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Review

Multiple downstream proarrhythmic targets for calmodulin kinase II: Moving beyond an ion channel-centric focus

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Abstract

The multifunctional Ca^{2+} calmodulin-dependent protein kinase II (CaMKII) has emerged as a pro-arrhythmic signaling molecule. CaMKII can participate in arrhythmia signaling by effects on ion channel proteins, intracellular Ca^{2+} uptake and release, regulation of cell death, and by activation of hypertrophic signaling pathways. The pleuripotent nature of CaMKII is reminiscent of another serine–threonine kinase, protein kinase A (PKA), which shares many of the same protein targets and is the downstream kinase most associated with β-adrenergic receptor stimulation. The ability of CaMKII to localize and coordinate activity of multiple protein targets linked to Ca^{2+} signaling set CaMKII apart from other "traditional" arrhythmia drug targets, such as ion channel proteins. This review will discuss some of the biology of CaMKII and focus on work that has been done on molecular, cellular, and whole animal models that together build a case for CaMKII as a pro-arrhythmic signal and as a potential therapeutic target for arrhythmias and structural heart disease.
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1. Introduction

The multifunctional Ca^{2+} calmodulin-dependent protein kinase II (CaMKII) is a serine–threonine kinase that was first identified by its dependence on Ca^{2+} and calmodulin (CaM) for activation [1]. The CaMKII holoenzyme consists of twelve monomers that are linked together through the C-terminus association domain. These association domains are capable of self-assembly, even in the absence of catalytic and regulatory domains [2]. In the absence of Ca^{2+} and CaM binding, the catalytic domains are sequestered and inactive by binding to the regulatory domain (Fig. 1) [3]. The regulatory domain contains a pseudo-substrate sequence centered around threonine 286/287 (precise numbering depends on the specific isoform) that engages the catalytic domain under resting conditions. When Ca^{2+}-bound CaM (Ca^{2+}/CaM) binds to the adjacent CaM binding sequence within the regulatory domain, it induces a conformational change in CaMKII that extends the catalytic domain and initiates enzymatic activity. The threonine in the 286/287 position is a preferred substrate for CaMKII and phosphorylation of threonine 286/287 causes a thousand-fold increase in the binding avidity of the CaMKII regulatory domain for CaM [4] and confers Ca^{2+} independent activity (i.e., activity that is present even in the absence of bound Ca^{2+}/CaM) [5–8]. The multimeric structure of CaMKII allows for progressive inter-subunit phosphorylation with high frequency Ca^{2+} stimulation or with prolonged Ca^{2+} transients, as occur during tachycardia or action potential prolongation. These intriguing structure–function properties of CaMKII activation have been demonstrated in a cell-free system [9] and also occur in heart with progressive action potential prolongation [10]. In contrast to other serine–threonine kinases such as PKA [11] and protein kinase C (PKC) [12], adaptor proteins for CaMKII have not been widely identified [13]. CaMKII associates with the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor subunit NR2B (accession

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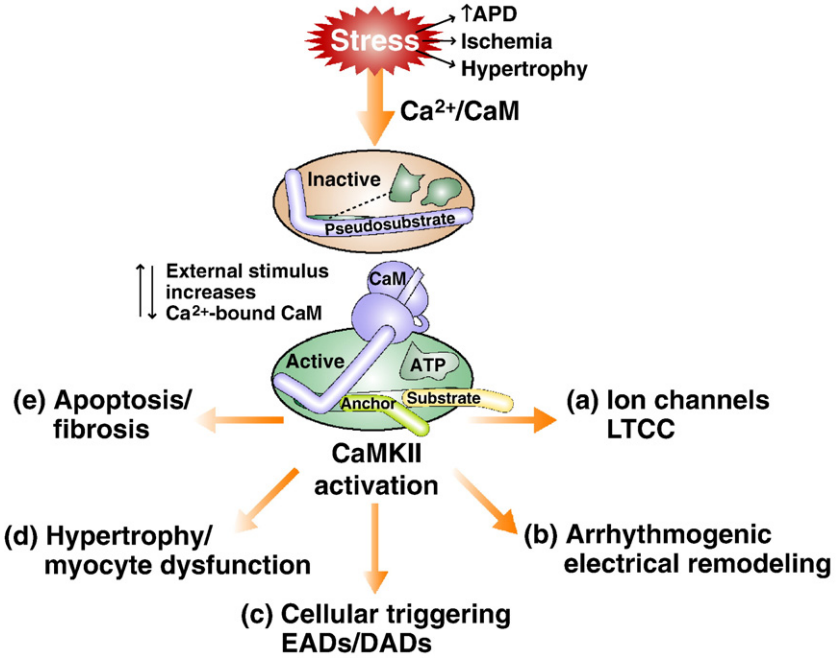

