Calmodulin-Dependent Protein Kinase II: Linking Heart Failure and Arrhythmias

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Abstract: Understanding relationships between heart failure and arrhythmias, important causes of suffering and sudden death, remains an unmet goal for biomedical researchers and physicians. Evidence assembled over the past decade supports a view that activation of the multifunctional Ca\(^{2+}\) and calmodulin-dependent protein kinase II (CaMKII) favors myocardial dysfunction and cell membrane electrical instability. CaMKII activation follows increases in intracellular Ca\(^{2+}\) or oxidation, upstream signals with the capacity to transition CaMKII into a Ca\(^{2+}\) and calmodulin-independent constitutively active enzyme. Constitutively active CaMKII appears poised to participate in disease pathways by catalyzing the phosphorylation of classes of protein targets important for excitation–contraction coupling and cell survival, including ion channels and Ca\(^{2+}\) homeostatic proteins, and transcription factors that drive hypertrophic and inflammatory gene expression. This rich diversity of downstream targets helps to explain the potential for CaMKII to simultaneously affect mechanical and electrical properties of heart muscle cells. Proof-of-concept studies from a growing number of investigators show that CaMKII inhibition is beneficial for improving myocardial performance and for reducing arrhythmias. We review the molecular physiology of CaMKII and discuss CaMKII actions at key cellular targets and results of animal models of myocardial hypertrophy, dysfunction, and arrhythmias that suggest CaMKII inhibition may benefit myocardial function while reducing arrhythmias. (Circ Res. 2012;110:1661-1677.)

Key Words: arrhythmias ■ calmodulin-dependent protein kinase II ■ heart failure ■ ion channels ■ remodeling

Despite important advances in drug and device therapy, cardiovascular disease remains a leading cause of death and suffering worldwide.\(^1\) Heart failure, a condition arising from multiple causes, occurs when the myocardium is unable to pump blood in sufficient volume to meet metabolic demands. Heart failure is responsible for almost 300,000 deaths\(^2\) and costs $39 billion dollars annually in the United States alone.\(^3\) Although heart failure is a mechanical problem, mostly of contraction (inotropy) but also of relaxation (lusitropy), many heart failure patients experience arrhythmias, an electrical problem in which cell membrane excitability is inadequately controlled. Ventricular arrhythmias (ventricular tachycardia and ventricular fibrillation) and atrial arrhythmias (sinus node dysfunction, atrial tachycardia, and atrial fibrillation) contribute to the increased incidence of sudden cardiac death in patients with heart failure and may themselves aggravate heart failure by disturbing the physiological relationship between heart rate and myocardial performance. Despite the clear association between heart failure and arrhythmias, it has proven difficult to develop widely applicable treatments that coordinately benefit both diseases. The association of heart failure and arrhythmias is a major challenge for clinicians, in part because myocardial performance-enhancing inotropic drugs increase the likelihood of sudden death,\(^4\) and because conventional ion channel antagonist antiarrhythmic medications are likely to impose proarrhythmic side effects on hypertrophied, scarred, and failing myocardium.\(^5\) Thus, an important goal for clinical cardiologists, their patients, industry, and society is to develop treatments that effectively target both heart failure and arrhythmias. It seems likely that a first step toward creating agents that can reduce arrhythmias and heart failure will be to identify cellular and molecular mechanisms that underlie both processes. This review considers established and emergent evidence that the multifunctional Ca\(^{2+}\) and calmodulin-dependent protein kinase II (CaMKII) is a signaling molecule that is uniquely positioned to promote the twin pathological phenotypes of heart failure and arrhythmias. In myocardium, CaMKII phosphorylates a diverse array of proteins involved in excitation–contraction coupling, cell death, and transcriptional activation of hypertrophy and inflammation. Excessive or unchecked CaMKII activity thereby promotes core “downstream” events and processes that contribute to heart failure and arrhythmias (Figure 1).

Original received March 29, 2012; revision received May 3, 2012; accepted May 8, 2012. In April 2012, the average time from submission to first decision for all original research papers submitted to Circulation Research was 12.79 days.

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Circulation Research is available at http://circres.ahajournals.org DOI: 10.1161/CIRCRESAHA.111.243956
Upstream Activator Signals for CaMKII

Loss of normal myocardial Ca$_{2+}$/CaM homeostasis and increased reactive oxygen species (ROS) are common “upstream” signals that contribute to arrhythmias and heart failure. However, concise molecular targets and signaling pathways connecting Ca$_{2+}$ and ROS to arrhythmias and heart failure have been elusive, whereas antioxidant therapies have had modest or no benefit, suggesting that improved knowledge of Ca$_{2+}$ and ROS-activated signals could lead to improved therapies. CaMKII is a serine threonine kinase that is abundant in myocardium and is activated by increased intracellular Ca$_{2+}$ and ROS. There are four CaMKII gene products (α-δ) that are homologous, share activation mechanisms, and are thought to coassemble as heteromultimers. The four CaMKII isoforms are differentially expressed in various cell types. For example, CaMKIIα and CaMKIIβ are relatively enriched in neuronal tissues and CaMKIIδ and CaMKIIγ are predominant in myocardium. However, to our knowledge, there are no differences in catalytic domain specificity for target proteins between CaMKII isoforms. There is a hypervariable region (yellow line in Figure 2) between the association domain and the C terminus of the regulatory domain that has a nuclear localization sequence in one CaMKIIδ splice variant (CaMKIIδ$_2$, or CaMKIIδ$_{a}$), but not in other CaMKIIδ splice variants (CaMKIIδ$_1$, or CaMKIIδ$_{b}$). However, the specific role of this sequence remains unclear in myocardium because recent data suggest that both CaMKIIδ$_{b}$ and CaMKIIδ$_{c}$ are present in nuclear and cytoplasmic compartments. It appears that signaling specificity for CaMKII is determined, at least in part, by the proximity of CaMKII to specific sources of Ca$_{2+}$ or ROS. Splice variants are relatively common in the hypervariable domain and produce different length linkers between the association and regulatory domains, leading to CaMKII variants with different sensitivities to activation by calcified calmodulin (Ca$_{2+}$/CaM). CaMKII variants with shorter linkers are relatively compact and is inaccessible, whereas CaMKII variants with longer linkers are relatively extended, resulting in a larger Ca$_{2+}$/CaM-accessible binding surface. An intriguing, but untested, possibility is that CaMKII splice variants with relatively longer hypervariable domain-encoded linkers are better-adapted to “fight or flight” physiology, but also are more likely to contribute to disease because they are more readily activated by Ca$_{2+}$/CaM and ROS. Each CaMKII monomer consists of an N-terminal catalytic domain and a C-terminal association domain that enables assembly of the holoenzyme. The regulatory domain resides between the catalytic and association domains (Figure 2). CaMKII is initially activated by binding Ca$_{2+}$/CaM, because Ca$_{2+}$/CaM binding to the C-terminal aspect of the regulatory domain (ie, the CaM-binding region) distorts and disables the negative regulatory effect of the autoinhibitory region to constrain the CaM-binding region) distorts and disables the negative regulatory effect of the autoinhibitory region to constrain the Ca$_{2+}$/CaM–dependent active enzyme. In the case of brief increases in intracellular Ca$_{2+}$, under low ROS conditions, CaMKII returns to an inactive conformation after Ca$_{2+}$/CaM unbinding. However, sustained increases in Ca$_{2+}$/CaM allow for...
binding.19 Oxidation of paired methionines (281/282) causes Drosophila melanogaster lifespan in flies (thionine pairs. Although CaMKII requires Ca2⁺/CaM–independent CaMKII activity similar to oxidized methionine oxidation both have the capacity to “lock” CaMKII into a Ca2⁺/CaM–autonomous conformation with sustained activity.20 In contrast to autophosphorylation, methionine oxidation does not cause CaM trapping, because oxidation of methionine (308 in CaMKII) reduces the affinity of Ca2⁺/CaM–CaMKII binding.

