Role of Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II in cardiac hypertrophy and heart failure

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Abstract

Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II (CaMKII), a critical transducer of Ca\textsuperscript{2+} signaling, is a multifunctional protein kinase which can phosphorylate a wide range of substrates and regulate numerous cellular functions. The \(\delta\) isoforms of CaMKII predominate in the heart and two splice variants of CaMKII, \(\delta\)\textsubscript{B} and \(\delta\)\textsubscript{C}, have been demonstrated to be present in the adult mammalian myocardium. The \(\delta\)\textsubscript{B} isoform contains a nuclear localization signal (NLS) that is absent from \(\delta\)\textsubscript{C}, and consequently, the two isoforms have different subcellular localization. Recent work from our laboratory and others has implicated CaMKII in the development of cardiac hypertrophy and heart failure. The specific roles of these CaMKII isoforms in regulating cardiac function appear to be determined by their subcellular localization. The nuclear \(\delta\)\textsubscript{B} isoform plays a key role in hypertrophic gene expression, whereas the cytoplasmic \(\delta\)\textsubscript{C} isoform can affect excitation–contraction (E–C) coupling through phosphorylation of Ca\textsuperscript{2+} regulatory proteins and may also transduce signals leading to apoptosis. In addition, the nuclear \(\delta\)\textsubscript{B} and the cytoplasmic \(\delta\)\textsubscript{C} isoforms of CaMKII are differentially regulated in pressure overload-induced cardiac hypertrophy. This review focuses on evidence that CaMKII plays an essential role in transcriptional activation associated with cardiac hypertrophy, as well as the aberrant Ca\textsuperscript{2+} handling and apoptosis that may contribute to heart failure. The hypothesis that CaMKII isoform selective activation, localization and substrate phosphorylation lead to specificity in the resultant signaling pathways is discussed.

Keywords: Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II; Cardiac hypertrophy; Gene expression; Heart failure; E–C coupling

1. Introduction

Ca\textsuperscript{2+} is arguably the most important second messenger in cardiac muscle. Changes in the intracellular Ca\textsuperscript{2+} concentration have both acute and chronic effects on cardiac function. The Ca\textsuperscript{2+} transients which accompany each cardiac cycle trigger cardiac muscle contraction, a process known as excitation–contraction (E–C) coupling. In contrast, more sustained or chronic changes in Ca\textsuperscript{2+} signaling can result in activation of gene expression, a process that has been referred to as excitation–transcription (E–T) coupling [1,2]. Increases in Ca\textsuperscript{2+} can transduce signals through various classes of Ca\textsuperscript{2+}-regulated enzymes, one of which is the Ca\textsuperscript{2+}/calmodulin-dependent protein kinase (CaM kinase or CaMK) family [3].

CaMKs are serine/threonine kinases that are regulated by Ca\textsuperscript{2+} liganded calmodulin (CaM). Myosin light chain kinase (MLCK) and phosphorylase kinase are Ca\textsuperscript{2+}/CaM-dependent protein kinases which are dedicated to a particular substrate (for review, see Ref. [4]). Elongation factor-2 (EF-2) kinase (originally termed CaMKIII) is also a kinase dedicated to the phosphorylation of a single substrate [5]. In contrast, CaMKI, CaMKII and CaMKIV are multifunctional CaMKs. CaMKII and CaMKIV are monomeric enzymes which are activated through phosphorylation by an upstream CaMK kinase (CaMKK) following their binding to Ca\textsuperscript{2+}/CaM [6,7]. CaMKI has broad tissue distribution and is cytoplasmic in mammalian cells [8], although a C-terminally truncated form of CaMKI can go to nucleus and phosphorylate and activate transcription factors when expressed in mammalian cells [9,10]. While CaMKI has...
been shown to be present in the heart, its function in this tissue remains to be determined since it is not upregulated during hypertrophy [11,12]. CaMKIV has restricted expression being found largely in neuronal tissues, T lymphocytes and testis [8] and undetectable in the heart [12,13]. CaMKIV, while present in the cytoplasm, is predominantly localized in the nucleus [14], implying a role for CaMKIV in transcriptional regulation and gene expression.

In contrast to CaMKI and CaMKIV, the ubiquitously expressed CaMKII is a homo- or heteromultimer of 6–12 subunits consisting of α, β, γ or δ subunits, each encoded by a separate gene [3,15]. The α and β subunits are mainly restricted to neuronal tissues while the γ and δ subunits are ubiquitous [16]. Alternative splice variants of the CaMKII subunits have been identified and several of them (i.e. αγ, γα and δα) contain nuclear localization signals (NLS) which target them to the nucleus. The subcellular localization of heteromultimers of CaMKII is determined by the ratio of the cytoplasmic to nuclear targeted subunits [17]. CaMKII is activated by binding to Ca2+/CaM and subsequent autophosphorylation [3]. Autophosphorylation generates a state of CaMKII that retains enzymatic activity even in the absence of Ca2+/CaM; this Ca2+/CaM-independent activity is referred to as autonomous activity.