Fig. 1. Proarrhythmic actions of CaMKII derive from activity at multiple cellular processes. Biological stress can increase intracellular Ca^{2+} to bind calmodulin (CaM) and activate CaMKII. Ca^{2+}/CaM binding activates CaMKII by displacing the autoinhibitory regulatory domain from the catalytic domain. Activated CaMKII can affect ion channels, induce arrhythmogenic electrical remodeling, trigger arrhythmia-initiating afterdepolarizations, stimulate hypertrophic transcriptional programs, and favor apoptosis and fibrosis.

numberBC113620) through a sequence that is homologous to the CaMKII autoinhibitory domain [14,15]. This finding led to the concept that CaMKII binding sites ("CaMK-CAPS") are embedded within target proteins rather than existing as separate protein entities, as is the case for PKA and PKC adapter proteins. Recently, a CaMK-CAP sequence was identified on the L-type Ca^{2+} channel β subunit (accession numberNM_000724). This sequence is homologous to the CaMKII autoinhibitory domain and appears to follow rules first identified for NR2B. Autophosphorylated (activated) CaMKII binds preferentially to the β subunit and this binding allows for targeting of a nearby amino acid (threonine 498) for phosphorylation [16].

2. L-type Ca^{2+} channels

CaMKII can directly affect a wide variety of ion channels. In general, ion channels consist of a pore forming protein or a set of proteins that establish a pore or passage way for ionic current through the outer cell membrane, but also consist of auxiliary proteins that form the multiprotein ion channel complex. CaMKII may regulate a variety of ion channels including sodium channels, potassium channels, chloride channels, intracellular Ca^{2+} release ryanodine receptor channels and the Na^{+}/Ca^{2+} exchanger through indirect influences on intracellular Ca^{2+} [17]. However, the effect of CaMKII signaling on L-type Ca^{2+} channels ($Ca_V1.2$) is most clearly implicated in arrhythmogenesis.

L-type Ca^{2+} channels are the primary portal for Ca^{2+} entry in ventricular myocardium and significantly contribute to Ca^{2+} entry in atrium and in the specialized conduction system as well as in impulse generating sinoatrial nodal cells. L-type Ca^{2+} channels consist of a pore-forming α-subunit, an auxiliary β-subunit, and α_2-delta (δ) subunit and (in some cases) a gamma (γ) subunit. Ca^{2+} channels, like other ion channel protein complexes, are physically linked to the cellular machinery through binding to cytoskeletal and other cytoplasmic proteins [18]. L-type Ca^{2+} channel openings are critical in heart for initiating sarcoplasmic reticulum Ca^{2+} release through ryanodine receptors that leads to contraction [19], for sculpting the action potential plateau [20], initiating early afterdepolarizations (see Section 4 below) [21], and for delivering Ca^{2+} that participates in signaling mechanisms for cell growth and cell death [22,23].

L-type Ca^{2+} channels (LTCCs) are voltage-gated, meaning that channel opening is primarily initiated by cell membrane depolarization, but opening probability is also regulated by phosphorylation and this is the mechanism by which CaMKII increases Ca^{2+} entry. CaMKII phosphorylation causes LTCCs to enter a high-activity gating mode (mode 2) [24] that is characterized by frequent long openings [25]. High activity gating is also seen with Ca^{2+} channel agonist drugs (used as research tools), with PKA phosphorylation [26], by stimulation with CaM binding agonist signals embedded in the C-terminus of Ca^{2+} channel α-subunit [27,28], and in patients with end-stage heart failure [29]. CaMKII can bind to multiple regions in Ca^{2+} channels including the α-subunit C-terminus [30,31] and the β-subunit [16]. We recently found that phosphorylation of a specific epitope (threonine 498 on the β-subunit) was

