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The promise of CaMKII inhibition for heart disease: preventing heart failure and arrhythmias

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Abstract

Introduction—Calcium-calmodulin-dependent protein kinase II (CaMKII) has emerged as a central mediator of cardiac stress responses which may serve several critical roles in the regulation of cardiac rhythm, cardiac contractility and growth. Sustained and excessive activation of CaMKII during cardiac disease has, however, been linked to arrhythmias, and maladaptive cardiac remodeling, eventually leading to heart failure (HF) and sudden cardiac death.

Areas covered—In the current review, the authors describe the unique structural and biochemical properties of CaMKII and focus on its physiological effects in cardiomyocytes. Furthermore, they provide evidence for a role of CaMKII in cardiac pathologies, including arrhythmogenesis, myocardial ischemia and HF development. The authors conclude by discussing the potential for CaMKII as a target for inhibition in heart disease.

Expert opinion—CaMKII provides a promising nodal point for intervention that may allow simultaneous prevention of HF progression and development of arrhythmias. For future studies and drug development there is a strong rationale for the development of more specific CaMKII inhibitors. In addition, an improved understanding of the differential roles of CaMKII subtypes is required.

Keywords

arrhythmia; CaMKII; heart failure; ischemia; mitochondria

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1. Biochemistry of CaMKII activation and target interaction

Calcium-calmodulin-dependent protein kinase II (CaMKII) is a dodecameric-holoenzyme formed from homo- or heterodimers of α -, β -, δ - or γ -subunits. CaMKII δ is the dominant cardiac isoform and the two abundant splice variants (δ_b and δ_c) that are found in the heart appear to display distinct localization profiles. CaMKII δ_b includes a nuclear localization sequence that is absent from mature CaMKII δ_c and confers a largely nuclear distribution [1]. The physiologic role of this localization remains to be fully characterized, but may be important for distinguishing specific pathophysiologic roles. Monomeric CaMKII δ is a 478 amino acid peptide in humans, with three key functional domains. The large N-terminal catalytic domain is tethered to an intermediate regulatory region, which is responsible for conferring calcified calmodulin (Ca₄-CaM) sensitivity, and is home to sites conferring at least three forms of kinase control [2,3]. The carboxy-terminal includes an association domain essential for oligomerization and the mature holoenzyme conformation.

Canonical kinase activation occurs by binding of Ca₄-CaM. This event appears to be a gatekeeper for remaining regulatory control by autophosphorylation at Threonine²⁸⁷ (Thr²⁸⁷) and Thr³⁰⁶, and oxidation at methionines 281 and 282 (Met^{281/282}). Binding of Ca₄-CaM to the regulatory region causes a large conformational change, which displaces the regulatory pseudo-substrate from its inactive position, where it shields the catalytic domain from binding of ATP and substrate [2]. Once active, the monomer is available to phosphorylate targets, including Thr²⁸⁷ of adjacent monomers in the dodecameric holoenzyme [2,4]. The key functional alteration accompanying Thr²⁸⁷ phosphorylation is a dramatic increase in Ca₄-CaM affinity, which prolongs activation as cytosolic Ca²⁺ declines during diastole [5].

Erickson *et al.* [3] showed that paired methionines (Meth^{281/282}) in the regulatory domain also undergo partially reversible oxidation and that this action was essential for increases in enzyme activity and apoptosis associated with H₂O₂ and angiotensin II challenge. Like phosphorylation at Thr²⁸⁷, Met^{281/282} oxidation has since been shown to first require activation by Ca₄-CaM [6]. A second phosphorylation of the regulatory domain at Thr³⁰⁶ acts to inactivate the kinase, and render it insensitive to activation by Ca₄-CaM [7]. It is not clear what role this phosphorylation plays in the heart, although it is important in modulating postsynaptic plasticity in neurons [2].

Finally, a key recent development in understanding of cardiac CaMKII biology has been the identification of several specific anchoring complexes that are essential for directing the kinase to some of its most important electrophysiological targets. As is described in further detail below, β_{1V} -Spectrin and synapse-associated protein 97 (SAP97) appear to be essential elements in macromolecular complexes that tether CaMKII in the vicinity of the sodium and potassium channel subunits Na_v1.5 and K_v4.3, respectively [8,9]. Up until the discovery of these interactions, it had been thought that CaMKII directly associated with its targets through specialized adapter sequences embedded in the target protein. These sequences were originally named CaMKII adapter sequences or 'CaMkaps' [10]. Unfortunately, this terminology may now be confused with that used for the A-kinase anchoring proteins (AKAPs), which like SAP97 and β_{1V} -Spectrin are separate proteins that serve to anchor

protein kinase A (PKA) in the immediate vicinity of its targets. As accessory proteins involved in CaMKII targeting continue to be discovered it may be necessary to revise the terminology applied to the embedded adaptor sequences to avoid confusion with proteins analogous to the AKAPs that could be considered as CaMKII anchoring proteins (CaMKAPs).

2. Pro-arrhythmic effects of CaMKII

A number of cellular behaviors are thought to be important precursors or initiators of tissue-level arrhythmia. The best established of these are early afterdepolarizations (EADs), delayed afterdepolarizations (DADs) and action potential (AP) duration alternans. The role of CaMKII in alternans is not well studied, however, substantial evidence exists to describe roles for CaMKII in afterdepolarizations of both types. EADs result from dis-coordinated current activation or reactivation during AP repolarization, whereas DADs, by definition, occur after repolarization is complete. While there is significant overlap in the mechanisms of these two classes of behavior [11,12], the range of cellular modifications capable of promoting EADs is, however, slightly broader than that for DADs. Although the dynamics of EADs are complex, it is generally true that any modulatory effects at sarcolemmal currents or on intracellular Ca^{2+} handling that reduce repolarization reserve also promote EADs [13]. With few exceptions, DADs appear to rely on spontaneous Ca^{2+} release (SCR) from the sarcoplasmic reticulum, secondary to cellular Ca^{2+} overload. For this reason, any perturbation capable of eliciting DADs, generally either induces Ca^{2+} overload, or reduces the limits at which overload is reached [14].