The first stage of methionine oxidation (methionine sulfoxide) is enzymatically reversible by methionine sulfoxide reductase A (MsRA), whereas the second stage (methionine sulfone) is irreversible. At this point it is unknown how much CaMKII becomes terminally methionine oxidized in normal myocardium or in disease. MsRA deletion increases susceptibility to death and heart failure from myocardial infarction, whereas MsRA overexpression prevents CaMKII oxidation and is protective against postmyocardial infarction cardiac rupture in mice.12 MsRA overexpression prolongs lifespan in flies (Drosophila melanogaster), whereas MsRA knock out increases mortality in mice and worms (Caenorhabditis elegans).23 These findings suggest that CaMKII is a critical target for the benefits of MsRA overexpression and for the detrimental effects of MsRA inhibition. CaMKII isoforms β, δ, and γ have regulatory domain methionine pairs (the numbering varies slightly between them), whereas CaMKIIα has a cysteine–methionine pair that appears to induce Ca2⁺/CaM–independent CaMKII activity similar to oxidized methionine pairs. Although CaMKII requires Ca2⁺/CaM binding for initial activation, oxidized CaMKII resets the Ca2⁺ sensitivity of CaMKII so that very low levels of intracellular Ca2⁺ are sufficient for activation,24 suggesting that conditions of high ROS may lead to increased CaMKII activity even at resting levels of Ca2⁺/CaM. Thus, autophosphorylation and oxidation both have the capacity to “lock” CaMKII into a Ca2⁺/CaM–autonomous conformation with sustained activity. Oxidized constitutively active CaMKII is now linked to heart failure,25 tachyarrhythmias,26–27 bradyarrhythmias,28 and postmyocardial infarction cardiac rupture,29 suggesting that CaMKII is an important ROS sensor for transduction of oxidative stress into clinically important disease phenotypes.7

**Myocardial Ultrastructure Determines the Consequences of Pathological CaMKII Activation**

Myocardial contraction is built around an elaborate membrane ultrastructure that places sarclolemmal domains enriched with ion channels in close approximation with sarcoplasmic reticulum (SR) Ca2⁺ release sites (Figure 3). This ultrastructure allows for feedback between intracellular Ca2⁺ and sarclolemmal excitability, a complex and exquisitely tuned set of relationships that are disturbed in patients with structural heart disease. One view is that the cell membrane system that supports the excitation–contraction coupling machinery also determines the association between heart failure, arrhythmias, and sudden cardiac death. CaMKII is enriched in the vicinity of the T-tubular sarclolemmal membrane invaginations29,30 that form the ultrastructural framework for closely (approximately 10 nm) approximating the SR Ca2⁺ release ryanodine receptor (RyR2) channels and sarclolemmal voltage-gated Ca2⁺ channels. The presence of CaMKII at CaV1.2 (the predominant voltage-gated Ca2⁺ channel in ventricular muscle) and at RyR2 may serve to coordinate and promote physiological activation of these excitation–contraction coupling proteins11 to increase the force–frequency relationship (ie, the tendency of myocardium...
to generate more tension at faster stimulation rates) and/or to engage fight or flight acceleration of heart rate. 

CaMKII is now recognized to bind to multiple voltage-gated ion channels (Ca\(^{2+}\), Na\(^+\), and K\(^+\)), some of which are discussed later in the context of disease. Although unproven, it seems likely that CaMKII coordinates physiological adaptations of membrane excitability to changes in intracellular Ca\(^{2+}\) and ROS. The enrichment of CaMKII in particular subcellular domains suggests that CaMKII is guided by targeting mechanisms. Some protein kinases use a strategy of anchoring proteins to achieve proximity to target proteins and to other regulatory proteins, such as phosphatases. For example, A-kinase anchoring proteins and receptors for activated C-kinase provide a structural basis for targeting protein kinase A and protein kinase C in a variety of tissues, including myocardium. In contrast, there is no known family of anchoring proteins for CaMKII. Instead, CaMKII likely identifies target proteins using regions that mimic the topology of its own regulatory domain autoinhibitory region (Figure 4). We identified short sequences with high homology to the CaMKII\(\beta\) subunit of CaV1.2 (bottom) and on \(\beta\)-spectrin, a cytoskeletal protein associated with Ankyrin G and the Na\(_{\infty}\)1.5 (top). These sequences are required for CaMKII to regulate CaV1.2 and Na\(_{\infty}\)1.5 currents.

**CaMKII Activity and Expression in Heart Failure**

CaMKII activation is a key part of a broader paradigm of interdependent pathological signals in heart failure and arrhythmia (also discussed in Systems Biology). Sympathetic nervous system activity is increased by a pressing need to maintain contractility in heart failure. Although activation of neurohumoral signaling pathways may be adaptive initially, chronic elevation of neurohumoral activity contributes to increased intracellular Ca\(^{2+}\) and ROS that are thought to promote disease progression. CaMKII is activated downstream to the three neurohormone agonists validated to be therapeutic targets for heart failure and arrhythmias. Most evidence for these pathways, \(\beta\)-adrenergic receptors, angiotensin II, and aldosterone, is for heart failure after myocardial infarction. Mice lacking \(\alpha\)-adrenoreceptors have development of sympathetic hyperactivity-induced heart failure, increased mortality, and elevated total and threonine 287-autophosphorylated CaMKII levels compared to wild-type (WT) animals. Dilated cardiomyopathy, exercise intolerance, and CaMKII activity in these mice were partially reversed by an angiotensin II receptor antagonist, further suggesting that the success of angiotensin II antagonist drug therapy is at least partially attributable to CaMKII inhibition. Several studies have shown the role of CaMKII in the causation and progression of cardiac hypertrophy—an independent risk factor for development of heart failure. 

CaMKII is increased at the transcriptional level, protein level, and in its activity in heart failure. Heart failure occurs in mice with transgenic CaMKII overexpression and myocardial infarction causing heart failure also occurs with increases in myocardial CaMKII activity and expression in mice, rabbits, dogs, and patients. CaMKII activation may be related to ventricular wall stress, because dogs with heart failure attributable to left bundle branch ablation and rapid pacing showed increased CaMKII activity in the lateral left ventricular wall compared with the septum. Furthermore, the increase in lateral wall CaMKII activity and expression was normalized by cardiac resynchronization (biventricular) pacing. Although little is known about the transcriptional programs that increase CaMKII expression in heart failure, calcineurin activity and/or expression increase in animal models and patients with structural heart disease and calcineurin promotes myocardial expression of CaMKII.