required for CaMKII activation of single L-type Ca^{2+} channels and for a dynamic macroscopic L-type Ca^{2+} current property called "facilitation" [16]. Facilitation is a pattern of increasing peak Ca^{2+} current and slowed inactivation that has been linked to physiological responses such as the 'treppe' phenomenon and pathophysiological responses such as afterdepolarizations (see Section 4). CaMKII can bind to the α subunit of the LTCC through shared calmodulin binding domains on the cytoplasmic C-terminus, and can also bind at the β-subunit through a motif that resembles the regulatory domain (a proposed "CaM-CAP"). Bona fide CaMKII phosphorylation sites leading to physiological or pathophysiological gating responses are presently unknown on the α-subunit, but threonine 498 on the β-subunit is necessary for increases in single LTCC opening as well as for dynamic facilitation responses in cardiomyocytes. Over expression of β-subunits has a dominant negative effect with regard to gating properties [32] and over expression of a β-subunit where threonine 498 is mutated to alanine eliminates the normal L-type Ca^{2+} current facilitation responses seen in adult cardiomyocytes in response to repetitive cell membrane depolarization [16]. Phosphorylation by CaMKII, by PKA and effects of other agonists that induce mode 2 gating, must dynamically re-order the LTCC complex to reduce the free energy of a conformation(s) that has a high opening probability. Understanding these dynamic conformational changes is a major goal for developing a mechanistic molecular understanding of these signals on cardiac excitation contraction, action potential physiology and arrhythmia-inducing afterdepolarizations.

3. Proarrhythmic electrical remodeling

Some patients with structurally normal hearts may be at increased risk for proarrhythmia due to rare genetic mutations in ion channel or ankyrin proteins [33]. Hearts from these patients or animal models of these diseases show prolonged action potential duration and disordered intracellular Ca^{2+} homeostasis, both conditions that favor activation of CaMKII. The patients at highest risk for life threatening arrhythmias have structural heart disease [34]. The most common form of structural heart disease in the northern and western hemispheres is related to adverse remodeling (i.e., hypertrophy, scar formation, chamber dilatation, and contractile dysfunction) that occurs following loss of viable myocardium due to myocardial infarction. Patients after myocardial infarction or in other forms of structural heart disease such as cardiac hypertrophy or tachycardia-induced cardiomyopathy undergo a stereotypical pattern of proarrhythmic electrical remodeling that includes QT interval and action-potential prolongation due to down-regulation of repolarizing K^+ currents and loss of normal Ca^{2+} homeostasis [35–37]. Electrical remodeling in structural heart disease is not unique to patients. It also occurs in 'large' animal models and even in mice [38,39].

Interestingly, transgenic over expression of CaMKII in mouse myocardium leads to cardiac hypertrophy, left ventricular chamber dilation, loss of normal contractile function, and proarrhythmic electrical remodeling. Mice with over expression of a splice variant of the predominant myocardial CaMKII isoform (CaMKIIδ, accession numberAY987011) that is preferentially resident in the cytoplasm ($CaMKII\delta_C$) are prone to arrhythmias and exhibit a phenotype of early mortality due to sudden cardiac death [40]. CaMKII is up-regulated in heart tissue from patients [41,42] and animal models [43] with structural heart disease (reviewed by Zhang) [44]. Ventricular myocytes from failing human hearts have L-type Ca^{2+} channels with enhanced opening probability [29]. Based upon these findings, we speculate that there may be an association between CaMKII up regulation, increased activity of Ca^{2+} channels and arrhythmias in heart failure [21].

Mice with myocardial delimited over expression of a CaMKII inhibitory peptide are resistant to electrical and Ca^{2+} homeostasis remodeling changes after myocardial infarction surgery [45]. These mice have CaMKII inhibition from the time of birth, due to the neonatal activation of the α myosin heavy chain promoter that is used to drive expression of the CaMKII inhibitory peptide. CaMKII over-expressing mice have increased activity of Ca^{2+} currents and heightened Ca^{2+} current facilitation, and so we anticipated that mice with chronic CaMKII inhibition would have reduced peak Ca^{2+} current. Instead, CaMKII inhibitor mice exhibited increased peak Ca^{2+} current due to hyperphosphorylation of the Ca^{2+} channel complex by PKA [46]. On the other hand, facilitation of Ca^{2+} current was nearly eliminated in these mice, suggesting that facilitation is a unique attribute of CaMKII signaling and not fully explained by mode 2 gating, which is seen both in response to CaMKII [24] and PKA [26]. Because increased inward Ca^{2+} current would tend to prolong the action potential, we also first considered that these mice would have prolonged QT intervals and action potential duration. To the contrary, optical action potential recordings revealed shortened repolarization, suggesting up regulation of alternative repolarizing potassium currents. Recently, we found that two repolarizing potassium currents — the fast component of the transient outward current (I_{tof} and the inward rectifier I_{K1}) were up-regulated in mice with chronic CaMKII inhibition [46]. Our understanding of the mechanism for K^+ channel up regulation in mice with myocardial CaMKII inhibition is incomplete, but does not appear to be related to the direct effects of CaMKII phosphorylation at these Ca^{2+} channel proteins. On the other hand, up regulation of these channels does appear to be part of a compensatory feedback regulation connecting the content of intracellular SR Ca^{2+} stores to repolarization. Mice with chronic CaMKII inhibition have reduced SR Ca^{2+} content compared to transgenic or wild-type controls [45], and this reduction in SR Ca^{2+} content appears to be linked to CaMKII signaling and phospholamban (PLN, accession numberNM_002667), because when PLN knockout mice are interbred with mice

