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CaMKII: 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 last 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-independeant, 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 reducing arrhythmias. Here we review the molecular physiology of CaMKII, 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.

Keywords

CaMKII; Arrhythmias; Heart Failure; Ion channels; Remodeling

Introduction

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]. While heart failure is a mechanical problem, mostly of contraction (inotropy) but also of relaxation (lusitropy), many heart failure patients suffer from arrhythmias, an electrical problem where cell

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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 will consider 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 (ECC), 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 (Fig 1).

Upstream activator signals for CaMKII

Loss of normal myocardial Ca^{2+} homeostasis and increased reactive oxygen species (ROS) are common ‘upstream’ signals that contribute to arrhythmias and heart failure [6,7] . However, concise molecular targets and signaling pathways connecting Ca^{2+} and ROS to arrhythmias and heart failure have been elusive, while antioxidant therapies have had modest or no benefit[8,8,9], 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[7]. There are four CaMKII gene products (α-δ), which are homologous, share activation mechanisms and are thought to co-assemble as heteromultimers. The four CaMKII isoforms are differentially expressed in various cells types. For example, CaMKII α and (β are relatively enriched in neuronal tissues and CaMKII δ and γ 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 Fig 2) between the association domain and the C terminus of the regulatory domain that has a nuclear localization sequence in one CaMKIIδ splice variant ($CaMKII\delta_3$ or $CaMKII\delta_B$) but not in other CaMKIIδ splice variants ($CaMKII\delta_2$ or $CaMKII\delta_C$)[10]. However, the specific role of this sequence remains unclear as recent data suggest that both $CaMKII\delta_B$ and $CaMKII\delta_C$ are present in nuclear and cytoplasmic compartments [11]. 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[11–13]. 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 Ca^{2+}/CaM inaccessible, while CaMKII variants with longer linkers are relatively extended resulting in a larger Ca^{2+}/CaM accessible binding surface[14]. 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 are also 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[15]. The regulatory domain resides between the catalytic and association domains (Fig 2). CaMKII is initially activated by binding Ca^{2+}/CaM, because Ca^{2+}/CaM binding to the C terminal aspect of the regulatory domain (i.e. the CaM-binding region) distorts and disables the negative regulatory effect of the autoinhibitory region to constrain the catalytic domain -converting inactive CaMKII to a 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 threonine 287 autophosphorylation (the specific numbering varies for each CaMKII isoform), leading to two important changes. The first is 'CaM trapping' where the binding affinity for Ca^{2+}/CaM-CaMKII increases by ~10^{516} . The second is that even after Ca^{2+}/CaM-CaMKII unbinding, CaMKII retains residual, Ca^{2+}/CaM-independent activity[17,18]. Autophosphorylation of threonine 306 can prevent CaM trapping by interfering with Ca^{2+}/CaM-CaMKII binding[19]. Oxidation of paired methionines (281/282) causes a similar Ca^{2+}/CaM-independent form of CaMKII[20] . In contrast to autophosphorylation, methionine oxidation does not cause CaM trapping, because oxidation of methionine (308 in CaMKIIδ) reduces the affinity of $^{2+}$ Ca^{2+}/CaM-CaMKII binding.

The first stage of methionine oxidation (methionine sulfuxide) is enzymatically reversible by methionine sulfoxide reductase A (MsrA), while 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 (MI)[20], while MsrA over-expression prevents CaMKII oxidation and is protective against post-MI cardiac rupture in mice[12]. MsrA over-expression prolongs lifespan in flies (*D. melanogaster*)[21], while MsrA knock out increases mortality in mice[22] and worms (*C elegans*)[23]. These findings suggest that CaMKII is a critical target for the benefits of MsrA over-expression 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), while CaMKII α has a cysteine-methionine pair that appears to induce Ca^{2+}/CaM-independent CaMKII activity, similar to oxidized methionine pairs. Although CaMKII requires Ca^{2+}/CaM binding for initial activation, oxidized CaMKII (ox-CaMKII) resets the Ca^{2+}-sensitivity of CaMKII so that very low levels of intracellular Ca^{2+} are sufficient for activation[24], suggesting that conditions of high ROS may lead to increased CaMKII activity even at resting levels of Ca^{2+}/CaM. Thus, autophosphorylation and oxidation both have the capacity to 'lock' CaMKII into a Ca^{2+}/CaM-autonomous conformation with sustained activity. Oxidized, constitutively active CaMKII is now linked to heart failure[20], tachyarrhythmias[25–27] , bradyarrhythmias[28] and post-myocardial infarction cardiac rupture,[12]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 sarcolemmal domains enriched with ion channels in close approximation with sarcoplasmic reticulum (SR) Ca^{2+} release sites (Fig 3). This ultrastructure allows for feedback between intracellular Ca^{2+} and sarcolemmal 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 ECC machinery also determines the association between heart failure, arrhythmias and sudden cardiac death. CaMKII is enriched in the vicinity of the T-tubular sarcolemmal membrane invaginations[29,30] that form the ultrastructural framework for closely (~10 nm) approximating the SR Ca^{2+} release ryanodine receptor (RyR2) channels and sarcolemmal voltage-gated Ca^{2+} channels. The

presence of CaMKII at $Ca_V1.2$ (the predominant voltage-gated Ca^{2+} channel in ventricular muscle) and at RyR2 may serve to coordinate and promote physiological activation of these ECC proteins[31] to increase the force-frequency relationship (i.e. the tendency of myocardium to generate more tension at faster stimulation rates)[32] and/or to engage 'fight or flight' acceleration of heart rate[33,34]. CaMKII is now recognized to bind to multiple voltage-gated ion channels (Ca^{2+}, Na^+ and K^+)[35], some of which will be 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[7]. The enrichment of CaMKII in particular subcellular domains[29,36] 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[37] and Receptors for Activated C Kinase[38] 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 may mimic the topology of its own regulatory domain autoinhibitory region (Figure 4). We identified short sequences with high homology to the CaMKIIδ autoinhibitory region on a wide variety of protein targets, and validated that these sequences are necessary for proarrhythmic effects of CaMKII on $Ca_V1.2$[39] and $Na_V1.5$[36]. Thus, CaMKII is enriched in at least one subcellular domain, marked by T-tubular membranes, that is a hotspot for arrhythmias and heart failure.

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 under 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 neurohormonal activity contributes to increased intracellular Ca^{2+} and ROS that are thought to promote disease progression [40]. CaMKII is activated downstream to the three neurohormone agonists validated to be therapeutic targets for heart failure and arrhythmias. Most evidence for these pathways – β adrenergic receptor[41], angiotensin II[20] and aldosterone[12] is for heart failure after myocardial infarction[42–44]. Mice lacking α-adrenoreceptors develop sympathetic hyperactivity-induced heart failure, increased mortality and had elevated total and threonine 287-autophosphorylated CaMKII levels compared to WT animals. Dilated cardiomyopathy, exercise intolerance and CaMKII activity in these mice were partially reversed by an angiotensin II receptor antagonist[45], further suggesting that the success of angiotensin II antagonist drug therapy is at least partially due to CaMKII inhibition. Several studies have shown the role of CaMKII in the causation and progression of cardiac hypertrophy[1,46–48], an independent risk factor for developing heart failure[2,49]. CaMKII is increased at the transcriptional level, protein level and in its activity in heart failure[3,50]. Heart failure occurs in mice with transgenic CaMKII over-expression[4,51] and myocardial infarction causing heart failure also occurs with increases in myocardial CaMKII activity and expression in mice[5,12], rabbits [6,7,52], dogs[8,8,9,53] and patients[7,54]. CaMKII activation may be related to ventricular wall stress, as dogs with heart failure due to left bundle branch ablation and rapid pacing showed increased CaMKII activity in the lateral left ventricular wall compared to the septum. Furthermore, the increase in lateral wall CaMKII activity and expression was normalized by cardiac resynchronization (biventricular) pacing[55]. Although little is known about the transcriptional programs that increase CaMKII expression in heart failure, calcineurin activity and/or expression increase in animal models[10,56] and patients with structural heart disease[11,57,58] and calcineurin promotes myocardial expression of CaMKII[11–13,59]. This example may point to a broader paradigm where interdependence between various pathological signals