CaMKII is thought to promote both of these types of events through broad molecular interactions with both sarcolemmal and intracellular targets. In this section, the authors discuss how the acute and chronic effects of CaMKII influence target behavior in a manner that would be expected to promote EADs, DADs or both.

2.1 L-type Ca^{2+} channel

CaMKII has been known to regulate Ca^{2+} channels for at least 20 years. As early as 1992, McCarron *et al.* [15] showed that CaMKII was responsible for the slight Ca^{2+} -dependent increase in peak Ca^{2+} current (I_{Ca}), and slowed I_{Ca} inactivation accompanying brief Ca^{2+} loading in smooth muscle cells. This phenomenon is known as Ca^{2+} -dependent I_{Ca} facilitation, and it has become the hallmark of CaMKII-dependent regulation of the L-type Ca^{2+} current (I_{CaL}). Parallel investigations by Yuan and Bers [16] and Anderson *et al.* [17] confirmed that blockade of CaMKII could eliminate this behavior in ventricular myocytes. Dzhura *et al.* [18] then engineered a constitutively active form of CaMK to show that phosphorylation of some complement of membrane proteins dramatically increases the open probability of single L-type Ca^{2+} channels (LCCs). This effect was mainly due to prolonged open time and a shift toward mode-2 gating strongly reminiscent of β -adrenergic [19] and dihydropyridine agonist modulation of LCCs [20]. At the authors' current state of knowledge, it is clear that CaMKII is capable of directly phosphorylating LCCs at a number of sites, and that this regulation gives rise to both the single-channel effects and I_{Ca} facilitation, but the precise phosphorylation events responsible for these actions remain

somewhat controversial [21]. Simultaneous investigations by Lee *et al.* [22] and Greuter *et al.* [23] identified different phosphorylation sites which they suggested to be responsible for the effects of CaMKII on I_{CaL} . Both studies utilized site-directed mutagenesis to ablate candidate sites. Lee *et al.* found serine 1512 (Ser¹⁵¹²) and Ser¹⁵⁷⁰ on the α_{2C} subunit to be critical, whereas Greuter *et al.* found phosphorylation of Thr⁴⁹⁸, and physical interaction between CaMKII and Thr⁴⁹³ of the β_{2a} subunit, to be required for both the macroscopic and single-channel signatures of CaM-KII phosphorylation [22,23]. Follow-up investigations from both groups provided further support for their respective original findings, but did little to reconcile the contexts or conditions that might explain their differing results [24,25]. It seems likely that all three of these sites are involved in CaMKII-dependent regulation of I_{CaL} , however the precise roles of each remain to be clarified, and may depend on contextual factors such as the expression of the β_{2a} splice variant.

Data describing transcriptional regulation of LCCs by CaMKII are sparse. The most direct study published to date suggests that CaMKII activity negatively regulates expression of the LCC α -subunit (Ca_v1.2) via the downstream regulatory element (DRE) [26]. This may be overcome by increased single channel activity during heart failure (HF), where CaMKII is hyperactive, but macroscopic I_{CaL} magnitude remains relatively unchanged [27].

During the AP, both the increased peak I_{CaL} and slowed inactivation accompanying CaMKII-dependent facilitation act to reduce repolarization reserve. As described above, this effect promotes EADs in and of itself [13]. Additionally, CaMKII may enhance I_{CaL} recovery from inactivation [13,28], which for any balance of currents during the AP plateau, would be expected to more readily permit I_{CaL} reactivation and initiate an EAD. Finally, all of these effects potentiate overall Ca²⁺ nflux via I_{CaL} , and thereby facilitate cellular Ca²⁺ overload. Thus, the effects of CaMKII at I_{CaL} are likely to be directly involved in initiating EADs, and indirectly involved in promoting both EADs and DADs, particularly when combined with other stimuli that induce Ca²⁺ overload, such as β -adrenergic challenge.

2.2 Sodium channel

Wagner *et al.* provided the first data to describe CaMKII-dependent modulation of myocardial sodium currents [29]. Functionally, this study showed that CaMKII elicits complex effects on gating of the fast sodium current (I_{Na}) including a negative shift in steady-state inactivation and enhancement of intermediate inactivation, but slowed fast inactivation and exaggerated late or persistent I_{Na} (I_{NaL}). These effects were consistent whether measured in rabbit myocytes acutely over-expressing CaMKII δ_c , or CaMKII δ_c transgenic mice, which develop and die from HF [30]. The majority of these effects were blocked by CaMKII inhibition after both chronic and acute CaMKII overexpression, although I_{NaL} enhancement was maintained in the mouse myocytes, suggesting that some remodeling process may contribute to this current component after prolonged CaMKII hyperactivity. While several subsequent studies have corroborated these findings, it should be noted that Aiba *et al.* [31] and Yoon *et al.* [32] have observed results, which, aside from enhancement of I_{NaL} , were precisely pposite to those of Wagner *et al.* The reasons for these discrepancies are not yet clear.

To define the molecular biochemistry of CaMKII-dependent regulation of I_{Na} , Hund *et al.* used a human genome screen and found that an interaction between CaMKII and β_{IV} -Spectrin is required for the effects of CaMKII at I_{Na} [8]. CaMKII localization at the intercalated disc is absent in mice expressing a β_{IV} -Spectrin (qv^{3J}) mutant that is CaMKII-binding incompetent, and these mice also lack all of the major functional effects of CaMKII described by Wagner *et al.* [29]. These data provide compelling evidence for the necessity of functional association between CaMKII and a macromolecular complex including $Na_v1.5$ at the intercalated disc.