with chronic CaMKII inhibition, there is no longer a reduction in the amount of SR Ca^{2+} [47]. When mice with chronic CaMKII inhibition are bred into a PLN null background they exhibit normal action potential durations and normalized I_{tof} and I_K [46]. Thus, chronic CaMKII inhibition not only can force resistance to electrical remodeling after myocardial infarction [45], but appears to cause a baseline shortening of the action potential duration due to up regulation of two repolarizing potassium currents, a phenomenon we have termed "reverse electrical remodeling" [46].

Over expression of a nuclear form of CaMK (CaMKIV, accession numberNM_001744) [48], not normally expressed in heart, causes marked QT interval and action potential prolongation due to reduced K^+ currents in mice with modest cardiac hypertrophy [38]. These mice have up-regulated CaMKII activity and expression, increased L-type Ca^{2+} channel opening probability, early afterdepolarizations and Torsade de Pointes. Injection of a CaMKII inhibitory drug (KN-93) reduced arrhythmias, while a more specific CaMKII inhibitory peptide (AC3-I) prevented afterdepolarizations and normalized L-type Ca^{2+} channel opening probability to control levels. These findings were the first to link cardiac hypertrophy to up regulation of CaMKII and arrhythmias.

Mice with over expression of LTCC ($Ca_V1.2$, accession numberNM_000719) α subunits develop cardiac hypertrophy [49]. When these mice are interbred with mice over-expressing a skeletal muscle form of the sarcoplasmic–endoplasmic reticulum Ca^{2+} ATPase (SERCA1a) they develop more marked hypertrophy, increased CaMKII expression, action potential prolongation, afterdepolarizations and arrhythmias [50]. On the other hand, these investigators found that a known CaMKII phosphorylation site on the SERCA regulatory protein phospholamban (threonine 17) was hypo-phosphorylated, suggesting that other uncharacterized compensatory mechanisms are active in this model. Mice with deletion of the atrial natriuretic peptide receptor show hypertension, hypertrophy and enhanced dispersion of right and left ventricular action potentials after atrioventricular node conduction block [51]. These mice had augmented intracellular Ca^{2+} transients and increased CaMKII expression. Importantly, the increased CaMKII expression levels correlated with propensity for ventricular tachycardia. Infusion of CaM (W-7) or CaMKII (KN-93) inhibitory drugs suppressed tachycardia in these mice. These findings highlight the central importance of intracellular Ca^{2+} as a trigger for electrical remodeling and add to evidence that CaMKII can be proarrhythmic by facilitation of L-type Ca^{2+} channels and electrical remodeling.

Calcineurin is a Ca^{2+}/CaM-activated phosphatase, and calcineurin over expression in cardiomyocytes confers a severe form of cardiomyopathy marked by cardiomyocyte hypertrophy, left ventricular dysfunction, dilation, and premature sudden death [52] due to enhanced arrhythmia susceptibility [53]. Calcineurin over-expressing mice have markedly increased myocardial CaMKII expression and activity, and acute injection of a CaMKII inhibitory drug (KN-93) markedly suppresses arrhythmias in these mice. Long-term CaMKII inhibition in calcineurin over-expressing mice, achieved by interbreeding with CaMKII inhibitory mice, caused a significant reduction in mortality that may be due to reduced susceptibility to cardiac arrhythmias [54].