ultimately produce severe, 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[14,60]. CaMKII protein levels and activity are markedly increased in calcineurin transgenic mice compared to WT littermates and that CaMKII inhibition improved mechanical function and reduced mortality, but with minimal impact on myocardial hypertrophy[15,59], suggesting that CaMKII inhibition could reduce heart failure and sudden death even in the face of forced over-expression of calcineurin. Taken together, these findings suggest that CaMKII may play a crucial role in myocardial hypertrophy and heart failure, while 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 due to either autophosphorylation of threonine 287 or oxidation of methionines 281 and 282 (Fig 1); these post-transcriptionally modified amino acids are located in the CaMKII regulatory domain (Fig 2). Autophosphorylated CaMKII levels were elevated in mice up to 7 days after transaortic constriction, a surgical model of acute pathological left ventricular afterload[16,51]. Autophosphorylated CaMKII[17,18] was increased in a pacing-induced rabbit model of left ventricular dysfunction and incessant ventricular tachycardia (VT storm)[61]. 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[19,61]. Angiotensin II[20] and aldosterone[12] appear to increase ROS by activating NADPH oxidase. We found that oxidized CaMKII levels were significantly elevated in mouse heart[12] and in the border zone of dog hearts days after myocardial infarction[21,53], suggesting that oxidized CaMKII is a transduction signal for the increased oxidant stress that accompanies common forms of structural heart disease[7,22]. Intriguingly, CaMKII oxidation is reversed by MsrA and mice lacking MsrA develop increased levels of oxidized CaMKII, myocardial apoptosis, adverse left ventricular remodeling[20,23], increased frequency of cardiac rupture and death after myocardial infarction[12,24]. In contrast, mice with myocardial transgenic MsrA over-expression show reduced mortality and are protected from aldosterone and myocardial infarction induced increases in oxidized CaMKII [12,20], suggesting that selectively targeted anti-oxidant 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. CaMKIIδ mRNA and protein were significantly elevated in hearts from patients with dilated cardiomyopathy, compared to hearts from donors without structural heart disease[25–27,62]. Left ventricular tissue from failing human hearts with dilated, non-ischemic cardiomyopathy showed increased CaMKII activity compared to non-failing hearts, while myocardial tissue from ischemic cardiomyopathy patients had a trend towards elevation in left ventricular CaMKII activity[28,63]. Protein levels of cytosolic and nuclear splice variants of CaMKIIδ were higher in failing human right and left ventricle than in controls, regardless of whether cardiomyopathy was due to ischemic or non-ischemic causes[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 to normal controls. We found that oxidized CaMKII levels are elevated in the right atrium of heart failure patients in the vicinity of the sinoatrial node. The increase in oxidized CaMKII is particularly pronounced in heart failure patients with co-existent sinoatrial (SA) node dysfunction, compared to those with similarly severe heart failure but no SA node dysfunction [7,28]. 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 multi-subunit protein complexes built around a transmembrane permeation pore. A full discussion of CaMKII and ion channels is beyond the scope of this review, but is addressed more completely in other recently published reviews [29,30,64]. CaMKII was first identified to increase L-type Ca^{2+} current (I_{Ca}), where CaMKII mediated phosphorylation induced a dynamic pattern of increasing peak current accompanied by slowing of inactivation[31,65–67]. 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 where CaMKII actions are thought to contribute to arrhythmias.

L-type Ca^{2+} Channels

CaMKII-catalyzed phosphorylation of L-type Ca^{2+} channels ($Ca_V1.2$) promotes entry into a highly active gating mode (mode 2), characterized by a high opening probability and prolonged opening events[32,68]. Mode 2 gating appears to underlie I_{Ca} facilitation, a dynamic pattern of increased peak I_{Ca} and slowed I_{Ca} inactivation, based on modeling studies[33,34,69] and because mutations that prevent mode 2 gating also reduce I_{Ca} facilitation[35,39]. Mode 2 gating[7,70] and I_{Ca} facilitation are hypothesized to be proarrhythmic by favoring early afterdepolarizations (EADs) [29,29,36,39,71]and SR Ca^{2+} leak[37,72], inward Na^+/Ca^{2+} exchanger current[38,73] and delayed afterdepolarizations (DADs)[39,74]. EADs and DADs of sufficient magnitude are able to trigger arrhythmias. Increased $Ca_V1.2$ current can contribute to action potential prolongation[36,75] and mode 2 gating is particularly proarrhythmic in the setting of action potential prolongation[40,76]. Mode 2 gating was first identified using BayK,[70], and BayK 8644 induces polymorphic ventricular tachycardia in an established rabbit model of QT interval prolongation and Torsade de Pointes[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, (e.g. long QT syndromes and cardiomyopathy). L-type Ca^{2+} channels are formed as a constellation of subunit proteins, including the α (pore-forming) and β (regulatory) subunits. The β subunit acts as a chaperone to increase α subunit membrane expression[12,79] and engages the α subunit to increase channel opening probability [30,39,42–44,80]. CaMKII does not appear to affect the chaperone activity of β subunits, because WT β subunit over-expression and over-expression of β subunit mutants lacking CaMKII binding and phosphorylation sites are equally effective at increasing I_{Ca} density in cultured adult cardiomyocytes [30,39,45]. CaMKII binding to β subunits is enhanced by Leu 493 and CaMKII-catalyzed phosphorylation of Thr 498 is critical for facilitation of I_{Ca} in isolated adult rat[30] and rabbit[39] ventricular myocytes. The Thr 498 phosphorylation site is conserved across β subunit isoforms, but was initially identified on isoform β_{2a}. The expression of the β_{2a} isoform is increased in failing human hearts[81], a condition marked by increased $Ca_V1.2$ opening probability[82], APD prolongation, loss of intracellular Ca^{2+} homeostasis[83], EADs[84] and excessive cardiomyocyte death[85]. Over-expression of WT β_{2a} in adult cardiomyocytes increases I_{Ca}, induces SR Ca^{2+} overload, and stimulates apoptosis by a pathway that involves CaMKII[82]. However, over-expression of a β_{2a} mutant lacking this CaMKII phosphorylation site (T498A) does not increase myocyte death or EADs[39], supporting the hypothesis that CaMKII phosphorylation of β_{2a} is a molecular mechanism for pathological membrane excitability and cardiomyocyte death.