This example may point to a broader paradigm in which interdependence between various pathological signals ultimately produce severe and progressive forms of heart failure and sudden death. Transgenic myocardial overexpression of a constitutively active form of calcineurin causes severe myocardial hypertrophy, mechanical dysfunction, and premature death in mice. CaMKII protein levels and activity are markedly increased in calcineurin transgenic mice compared to WT littermates and CaMKII inhibition improved mechanical function and reduced mortality, but with minimal impact on myocardial hypertrophy, suggesting that CaMKII inhibition could reduce heart failure and sudden death even in the face of forced overexpression of calcineurin. Taken together, these findings suggest that CaMKII may play a crucial role in myocardial hypertrophy and heart failure, whereas core clinically validated pathways that drive progression of heart failure and sudden death after myocardial infarction converge to activate CaMKII in myocardium.
The mechanism of CaMKII hyperactivity in heart failure is likely attributable to either autophosphorylation of threonine 287 and/or oxidation of methionines 281 and 282 (Figure 1); these posttranscriptionally modified amino acids are located in the CaMKII regulatory domain (Figure 2). Autophosphorylated CaMKII levels were elevated in mice up to 7 days after transaortic constriction, a surgical model of acute pathological left ventricular afterload.\(^\text{51}\) Autophosphorylated CaMKII\(^\text{17,18}\) was increased in a pacing-induced rabbit model of left ventricular dysfunction and incessant ventricular tachycardia (ventricular tachycardia storm).\(^\text{64}\) Rabbits treated chronically with a calmodulin antagonist, W-7, had improved left ventricular function, arrhythmia termination, and restoration of autophosphorylated CaMKII to control levels. These recent findings provided the first functional evidence in a “large” animal model that excessive CaMKII activation was causal in myopathy and arrhythmias.\(^\text{63}\) Angiotensin II\(^\text{59}\) and aldosterone\(^\text{12}\) appear to increase ROS by activating NADPH oxidase. We found that oxidized CaMKII levels were significantly elevated in mouse heart\(^\text{12}\) and in the border zone of dogs hearts days after myocardial infarction,\(^\text{53}\) suggesting that oxidized CaMKII is a transduction signal for the increased oxidant stress that accompanies common forms of structural heart disease.\(^\text{13}\) Intriguingly, CaMKII oxidation is reversed by MsrA, and mice lacking develop increased levels of oxidized CaMKII, myocardial apoptosis, adverse left ventricular remodeling,\(^\text{20}\) increased frequency of cardiac rupture, and death after myocardial infarction.\(^\text{12}\) In contrast, mice with myocardial transgenic MsrA overexpression show reduced mortality and are protected from aldosterone and myocardial infarction–induced increases in oxidized CaMKII,\(^\text{12}\) suggesting that selectively targeted antioxidant therapy could protect against excessive CaMKII oxidation and increased mortality after myocardial infarction.

One-time static measurements of CaMKII have been made in humans with heart failure and have provided potential insights into the role of CaMKII in cardiomyopathy. CaMKII\(\delta\) mRNA and protein were significantly elevated in hearts from patients with dilated cardiomyopathy, compared with hearts from donors without structural heart disease.\(^\text{62}\) Left ventricular tissue from failing human hearts with dilated nonischemic cardiomyopathy showed increased CaMKII activity compared with nonfailing hearts, whereas myocardial tissue from ischemic cardiomyopathy patients had a trend toward elevation in left ventricular CaMKII activity.\(^\text{63}\) Protein levels of cytosolic and nuclear splice variants of CaMKII\(\delta\) were higher in failing human right and left ventricle than in controls, regardless of whether cardiomyopathy was attributable to ischemic or nonischemic causes.\(^\text{12,54}\) We recently measured oxidized CaMKII levels in the right atrium of heart failure patients with or without sinus node dysfunction and compared them with normal controls. We found that oxidized CaMKII levels are elevated in the right atrium of heart failure patients in the vicinity of the sinoatrial (SA) node. The increase in oxidized CaMKII is particularly pronounced in heart failure patients with coexistent SA node dysfunction, compared with those with similarly severe heart failure but no SA node dysfunction.\(^\text{28}\) Thus, a combination of data from animal models and from failing human myocardium suggests that CaMKII activity and expression are increased in injured and failing hearts.

CaMKII Target Proteins

Ion channels are multisubunit protein complexes built around a transmembrane permeation pore. A full discussion of CaMKII and ion channels is beyond the scope of this review, but it is addressed more completely in other recently published reviews.\(^\text{64}\) CaMKII was first identified to increase L-type Ca\(^{2+}\) current (\(\text{I}_{\text{Ca,L}}\)), in which CaMKII-mediated phosphorylation induced a dynamic pattern of increasing peak current accompanied by slowing of inactivation.\(^\text{65–66}\) However, CaMKII now appears to influence myocardial cell membrane excitability by affecting most or all known voltage-gated ion channels. What follows is a brief overview of CaMKII actions on ion channels in which CaMKII actions are thought to contribute to arrhythmias.

L-Type Ca\(^{2+}\) Channels

CaMKII-catalyzed phosphorylation of L-type Ca\(^{2+}\) channels (\(\text{Ca}_{\text{v}}1.2\)) promotes entry into a highly active gating mode (mode 2), characterized by a high opening probability and prolonged opening events.\(^\text{68}\) Mode 2 gating appears to underlie \(\text{I}_{\text{Ca,L}}\) facilitation, a dynamic pattern of increased peak \(\text{I}_{\text{Ca}}\) and slowed \(\text{I}_{\text{Ca}}\) inactivation, based on modeling studies\(^\text{69}\) and because mutations that prevent mode 2 gating also reduce \(\text{I}_{\text{Ca}}\) facilitation.\(^\text{39}\) Mode 2 gating\(^\text{70}\) and \(\text{I}_{\text{Ca}}\) facilitation are hypothesized to be proarrhythmic by favoring early afterdepolarizations\(^\text{29,39,71}\) and SR Ca\(^{2+}\) leak,\(^\text{22}\) inward Na\(^+\)/Ca\(^{2+}\) exchanger current,\(^\text{22}\) and delayed afterdepolarizations.\(^\text{39,74}\) Early afterdepolarizations and delayed afterdepolarizations of sufficient magnitude are able to trigger arrhythmias. Increased \(\text{Ca}_{\text{v}}1.2\) current can contribute to action potential prolongation\(^\text{75}\) and mode 2 gating is particularly proarrhythmic in the setting of action potential prolongation.\(^\text{76}\) Mode 2 gating was first identified using BayK 8644, a dihydropyridine \(\text{Ca}_{\text{v}}1.2\) agonist drug.\(^\text{70}\) BayK 8644 induces polymorphic ventricular tachycardia in an established rabbit model of QT interval prolongation and Torsade de Pointes.\(^\text{77,78}\) Suggesting that enhancement of mode 2 gating is sufficient to trigger life-threatening ventricular arrhythmias in the setting of proarrhythmic QT interval prolongation, (eg, long QT syndromes and cardiomyopathy). L-type Ca\(^{2+}\) channels are formed as a constellation of subunit proteins, including the \(\alpha\) (pore-forming) and \(\beta\) (regulatory) subunits. The \(\beta\) subunit acts as a chaperone to increase \(\alpha\) subunit membrane expression\(^\text{79}\) and engages the \(\alpha\) subunit to increase channel opening probability.\(^\text{30,39,80}\) CaMKII does not appear to affect the chaperone activity of \(\beta\) subunits, because WT \(\beta\) subunit overexpression and overexpression of \(\beta\) subunit mutants lacking CaMKII binding and phosphorylation sites are equally effective at increasing \(\text{I}_{\text{Ca,L}}\) density in cultured adult cardiomyocytes.\(^\text{30,39}\) CaMKII binding to \(\beta\) subunits is enhanced by Leu 493, and CaMKII-catalyzed phosphorylation of Thr 498 is critical for facilitation of \(\text{I}_{\text{Ca,L}}\) in isolated adult rat\(^\text{46}\) and rabbit\(^\text{9}\) ventricular myocytes. The Thr 498 phosphorylation site is conserved across \(\beta\) subunit isoforms but was initially identified on isoform \(\beta_{2a}\). The expression of the \(\beta_{2a}\) isoform is increased in failing human hearts,\(^\text{81}\) a condition marked by increased...
CaV1.2 opening probability, action potential duration prolongation, loss of intracellular Ca2+ homeostasis, early afterdepolarizations, and excessive cardiomyocyte death. Overexpression of WT β2a in adult cardiomyocytes increases I_{Ca}, induces SR Ca2+ overload and stimulates apoptosis by a pathway that involves CaMKII. However, overexpression of a β2a mutant lacking this CaMKII phosphorylation site (T498A) does not increase myocyte death or early afterdepolarizations, supporting the hypothesis that CaMKII phosphorylation of β2a is a molecular mechanism for pathological membrane excitability and cardiomyocyte death.