Taken together, these findings suggest that the LTCC complex is an important target for CaMKII and that inhibition of CaMKII signaling to LTCCs might reduce arrhythmias related to an increased probability of LTCC opening. The pleuripotent nature of CaMKII signaling to ion channel [55] and non-ion channel proteins strongly suggests that increases in LTCC opening are not the only mechanism for proarrhythmia by CaMKII. These findings also reveal that the compensatory responses to chronic CaMKII inhibition are not easily predicted and suggest, should effective drug therapy for chronic proarrhythmic disease conditions ultimately develop, patients on chronic CaMKII inhibitor therapy might be anticipated to exhibit compensatory responses, such as reverse electrical remodeling.

Atrial fibrillation is the most common arrhythmia and is associated with increased mortality, stroke and heart failure [56]. Atrial fibrillation shows increased ryanodine receptor Ca^{2+} 'leak' and hyperphosphorylation [57] and electrical remodeling in atrial fibrillation leads to abbreviated action potentials and enhanced tissue dispersion of repolarization, in part due to reduced L-type Ca^{2+} current and increased inward rectifier K^+ current [58]. CaMKII is up-regulated in atrial tissue from patients with atrial fibrillation [59] and recent studies show that the CaMKII site on phospholamban (threonine 17) is hyperphosphorylated in human atrial tissue with atrial fibrillation compared to control patients in normal sinus rhythm [60]. At present, it is unknown if CaMKII inhibition can affect atrial fibrillation. Patients with atrial fibrillation can often be successfully managed by controlling the rate of impulse transmission through the atrioventricular node (AVN) to slow the ventricular response rate [61]. CaMKII activity seems to be important for stimulating AVN impulse transmission at baseline and in response to catecholamine stimulation, because mice with cardiac CaMKII inhibition have slowed AVN transmission and prolonged PR intervals at baseline and after isoproterenol compared to control animals [62]. This interesting finding suggests that CaMKII inhibition could be used for ventricular rate control in the setting of atrial fibrillation [63].

4. Cellular arrhythmia triggering mechanisms: EADs and DADs

Cellular arrhythmia mechanisms are thought to be important as initiating mechanisms for focal arrhythmias (such as ectopic atrial tachycardias, atrial fibrillation and some forms of catecholamine sensitive ventricular tachycardia such as right ventricular outflow-tract tachycardia) and for initiating impulses that lead to arrhythmias sustained by reentrant circuits such as ventricular tachycardia in post

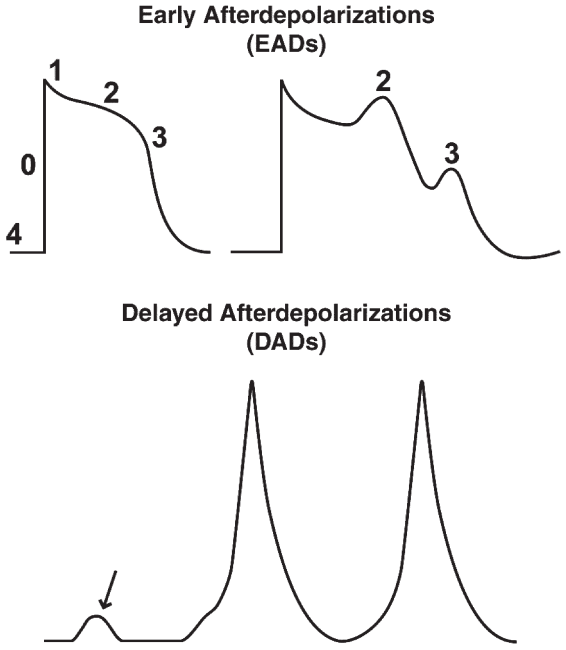

Fig. 2. Cellular arrhythmia mechanisms. Early afterdepolarizations (EADs) are oscillations in the cell membrane potential (ordinate) that occur during to action potential repolarization. EADs are often denoted as phase 2 or phase 3 to indicate their association with numbered phases of the action potential. Delayed afterdepolarizations (DADs) are oscillations in the cell membrane potential that occur after action potential repolarization and are shown here in isolation and initiating action potentials. Both EADs and DADs can depolarize the cell membrane to the threshold for repetitive action potential initiation.