Voltage-gated Na^+ Channels

Failing myocardium is marked by an electrical remodeling process where reduction in net repolarizing inward current leads to action potential and QT interval prolongation. Although

cardiac voltage-gated Na^+ channels (mostly $Na_V1.5$) open and close rapidly (1–10 ms), a persistent (late) component of Na^+ current (I_{NaL}), appears to contribute to action potential prolongation in a rare form of the long QT syndrome (LQT3)[86] and in myocytes isolated from failing hearts[87]. Evolving evidence suggests that CaMKII associates with and phosphorylates cardiac Na^+ channels[25,88]. CaMKII binding motifs have been localized to the inter domain I–II linker and the C-terminal region[36,88–90]. Interestingly, CaMKII phosphorylation has contrasting effects on I_{Na}, as it enhances late non-inactivating I_{NaL} but also decreases the availability of $Na_V1.5$, a phenocopy of I_{Na} alterations seen during ischemia and heart failure and in LQT3[90,91]. CaMKII inhibition reverses heart failure-induced increases in I_{NaL}[36,88], suggesting that $Na_V1.5$ is an important target for the proarrhythmic actions of CaMKII.

Voltage-gated K^+ Channels

Voltage-gated K currents (I_K) are the major driving force for myocardial membrane repolarization. I_K is due to multiple ion channel proteins with diverse kinetic properties. Collectively, I_K plays an important role in determining the action potential configuration and duration and multiple genetic long QT syndromes linked to arrhythmic sudden death are due to biophysical and trafficking defects in I_K. Furthermore, reduction in I_K 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})[92–95] and inwardly rectifying (I_{K1}) K^+ current[96]. I_{to} is comprised of $I_{to,fast}$ ($K_V4.2$/ $K_V4.3$) and $I_{to,slow}$ ($K_V1.4$), and site-directed mutagenesis studies have identified $K_V4.3$ S550[95] and $K_V1.4$ Thr602[97] as potential phosphorylation targets for CaMKII. Increased CaMKII activity has been shown to slow I_{to} inactivation and 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[98], likely in part by downregulation of $I_{to,fast}$ and upregulation of $I_{to,slow}$, events that occur in failing cardiomyocytes[96]. Heart failure is also characterized by reduction in I_{K1}, responsible for maintaining stable resting membrane potential. Interestingly, chronic CaMKII over-expression also leads to downregulation of I_{K1} and conversely, chronic CaMKII inhibition increases I_{K1} current[96]. Thus, reduction in various K^+ currents contributes to proarrhythmic action potential prolongation in long QT syndromes and heart failure. CaMKII over-expression also causes reduced repolarizing K^+ current and CaMKII inhibition shortens action potentials[41], likely by increasing K^+ currents. However, CaMKII actions are complex and appear to include transcriptional and post-transcriptional 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.

Ca^{2+} cycling proteins

Ryanodine receptor (RyR2), phospholamban (PLN) and SR Ca-ATPase (SERCA) are well-known regulators of SR calcium uptake and release and RyR2[99] and PLN[100] 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 Ca^{2+} content and SR Ca^{2+} release during systole [83,101–103]. Low SR calcium content may be a result of decreased uptake of Ca^{2+} by SERCA and/or increased diastolic SR calcium release (‘leak’). Abundant evidence now supports an important role of CaMKII in promoting heart failure and arrhythmias[104]. RyR2 is a SR Ca^{2+} release channel that is activated by a trigger of Ca^{2+} from I_{Ca} [105]. RyR2 phosphorylation by protein kinase A and CaMKII both enhance I_{Ca} and RyR2 Ca^{2+} release. CaMKII helps coordinate this physiological process of Ca^{2+}-induced Ca^{2+} release by phosphorylation of $Ca_V1.2$ and RyR2. However, in failing myocytes the cell membrane ultrastructure supporting Ca^{2+}-induced Ca^{2+} release is distorted[106] and CaMKII hyperphosphorylation of $Ca_V1.2$ and RyR2 becomes arrhythmogenic. CaMKII

hyperphosphorylation of serine 2814 on RyR2 promotes RyR2 Ca^{2+} leak and arrhythmia-triggering delayed afterdepolarizations (DADs)[72] while depleting SR Ca^{2+} to impair inotropy[99,107–110]. 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- Ca^{2+} leak and arrhythmias [111–113]. During the normal cardiac cycle, intracellular Ca^{2+} is either pumped back into the SR (by SERCA) or extruded from the cytoplasm by the Na^{+}/Ca^{2+} exchange protein (NCX1). Excess Ca^{2+} in diastole increases the likelihood for DADs due to the electrogenic action of NCX1. Interestingly, the pathological actions of CaMKII to favor DADs in atrial[114] and ventricular[74] myocardium mirror the physiological role of CaMKII to promote RyR2 Ca^{2+} leak and increased automaticity in SA nodal pacemaker cells[33,34]. While the role of CaMKII in RYR dysfunction in heart failure is controversial and in early studies mice with genetically altered RyR, resistant to CaMKII phosphorylation, are not protected from heart failure[32], pathological effects of CaMKII were recently shown in knock-in mice with RyR genetically modified to mimic RyR phosphorylation by CaMKII (Ser 2814 Asp)[115]. 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^{2+} release.

CaMKII regulates SR Ca^{2+} content by affecting SR Ca^{2+} uptake, as shown by the findings that myocardial CaMKII inhibition reduces SR Ca^{2+} content in ventricular myocytes[116] and sinoatrial nodal pacemaker cells[33]. PLN appears to be a target for CaMKII effects on SR Ca^{2+} content because knock out of PLN reverses the reduction in SR Ca^{2+} content in mice with myocardial CaMKII inhibition[117]. PLN is a negative regulator of SERCA[118] but PLN phosphorylation by PKA (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^{2+} filling in mice with cardiomyopathy and sudden death due to over-expression of CaMKII[119]. However, PLN ablation leads to premature death and mitochondrial Ca^{2+} over-load, suggesting that increasing SR Ca^{2+}, at least in the presence of excessive CaMKII activity, is disadvantageous. Whether or not CaMKII directly catalyzes SERCA phosphorylation and its physiological significance remain controversial[120,121]. Thus, CaMKII appears to act as a coordinating signal that favors SR Ca^{2+} flux, by enhancing SR Ca^{2+} uptake and release. These actions are likely important for myocardial ECC but are corrupted in cardiomyopathy and contribute to myocardial dysfunction and arrhythmias.

Inflammation and hypertrophy

There is 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 (IL-6) and −8 (IL-8), compared to normal controls. Human atrial biopsies 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[122] and chemokines, like IL-8 and MCP-1, are elevated in patients with sudden death and ventricular fibrillation [123], suggesting life-threatening ventricular arrhythmias and sudden death are favored by a pro-inflammatory state. CaMKIV affects inflammatory responses by aiding T-cell development, regulating IL-2 activity in T-cells and by modulating the activity of transcription factors[124]. However, the role of CaMKII in promoting inflammatory responses in myocardium is a newer finding. The α-isoform of CaMKII is present in macrophages where it promotes toll-like receptor (TLR) 4, 9, 3-triggered production of IL-6, tumor necrosis factor-alpha, and interferon-alpha/beta (IFN-

alpha/beta) [125]. CaMKII also regulates the localization of MHC-class II proteins in human dendritic cells [126]. The pro-inflammatory 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 pro-inflammatory genes regulated by CaMKII in cardiomyocytes, by comparing mRNA levels between control mice and mice with myocardial-delimited CaMKII inhibition due 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 (CFB), 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 MI[127]. The increase in CFB expression in myocardium by CaMKII is mediated through NF-κB, which has been shown to be activated by CaMKII in neuronal cells[128] . More recently, we found that oxidized CaMKII is increased in response to lipopolysaccharide (LPS) a TLR-4 agonist in myocardium[129] , suggesting that CaMKII activity is increased by inflammatory signals that increase oxidative stress, while at the same time CaMKII can act as a feed forward signal to augment transcription of inflammatory genes.