As for the L-type channel, the specific phosphorylation events and sites required for CaMKII regulation of I_{Na} remain under debate. Generally, it is agreed that the I-II linker region of $Na_v1.5$ contains the important sites of regulatory phosphorylation by CaMKII, and that multiple sites may be involved. Both Hund *et al.* [8] and Koval *et al.* [33] used *in vivo* genetic techniques to implicate Ser⁵⁷¹ as a key site for all previously observed functional outcomes, and an unbiased mass-spectrometry study of purified $Na_v \alpha$ confirms that this site may be basally phosphorylated in the cardiac myocyte. However, these findings should be tempered by recent *in vitro* findings, which have been unable to replicate CaMKII-dependent phosphorylation at Ser⁵⁷¹ [34]. It is also currently not clear whether CaMKII activity regulates $Na_v1.5$ expression.

The pro-arrhythmic effects of CaMKII-dependent regulation at I_{Na} have commonly been compared with the simultaneous long QT syndrome (subtype 3, LQT3) and Brugada-like phenotypes associated with the 1795insD mutation in $Na_v1.5$ [29,34,35]. Gain-of-function effects associated with the LQT3 phenotype are thought to be due largely to enhanced I_{NaL} , which, like enhanced I_{CaL} function, reduces repolarization reserve and prolongs the AP. The loss-of-function effects associated with the Brugada-like phenotype (slowed conduction and increased QRS duration) are more readily attributed to reduced channel availability accompanying left shifted steady-state inactivation, and slowed recovery from inactivation.

2.3 K⁺ channels

CaMKII is clearly involved in regulation of the transient outward (I_{to}) and inward rectifier (I_{K1}) potassium currents, and more so than for either the LCC or sodium channels, these influences appear to involve modulation of both channel expression and gating. For this reason, distinguishing the roles of disease-based remodeling and CaMKII hyperactivity on K⁺ channel regulation has become an important and somewhat onerous task. While CaMKII may also regulate other important myocardial repolarizing currents, the authors constrain this section to focus on the I_{to} and I_{K1} components of the potassium current channels, primarily because the CaMKII-dependent effects at these two currents are established and heavily implicated in disease [36].

The first indication that CaMKII regulates potassium currents was provided by Li *et al.* [37], in a study that employed chronic cardiac-specific overexpression of the CaMKII inhibitory peptide AC3-I. These authors observed potentiation of I_{to} and I_{K1} that could not be replicated with acute AC3-I-based antagonism, thus suggesting that the functional outcomes of chronic CaMKII inhibition resulted from chronic abrogation of a CaMKII-dependent effect. However, it was unclear from this study how CaMKII activity was suppressing either

current because expression of the major channel subunits for both I_{to} and I_{K1} were largely unchanged. Subsequent investigations have helped to clarify this point [9,38,39]. Xiao *et al.* studied downregulation of I_{to} in rapidly paced canine cells, and observed that inhibition of CaMKII, calmodulin or Ca^{2+} entry via I_{CaL} , during 24 h of pacing eliminated negative remodeling of I_{to} and repression of $K_{v4.3}$ [38]. Wagner *et al.* repeated the design of their 2006 study of I_{Na} by examining the CaMKII-dependent changes to K^+ currents in myocytes from CaMKII δ_c transgenic mice and rabbit myocytes acutely transfected with CaMKII δ_c [39]. Their data suggest that acute effects of CaMKII potentiate both I_{to} and I_{K1} , but that under chronic CaMKII overexpression there is transcriptional repression of $K_{v4.2/3}$ and $K_{ir2.1}$, which reduces overall I_{to} and I_{K1} , respectively. Finally, El-Haou *et al.* showed that the primary acute effect of CaMKII at I_{to} , is to slow inactivation of the larger fast component of the current ($I_{to,f}$) [9]. This may explain why Wagner *et al.* observed acute potentiation of the slow component of I_{to} , even though this current appears to only rarely be present in murine myocytes [40]. Findings from other groups support this CaMKII-dependent slowing of $I_{to,f}$ inactivation [41,42], although it should be noted that these investigations used KN-93 to antagonize CaMKII, and this drug has also been shown to inhibit K^+ channels in a non-specific manner [43,44].

Together these data suggest that CaMKII exerts important acute regulatory effects at I_{to} , which tend to slow current inactivation and enhance recovery from inactivation, thereby increasing repolarization reserve. However, over as little as 24 h, increased CaMKII activity is capable of repressing expression of I_{to} and $K_{v4.3}$, in a manner consistent with HF remodeling. Similar effects occur for I_{K1} , although the precise time-course of CaMKII-dependent current repression is not known, and may be secondary to broader HF remodeling. The pro-arrhythmic effects of these changes are again likely to arise from an overall reduction in repolarization reserve, and a flattening in the trajectory of late repolarization in myocytes. Chronic changes at I_{K1} would also be expected to destabilize resting membrane potential, such that any perturbing current, for example due to SCR, would be expected to create a larger DAD and more easily result in a spontaneous AP.

3. Ca^{2+} -handling targets

CaMKII is known to phosphorylate or otherwise regulate a large number of Ca^{2+} -handling proteins, including all of the most important contributors to whole cell Ca^{2+} homeostasis. Here, the authors discuss the actions of CaMKII at the ryanodine receptor (RyR), the sarcoendoplasmic reticulum Ca^{2+} ATPase (SERCA)/phospholamban (PLN) axis and Na^+ / Ca^{2+} exchanger (NCX).