myocardial infarction border zone scar areas [64,65]. These triggers are due to increased net inward current and have been grouped into two broad categories (Fig. 2): early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs). EADs are named because they occur early relative to the completion of action potential repolarization. These occur during the action potential plateau or during the early phases of action potential resolution (so-called phase II and III) and are most often associated with repetitive L-type Ca^{2+} channel openings [66]. Signaling events that favor mode 2 gating such as catecholamine stimulation, L-type Ca^{2+} agonist drugs, and CaMKII are all associated with generation of EADs [10,67,68]. The enhanced susceptibility of cells to EAD formation during action potential prolongation appears to be related to the fact that the cell membrane potential during the action potential plateau resides within a window current voltage range. This window current range is defined by the overlap between activation and steady-state inactivation, and typically ranges between −40 and +10 mV for L-type Ca^{2+} channels. The window current range can be "opened" by stimulation with Ca^{2+} channel agonists, because they shift the inactivation curve to the right and shift the activation curve to more negative cell membrane potentials. CaMKII activity is enhanced even in a cell-free system during prolonged activating Ca^{2+} pulses [9], which mimic the cellular Ca^{2+} transient response to action potential prolongation. In fact, prolongation of the cardiac action potential with K^+ channel blocking drugs is sufficient to enhance Ca^{2+} entry through L-type Ca^{2+} channels and, in turn, activate the Ca^{2+} independent fraction of total CaMKII activity [10]. Under some experimental conditions EADs are independent of the release of intracellular Ca^{2+} stores [69], but we and others have found that blocking SR Ca^{2+} cycling prevents the development of EADs and arrhythmias [70,71]. One possible reason for the blockade of EADs and arrhythmias by inhibitors of SR Ca^{2+} cycling is that SR Ca^{2+} release is the predominant source of Ca^{2+} for CaMKII activation [27,72].

CaMKII inhibitory drugs or dialysis of highly specific CaMKII inhibitory peptides (modeled after the autoinhibitory region of the regulatory domain) effectively inhibit EAD formation and arrhythmias even without shortening action potentials or QT intervals [10,70,73,74]. Although CaMKII activity may have other targets for activating EADs than L-type Ca^{2+} channels, enhancing L-type Ca^{2+} channel activity is sufficient to induce EADs [67] and the polymorphic tachycardia Torsade de Pointes [73].

Delayed afterdepolarizations are named because they take place after repolarization of the action potential to baseline. These afterdepolarization are linked to conditions that favor SR Ca^{2+} overload. A common example of a circumstance favoring DADs occurs during digitalis toxicity [75], where Ca^{2+} loading occurs due to poisoning of the sodium gradient across the cell membrane and secondary reduction in the ability of the electrogenic sodium Ca^{2+} exchanger to clear cytoplasmic Ca^{2+} to the extracellular space during its forward mode of operation [76].

CaMKII inhibition leads to a reduction in SR Ca^{2+} content, at least in structurally normal myocardium [45], and this is one potential mechanism for CaMKII inhibition reducing DADs [77]. On the other hand, DADs can occur simultaneously with action potential prolongation and EADs [78] so that CaMKII could contribute to SR Ca^{2+} overload through enhanced cellular Ca^{2+} entry by way of L-type Ca^{2+} channels during EADs [72]. When artificially prolonged action potential wave forms are used as voltage clamp commands, cell membranes exhibit a transient inward current that is blocked by dialysis of a CaMKII inhibitory peptide [77]. These studies prove that Ca^{2+} loading during a prolonged action potential is capable of overloading the SR with Ca^{2+} and producing currents likely to be responsible for DADs. May many inward currents have been considered candidates for generating DADs, such as Ca^{2+} activated chloride currents, non-selective Ca^{2+} activated cation currents and non-inactivating voltage independent Ca^{2+} channel opening. However, most data point to the sodium–Ca^{2+} exchanger as the source of inward current for DADs [76,77].

Cellular arrhythmia triggers are a plausible initiating mechanism for arrhythmias, but in contrast to macro reentrant circuits, which are relatively easy to measure in an integrated preparation such as a whole heart or even a

patient, it is not possible to directly measure cellular afterdepolarizations in situ. Some studies in patients during arrhythmia and aneurysmectomy surgery, however, have pointed to a potential role for initiation of reentrant arrhythmias by highly focal triggers that are consistent with initiation by earlier delayed afterdepolarizations [64,65,79]. Furthermore, it is well accepted that conditions such as excessive QT interval prolongation (essentially a read-out of action potential prolongation) and Ca^{2+} overload are associated with both afterdepolarizations and with enhanced susceptibility to arrhythmias in patients. In this regard, a rabbit model of excessive QT interval prolongation and Torsade de Pointes shows that CaM or CaMKII inhibitors can prevent arrhythmias, suppress prominent secondary U waves that are linked to afterdepolarizations, and prevent arrhythmias without shortening the QT interval [73,74]. These same conditions suppress early afterdepolarizations in cellular and isolated heart studies.