We used AC3-I mice with myocardial-targeted CaMKII expression as a probe to test the breadth of CaMKII-regulated transcription after myocardial infarction. We found that nearly half of the significantly up- or down-regulated mRNAs interrogated by our 8,500 element array were regulated by CaMKII expression three weeks after myocardial infarction, suggesting CaMKII activation has important consequences for transcriptional reprogramming after myocardial infarction[127] . Because myocardial CaMKII inhibition appears to be protective in the setting of MI, at least in mice[12,20,41], it seems likely that the preponderance of CaMKII-modulated transcriptional events after MI are ultimately maladaptive. To date, we have examined two CaMKII targets identified in this gene array in detail. The first was increased CFB expression that was down stream to CaMKII-dependent increases in NF-κB activity[127]. We also identified increased expression *of Mmp9*, a gene encoding matrix metalloproteinase 9 (MMP9). Recently, we found that aldosterone infusion led to non-genomic activation of CaMKII by oxidation and oxidized CaMKII enhanced *Mmp9* expression causing increased mortality due to cardiac rupture by activating the transcription factor myocyte enhancer factor 2 (MEF2) [12]. 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 MMP9, 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 post-myocardial 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 [41], angiotensin II signaling[20], and aldosterone[12].

Electrocardiographic left ventricular hypertrophy is an independent risk factor for sudden cardiac death[130]. 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 HDACs, especially HDAC4 and HDAC5, derepress *M*EF-2 mediated gene expression[131].

CaMKδ phosphorylates HDAC4 preferentially compared to HDAC5, leading to activation of hypertrophic genes. CaMKIIδ knock out mice with pressure overload due to aortic banding have attenuated cardiac hypertrophy and less phosphorylated HDAC4 compared to controls[46]. Other labs have also shown that while HDAC5 phosphorylation does not change in CaMKIIδ knock out mice subjected to pressure overload compared to controls, and that these mice are protected from progressive left ventricular dilatation, myocardial decompensation and heart failure but not from hypertrophy[132]. Calcineurin, a Ca^{2+} and calmodulin-dependent phosphatase, is a powerful prohypertrophic signal by dephosphorylating the nuclear factor for activated T cells (NFAT), leading to increased nuclear localization of NFAT and transcription of hypertrophic genes[60]. On one hand, CaMKII decreases transcription of calcineurin, a regulator of the NFAT transcription factor involved in cardiac hypertrophy [133]. On the other hand, calcineurin over-expression increases myocardial CaMKII expression. We found that CaMKII inhibition in mice with transgenic myocardial over-expression of calcineurin reduced cardiac arrhythmias, improved myocardial function and reduced mortality without a major effect on hypertrohpy [59]. Taken together, we interpret these findings to suggest that a complex interplay between hypertrophic, inflammatory and likely yet to be identified transcriptional pathways are activated directly or indirectly by CaMKII. The potential complexity of these interactions is highlighted by a recent study showing that the role of CaMKII to promote pathological hypertrophy appears to be antagonized by protein kinase A (PKA), a protein kinase that is responsive to cyclic AMP and that is activated by catecholamines. Although mice with transgenic myocardial PKA over-expression showed myocardial hypertrophy[134], PKA leads to an inhibitory, CaMKII-insensitive, cleavage product of HDAC4 that antagonizes hypertrophic actions of CaMKII[135]. Despite these complexities, we anticipate that the net effect of CaMKII in these pathways varies over time and differentially couples to upstream pathological stress signals, but in the aggregate down stream CAMKII actions are maladaptive and so are candidate targets for future antiarrhythmic and anti-cardiomyopathic therapies.

Cell death and Fibrosis

CaMKII induced apoptosis appears to play an instrumental role in both the development of heart failure after myocardial infarction and in the transition from compensated hypertrophy to decompensated cardiomyopathy in non-ischemic animal models. CaMKII induces apoptosis by an array of downstream mechanisms, some of which involve activation of pro-apoptotic proteases and others that involve transcriptional control on pro-apoptotic pathways. CaMKII can activate AP24, a pro-apoptotic protease, leading to DNA fragmentation[136]. CaMKII can also directly phosphorylate and activate pro-apoptotic factor BCL10. In addition, CaMKII can increase expression of pro-apoptotic genes that are downstream of the MAP kinase kinase TAK1[137], and ASK 1[138]. Recently, CaMKII has been implicated in mitochondria dependent pro-death pathways. Activation of CaMKII by a $Ca_V1.2$ and SR Ca^{2+} release dependent pathway increases mitochondrial-triggered myocyte death[82]. Endoplasmic reticulum (ER) stress triggers an increase in cytosolic calcium 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[139]. CaMKII-dependent phosphorylation of PLN triggers mitochondria-dependent cell death[140], consistent with the effects of PLN knock out in CaMKIIδ transgenic mice where mitochondrial Ca^{2+} and myocardial apoptosis are increased[119]. Transgenic mice over-expressing mActin develop dilated cardiomyopathy with four chamber enlargement, cardiac dysfunction, increased total and auto-phosphorylated 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[141]. Our group showed that mice with myocardial

CaMKII inhibition due to transgenic expression of AC3-I were resistant to apoptosis by angiotensin II infusion[20], and 2 hours and 5 hours after myocardial infarction[116]. The AC3-I transgenic mice were protected from myocardial infarction-induced systolic dysfunction compared to WT and transgenic control mice[41]. 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[142]. 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 due exclusively to increased intracellular Ca^{2+} that followed protein kinase A-dependent phosphorylation of Ca^{2+} homeostatic proteins. However, Zhu et al showed that β adrenergic receptor stimulation with isoproterenol can promote cardiomyocyte cell death by activating CaMKII, independent of PKA in vitro[143]. 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 Ca^{2+} uptake[116], by Ca^{2+} loading the SR even when CaMKII was inhibited. β adrenergic receptor type 1 knock out mice are protected from increased CaMKII activity, and cell death after myocardial infarction[144], consistent with findings that show CaMKII binds selectively to type 1 β adrenergic receptors[13]. 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[20]. Subsequently other investigators showed that angiotensin II caused myocyte death mediated by ROS- dependent CaMKII activation causing p38 MAPK activation[24]. 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 Ca^{2+} channel $Ca_V1.2$. CaMKII-catalyzes phosphorylation of threonine 498 on β_{2a}, leading to increased Ca^{2+} current, increased sarcoplasmic reticulum Ca^{2+}, arrhythmia-initiating afterdepolarizations and cell death. Both afterdepolarizations and cell death could be reduced or prevented by CaMKII inhibition, inhibition of sarcoplasmic reticulum Ca^{2+} release or by elimination of the β_{2a} CaMKII phosphorylation site[39]. Mice with transgenic myocardial over-expression of CaMKIIδ show increased myocardial apoptosis, afterdepolarizations, arrhythmias and premature death[145]. We interpret these findings to suggest that pro-death actions of excessive CaMKII activity are likely to promote heart failure and contribute to pro-arrhythmic 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 the 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)[146]. In the last two decades our understanding of this process has been refined by experimental evidence describing the role of intracellular calcium cycling in pacemaker activity. Coordinated action between SERCA and RyR in SAN cells causes rhythmic calcium 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[147]. Localized calcium release events