The ability of CaMKII to phosphorylate the cardiac RyR isoform (RyR2) has been known for some time [45,46], although the specific phosphorylation site, and functional outcomes have been debated. In 2004, Wehrens *et al.* convincingly demonstrated that CaMKII phosphorylates recombinant RyR2 at Ser^{2814/5}, and potentially other sites in the native channel [47]. The observed functional outcome of Ser^{2814/5} phosphorylation was to increase the open probability of the receptor. This disagreed with some prior findings [45,46], but agrees well with observations in the intact cell, where CaMKII activity increases both diastolic spark frequency [48,49], SR Ca^{2+} leak [50,51], and fractional release [48,49,52],

even at sharply reduced SR Ca^{2+} load. Subsequent investigations have suggested that this phosphorylation is central to HF-associated RyR dysfunction [51,53], and is specifically required for arrhythmogenic spontaneous SR Ca^{2+} release during HF [52,54].

CaMKII also phosphorylates PLN at Thr¹⁷ and this site is the most commonly used marker of endogenous CaMKII activity. Like PKA-mediated phosphorylation of PLN at Ser¹⁶, CaMKII action at PLN disinhibits SERCA and accelerates SR Ca^{2+} reuptake. The role of this phosphorylation specifically in the context of arrhythmogenesis is not clear, although, interestingly, PLN is required for CaMKII-specific effects in the above-mentioned remodeling of I_{to} [37]. CaMKII also acutely phosphorylates SERCA [55], but the physiological importance of this action is not clearly understood [56].

An important potential arrhythmogenic effect of chronic CaMKII hyperactivity may be the coordination of HF-associated changes in expression of NCX and SERCA. Generally, NCX expression increases during HF and SERCA expression decreases, both of which serve to shift diastolic Ca^{2+} fluxes in favor of Ca^{2+} extrusion. This contributes to SR Ca^{2+} depletion during HF, but also increases arrhythmogenic current generation during acute Ca^{2+} overload [27]. Recent studies have suggested that these transcriptional effects may be orchestrated by CaMKII [57,58]. If confirmed, this mechanism would constitute one of the most important CaMKII-mediated pro-arrhythmic outcomes.

4. Evidence for the role of CaMKII in experimental and clinical arrhythmia

CaMKII has been shown to play a role in a number of arrhythmia-susceptible phenotypes with a broad range of underlying etiologies. In the context of structural heart disease, CaMKII inhibition reduces susceptibility to ventricular arrhythmia in the calcineurin [59], and CaMKII δ_c transgenic models of dilated cardiomyopathy [29,60], and in the hypertrophic CaMKIV transgenic mouse [61]. In and of itself, murine knock-in of a RyR mutant mimicking constitutive CaMKII phosphorylation at Ser²⁸¹⁴ has also been shown to result in HF and susceptibility to ventricular tachycardia (VT [52]). Work with larger animals has extended these findings to other forms of acquired heart disease. Models of long-QT-associated arrhythmia induced by AV nodal ablation have been developed in both the rabbit [62,63] and dog [64], and CaMKII appears to play a role in the arrhythmia-susceptibility in both species. Recently, a series of studies have shown that CaMKII is also involved in the etiology of atrial fibrillation (AF), which is the most common clinical arrhythmia [65]. Using two different mouse models [66,67], and most recently studies with human tissues [65,67,68], the AF phenotype has been shown to be associated with CaMKII hyperactivity and disruptions to Ca^{2+} handling very similar to those apparent in the ventricle.

Additionally, CaMKII appears to play some role in several arrhythmia-susceptible congenital diseases. CaMKII inhibition reduces VT incidence in the well-described RyR2^{R4996C +/-} mouse model of catecholaminergic polymorphic ventricular tachycardia (CPVT [69]), and also in the ankyrin-B-deficient CPVT mouse [70]. At least one variant of Timothy syndrome has also been shown to exhibit sensitivity to CaM-KII blockade [71]. Finally, CaMKII has also been shown to play a role in drug-induced arrhythmias elicited in

otherwise disease-free animals. A series of studies employing a rabbit model of experimental long-QT syndrome by simultaneous clofilium/methoxamine challenge suggests that acute CaMKII activity/hyperactivity is sufficient to elicit arrhythmia during simultaneous pharmacologic reduction of repolarization reserve and α -adrenergic challenge.

5. Evidence for a role for CaMKII in myocardial infarction

CaMKII activity is increased in experimental models of ischemia/reperfusion (I/R) injury as well as in patients after myocardial infarction (MI) [72,73]. Furthermore, several lines of evidence have shown that excessive CaMKII activation promotes cell death, suggesting that this CaMKII activation during I/R could promote infarct formation [21]. Initial studies in cultured cardiomyocytes indicated that CaMKII is required to ameliorate SR Ca^{2+} mishandling and restore mechanical recovery of the infarct zone, suggesting that CaMKII activation is protective during I/R [72]. However, the sustained activation of CaMKII that occurs during reperfusion has now robustly been shown to promote cardiomyocyte apoptosis and necrosis, stimulate infarct formation and deteriorate functional recovery after ischemic injury [74–76]. Although CaMKII can activate mitochondria-independent pathways of apoptosis [77], mitochondrial pathways of cell death appear to be the primary mechanism of CaMKII-mediated cell death during I/R [74–76]. In particular, CaMKII activation promotes mitochondrial Ca^{2+} overload through increased cytosolic Ca^{2+} and phosphorylation of the mitochondrial Ca^{2+} uniporter (MCU), thus promoting cytochrome *c* release, mitochondrial swelling and permeability transition pore opening [74,76]. CaMKII therefore appears to promote both apoptotic and necrotic cell death after I/R. Importantly, mice with mitochondrial-restricted expression of a CaMKII inhibitory peptide displayed reduced infarct formation after *in vivo* I/R injury through a pathway involving the MCU. These results further support the notion that the mitochondrial effects of CaMKII are major contributors to pathogenic pathways responsible for CaMKII effects during ischemic injury [74]. Recent studies from the authors' group also clearly implicated CaMKII-mediated activation of nuclear factor- κB (NF- κB) and the transcriptional regulation of pro-inflammatory gene expression in ischemia reperfusion injury as suggested by others [78,79].

