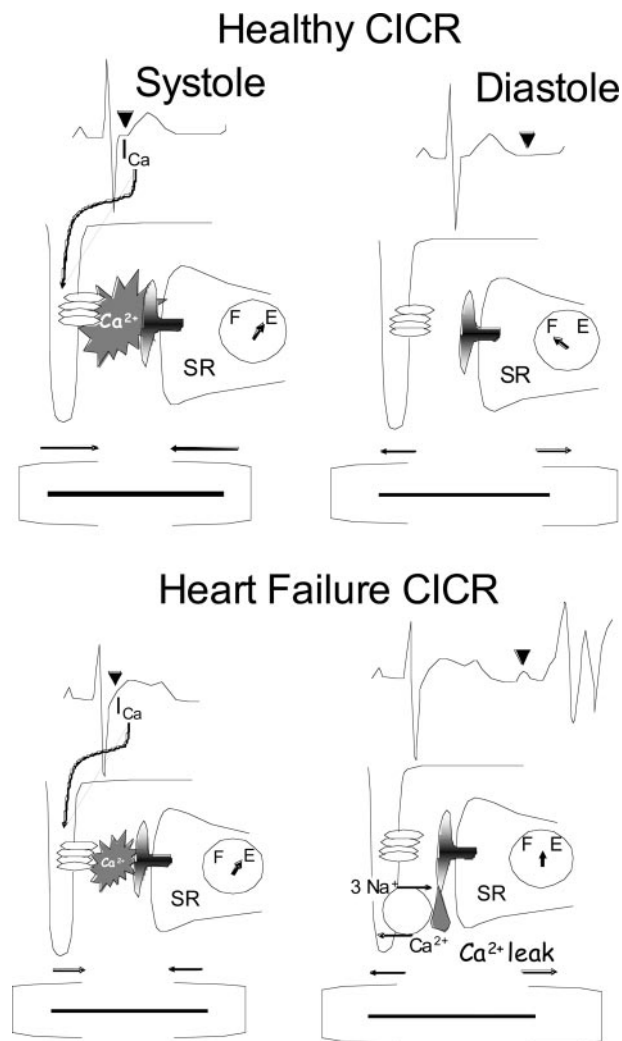
The key clinical phenotypes of contractile dysfunction and electrical instability in heart failure involve problems with Ca²⁺_i homeostasis. Broad changes in Ca²⁺_i-handling proteins

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Ca²⁺-induced Ca²⁺ release (CICR) in health and disease. Each heart beat is initiated by cell membrane depolarization that opens Ca²⁺ channels. The Ca²⁺ current (I_{Ca}) induces ryanodine receptor (RyR) opening that allows release of myofilament activating Ca²⁺ for contraction. In healthy CICR, RyRs close during diastole while Ca²⁺ is removed from the cytoplasm by uptake into the sarcoplasmic reticulum (SR). In heart failure the SR has reduced Ca²⁺ content so that the amount of Ca²⁺ released to the myofilaments is smaller than in health. RyR hyperphosphorylation by CaMKII promotes repetitive RyR openings leading to a Ca²⁺ leak in diastole. This leak contributes to the reduction in SR Ca²⁺ content and can engage the electrogenic Na⁺-Ca²⁺ exchanger to trigger afterdepolarizations and arrhythmias.

can occur in various heart failure models, but in general heart failure is marked by a reduction in the capacity for SR Ca²⁺ uptake, enhanced activity of the sarcolemmal Na⁺-Ca²⁺ exchanger, and reduction in CICR-coordinated SR Ca²⁺ release. On the other hand, the opening probability of individual LTCCs is increased in human heart failure,¹⁴ suggesting that posttranslational modifications may also be mechanistically important for understanding these Ca²⁺ disturbances at Ca²⁺ homeostatic proteins.

Is Heart Failure a Disease of Enzymatic Over-Activity?

Heart failure is marked by hyper-adrenergic tone, and beta adrenergic receptor antagonist drugs (beta blockers) are a

mainstay of therapy for reducing mortality in heart failure patients. The Marks group pioneered the concept that RyRs are hyperphosphorylated by PKA in patients with heart failure and showed that successful therapies, ranging from beta blockers to left ventricular assist devices, reduce RyR phosphorylation in step with improved mechanical function. They have developed a large body of evidence in patients and in animal models that PKA phosphorylation of Ser2809 on cardiac RyRs destabilizes binding of FK12.6 to RyRs and promotes increased RyR opening that causes an insidious Ca²⁺ leak. This leak is potentially problematic because it can reduce SR Ca²⁺ content (to depress inotropy), engage pathological Ca²⁺-dependent transcriptional programs (to promote myocyte hypertrophy), and activate arrhythmia-initiating afterdepolarizations (to cause sudden death). Indeed, RyR hyperphosphorylation can produce arrhythmias as well as mechanical dysfunction, whereas a drug that prevents FK12.6 dissociation from RyR also reduces or prevents arrhythmias.¹⁵ Taken together these findings make a strong case that RyR hyperphosphorylation (a result of net excess kinase activity) is a central event in heart failure and sudden death.