Voltage-Gated Na+ Channels
Failing myocardium is marked by an electric remodeling process in which reduction in net repolarizing outward current leads to action potential and QT interval prolongation. Although cardiac voltage-gated Na+ channels (mostly NaV1.5) open and close rapidly (1–10 ms), a persistent (late) component of Na+ current (I_{NaL}) contributes to action potential prolongation in a rare form of the long QT syndrome and in myocytes isolated from failing hearts. Interestingly, CaMKII phosphorylation has contrasting effects on I_{NaL} because it enhances late nonactivating I_{NaL}, but also decreases the availability of NaV1.5, a phenocopy of NaV alterations seen during ischemia and heart failure and in long QT and Brugada syndromes. CaMKII inhibition reverses heart failure–induced decreases in I_{Na}, suggesting that NaV1.5 is an important target for the proarrhythmic actions of CaMKII.

Voltage-Gated K+ Channels
Voltage-gated K currents (IK) are the major driving force for myocardial membrane repolarization. IK is caused by multiple ion channel proteins with diverse kinetic properties. Collectively, IK plays an important role in determining the action potential configuration, and duration and multiple genetic long QT syndromes linked to arrhythmic sudden death are attributable to biophysical and trafficking defects in IK. Furthermore, reduction in IK is a consistent feature of cardiomyopathy that contributes to proarrhythmic action potential and QT interval prolongation. CaMKII activates the transient outward K+ current (I_{to}) and inwardly rectifying (IK1) K+ current. IK comprises I_{to,fast} (Kv4.2/Kv4.3) and I_{to,slow} (Kv1.4), and site-directed mutagenesis studies have identified Kv4.3 S550 and Kv1.4 Thr602 as potential CaMKII phosphorylation targets for CaMKII. Increased CaMKII activity has been shown to slow I_{to}, inactivation and to accelerate recovery, with an overall effect of shortening the action potential and decreasing the refractory period. Chronic CaMKII activation leads to increased action potential duration, likely partly by downregulation of I_{to,fast} and upregulation of I_{to,slow} events that occur in failing cardiomyocytes. Heart failure is also characterized by reduction in IK1, which is responsible for maintaining stable resting membrane potential. Interestingly, chronic CaMKII overexpression also leads to downregulation of IK1 and, conversely, chronic CaMKII inhibition increases IK1 current. Thus, reduction in various K+ currents contributes to proarrhythmic action potential prolongation in long QT syndromes and heart failure. CaMKII overexpression also causes reduced repolarizing K+ current and CaMKII inhibition shortens action potentials, likely by increasing K+ currents. However, CaMKII actions are complex and appear to include transcriptional and posttranscriptional effects that defy simple comprehensive models of CaMKII activity, action potential, and QT interval duration. These findings suggest that cardiomyopathic changes in K+ current are at least partially the result of increased CaMKII activity in heart failure.

Ca2+ Cycling Proteins
The RyR2, phospholamban (PLN), and SR Ca-ATPase (SERCA) are well-known regulators of SR Ca2+ uptake and release and RyR2 and PLN are highly validated CaMKII targets. Although the exact mechanisms are yet to be defined, it is generally agreed that in failing cardiomyocytes there is a decrease in SR Ca2+ content and SR Ca2+ release during systole. Low SR Ca2+ content may be a result of decreased uptake of Ca2+ by SERCA and/or increased diastolic SR Ca2+ release ("leak"). Abundant evidence now supports an important role of CaMKII in promoting heart failure and arrhythmias by actions on SR Ca2+ uptake and release. RyR2 is a SR Ca2+ release channel that is activated by a trigger of Ca2+ from I_{Ca} and RyR2 phosphorylation by protein kinase A and CaMKII both enhance I_{Ca} and RyR2 Ca2+ release. CaMKII helps coordinate this physiological process of Ca2+-induced Ca2+ release by phosphorylation of CaV1.2 and RyR2. However, in failing myocytes the cell membrane ultrastructure supporting Ca2+-induced Ca2+ release is distorted and CaMKII hyperphosphorylation of CaV1.2 and RyR2 becomes arrhythmogenic. CaMKII hyperphosphorylation of serine 2814 on RyR2 promotes RyR2 Ca2+ leak and arrhythmia-triggering delayed afterdepolarizations while depleting SR Ca2+ to impair inotropy. Excessive and unbalanced β-adrenergic stimulation in heart failure could potentially activate CaMKII by cAMP–EPAC–dependent and cAMP-independent mechanisms to hyperphosphorylate RyR, causing SR-Ca2+ leak and arrhythmias. During the normal cardiac cycle, most intracellular Ca2+ is either pumped back into the SR (by SERCA) or extruded from the cytoplasm by the Na+/Ca2+ exchange protein (NCX1). Excess Ca2+ in diastole increases the likelihood for delayed afterdepolarizations attributable to the electrogenic action of NCX1. Interestingly, the pathological actions of CaMKII to favor delayed afterdepolarizations in atrial and ventricular myocardium mirror the physiological role of CaMKII to promote RyR2 Ca2+ leak and increased automaticity in SA nodal pacemaker cells. While the role of CaMKII in RYR dysfunction in heart failure is controversial, and although in early studies mice with genetically altered RyR resistant to CaMKII phosphorylation are not protected from heart failure, pathological effects of CaMKII were recently shown in knock-in mice with RyR genetically modified to mimic RyR phosphorylation by CaMKII (Ser 2814 Asp). After aortic banding, RyR (Ser 2814 Asp) mice are more likely to develop heart failure and...
arrhythmias. Conversely, mice engineered with CaMKII phosphorylation-resistant RyR2 (Ser 2814 Ala) were protected from heart failure and arrhythmia after aortic banding surgery. These and other findings support a view that CaMKII promotes arrhythmias and myocardial mechanical dysfunction, in part, by effects on SR Ca\textsuperscript{2+} release.

CaMKII regulates SR Ca\textsuperscript{2+} content by affecting SR Ca\textsuperscript{2+} uptake, as shown by the findings that myocardial CaMKII inhibition reduces SR Ca\textsuperscript{2+} content in ventricular myocytes\textsuperscript{116} and sinoatrial nodal pacemaker cells.\textsuperscript{33} PLN appears to be a target for CaMKII effects on SR Ca\textsuperscript{2+} content because knockout of PLN reverses the reduction in SR Ca\textsuperscript{2+} content in mice with myocardial CaMKII inhibition.\textsuperscript{117} PLN is a negative regulator of SERCA;\textsuperscript{118} but PLN phosphorylation by protein kinase A (at serine 16) or CaMKII (at threonine 17) can reduce the inhibitory efficacy of PLN, leading to increased SERCA activity. Loss of PLN increases SR Ca\textsuperscript{2+} filling in mice with cardiomyopathy and sudden death attributable to overexpression of CaMKII.\textsuperscript{119} However, PLN ablation leads to premature death and mitochondrial Ca\textsuperscript{2+} overload, suggesting that increasing SR Ca\textsuperscript{2+}, at least in the presence of excessive CaMKII activity, is disadvantageous. Whether CaMKII directly catalyzes SERCA phosphorylation and its physiological significance remains controversial.\textsuperscript{120,121}

Thus, CaMKII appears to act as a coordinating signal that favors SR Ca\textsuperscript{2+} flux by enhancing SR Ca\textsuperscript{2+} uptake and release. These actions are likely important for myocardial excitation–contraction coupling but are corrupted in cardiomyopathy and contribute to myocardial dysfunction and arrhythmias.