Intriguingly, CaMKII inhibition was not found to affect infarct formation after permanent MI, underscoring the pathogenic significance of the CaMKII-activation that occurs during reperfusion [80]. CaMKII has, however, been implicated in myocardial rupture after permanent MI, indicating that CaMKII is activated during permanent MI and involved in the regulation of infarct healing [81]. Together these findings clearly demonstrate that CaMKII activation during I/R is detrimental and suggests that CaMKII-inhibiting drugs could improve outcome after MI.

6. Evidence for a role for CaMKII in cardiac hypertrophy

In addition to the acute effects of CaMKII on cardiac rhythm, excitation–contraction coupling and hypoxic stress, CaMKII has also been implicated in the regulation of chronic adaptation of the heart. In this section, the authors discuss the role of CaMKII in cardiac remodeling, hypertrophy and dysfunction.

Sustained increases in cardiac workload induce cardiac hypertrophy, a physiological response of the heart that acts to reduce the increased left ventricular wall stress. During cardiac diseases, hypertrophy may become 'pathologic' and propagate into cardiac dysfunction or overt HF. Indeed, left ventricular hypertrophy is an independent predictor of mortality, largely because chronically hypertrophied hearts eventually remodel into dilated cardiomyopathy [82]. Pathological hypertrophy is characterized by extensive structural remodeling, including increased cell size, changes in protein synthesis, myofilament reorganization and re-expression of fetal genes such as atrial natriuretic factor (ANF), brain natriuretic peptide (BNP), β -myosin heavy chain (MHC) and α -skeletal actin [83]. Many of these molecular events can be induced in isolated cardiomyocytes through stimulation of G-protein-coupled receptors (GPCRs), in particular α -adrenergic receptors and receptors for angiotensin-II or endothelin-1, which are coupled to the heterotrimeric G protein, Gq [84]. This is phenocopied in mice in which cardiac-specific overexpression of the α -subunit of Gq (G α_q) causes marked cardiac hypertrophy that rapidly propagates into HF in the absence of agonists or cardiac stress [85].

Initially, a body of evidence suggested that CaMKII plays a major role in cardiac hypertrophy. First, studies using the neonatal rat ventricular myocyte (NRVM) model of hypertrophy have consistently shown that CaMKII contributes to hypertrophy downstream of multiple GPCR agonists [84,86–92]. Forced expression of wild-type or constitutively activated CaMKII also elicits strong hypertrophic responses in NRVMs [93]. Second, cardiac-specific overexpression of CaMKII induces cardiac hypertrophy in mice, including an increase in cardiomyocyte size and the induction of a fetal gene program [30,94,95]. CaMKII regulates hypertrophy, at least in part, through phosphorylation of class 2 histone deacetylases 4 and 5 (HDAC4 and HDAC5), causing their nucleo-cytoplasmic shuttling and thereby de-repressing myocyte enhancing factor-2 (MEF2) [87,96]. Third, CaMKII expression and activity are increased in the hearts of patients with cardiac hypertrophy [21,30]. These observations strongly suggest that CaMKII is a critical regulator of cardiac hypertrophy. Surprisingly, however, the reciprocal experiments provide a more nuanced view of the role of CaMKII in cardiac hypertrophy.

The effect of CaMKII inhibition in relevant models of cardiac disease was first tested using the AC3-I overexpressing mouse described above (Section 2.3) [80]. Intriguingly, cardiac-specific inhibition of CaMKII did not attenuate hypertrophy development in mice with HF after MI. The authors' laboratory developed mice in which the predominant cardiac CaMKII isoform, CaMKII δ , was genetically deleted. When these mice were subjected to transverse aortic constriction (TAC), CaMKII δ knockout (CaMKII δ -KO) mice developed a similar degree of cardiac hypertrophy compared with their wild-type littermates [97], and their hypertrophic response to chronic isoproterenol infusion was also unaltered [98]. A distinct CaMKII δ -KO line generated by another group showed normal echocardiographic hypertrophy after TAC, although they did observe reduced cardiac weight and cardiomyocyte size in CaMKII δ -KO mice [99]. Thus, although CaMKII has clear prohypertrophic effects *in vitro* and *in vivo*, it does not appear to be required for hypertrophy in models of heart disease. It is likely that an increase in the activity of complementary signaling molecules such as calcineurin or protein kinase D compensate for the loss of CaMKII [97,99].

7. Evidence of a role for CaMKII in heart failure

HF is the final common end point of heart disease and accounts for substantial morbidity, mortality and healthcare expenditure [100]. While a wide range of distinct cardiac insults can lead to HF development, the pathophysiological consequences of HF are remarkably similar. The most notable and well-described consequence of cardiac dysfunction is the excessive activation of the sympathetic nervous system and the rennin–angiotensin–aldosterone (RAAS) systems, also called the neurohumoral response. Although activation of these neurohumoral-signaling pathways is initially adaptive, sustained neurohumoral activity eventually becomes toxic and promotes disease progression through pathways associated with increased cytosolic Ca^{2+} and increased cardiomyocyte reactive oxygen species (ROS) production [27,101]. As these are conditions that favor CaMKII activation, it is not surprising that myocardial CaMKII activity is markedly increased in murine and human HF [21]. Accordingly, there is a large body of evidence that implicates CaMKII in HF development [21].