(LCRs) 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 $I_{Ca,L}$ amplitude and SAN action potential formation[148]. 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 calcium content, SR Ca^{2+} release and inward I_{NCX}, leading to enhancement of the diastolic depolarization rate (DDR). In contrast, CaMKII did not appear to affect the HCN4 mediated pacemaker current (I_f). We also showed that mice with genetic CaMKII inhibition failed to increase diastolic calcium sparks, SR calcium and DDR in response to isoproterenol, even though their resting heart rates were similar to wild type mice[33]. Recent work from our lab shows that CaMKII activity is not only important in β adrenergic receptor stimulation induced heart rate response, but is also necessary for catecholamine independent chronotropic competence. We activated Ca^{2+}-dependent pacing mechanisms directly by applying the L-type Ca^{2+} 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_f, suggesting that Ca^{2+}-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 calcium release and I_{NCX} when treated with BayK 8644 compared to WT controls, even though Bay K increased I_{Ca} equally in all SAN cells [34]. These findings suggested that CaMKII contributes to heart rate by a series of events that promote SR Ca^{2+} release and I_{NCX} in SAN cells.

While 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 HR and activity related heart rate increases[28]. Interestingly, human patients with heart failure and SA node dysfunction had increased oxidized CaMKII in the right atria compared to patients with heart failure but no SA node dysfunction. Mice with genetic cardiac CaMKII inhibition and mice lacking a critical subunit (p47) of important NADPH 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 due to loss of SA node cells relative to surrounding atrial myocardium, leading to sinus node dysfunction ([Fig 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[149]and SA node dysfunction[150]. CaMKII is activated by upstream signaling pathways including catecholamines [41,151], the renin-angiotensin-aldosterone system (RAAS)[12,20] and reactive oxygen species[20,25,28] that are critical to structural and electrical remodeling in heart failure[152] and atrial fibrillation[153]. Most fundamental evidence of the role of CaMKII in atrial fibrillation comes from mouse models where 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[92,114] and show increased phosphorylation of downstream target proteins RyR2[154] and PLN[155]. Interestingly, atrial myocytes from patients with atrial fibrillation also show increased spontaneous Ca^{2+} spark frequency that is normalized by CaMKII inhibition[156].

In many cases, AF is thought to originate in pulmonary vein ostia in the left atrium[157] and in isolated rabbit pulmonary vein tissue isoproterenol stimulates non-sustained spontaneous arrhythmias[158], that are suppressed by the CaMKII inhibitor, KN-93[158]. Transgenic mice expressing the CaMKII inhibitory peptide AC3-I[41] or knock-in mice with CaMKII resistant RyR2 Ser 2814 Ala[114] 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 higher atrial fibrillation as compared to wild type mice[114]. Mice with cardiac-specific overexpression of CREM-IbΔC-X develop spontaneous atrial fibrillation and atrial myocytes isolated from these mice show increased phosphorylation of RyR2 at Ser 2814[159]. 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[160]. Cross-breeding FKBP12.6 knock-out mice with Ser 2814 Ala knock-in mice inhibits the SR calcium leak normally present in FKBP $12.6^{-/-}$ atrial myocytes and decreases atrial fibrillation[160]. 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 transgenic 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 anti-arrhythmic 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 anti-arrhythmic 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 encoding for the cardiac SR Ca^{2+} release channel 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 has recently been shown to completely eliminate stress-induced arrhythmias and triggered activity in the R4496C mouse model of CPVT[161].

Timothy syndrome (also referred to as long-QT syndrome 8) is a rare autosomal dominant disease caused by mutations in L-type Ca^{2+} channel ($Ca_v1.2$) and characterized by ventricular tachycardia and sudden death[162]. At the cellular level, $Ca_v1.2$ Timothy syndrome mutations result in impaired $Ca_v1.2$ 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[98,163]. Recent studies expressed a $Ca_v1.2$ Timothy

syndrome mutant (Gly 406 Arg) 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 calcium content and SR calcium transients and prevented arrhythmogenic afterdepolarizations in myocytes expressing the Timothy syndrome mutant $Ca_V1.2$[164]. 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 (e.g. CPVT, Timothy syndrome, other long-QT syndromes) may respond well 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[163]. 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[59]. Finally, CaMKII inhibition prevents cellular afterdepolarizations and isoproterenol-induced arrhythmias in vivo in transgenic mice over-expressing $CaMKII\delta_C$ with hypertrophic and dilated cardiomyopathy[51,145]. 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/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 following aortic banding surgery[115]. Consistent with this finding, CaMKII inhibition has been shown to reduce RyR phosphorylation and SR Ca^{2+} leak in non-ischemic rabbit model of heart failure[72]. CaMKII inhibition has also been shown to be effective in rabbit chronic AV block models of acquired long-QT and electrical storm[61,165].

The anti-arrhythmic benefit of CaMKII inhibition has also been explored in ischemic heart disease. Importantly, transgenic inhibition of CaMKII prevents structural remodeling after myocardial infarction[41]. Furthermore, elevated levels of autophosphorylated and oxidized CaMKII have been reported in a well-established canine model of arrhythmias due 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[53,166]. Taken together these studies in both non-ischemic and ischemic animal models highlight the therapeutic anti-arrhythmic 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 electrical dysfunction in the failing heart, it will be important to consider alternative approaches that are well-suited for studying large and multi-scalar systems. Mathematical modeling has been applied for over 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[167]. 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[53,69,166,168–177]. Furthermore, the multimeric nature of the CaMKII holoenzyme and its multiple modes of regulation (e.g. Ca^{2+}/calmodulin, autophosphorylation, oxidation) lend themselves to a computational approach that is capable of answering questions difficult or even impossible to address in an experiment.

Systems biology has already generated important insights into the behavior and function of CaMKII in excitable cells. Early mathematical modeling studies demonstrated that CaMKII at the neuronal post-synaptic density is a critical mediator of long-term memory[172,173,176]. Specifically, these studies showed that due to its unique structural and biophysical properties, CaMKII operates as a molecular “switch” that can store information in a stable manner. While 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[53,69,166,169,171,174,175,178]. These detailed models have advanced the field by helping to integrate the extensive and growing literature on CaMKII regulatory network in 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[53,69,166,171,174,178,179]. In fact, computer simulations have shown that CaMKII is ideally suited to detect pacing frequency (e.g. heart rate), and may effectively store a cell’s pacing history, 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 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 heart. Computational biology has also helped identify potential new CaMKII targets and interacting proteins[36]. A computational screen of the human genome using a consensus CaMKII binding sequence found in the b_{2a} subunit of the L-type Ca^{2+} channel generated a select list of potential binding partners, including cytoskeletal, mitochondrial, nuclear and membrane proteins[36]. One of these proteins, the actin-associated polypeptide b_{IV}-spectrin, was validated as a CaMKII anchoring protein (CaMKAP) *in vivo* that targets CaMKII to the cardiomyocyte intercalated disc[36].