Taken together, these findings show that CaMKII can facilitate EADs and DADs and plausibly link these afterdepolarizations to arrhythmias in animal models. Further studies and possibly newer 'mapping' techniques applicable to patients will be necessary before afterdepolarizations are definitively linked to arrhythmias in patients.

5. Myocardial hypertrophy and cardiomyocyte dysfunction

Cardiac hypertrophy and myocardial dysfunction are key components of structural heart disease in patients and in animal models. These states also go hand in hand with a pattern of electrical remodeling that confers an enhanced susceptibility to arrhythmia, as discussed above. Myocardial hypertrophy can be an adaptive initial response to some stressors, such as increased afterload, sustained catecholamine stimulation, or enhanced demand of surviving myocytes after myocardial infarction, so understanding biological signals that potentially differentiate adaptive from maladaptive, proarrhythmic responses to cardiac stress is a fundamental goal for cardiology. Multiple signals are involved in hypertrophic signaling and it is increasingly clear that signaling networks are highly interconnected. Perturbations in one signaling system are likely to have repercussions in other signaling cascades. Despite the complexity of signaling and the limitations of our current knowledge, it is clear that CaMKII is an important hypertrophic signaling molecule in heart. CaMKIIδ over expression causes cardiomyocyte hypertrophy, electrical remodeling and arrhythmias [40,80]. CaMKII can do this through a variety of candidate transcription signaling pathways, some of which are discussed in detail in this special issue. The myocyte enhancer factor II is a transcription factor that is negatively regulated by class II histone deacetylase (HDAC). HDAC phosphorylation leads to an unbinding or derepression of HDAC from the transcription factor myocyte enhancer factor 2 (MEF2), allowing for activation of MEF2 dependent genes that are associated with hypertrophy [81]. HDAC (accession numberNM_006037) can be phosphorylated by CaMKII, and its binding to MEF2 can also be disabled by binding to Ca^{2+}/CaM [82]. This role of HDAC as a negative regulator of MEF2 appears to be distinct from its enzymatic role as a histone deacetylase [83]. Because CaMKII can phosphorylate HDAC to derepress MEF2-mediated transcription and also contribute to enhanced Ca^{2+} flux that might increase the availability of Ca^{2+}/CaM to bind HDAC in the nucleus, CaMKII could effect transcription through the MEF2 signaling pathway via two distinct mechanisms.

CaMKII also positively regulates NFκB (nuclear factor for κB, accession numberNM_021975). CaMKII can act as an upstream kinase (Iκ kinase) by phosphorylating the negative regulatory protein IκB [84]. IκB phosphorylation derepresses NFκB by unbinding and allowing consequent migration of NFκB to the nucleus to activate transcription. The role in NFκB signaling in myocardium is an area of active investigation [85]. However, at this time NFκB activation is not known to result in a proarrhythmic electrical remodeling phenotype.

CaMKII can regulate the CRE (cyclic AMP response binding element) binding protein (CREB, accession numberNM_004379) and the homologous transcription factor, ATF1 (accession numberNM_005171) [86]. Over expression of a dominant negative form of CREB (Ser133Ala) causes a very severe cardiomyopathy marked by sudden death [87]. The serine 133 site is a target for a number of protein kinases, including CaMKII. On the other hand, the serine 142 site appears to be exclusively phosphorylated by CaMKII, and phosphorylation reduces the activity of CREB [88]. Thus, CaMKII could function as an anti-hypertrophic signal by negatively regulating CREB-mediated transcription, although this has not been validated in cardiomyocytes or *in vivo*.

CaMKII is up regulated in many models of cardiac hypertrophy and appears to contribute to the loss of Ca^{2+} homeostasis that is a hallmark of cardiac hypertrophy. When CaMKII is transgenically over expressed in mouse heart, it leads to a prolonged Ca^{2+} transient, enhanced L-type Ca^{2+} current facilitation, increased ryanodine receptor Ca^{2+} leak (measured as increased sparks), and loss of myocyte contractile function [80]. Increased ryanodine receptor leak is associated with structural heart disease in patients [89] and in animal models [90], due to PKA hyperphosphorylation. CaMKII also increases ryanodine receptor (accession numberNM_001035) opening [91] and CaMKII causes increased ryanodine receptor leak in a rabbit model of structural heart disease, DADs, arrhythmias and sudden death [43], suggesting that CaMKII may play a role in the SR Ca^{2+} release process in cardiac hypertrophy. Viral over expression of CaMKIIδ also causes increased ryanodine receptor Ca^{2+} leak in isolated ventricular myocytes, even before signs of hypertrophy are manifest [92], suggesting that increased CaMKII activity may drive an increased ryanodine receptor leak that leads to the development of