The authors' laboratory was the first to demonstrate that *in vivo* CaMKII activation can cause HF as evidenced by mice with cardiomyocyte-specific overexpression of the predominant cardiac isoform of CaMKII, CaMKII δ [30,94]. Overexpression of the delta C isoform of CaMKII (CaMKII δ_c) caused cardiac hypertrophy that rapidly transitioned into an overt HF phenotype with severely disrupted Ca^{2+} handling, malignant arrhythmias, rapid deterioration of cardiac function and premature death [30]. Using loss of function experiments on CaMKII δ -KO, the authors subsequently demonstrated reduced left ventricular dilatation and contractile dysfunction after TAC [97,99]. CaMKII δ -KO mice are also resistant to HF induced by isoproterenol toxicity and overexpression of G α_q [98]. AC3-I overexpressing mice also display reduced HF development after a variety of cardiac insults including infusion of isoproterenol, angiotensin-II, aldosterone and permanent MI [3,80,81]. Furthermore, treatment of mice with the CaMKII-inhibitor KN-93 attenuated HF development in a genetic model of dilated cardiomyopathy, without affecting cardiac function in control mice [77]. Together these results unequivocally demonstrate that CaMKII is a critical mediator of HF development under a variety of pathological conditions and suggests CaMKII as an attractive therapeutic target in HF.

8. Mechanisms of CaMKII-induced heart failure

Several mechanisms have been proposed to explain the actions of CaMKII in promoting HF, including defective Ca^{2+} cycling, pathological gene transcription, mitochondrial dysfunction, myocardial fibrosis and inflammation [21]. A hallmark of failing cardiomyocytes is a decrease in diastolic SR Ca^{2+} content and a consequent reduction of SR Ca^{2+} release during systole [102,103]. As described above, CaMKII regulates multiple proteins involved in cardiac Ca^{2+} cycling and sustained activation of CaMKII results in alterations to Ca^{2+} cycling that closely resemble HF [48,104,105]. Most prominently, sustained CaMKII activation results in reduced SR Ca^{2+} load in the presence of activated SR Ca^{2+} release [49]. Accordingly, the reduced HF development in CaMKII δ -KO mice after TAC is associated with partial normalization of cardiac Ca^{2+} handling defects [97]. It is

therefore reasonable to propose that the effects of CaMKII on cardiac Ca^{2+} cycling are responsible for its pathogenic effects in HF [106].

To interrogate the role of defective Ca^{2+} handling in CaMKII δ -induced HF, the authors crossed CaMKII δ_c transgenic mice with mice in which PLN was deleted, expecting that enhancement of SR Ca^{2+} load due to PLN KO would at least partially restore contractile function. As expected, PLN KO normalized SR Ca^{2+} cycling in CaMKII δ_c transgenic mice, but surprisingly worsened the HF phenotype [107]. Cardiomyocytes from CaMKII δ_c transgenic/PLN KO mice displayed increased diastolic Ca^{2+} sparks, mitochondrial Ca^{2+} overload and poor viability, suggesting that normalization of SR Ca^{2+} load without simultaneous reduction in the signals enhancing SR Ca^{2+} leak, can be highly toxic to cardiomyocytes. As described above, CaMKII-mediated activation of RyRs is thought to be responsible for the increased diastolic Ca^{2+} leak that has been associated with diastolic Ca^{2+} sparks and arrhythmogenesis. Several lines of evidence have suggested that hyperphosphorylation of RyR by CaMKII also induces cardiac dysfunction [108]. To further investigate the role of RyR phosphorylation by CaMKII in HF development and arrhythmogenesis, a line of knock-in mice expressing RyR2 with a mutation mimicking constitutive RyR2 phosphorylation at the primary CaMKII site (RyR) and another line expressing RyR2 resistant to CaMKII phosphorylation (S2814A) were generated [52]. Intriguingly, mice with constitutively phosphorylated RyR displayed accelerated HF development after TAC or isoproterenol infusion [52], whereas CaMKII-resistant mutants were protected from these insults [52,53]. Earlier studies using a different mouse line with CaMKII-resistant RyRs also showed reduced cardiac performance after TAC [109]. These findings underscore the pathogenic effects of CaMKII on SR Ca^{2+} cycling, but also indicate that defective Ca^{2+} cycling alone is not sufficient to explain CaMKII-induced HF.

In addition to post-translational modifications of ion channels and calcium handling proteins, CaMKII activation has effects on cardiac gene transcription and CaMKII-mediated transcriptional reprogramming has been implicated in the pathophysiology of HF. The best-described pathway by which CaMKII mediates altered gene transcription involves phosphorylation of HDAC4 and HDAC5. Phosphorylation results in nucleo-cytoplasmic shuttling of HDAC4 and 5, which de-represses MEF2-mediated gene transcription. While this pathway has primarily been implicated in CaMKII-mediated hypertrophy, a recent study showed that the CaMKII-MEF2 axis is activated downstream of aldosterone in cardiomyocytes and promotes cardiac fibrosis and left ventricular rupture after MI [81]. The pro-fibrotic effects of CaMKII were mediated, at least in part, through increased matrix metalloproteinase 9 transcription, downstream of MEF2 [81]. Recent studies have shown the ability of NF- κ B to increase inflammatory cytokines that activate CaMKII and that CaMKII activates NF- κ B, which thus would provide an auto-amplification loop for cardiac inflammatory signaling [78]. The complete pathogenic significance of NF- κ B activation in HF remains to be established.