**Inflammation and Hypertrophy**

There is a substantial body of literature that links inflammation to cardiovascular disease, including heart failure and arrhythmias. Human patients with atrial fibrillation have higher blood levels of C-reactive protein, interleukin-6 and interleukin-8, compared with normal controls. Human atrial biopsy samples from patients with atrial fibrillation and animal models with atrial fibrillation have inflammatory infiltrates, suggesting an association between atrial fibrillation and inflammation. Markers of inflammation such as high-sensitivity C-reactive protein\textsuperscript{122} and chemokines, like interleukin-8 and monocyte chemoattractant protein-1, are elevated in patients with sudden death and ventricular fibrillation,\textsuperscript{123} suggesting life-threatening ventricular arrhythmias and sudden death are favored by a proinflammatory state. CaMKIV affects inflammatory responses by aiding T-cell development, regulating interleukin-2 activity in T-cells and by modulating the activity of transcription factors.\textsuperscript{124} However, the role of CaMKII in promoting inflammatory responses in myocardium is a newer finding. The α-isofrom of CaMKII is present in macrophages where it promotes Toll-like receptor-4–triggered, Toll-like receptor-9–triggered, and Toll-like receptor-3–triggered production of interleukin-6, tumor necrosis factor-α, and interferon-α/β.\textsuperscript{125} CaMKII also regulates the localization of major histocompatibility complex class II proteins in human dendritic cells.\textsuperscript{126} The proinflammatory actions of CaMKII in conventional inflammatory cells appear to be recapitulated, at least in part, in cardiomyocytes. We used a microarray to identify a cluster of proinflammatory genes regulated by CaMKII in cardiomyocytes by comparing mRNA levels between control mice and mice with myocardial-delimited CaMKII inhibition attributable to expression of a CaMKII inhibitor peptide (AC3-I) that resembles the CaMKII regulatory domain at baseline and after myocardial infarction. We found that complement factor B, a critical component of the alternative complement fixation pathway, was induced in cardiomyocytes and contributed to membrane injury by assembly into a membrane attack complex. Genetic inhibition of complement factor B reduced mortality and protected against adverse structural remodeling in hearts after myocardial infarction.\textsuperscript{127} The increase in complement factor B expression in myocardium by CaMKII is mediated through nuclear factor-κB, which has been shown to be activated by CaMKII in neuronal cells.\textsuperscript{128} More recently, we found that oxidized CaMKII is increased in response to lipopolysaccharide, a Toll-like receptor-4 agonist in myocardium,\textsuperscript{129} suggesting that CaMKII activity is activated by inflammatory signals that increase oxidative stress, although at the same time CaMKII can act as a feed-forward signal to augment transcription of inflammatory genes.

We found that nearly half of the significantly upregulated or downregulated mRNAs interrogated by our 8500 element array were regulated by CaMKII expression 3 weeks after myocardial infarction, suggesting CaMKII activation has important consequences for transcriptional reprogramming after myocardial infarction.\textsuperscript{127} Because myocardial CaMKII inhibition appears to be protective in the setting of myocardial infarction, at least in mice,\textsuperscript{12,20,41} it seems likely that the preponderance of CaMKII-modulated transcriptional events after myocardial infarction are ultimately maladaptive. To date, we have examined two CaMKII targets identified in this gene array in detail. The first was increased complement factor B expression that was downstream to CaMKII-dependent increases in nuclear factor-κB activity.\textsuperscript{127} We also identified increased expression of Mmp9, a gene encoding matrix metalloproteinase-9. Recently, we found that aldosterone infusion led to nongenomic activation of CaMKII by oxidation and oxidized CaMKII enhanced Mmp9 expression causing increased mortality attributable to cardiac rupture by activating the transcription factor myocyte enhancer factor-2.\textsuperscript{13} When aldosterone was infused in mice to levels measured in high-risk patients with heart failure resulting from myocardial infarction, at the time of myocardial infarction surgery we found excessive early mortality that was mostly the result of cardiac rupture. Although invading cells are a major source of matrix metalloproteinase-9, we found that mice with myocardial-delimited expression of AC3-I or MsrA were significantly protected from myocardial rupture, suggesting that CaMKII-dependent transcription of Mmp9 in heart muscle cells made a critical contribution to enhancing vulnerability to postmyocardial cardiac rupture. Three studies now show that CaMKII activation is critical for pathological responses of each of the therapeutically validated neurohumoral pathways that promote heart failure and sudden death, β-adrenergic receptor signaling,\textsuperscript{41} angiotensin II signaling,\textsuperscript{20} and aldosterone.\textsuperscript{12}
Electrocardiographic left ventricular hypertrophy is an independent risk factor for sudden cardiac death. The relationship of left ventricular hypertrophy to supraventricular arrhythmias like atrial fibrillation is also well-established. The relationship between hypertrophy and arrhythmias could be favored by relative myocardial ischemia caused by the thickened ventricular wall, modified myocardial substrate related to fibrosis, and electrophysiological alterations in the hypertrophied myocardial cell. CaMKII appears to play an important role in ischemic and pressure overload induced hypertrophy, primarily by transcriptional regulation of hypertrophic genes. CaMKII-mediated phosphorylation of class II histone deacetylases (HDACs), especially HDAC4 and HDAC5, derepress myocyte enhance factor-2–mediated gene expression. Other laboratories also have shown that load attributable to aortic banding have attenuated cardiac expression. CaMKII HDAC5, derepress myocyte enhance factor-2–mediated gene expression. CaMKII-mediated phosphorylation of class II histone deacetylases (HDACs), especially HDAC4 and HDAC5, derepress myocyte enhance factor-2–mediated gene expression. CaMKIIδ phosphorylates HDAC4 preferentially compared with HDAC5, leading to activation of hypertrophic genes. CaMKIIδ knockout mice with pressure overload attributable to aortic banding have attenuated cardiac hypertrophy and less phosphorylated HDAC4 compared with controls. Other laboratories also have shown that while HDAC5 phosphorylation does not change in CaMKIIδ knockout mice subjected to pressure overload compared with controls, and that these mice are protected from progressive left ventricular dilatation, myocardial decompensation, and heart failure, but not from hypertrophy. Calcineurin, a Ca2+/calmodulin-dependent phosphatase, is a powerful prohypertrophic signal by dephosphorylating the nuclear factor for activated T-cells, leading to increased nuclear phosphorylation of PLN triggers mitochondria-dependent cell death, consistent with the effects of PLN knockout in CaMKIIδ transgenic mice in which mitochondrial Ca2+/Ca2+ and myocardial apoptosis are increased. Transgenic mice overexpressing mActin develop dilated cardiomyopathy with four-chamber enlargement, cardiac dysfunction, increased total and autophosphorylated CaMKII, increased expression of p53, and increased cardiomyocyte death. These maladaptive changes were ameliorated by CaMKII inhibition with KN-93 or interbreeding with AC3-I transgenic mice. Our group showed that mice with myocardial CaMKII inhibition attributable to transgenic expression of AC3-I were resistant to apoptosis by angiotensin II infusion and 2 hours and 5 hours after myocardial infarction. The AC3-I transgenic mice were protected from myocardial infarction–induced systolic dysfunction compared to WT and transgenic control mice. In a Langendorff-perfused isolated heart preparation, ischemia-reperfusion injury caused an increase in CaMKII activity, increased caspase 3 activity, and myocardial apoptosis, all of which were ameliorated by the addition of CaMKII inhibitory drug KN-93. We interpret these findings, in diverse models, to suggest that CaMKII-triggered apoptosis contributes to adverse left ventricular remodeling and mechanical dysfunction.