heart failure and arrhythmias. Genetic CaMKII inhibition confers a resistance to cardiac hypertrophy from excessive and sustained catecholamine stimulation [45], suggesting that hypertrophy could be prevented through a CaMKII inhibitory strategy. On the other hand, CaMKII inhibition significantly restored myocardial function in calcineurin cardiomyopathy (linked to NFAT/GATA4 signaling) with very little effect on reducing the consequent hypertrophy [54], suggesting that CaMKII over activity may have deleterious effects on myocyte function that are independent of cardiac hypertrophy.

Patients with structural heart disease are susceptible to arrhythmias and sudden death [34]. This is a critical problem for public health and for cardiovascular medicine. An interesting and growing body of evidence suggests that CaMKII signaling engenders both structural/mechanical phenotypes as well as proarrhythmic electrical phenotypes. This evidence raises the tantalizing suggestion that CaMKII signaling may link these two troubling phenotypes and that inhibition of CaMKII potentially could treat both arrhythmias and cardiac hypertrophy/mechanical dysfunction. Before this hypothesis can be tested, it will be important to have highly potent/high specificity drugs and to determine if systemic CaMKII inhibition would not have unforeseen side effects.

6. Cell survival and generation of a pro-arrhythmic substrate

CaMKII initiates a caspase-3 (accession numberNM_004346) dependent apoptotic signaling pathway in isolated cardiomyocytes. Cardiomyocytes exposed chronically to isoproterenol developed apoptosis linked to release of Ca^{2+} from the SR stores and activation of CaMKII [93]. Curiously, this signaling pathway appeared to be independent of PKA activation. CaMKII inhibitors (either pharmacological or peptide based) effectively inhibited apoptosis under these conditions, as was a strategy of depleting the SR Ca^{2+} stores. These findings have recently been validated *in vivo* using mice with genetic CaMKII inhibition [22]. These mice were resistant to the apoptotic effects of ischemia and high dose isoproterenol. It appeared that these *in vivo* results were also linked to SR Ca^{2+} stores because when these mice were interbred with mice lacking phospholamban, they had increased SR Ca^{2+} content [47], and were no longer resistant to apoptosis initiated by myocardial infarction. In contrast, CaMKII inhibition continued to confer resistance to apoptosis due to isoproterenol injection in phospholamban knock out mice, suggesting that CaMKII may connect to phospholamban-dependent and -independent apoptotic signaling pathways. While apoptosis is typically linked to cell death without fibrosis, in some systems apoptosis can be associated with fibrosis and so might contribute to scarring and proarrhythmic delay in conduction and reentry circuits after myocardial infarction [94,95].

CaMKII is activated during β-adrenergic receptor signaling, and appears to be a downstream signal that is required for pathological aspects of sustained β-adrenergic signaling including hypertrophy, myocardial dysfunction, and apoptosis [45,22]. CaMKII itself is necessary for some of the dynamic intracellular Ca^{2+} responses to pacing [47]. We recently found that mice subjected to daily high doses of isoproterenol developed myocardial hypertrophy, dysfunction [45] and subendocardial fibrosis (unpublished data). On the other hand, mice with CaMKII inhibition were significantly resistant to hypertrophy, mechanical dysfunction [45] and subendocardial fibrosis (unpublished data). These findings suggest that CaMKII can modify cell death, at least in models of ischemia and catecholamine toxicity. In some cases, this may contribute to fibrosis or excessive apoptosis that could lead to compensatory hypertrophy of surviving cells that would together contribute to a proarrhythmic tissue substrate.

7. Conclusions

CaMKII was recognized in the late 1990s as being a potential pro-arrhythmic signaling molecule based on its ability to enhance Ca^{2+} current facilitation and EADs and DADs. It has become increasing clear that multiple targets and molecular, cellular, and tissue mechanisms may be important for the pro-arrhythmic signaling features of CaMKII. Most intriguing, perhaps, is the suggestion that CaMKII can dually contribute to pro-arrhythmic electrical remodeling and cardiac hypertrophy, and therefore, supplies a molecular rationale for the regular occurrence of these two terrible disease phenotypes.

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