The authors recently addressed the pathogenic significance of CaMKII-induced transcriptional responses by interrogating transcriptional changes in the Gq-transgenic mice crossed with CaMKII δ -KO mice [110]. Interestingly, the expression of a large number of mitochondrial genes was altered in Gq-transgenic mice, and the expression of 41% of these

genes was normalized by CaMKII deletion. Mitochondria from Gq-transgenic mice displayed increased markers of oxidative stress and impaired respiration, linked to reduced expression of uncoupling protein 3 (UCP3), a critical suppressor of mitochondrial superoxide generation. Intriguingly, Gq-induced mitochondrial dysfunction was improved by combined CaMKII δ ablation. Moreover, the protective effects of CaMKII δ inhibition on Gq-induced mitochondrial dysfunction in isolated cardiomyocytes were abrogated by knock down of UCP3. CaMKII-mediated transcriptional reprogramming of mitochondria thus appears to contribute to mitotoxicity in decompensating hypertrophy, at least in part through repression of UCP3. As described above, CaMKII is present in the mitochondria and contributes to CaMKII-mediated I/R injury through phosphorylation of the MCU [74,76]. CaMKII might therefore also exert transcription-independent effects on mitochondrial function that also contribute to HF development.

Finally, CaMKII regulates cardiomyocyte apoptosis through a variety of downstream mechanisms [21,111]. It is generally accepted that cardiomyocyte apoptosis contributes to the pathophysiology of HF and increased CaMKII activity has consistently been linked to cardiomyocyte apoptosis in HF models [21,111]. It is therefore likely that the pro-apoptotic properties of CaMKII promote HF development. Since CaMKII-mediated apoptosis and necrosis predominantly ensue through mitochondrial pathways, the mitochondrial effects of CaMKII might emerge as principal targets for therapy in the future.

9. Integrating the mechanisms of CaMKII-mediated pathogenesis

The interactions by which CaMKII is thought to promote or ameliorate cardiac pathology are summarized in Figure 1. CaMKII exerts effects across broad scales of time (channel gating to protein expression), and is active in cellular compartments with dramatically different $[Ca^{2+}]$ profiles (dyadic cleft vs nucleus vs mitochondria), efforts to quantitatively integrate CaMKII activity are essential for understanding the outcomes of acute and chronic CaMKII blockade. Mathematical models of ion channel function have been a stalwart of electrophysiological research since the birth of the field, and many detailed models exist to describe cardiac-specific electrophysiology and Ca^{2+} handling [112]. A decade ago, the authors' group extended this family of models by integrating a mathematical representation of β -adrenergic signaling into an existing model of the guinea-pig myocyte [113–116]. Over the last 10 years, similar models have been developed to integrate CaMKII signaling alone [117,118], and combined CaMKII/ β -adrenergic signaling [119,120].

Most recently a normalized network model of hypertrophic signaling was developed, based on existing literature, to define the dynamic relationships between signaling events that occur during various forms of hypertrophy [121]. While this approach is in its infancy, the agreement between experiment and model in terms of overall hypertrophic outcomes was quite good, and achieved by comparatively simple representations of specific molecular interactions.

In principle, models of these two types are, or will soon be, capable of interrogating drug-specific effects of acute and chronic CaMKII inhibition, and are therefore likely to allow more rapid progress toward a clinically viable CaMKII antagonist.

10. Expert opinion: CaMKII as a therapeutic target

As has been noted previously [122,123], a significant motivation for pursuing CaMKII as an anti-arrhythmic target is the lack of success of ion channel blockers in preventing sudden cardiac death (SCD). In fact, most ion channel blockers are contraindicated in patients with HF because they increase the incidence of SCD [124,125]. Since HF patients display an extremely high incidence of arrhythmias and SCD, there is an urgent need for novel anti-arrhythmic drugs that are safe in HF patients. CaMKII is a nodal regulator of cardiac electrophysiology and Ca^{2+} -handling and plays an active role in arrhythmias of widely varying molecular etiologies. Together, these considerations suggest that CaMKII is poised to offer broader and more integrated anti-arrhythmic potential than any other ion channels or intracellular proteins. Importantly, abundant evidence also indicates that CaMKII may be directly involved in the pathogenesis of I/R injury and promotes HF development downstream of multiple cardiac insults including hypertension, valve disease, MI, excessive neurohumoral activation and chemotherapy [3]. Importantly, CaMKII activation is increased in hearts from patients suffering from various types of heart disease and sustained CaMKII activation has been shown to underlie several other prevalent human heart diseases, including sinus node dysfunction, diabetic cardiomyopathy and diastolic dysfunction [126–128]. To date, pharmacological inhibition of CaMKII in mice and in isolated human cardiomyocytes has been shown to attenuate maladaptive cardiac remodeling and reduce arrhythmias in distinct, clinically relevant models of cardiac disease [77,80,129]. CaMKII appears to be involved in the pathogenesis of several of the most clinically relevant cardiac diseases and pharmacological CaMKII inhibition strategies in rodents appears to be both feasible and effective. CaMKII therefore provides a promising nodal point for intervention that may simultaneously prevent arrhythmias and attenuate HF development.

Would this strategy allow us to run with the hare and hunt with the hounds? There are several gaps in our knowledge that may impede effective drug design and development. First and foremost, we still lack a small molecule inhibitor with characteristics that allow reliable study of CaMKII, let alone for potential therapeutic targeting. Cell-impermeant peptides, such as AIP [39,130], have been used in patch-clamp experiments, but genetic approaches remain the cleanest tools for scientific purposes. The most commonly applied pharmacological antagonist is the methoxybenzenesulfonamide, KN-93 [131]. This compound competes for the $\text{Ca}_4\text{-CaM}$ binding site of CaMKII with a half maximal concentration between 0.37 and 2.58 μM [17,132]. Unfortunately, both this compound and its putatively ‘inactive’ congener (KN-92) inhibit a wide variety of potassium currents and the I_{CaL} at concentrations in the nM range [17,132]. Vastly superior antagonists will need to be developed if CaMKII targeting is to be considered a viable therapeutic strategy. A finer point related to CaMKII inhibition strategies is subtype specificity, as there is a very high homology of the regulatory and catalytic domains of the CaMKII δ subtypes. Through genetic manipulation these isoforms have been shown to exert opposing effects on cardiomyocytes survival *in vitro* [133]. The authors recently crossed CaMKII δ_c and CaMKII δ_b transgenic mice with CaMKII δ -KO mice to generate mice with only one CaMKII δ splice variant [134,135]. Intriguingly, loss of CaMKII δ_b markedly accelerated HF development in CaMKII δ_c transgenic mice whereas loss of δ_c reduced infarct development