CaMKII is activated by catecholamine stimulation, and initially it was thought that CaMKII activation was exclusively attributable to increased intracellular Ca2+ that followed protein kinase A–dependent phosphorylation of Ca2+-homeostatic proteins. However, Zhu et al. showed that β-adrenergic receptor stimulation with isoproterenol can promote cardiomyocyte cell death by activating CaMKII, independent of protein kinase A in vitro. We found AC3-I mice were significantly resistant to isoproterenol-induced apoptosis in vivo. This resistance to isoproterenol-induced apoptosis was reduced in mice with myocardial AC3-I expression but lacking PLN, a negative regulator of sarcoplasmic reticulum Ca2+ uptake, by Ca2+ loading the SR even when CaMKII was inhibited. β-adrenergic receptor type 1 knockout mice are protected from increased CaMKII

**Cell Death and Fibrosis**

CaMKII-induced apoptosis appears to play an instrumental role in the development of heart failure after myocardial infarction and in the transition from compensated hypertrophy to decompensated cardiomyopathy in nonischemic animal models. CaMKII induces apoptosis by a variety of downstream mechanisms, some of which involve activation of proapoptotic proteases and others involve transcriptional control on proapoptotic pathways. CaMKII can activate AP24, a proapoptotic protease, leading to DNA fragmentation. CaMKII also can directly phosphorylate and activate proapoptotic factor BCL110. In addition, CaMKII can increase expression of proapoptotic genes that are downstream of the mitogen-activated protein kinase kinase TAK1 and ASK1. Recently, CaMKII has been implicated in mitochondrial-dependent prodeath pathways. Activation of CaMKII by a Ca2+/Ca2+ release–dependent pathway increases mitochondrial-triggered myocyte death. Endoplasmic reticulum stress triggers an increase in cytosolic Ca2+/Ca2+ and ROS that activates CaMKII, at least in part by oxidation, which can associate with mitochondria, where it promotes release of cytochrome C, loss of mitochondrial membrane potential, and mitochondrial-dependent apoptosis. CaMKII-dependent phosphorylation of PLN triggers mitochondria-dependent cell death, consistent with the effects of PLN knockout in CaMKIIδ transgenic mice in which mitochondrial Ca2+/Ca2+ and myocardial apoptosis are increased. Transgenic mice overexpressing mActin develop dilated cardiomyopathy with four-chamber enlargement, cardiac dysfunction, increased total and autophosphorylated CaMKII, increased expression of p53, and increased cardiomyocyte death. These maladaptive changes were ameliorated by CaMKII inhibition with KN-93 or interbreeding with AC3-I transgenic mice. Our group showed that mice with myocardial CaMKII inhibition attributable to transgenic expression of AC3-I were resistant to apoptosis by angiotensin II infusion and 2 hours and 5 hours after myocardial infarction. The AC3-I transgenic mice were protected from myocardial infarction–induced systolic dysfunction compared to WT and transgenic control mice. In a Langendorff-perfused isolated heart preparation, ischemia-reperfusion injury caused an increase in CaMKII activity, increased caspase 3 activity, and myocardial apoptosis, all of which were ameliorated by the addition of CaMKII inhibitory drug KN-93. We interpret these findings, in diverse models, to suggest that CaMKII-triggered apoptosis contributes to adverse left ventricular remodeling and mechanical dysfunction.

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activity and cell death after myocardial infarction, consistent with findings that show CaMKII binds selectively to type 1 β-adrenergic receptors. Recently, an alternative pathway for activating CaMKII after myocardial infarction has been shown by our group. This pathway involved the activation of CaMKII by oxidation of the methionine 281/282 residues in the regulatory domain. We found that myocardial infarction or angiotensin II caused increased oxidized CaMKII leading to cell death and mechanical dysfunction. Subsequently, other investigators showed that angiotensin II caused myocyte death mediated by ROS-dependent CaMKII activation causing p38 MAPK activation. Thus, a variety of upstream signals converge on CaMKII to increase apoptosis, mechanical dysfunction, and premature death.

The role of CaMKII to increase cell death may be linked to arrhythmias. We recently found that CaMKII activation can cause cellular arrhythmias and death by phosphorylating an accessory β subunit of the voltage-gated Ca²⁺ channel CaV1.2. CaMKII catalyzes phosphorylation of threonine 498 on β²a, leading to increased Ca²⁺ current, increased sarcoplasmic reticulum Ca²⁺, arrhythmia-initiating afterdepolarizations and cell death. Both afterdepolarizations and cell death could be reduced or prevented by CaMKII inhibition, inhibition of sarcoplasmic reticulum Ca²⁺ release, or by elimination of the β²a, CaMKII phosphorylation site. Mice with transgenic myocardial overexpression of CaMKIIβ show increased myocardial apoptosis, afterdepolarizations, arrhythmias, and premature death. We interpret these findings to suggest that prodeath actions of excessive CaMKII activity are likely to promote heart failure and to contribute proarrhythmic tissues substrates.

Sinus Node Physiology and Dysfunction

The rhythm in the mammalian heart is set by autonomous excitation of the specialized pacemaker cells in the SA node. In conditions requiring increased cardiac output, the heart ramps up its beating rate while maintaining rhythm. Until recently, the spontaneous action potentials in SA node cells were thought to be exclusively governed by a hyperpolarization-activated cyclic nucleotide-gated channels (HCN4). In the past two decades, our understanding of this process has been refined by experimental evidence describing the role of intracellular Ca²⁺ cycling in pacemaker activity. Coordinated action between SERCA and RyR in sino-atrial node (SAN) cells causes rhythmic Ca²⁺ oscillations, setting up a series of events leading to a net positive charge in the cell in late diastole, culminating in action potential initiation and enhanced automaticity.

Localized Ca²⁺ release events were shown by Vinogradova et al to be dependent on CaMKII activity, because inhibition of CaMKII by AIP, an inhibitory peptide, or KN-93 led to decreased ICa,L amplitude and SAN action potential formation. We recently showed that CaMKII plays an essential role in increasing heart rate in response to fight or flight, mediating catecholamine-induced heart rate increases by increasing SR Ca²⁺ content, SR Ca²⁺ release, and inward INa, leading to enhancement of the diastolic depolarization rate. In contrast, CaMKII did not appear to affect the HCN4-mediated pacemaker current (I). We also showed that mice with genetic CaMKII inhibition failed to increase diastolic Ca²⁺ sparks, SR Ca²⁺, and diastolic depolarization rate in response to isoproterenol, even though their resting heart rates were similar to those of WT mice. Recent work from our laboratory shows that CaMKII activity not only is important in β-adrenergic receptor stimulation–induced heart rate response but also is necessary for catecholamine-independent chronotropic competence. We activated Ca²⁺-dependent pacing mechanisms directly by applying the L-type Ca²⁺ channel agonist Bay K8644 directly to SAN cells and Langendorff-perfused isolated hearts. BayK 8644 increased heart rate in a dose-dependent fashion over a similar dynamic range as isoproterenol, but without increasing I, suggesting that Ca²⁺-dependent mechanisms were fully capable of generating physiological fight or flight heart rate responses. SAN cells and isolated hearts from mice with genetic CaMKII inhibition were refractory to heart rate increases and had reduced late diastolic intracellular Ca²⁺ release and INa when treated with BayK 8644 compared with WT controls, even though Bay K increased ICa equally in all SAN cells. These findings suggested that CaMKII contributes to heart rate by a series of events that promote SR Ca²⁺ release and INa in SAN cells.