Systems biology has also 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[39,53,69,164,166,169,179]. Following 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 autophosphorylation) as a nexus for pro-arrhythmic changes in mechanical and electrical function[53,166]. 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 Ca^{2+} channel and/or RyR2 SR Ca^{2+} release channel[39,179,180]. 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[28]. Thus, models illustrate the multi-factorial 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?* It is 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[175] . Alternatively, CaMKAPs such as b_{IV}-spectrin may define subcellular CaMKII signaling domains[36]. To what extent do these pathways fine-tune CaMKII regulation within the cell? *How does CaMKII transform from an adaptive to 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 (e.g. 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 (e.g. apoptosis, mitochondrial energetics and pacemaking), as well as in other organ systems (e.g. brain, vasculature, pancreas).

Summary

Understanding of the roles for CaMKII in physiology and disease in 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 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 where elevated ROS and disrupted Ca^{2+} homeostasis are features of disease.

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Non-standard Abbreviations and Acronyms

Ca^{2+}/CaM	Calcified Calmodulin
CFB	Complement factor B
CPVT	Catecholaminergic polymorphic ventricular tachycardia
DAD	Delayed afterdepolarization
DDR	Diastolic depolarization rate
EAD	Early afterdepolarizations
ECC	Excitation-contraction coupling
HR	Heart rate

MMP9	Matrix metalloproteinase 9
MsrA	Methionine sulfoxide reductase A
NFAT	Nuclear factor for activated T cells
ox-CaMKII	Oxidized Calmodulin Kinase II
PLN	Phospholamban
ROS	Reactive oxygen species
RyR2	Ryanodine receptor
SA Node	Sinoatrial node
SERCA	Sarcoplamic endoplamic reticulum calcium ATPase
SR	Sarcoplasmic reticulum

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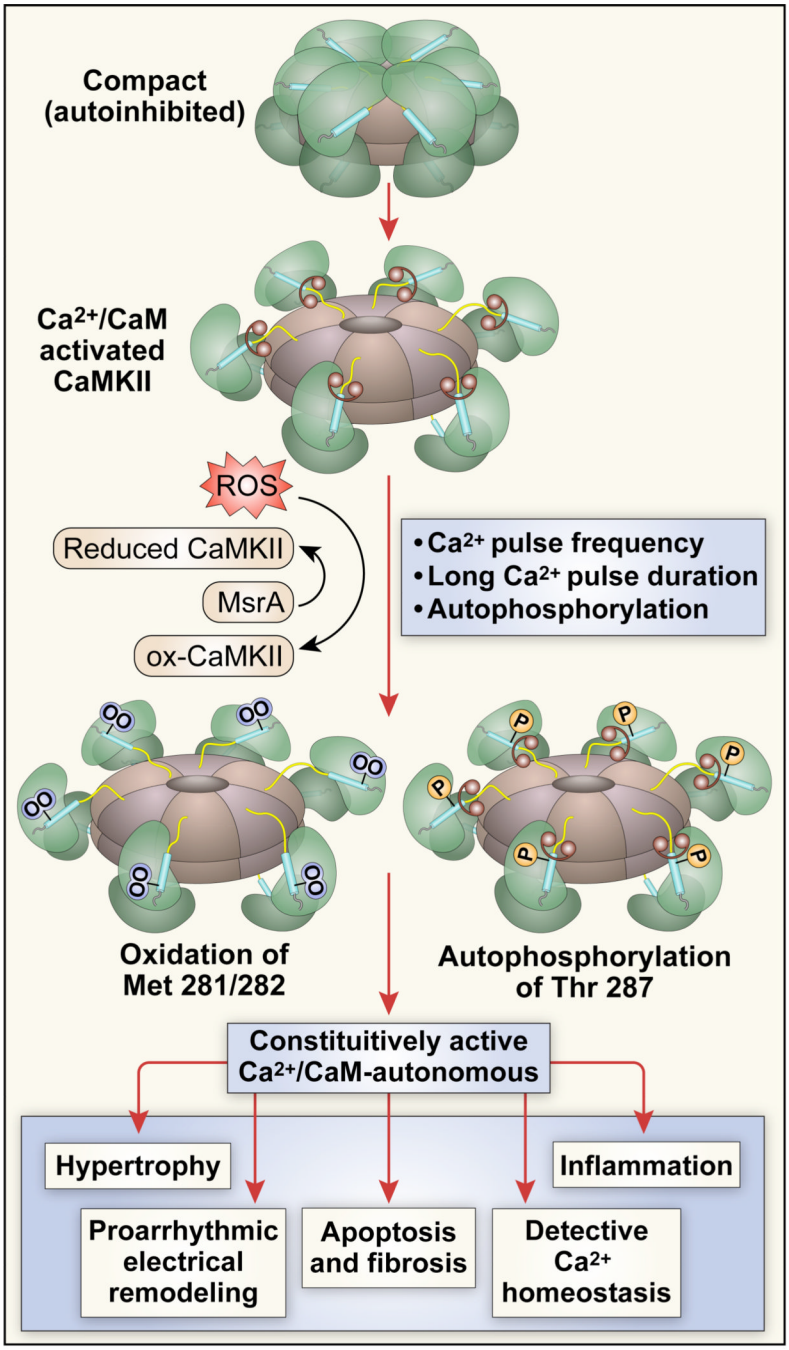

Figure 1. CaMKII becomes constitutively active by autophosphorylation and/or oxidation and constitutively active CaMKII promotes core events important for heart failure and arrhythmias

The CaMKII holoenzyme consists of two hexameric stacked rings. Under resting conditions the catalytic subunit is conformationally constrained so that CaMKII is inactive (top). CaMKII is initially activated when Ca^{2+} bound calmodulin (Ca^{2+}/CaM, dumbbell shapes) bind to the regulatory domain (blue segments) causing a more extended conformation where the catalytic domain (green) becomes accessible to substrate proteins and ATP. Sustained increases in Ca^{2+} lead to autophosphorylation at Thr 287, enhancing the avidity of CaM binding but also inducing a Ca^{2+}/CaM-independent form of CaMKII after CaM unbinding. Oxidation of Met 281/282 induces a Ca^{2+}/CaM-independent form of CaMKII without CaM

trapping. Increased levels of autophosphorylated or oxidized CaMKII favor heart failure and arrhythmias, at least in part, by activating hypertrophic and inflammatory gene programs, inducing proarrhythmic electrical remodeling, increasing myocyte death and fibrosis and defective intracellular Ca^{2+} homeostasis.