Not all findings point to hyperphosphorylation of RyR by PKA and subsequent FK12.6 dissociation as critical determinants of heart failure¹⁶ and arrhythmias.¹⁷ For example, studies in isolated and permeabilized ventricular myocytes failed to show an increase in RyR openings, called sparks, which are monitored by photoemission of a Ca²⁺-sensitive fluorescent dye.¹⁸ FKBP12.6 dissociation is not universally reported to follow RyR phosphorylation by PKA.¹⁹ Furthermore, FKBP12.6 binding to RyR is not affected during catecholamine stimulation that results in arrhythmias in a mouse model of catecholamine-induced ventricular tachycardia,^{20,21} a genetic disorder of hypersensitive RyR Ca²⁺ release. These findings challenge the PKA hypothesis and make room, conceptually, to consider the role of additional signals for modulating RyR activity in heart disease.

Both PKA and CaMKII may phosphorylate Ser2809, but recently CaMKII was found to exclusively phosphorylate Ser2815 and this phosphorylation caused increased RyR opening.¹² However, the PKA and CaMKII responses may be mechanistically distinct because CaMKII evoked increased RyR opening in the absence of FK12.6 dissociation. These findings together with the fact that CaMKII activity is recruited under conditions of increased PKA activity suggest that CaMKII might also be important in regulating RyRs in heart failure.

The article by Ai et al shows that expression of a CaMKII splice variant that is resident in cytoplasm (CaMKII δ c) was increased, and there was enhanced phosphorylation of the recently identified CaMKII site (Ser2815) on RyR. Both Ser2815 and the PKA site (Ser2809) were hyperphosphorylated in failing hearts, but phosphorylation of the CaMKII site was greater than the PKA site. Because both Ser2809 and Ser2815 can increase RyR openings, it seemed likely that PKA and CaMKII would work together to increase Ca²⁺ leak. Surprisingly, CaMKII inhibition but not PKA inhibition suppressed the leak. These experiments were performed with meticulous attention to matching SR Ca²⁺ load, a technically difficult accomplishment that is not performed by most

groups evaluating SR Ca^{2+} release. Thus, differences in the SR intraluminal Ca^{2+} could not account for these findings. Although these experiments were carefully controlled, one potential limitation is that the experiments relied exclusively on CaMKII and PKA inhibitor drugs that are notorious for nonspecific actions at ion channel proteins. They also showed that the ratio of inositol tris phosphate receptors (IP_3R) to RyRs was increased in failing left ventricular myocytes. IP_3R are important for regulating Ca^{2+}_i in many cells types, including atrial myocytes, but their role in ventricle remains uncertain. The finding that the IP_3R are increased at the expense of RyR suggests that Ca^{2+}_i release sites are fundamentally reordered in heart failure but leaves the impact of this change untested. IP_3R are also a target for CaMKII, so interesting questions remain about the potential role for this channel and CaMKII in heart failure, at least in this model.

What We Learned and What We Need to Know

CaMKII activity seems to be part and parcel of the adrenergic signaling seen in structural heart disease. This work shows us that CaMKII can contribute directly to increased SR Ca^{2+} leak in a clinically relevant model of heart failure that is marked by arrhythmias and sudden death.²² Acute experiments with CaMKII inhibitory drugs strongly suggest that SR Ca^{2+} leak is principally linked to CaMKII rather than PKA activity. Excessive SR Ca^{2+} release can activate inward (forward mode) $\text{Na}^+-\text{Ca}^{2+}$ exchanger current to cause delayed afterdepolarizations and arrhythmias and CaMKII inhibition can prevent these inward $\text{Na}^+-\text{Ca}^{2+}$ exchanger currents.²³ An important next step toward translating these findings will be to evaluate the effects of chronic CaMKII inhibition in this model to see whether it reverses cardiac dysfunction, arrhythmias, and whether chronic CaMKII inhibitor therapy can stop the RyR leak to refill the SR. It will be necessary to have improved pharmacological agents with fewer nonspecific effects to convincingly perform these experiments. These future experiments will tell us whether CaMKII inhibition is a potentially viable therapy for structural heart disease and arrhythmias in a non-genetic non-mouse model. We need to know whether CaMKII inhibition is really a highly-specific form of beta blockade that can preserve inotropic responses to catecholamines while preventing the adverse consequences of catecholamines in heart failure.⁵

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