Although CaMKII activity is essential for the proper functioning of the cardiac pacemaker cells, excess CaMKII has deleterious effects resulting in SA node dysfunction. Recently, we showed that angiotensin II, a neurohormone found in excess in heart failure, when infused into mice, increased SA nodal oxidized CaMKII, increased SA node cell death, and decreased resting heart rate and activity-related heart rate increases. Interestingly, human patients with heart failure and SA node dysfunction had increased oxidized CaMKII in the right atria compared with patients with heart failure but no SA node dysfunction. Mice with genetic cardiac CaMKII inhibition and mice lacking a critical subunit (p47) of important myocardial NADPA oxidases were protected from angiotensin II–induced SA node cell death, fibrosis, and dysfunction, indicating that NADPH oxidase–mediated oxidation of CaMKII plays an essential role in angiotensin II–induced toxicity in the SA node. We also showed that SA node targeted CaMKII inhibition was sufficient to protect against SA node effects of angiotensin II infusion. We developed a computational model that showed that angiotensin II–induced cell death in the SA node caused defective impulse formation and propagation by creating a source-sink mismatch attributable to loss of SA node cells relative to surrounding atrial myocardium, leading to sinus node dysfunction (Figure 5). These data suggest that although CaMKII is critical for cardiac pacemaking and maintenance of a normal sinus rhythm, excess CaMKII, especially in conditions of increased oxidant stress, such as heart failure and/or angiotensin II infusion, promotes cardiac arrhythmias.

Atrial Fibrillation

Emerging evidence suggests that CaMKII may play an important role in modulating signaling pathways leading to atrial fibrillation, a rhythm that is particularly common in patients with heart failure and SA node dysfunction. CaMKII is activated by upstream signaling pathways, including catecholamines, the renin-angiotensin-aldosterone system, and inflammatory cytokines.
system, and ROS, that are critical to structural and electric remodeling in heart failure and atrial fibrillation. Most fundamental evidence of the role of CaMKII in atrial fibrillation comes from mouse models in which molecular hypotheses can be directly tested. However, it is important to examine this evidence in the context of the clinical disease and data acquired from clinical and translational studies. Atrial myocytes from patients with atrial fibrillation express elevated levels of CaMKII and show increased phosphorylation of downstream target proteins RyR2 and PLN. Interestingly, atrial myocytes from patients with atrial fibrillation also show increased spontaneous Ca$^{2+}$ spark frequency that is normalized by CaMKII inhibition.

In many cases, atrial fibrillation is thought to originate in pulmonary vein ostia in the left atrium, in isolated rabbit pulmonary vein tissue, isoproterenol stimulates nonsustained spontaneous arrhythmias that are suppressed by the CaMKII inhibitor, KN-93. Transgenic mice expressing the CaMKII inhibitory peptide AC3-I or knock-in mice with CaMKII-resistant RyR2 Ser 2814 Ala have proved to be useful in testing the role of CaMKII and SR calcium release in atrial fibrillation without potential off-target actions of currently available small-molecule CaMKII inhibitors. RyR2 R176Q knock-in mice have enhanced diastolic SR Ca$^{2+}$ leak, and rapid atrial pacing increases phosphorylation of RyR2 Ser 2814, a validated CaMKII site, leading to significantly more atrial fibrillation as compared to WT mice. Mice with cardiac-specific overexpression of CREM-Ib ΔC-X have spontaneous atrial fibrillation, and atrial myocytes isolated from these mice show increased phosphorylation of RyR2 at Ser 2814. FKBP12.6 is a protein that is hypothesized to stabilize RyR2 and mice lacking FKBP12.6 are prone to spontaneous and pacing-induced atrial fibrillation. Taken together, these studies suggest an important role of CaMKII activation and subsequent RyR2 phosphorylation in contributing to atrial fibrillation.

**Ventricular Arrhythmias**

CaMKII inhibition, through pharmacological and/or genetic means, has been shown to prevent or suppress ventricular arrhythmias in a variety of animal models of both acquired and congenital cardiac disease. In fact, the antiarrhythmic benefit of targeted disruption in CaMKII signaling extends across a relatively broad range of disorders with unique phenotypic presentations.

**Congenital Arrhythmia Disorders**

CaMKII has emerged as a novel antiarrhythmic target in several forms of congenital heart disease that, for the most part, confer arrhythmia susceptibility by interfering with normal intracellular Ca$^{2+}$ cycling. Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmia syndrome characterized by stress-induced arrhythmias in a structurally normal heart without any electrocardiographic indications under resting conditions. Genetic mutations in RyR2 are the primary cause of CPVT. The role of CaMKII in regulating Ca$^{2+}$ cycling and its involvement in the β-adrenergic receptor signaling cascade make it a logical therapeutic target in CPVT. Consistent with this hypothesis, pharmacological CaMKII inhibition recently has been shown to completely eliminate stress-induced arrhythmias and triggered activity in the R4496C mouse model of CPVT.

Timothy syndrome (also referred to as long-QT syndrome 8) is a rare autosomal-dominant disease caused by mutations in the L-type Ca$^{2+}$ channel (Ca$_{1.2}$) and is characterized by ventricular tachycardia and sudden death. At the cellular level, Ca$_{1.2}$ Timothy syndrome mutations result in impaired Ca$_{1.2}$ voltage-dependent inactivation and inappropriate Ca$^{2+}$ entry that, in turn, activates intracellular signaling cascades, including CaMKII. In some critical aspects, CaMKII activation phenocopies Timothy syndrome by promoting action potential prolongation and arrhythmias. Recent studies expressed a Timothy syndrome mutant (Gly 406 Arg) Ca$_{1.2}$ in adult rat ventricular myocytes to examine
the therapeutic potential of CaMKII inhibition in Timothy syndrome. Consistent with the proposed link between increased Ca\(^{2+}\) entry, CaMKII activation, and arrhythmias, CaMKII inhibition normalized action potential duration, SR Ca\(^{2+}\) content, and SR Ca\(^{2+}\) transients, and prevented arrhythmogenic afterdepolarizations in myocytes expressing the Timothy syndrome mutant Ca\(_{\alpha}1.2\). These findings support the idea that CaMKII represents a promising therapeutic target for preventing arrhythmias associated with Timothy syndrome, similar to CPVT. Furthermore, it is interesting to consider the likelihood that, in a broader sense, genetic arrhythmias associated with inappropriate Ca\(^{2+}\) cycling (eg, CPVT, Timothy syndrome, other long-QT syndromes) may respond favorably to CaMKII inhibition.

**Common Forms of Acquired Ventricular Arrhythmias**

Of course, arrhythmias linked to defects in Ca\(^{2+}\) signaling are not restricted to rare congenital forms of heart disease, and CaMKII inhibition has been shown to prevent ventricular arrhythmias in a variety of animal models of acquired cardiac disease. Early studies showed that CaMKII inhibition prevented arrhythmias and afterdepolarizations in transgenic mice with cardiac hypertrophy induced by overexpression of constitutively active CaMKIV. For uncertain reasons, the CaMKIV transgenic mice also have elevated levels of myocardial CaMKII. Similarly, improved left ventricular function and decreased arrhythmias were observed with CaMKII inhibition in calcineurin-overexpressing mice with severe cardiomyopathy. Finally, CaMKII inhibition prevents cellular afterdepolarizations and isoproterenol-induced arrhythmias in vivo in transgenic mice overexpressing CaMKII\(\theta\) with hypertrophic and dilated cardiomyopathy. Going forward, it will be interesting to dissect the specific contributions of individual CaMKII targets to better-understand the beneficial effects of CaMKII inhibition in hypertrophy and heart failure. For example, recent work has shown that transgenic mice lacking the CaMKII phosphorylation site on the ryanodine receptor SR Ca\(^{2+}\) release channel are resistant to pacing-induced ventricular arrhythmias after aortic banding surgery. Consistent with this finding, CaMKII inhibition has been shown to reduce RyR phosphorylation and SR Ca\(^{2+}\) leak in a nonischemic rabbit model of heart failure. CaMKII inhibition has also been shown to be effective in rabbit chronic atrioventricular block models of acquired long-QT and electrical storm.