in response to *ex vivo* I/R. The CaMKII δ splice variants therefore appear to play distinct and opposing roles in maladaptive responses in HF or I/R injury. While this suggests that pharmacologic strategies that specifically target the more ‘malignant’ CaMKII δ_c could offer greater protection than indiscriminate CaMKII inhibitors, such strategies would not be feasible given subtype structural homology. Possibly this could be achieved through targeting specific localized interactions, such as those of the CaMKII δ_c anchoring proteins like SAP97 and β_{IV} -Spectrin. At the very least, distinct subtype-specific effects should be considered when developing CaMKII modulating drugs, as the potentially beneficial effects of CaMKII are currently likely to be underestimated. Additionally, avoiding inhibition of the CaMKII isoforms in the brain (primarily CaMKII α) is likely to be more easily achieved by designing compounds that do not enter the central nervous system.

Finally, CaMKII is activated downstream of multiple neurohumoral agonists for which effective antagonists are already available and have proven their efficacy in randomized controlled clinical trials. These include angiotensin-converting enzyme inhibitors, β -blockers, aldosterone antagonists and ivabradine [125]. There is currently no evidence that CaMKII inhibition is beneficial when added to these contemporary evidence-based therapies. Indeed, the profile of CaMKII pathophysiology in the heart closely resembles conditions of increased sympathetic tone, and it will be particularly important to determine if CaMKII inhibition offers clinically relevant improvements when tested in addition to β -blockers.

Future studies are required to improve the understanding of the functions of CaMKII in physiology and heart disease. In addition, we should strive to develop therapies that will allow us to separate the physiological effects from the pathogenic effects of CaMKII. Nevertheless, an overwhelming body of evidence suggests that CaMKII inhibitors may become the next evidence-based strategy for heart disease.

Acknowledgments

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135. Westenbrink BD, Gray CB, Xiang S, et al. Abstract 18536: CaMKII- δ splice variants exert differential effects on heart failure development and myocardial ischemia/reperfusion injury. *Circulation.* 2012; 126:A18536.

Article highlights

- Calcium-calmodulin-dependent protein kinase II (CaMKII) is a nodal regulator of cardiac electrophysiology and Ca^{2+} -handling and plays an active role in arrhythmias of widely varying molecular etiologies.
- Arrhythmias are responsible for considerable morbidity and mortality in heart failure (HF) patients and targeting CaMKII could offer broader and more integrated anti-arrhythmic potential than any other ion channel or intracellular proteins.
- CaMKII activation during ischemia/reperfusion (I/R) injury promotes infarct formation.
- Sustained CaMKII activation promotes HF development through multiple pathways, including aberrant Ca^{2+} handling, mitochondrial dysfunction, inflammation and fibrosis.
- CaMKII provides a promising nodal point for intervention that may simultaneously prevent HF progression and arrhythmias.

This box summarizes key points contained in the article.

Figure 1.

The interactions by which CaMKII is thought to promote or ameliorate cardiac pathology can broadly be classified into those that involve acute phosphoregulation (**A**), or transcriptional control (**B**). For the targets that are acutely activated, site-directed mutagenesis of the CaMKII phosphorylation site suggests that several may significantly contribute to CaMKII-dependent pathological outcomes. In particular, disruption of phosphorylation at Ser⁵⁷¹ of Na_v1.5 (depicted simply as I_{Na}), and several sites on the cardiac L-type calcium channel (I_{CaL}), has been shown to abrogate pro-arrhythmic effects of CaMKII. RyR2 phosphorylation at Ser^{2814/5} appears to be a potent modulator of CaMKII-associated myocardial remodeling and disease progression. Additionally, recent work has highlighted direct regulation of the mitochondrial calcium uniporter by CaMKII, and described a role for CaMKII phosphorylation of MCU at Ser⁵⁷ and/or Ser⁹² in Ca²⁺-induced mitotoxicity and tissue death. Several beneficial effects of direct CaMKII activity have also been described. In particular, CaMKII acutely facilitates I_{K1} and $I_{to,f}$, and these actions would be expected to counteract effects at both I_{Na} and I_{CaL} . Thus, the importance of integrating CaMKII effects across its many different targets should be carefully and quantitatively addressed when proposing acute CaMKII blockade as potential anti-arrhythmic strategy. Additionally in this panel, the authors depict two mechanisms by which CaMKII exerts transcriptional regulation in the myocyte: through phosphorylation of

HDAC4/5, which appears to uniquely depend on perinuclear calcium signaling, and is prevented in mutated HDAC, and through phosphorylation of IKK, an upstream regulator of NF- κ B signaling. Panel B shows the outcomes of this and other transcriptional mechanisms controlled by CaMKII. Expression of the α -subunits of both I_{K1} and I_{to-f} are subject to CaMKII control, and are repressed within hours to several days of applying CaMKII-activating stimuli. These are both loss of function outcomes, and have been successfully prevented or restored by peptide-based inhibition of the kinase. Additional transcriptional targets have been reported including the calcium regulatory proteins NCX1, SERCA2 and Cav_{1.2}, the mitochondrial protein UCP3, and inflammatory cytokines as well as fetal genes characteristic of hypertrophy. It is not yet clear whether these effects can be rectified by pharmacologic or site-directed inhibition of CaMKII actions on particular transcription factors but many are attenuated by CaMKII gene deletion or inhibition. Thus, dysregulated expression of these targets also offers potential for correction by specific pharmacologic antagonists of CaMKII.