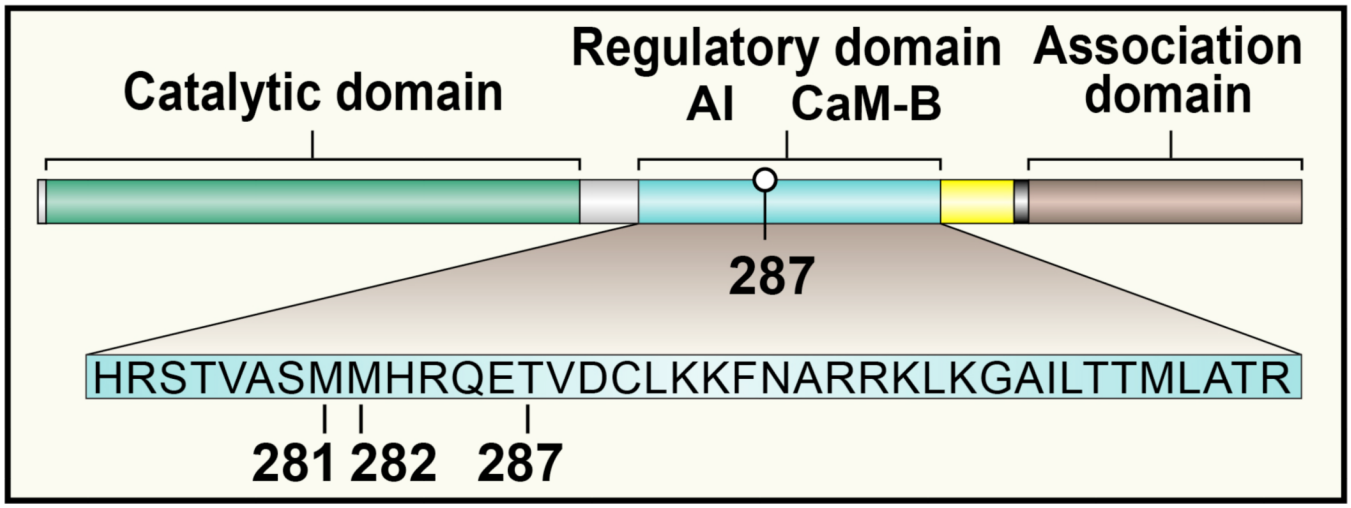

Figure 2. The domain structure of a CaMKII monomer

Each CaMKII monomer has a C terminus association domain and an N terminus catalytic domain. The internal regulatory domain consists of a C terminal side Ca^{2+}/CaM binding region (CaM-B) and an N terminal side autoinhibitory region (AI). Oxidation at Met 281/282 or autophosphorylation at Thr 287 prevent reassociation of the catalytic domain and the AI sequence, leading to constitutive, Ca^{2+}/CaM-independent CaMKII activity.

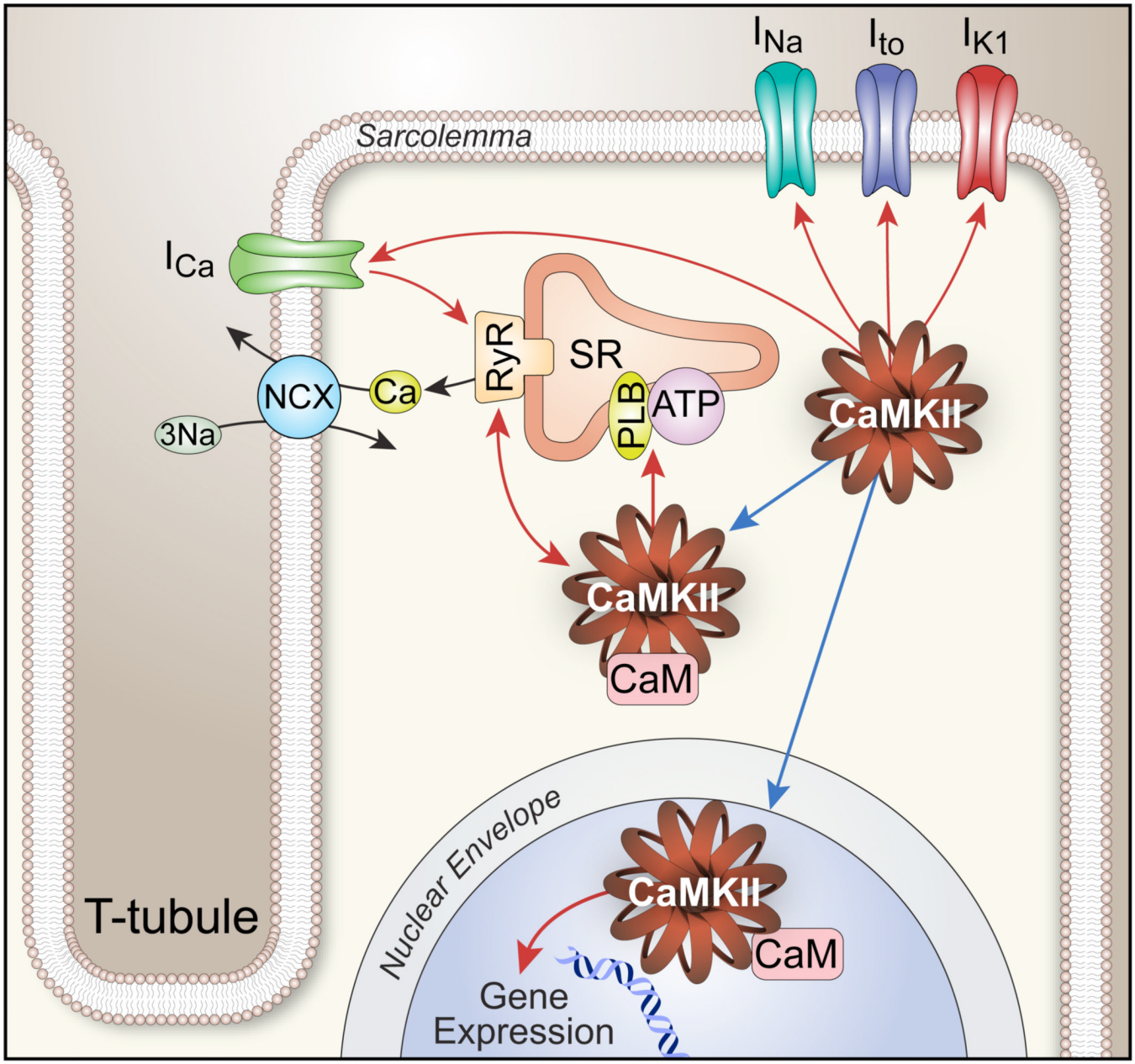

Figure 3. Myocardial cell ultrastructure creates close associations between CaMKII targets important for membrane excitability and intracellular Ca^{2+} homeostasis

Activated CaMKII enhances cellular Ca^{2+} entry by catalyzing phosphorylation of voltage-gated Ca^{2+} channels (mostly $Ca_V1.2$ in ventricle) and ryanodine receptor (RyR2) Ca^{2+} release channels. $Ca_V1.2$ and RyR2 are brought into close association (~10 nm) by $Ca_V1.2$ enrichment on T-tubular sarcolemmal invaginations. CaMKII phosphorylation of voltage-gated Na^+ channels (mostly $Na_V1.5$ in ventricle) increases subsarcolemmal Na^+ concentration that disadvantages the electrochemical driving force for Ca^{2+} extrusion from the cell by the Na^+/Ca^{2+} exchanger (NCX), leading to increased intracellular Ca^{2+} concentration.