The antiarrhythmic benefit of CaMKII inhibition also has been explored in ischemic heart disease. Importantly, transgenic inhibition of CaMKII prevents structural remodeling after myocardial infarction. Furthermore, elevated levels of autophosphorylated and oxidized CaMKII have been reported in a well-established canine model of arrhythmias attributable to myocardial infarction. Parallel computational studies indicate that CaMKII overactivity contributes to the arrhythmogenic substrate after myocardial infarction, with CaMKII inhibition improving Ca\(^{2+}\) cycling and voltage-gated Na\(^{+}\) channel function in the infarct border zone. Taken together, these studies in both nonischemic and ischemic animal models highlight the therapeutic antiarrhythmic potential of CaMKII across a broad spectrum of acquired cardiac disease states.

**Systems Biology and CaMKII Signaling**

As we attempt to elucidate the critical pathways linking mechanical and electric dysfunction in the failing heart, it will be important to consider alternative approaches that are well-suited for studying large and multiscale systems. Mathematical modeling has been applied for more than half a century to provide important insight into fundamental processes governing complex biological systems. Recent advances in biology (particularly in genetics) along with technological advances in computing have elevated the profile of systems level approaches capable of integrating and analyzing large networks of data. With its vast network of substrates and interacting proteins, CaMKII is an attractive target for a systems biology approach that combines “wet bench” science with computational biology and mathematical modeling to develop a quantitative understanding of CaMKII signaling pathways. Furthermore, the multi-numeric nature of the CaMKII holoenzyme and its multiple modes of regulation (eg, Ca\(^{2+}\)/calmodulin, autophosphorylation, oxidation) lend themselves to a computational approach that is capable of answering questions difficult or even impossible to address in a biological experiment.

Systems biology already has generated important insights into the behavior and function of CaMKII in excitable cells. Early mathematical modeling studies demonstrated that CaMKII at the neuronal postsynaptic density is a critical mediator of long-term memory. Specifically, these studies showed that because of its unique structural and biophysical properties, CaMKII operates as a molecular “switch” that can store information in a stable manner. Although early computational studies focused on the role of CaMKII in neuronal function, recent efforts have analyzed CaMKII dynamics and function in heart. Notably, mathematical models of CaMKII activity have been successfully incorporated into whole-cell models of the cardiac action potential to investigate CaMKII function in the broader context of the intact cell. These detailed models have advanced the field by helping to integrate the extensive and growing literature on CaMKII regulatory network in the heart, and by increasing our understanding of CaMKII function in both normal and diseased states. For example, computational models have predicted an important role for CaMKII in the normal response of cardiac excitability and contractility to changes in pacing rate. In fact, computer simulations have shown that CaMKII is ideally suited to detect pacing frequency (eg, heart rate), and may effectively store the pacing history of a cell, similar to its role in neurons. The key features that allow CaMKII to sense pacing are its sensitivity to Ca\(^{2+}\)/CaM and the ability for subunits to phosphorylate neighboring subunits (autophosphorylation). These studies address a critical outstanding question related to CaMKII in the heart. Namely, with mounting evidence supporting a view of CaMKII as a purveyor of dysfunction in disease, why do cardiomyocytes express CaMKII at all? Dynamic modeling is
just one area in which systems level approaches have contributed to our understanding of CaMKII function in the heart. Computational biology also has helped identify potential new CaMKII targets and interacting proteins. A computational screen of the human genome using a consensus CaMKII-binding sequence found in the β2a subunit of the L-type \( \text{Ca}^{2+} \) channel generated a select list of potential binding partners, including cytoskeletal, mitochondrial, nuclear, and membrane proteins. One of these proteins, the actin-associated polypeptide \( \beta_{\text{IV}} \)-spectrin, was validated as a CaMKII anchoring protein in vivo that targets CaMKII to the cardiomyocyte intercalated disc.

Systems biology also has identified important roles for CaMKII in abnormal cell function in the diseased heart. Specifically, computational models have predicted a role for CaMKII overactivity in creating a substrate for arrhythmias in both congenital and acquired heart disease. After myocardial infarction, extensive ion channel and action potential remodeling is a hallmark of infarct border zone regions where arrhythmias are highly localized. Computer models have helped identify CaMKII overactivity (downstream of increased oxidation and auto-phosphorylation) as a nexus for proarrhythmic changes in mechanical and electric function. Similarly, other studies have shown that CaMKII may promote inappropriate membrane depolarizations (afterdepolarizations) that serve as arrhythmia triggers through direct phosphorylation of the L-type \( \text{Ca}^{2+} \) channel and/or \( \text{RyR2 SR Ca}^{2+} \) release channel. More recently, computational modeling has shown that loss of SA node cells secondary to CaMKII overactivity can produce SA node dysfunction by changing the source–sink relationship between the primary pacemaker and surrounding atrial tissue. Thus, models illustrate the multifactorial way in which CaMKII can regulate not only the trigger but also the substrate for life-threatening arrhythmias.

Going forward, systems biology will undoubtedly play a critical role in addressing some of the important unanswered questions in CaMKII pathophysiology. For example, how does CaMKII coordinate the heart’s fight-or-flight response to β-adrenergic receptor stimulation? Is it clear that CaMKII regulates the response of working myocardium to increases in heart rate, but how does CaMKII regulate heart rate itself? How is fidelity of CaMKII signaling maintained within the cell? Dedicated CaM tethered to substrates is one potential mechanism for controlling CaMKII signaling. Alternatively, CaMKII anchoring proteins such as \( \beta_{\text{IV}} \)-spectrin may define subcellular CaMKII signaling domains. To what extent do these pathways fine-tune CaMKII regulation within the cell? How does CaMKII transform from an adaptive to a maladaptive signaling node in disease? Clearly, there is overlap between the cellular pathways involved in regulating normal heart function and disease. What determines the breaking point when a physiological CaMKII response becomes detrimental? Can we eliminate or mitigate the negative elements of CaMKII signaling while preserving heart function (eg, response to β-adrenergic receptor stimulation)? Finally, with the ever-expanding list of CaMKII targets, mathematical models will become increasingly important for understanding broader roles for CaMKII in cardiomyocytes (eg, apoptosis, mitochondrial energetic, and pacemaking) as well as in other organ systems (eg, brain, vasculature, pancreas).

Summary
Understanding of the roles for CaMKII in physiology and disease in the heart has advanced dramatically over the past decade. Accumulated information is now sufficient to show CaMKII is a validated molecular mechanism and a potential therapeutic target for heart failure and arrhythmias. Much remains to be learned about how CaMKII integrates oxidant stress and \( \text{Ca}^{2+} \) to target specific proteins in cardiovascular and other systems. Findings in the cardiovascular system may be relevant to other diseases in other systems in which elevated ROS and disrupted \( \text{Ca}^{2+} \) homeostasis are features of disease.

Acknowledgments
The authors are grateful for artistic contributions of Mr. Shawn Roach.

Sources of Funding
This work was funded in part by National Institutes of Health (NIH) grants R01 HL 079031, R01 HL 096652, R01 HL 113001, and R01 HL 070250, the University of Iowa Research Foundation and the Fondation Leducq Transatlantic Alliance for CaMKII Signaling. P.D. Swaminiathan’s salary was supported by a Kenneth M. Rosen Fellowship and Max Schaldach Fellowship from the Heart Rhythm Society and a University of Iowa Cardiovascular Center Interdisciplinary Research Fellowship. A. Purohit’s salary was supported by American Heart Association postdoctoral fellowship grant 10POST3620047. T.J. Hund has support from NIH grant R00 HL 096805 and the Gilead Sciences Research Scholars Program in Cardiovascular Disease.

Disclosures
Dr Anderson is a cofounder of Allosteros Therapeutics and an inventor on patents claiming to treat heart failure and arrhythmias by CaMKII inhibition.

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_Circ Res._ 2012;110;1661-1677
doi: 10.1161/CIRCRESAHA.111.243956
_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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