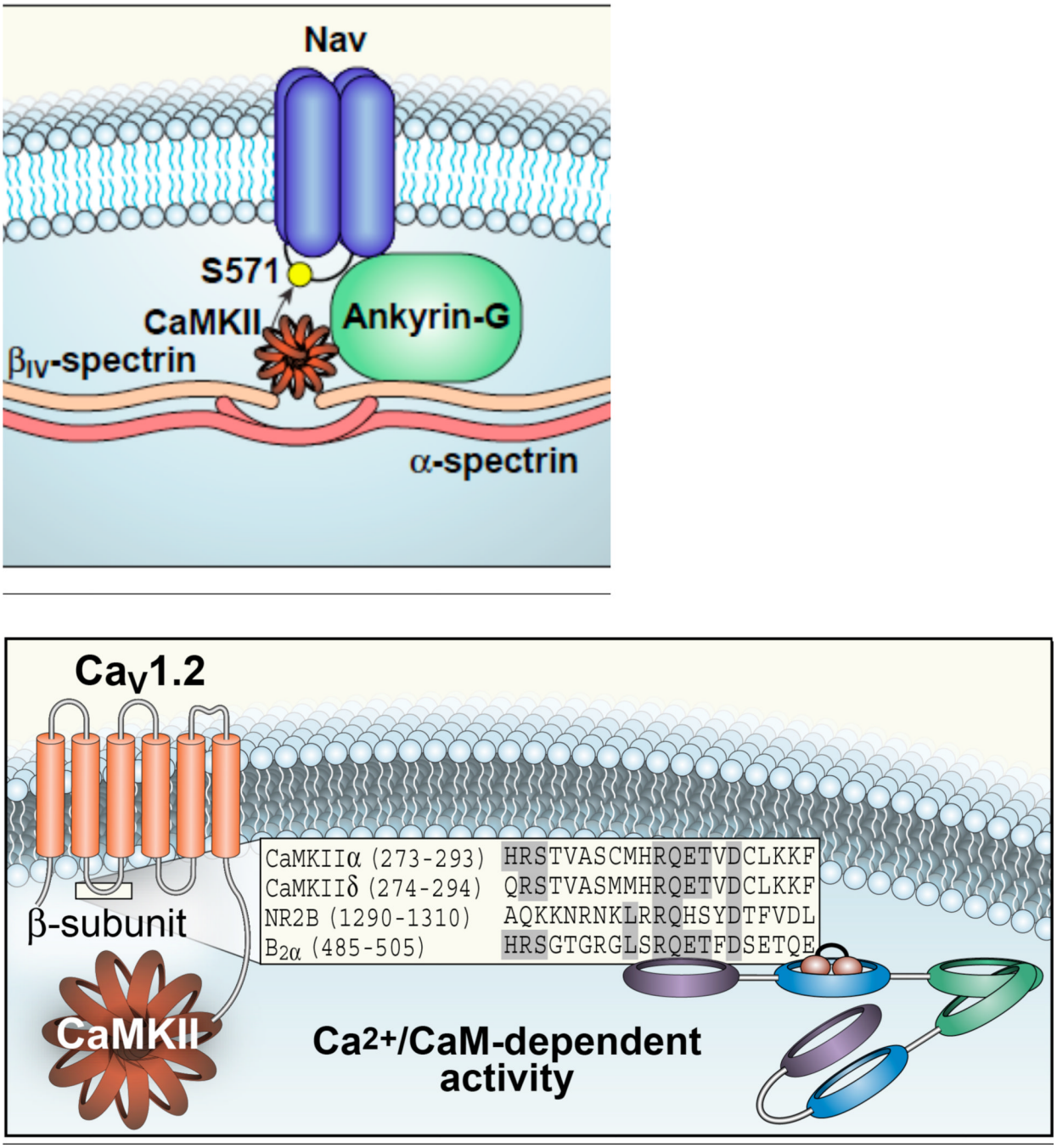

Figure 4. CaMKII anchoring at $Ca_V1.2$ and $Na_V1.5$

CaMKII associates with two important voltage-gated ion channels by catalytic domain binding to a recognition sequence that resembles the autoinhibitory region of the CaMKII regulatory domain. A sequence conserved across validated CaMKII targets is present on the β subunit of CaV1.2 (bottom panel) and on β_{IV} spectrin, a cytoskeletal protein associated with Ankyrin G and the $Na_V1.5$ (top panel). These sequences are required for CaMKII to regulate $Ca_V1.2$ and $Na_V1.5$ currents.

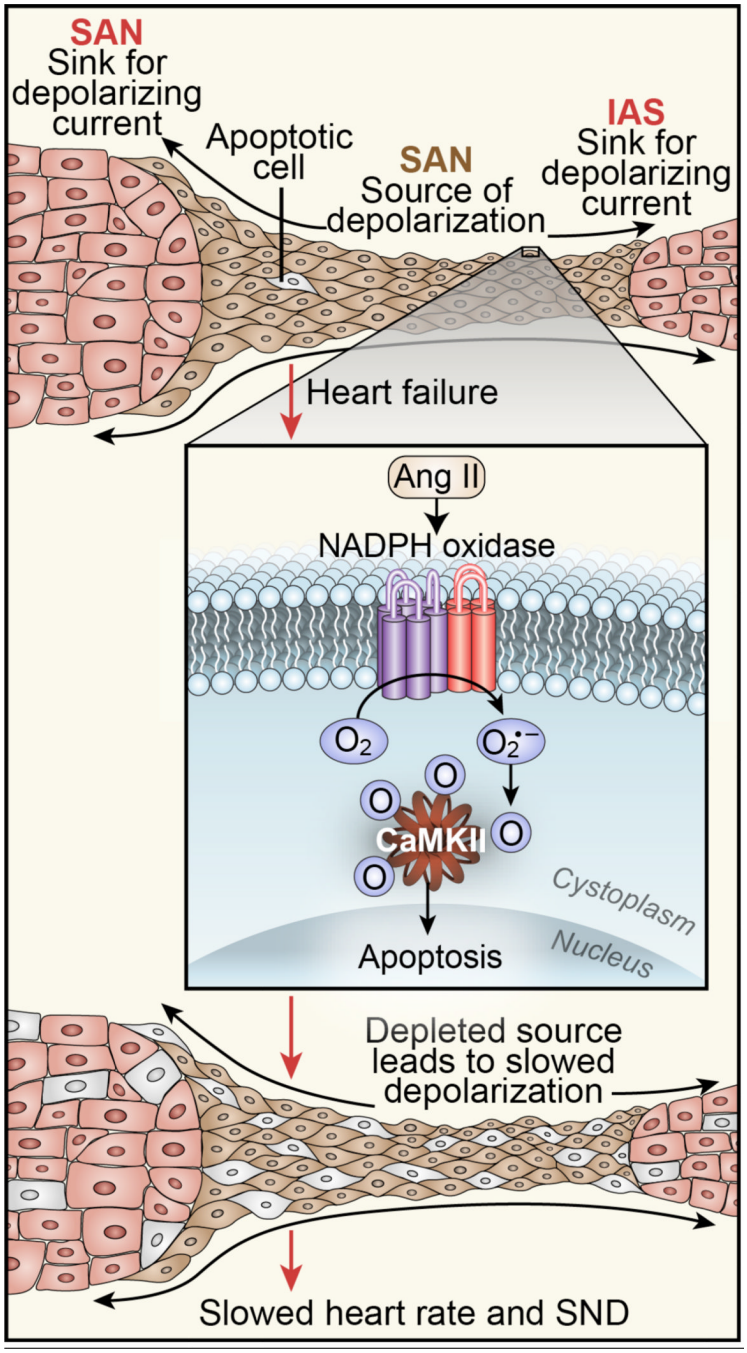

Figure 5. Oxidized CaMKII leads to SA node cell death and SA node dysfunction

Under physiological conditions (top) the SA node initiates each heart beat by inducing an inward current of sufficient magnitude to depolarize surrounding atrial myocardium with high fidelity. Activation of NADPH oxidase by angiotensin II leads to CaMKII activation by oxidation and excessive oxidized CaMKII leads to SA node cell apoptosis. SA node dysfunction occurs when SA node cell loss beyond a critical threshold prevents high fidelity capture of surrounding myocardium (bottom).