Numerous studies demonstrate that CaMKII can phosphorylate a range of substrates and regulate many aspects of cellular functions. Recent work from our laboratory and others has implicated CaMKII in cardiovascular disease. This review focuses on how CaMKII activation, localization and isoform specific signaling pathways regulate chronic alterations in gene expression such as those seen in cardiac hypertrophy, as well as phosphorylation of proteins that are involved in transcriptional regulation and gene expression. Recent work from our laboratory and others has implicated CaMKII in cardiovascular disease. This review focuses on how CaMKII activation, localization and isoform specific signaling pathways regulate chronic alterations in gene expression such as those seen in cardiac hypertrophy, as well as phosphorylation of proteins that are involved in acute E–C coupling and may be dysregulated in heart failure.

2. CaMKII in the heart

2.1. Isoforms of CaMKII in the heart

The ubiquitous expression of δ and γ subunits of CaMKII have been demonstrated by Northern blot analysis to be present in the heart [16]. In contrast, neither α nor β subunits of CaMKII are expressed in the heart. Since the γ subunit is only expressed at low levels in cardiomyocytes [18], the δ isoform of CaMKII is accepted to be the predominant isoform in the heart [19–21], including in human myocardium [22]. This CaMKII isoform was the first to be cloned from the heart (two splice variants of CaMKIIδ, see below) [19]. Soon after that, Baltas et al. [20] demonstrated that the cardiac sarcoplasmic reticulum (SR) phospholamban kinase is also a CaMKIIδ isoform.

2.2. Domain structure and activity regulation of CaMKII

As discussed earlier, CaMKII is encoded by four distinct genes (α, β, γ and δ) in mammals. The corresponding CaMKII subunits are highly conserved among different mammalian species. Each CaMKII subunit contains three structural domains (catalytic, regulatory and association) which are conserved among the different subunits. The N-terminus, the kinase catalytic domain, is followed by a regulatory region with an autoinhibitory domain and a CaM binding site. The C-terminus is the association domain responsible for subunit assembly and perhaps also for interaction with other proteins (for review, see Ref. [23]). A schematic representation showing the domain structure of the CaMKIIδ subunit is shown in Fig. 1A. In its basal state, CaMKII catalytic activity is inhibited by the autoregulatory domain, which prevents substrates from binding. Binding of Ca2+/CaM induces a conformational change which activates CaMKII by disrupting the interaction of the autoinhibitory domain with the ATP and protein substrate binding sites in the catalytic domain of the kinase. The autophosphorylation sites within the autoregulatory domain of the δ isoform are Thr287 (the same in β and γ, and Thr286 in α) and Thr306/Thr307 (the same in β and γ, and Thr305/Thr306 in α). Autophosphorylation at Thr287 (or Thr286 in α) in the autoinhibitory domain of CaMKII occurs once the enzyme is activated by Ca2+/CaM binding and will generate autonomous activity of CaMKII. Once the kinase is phosphorylated at Thr287 and Ca2+/CaM dissociates, autophosphorylation can occur at Thr306/Thr307 in the CaM binding site. This blocks Ca2+/CaM rebinding, preventing further enzyme activation [23]. Half maximal activation of CaMKII occurs at 0.5–1 μM free Ca2+ [4], a concentration similar to free systolic [Ca2+] during cardiac contraction. However, whether CaMKII is activated in a beat-to-beat manner is not known [2]. At saturating levels of Ca2+, half maximal activity of CaMKII requires 25–100 nM CaM, a low affinity compared to other Ca2+/CaM-regulated enzymes such as MLCK and calcineurin. Since the amount of CaM in cardiomyocytes is thought to be limiting [2], the affinity of these target enzymes for binding to CaM is another factor that could determine their differential activation. CaMKII activity can also be negatively regulated by dephosphorylation of autophosphorylated CaMKII by protein phosphatases, including PP1, PP2A, PP2C and a new family of protein phosphatases that shows strict substrate specificity for the family of multifunctional CaMKs (CaMKI, II and IV) (for review, see Ref. [24]).

2.3. CaMKII isoforms, splice variants and subcellular localization

As mentioned above, CaMKII α, β, γ or δ subunits undergo alternative splicing, generating splice variants, which differ in regions between the CaM binding and association domains or within the association domain (for review, see Ref. [4]). To date, 13 splice variants of the δ subunit of CaMKII have been identified and the domain structure of the cardiac CaMKIIδ splice variants is shown in Fig. 1B. Both δα (δ1) and δε (δ2) splice variants have been
shown to be present at the protein level in the adult mammalian myocardium [19,20,25]. The δB subunit contains an 11-amino-acid NLS that is absent from δC. Because of this difference, CaMKII comprised predominantly of δB subunits localizes to the nucleus while CaMKII comprised of δC subunits localizes to the cytoplasm [17,19,26]. Based on RT-PCR analysis, transcripts for δH (δ8) and δI (δ9) subunits are also present in adult cardiac tissue [22,25], while δD (δ4) isoform appears to be present as an embryonic isoform in the heart [25]. Transcript expression of the γ subunit is seen in neonatal rat cardiomyocytes [18]; however, CaMKIIγ mRNA and protein are virtually undetectable in adult mouse ventricles except following transverse aortic constriction (TAC) [12].

The significance of the diversity of CaMKII isoforms and their splice variants in regulating cardiac function has not been adequately explored. The existence of these variants suggests that they have distinct properties. Prominent amongst these is their different subcellular localization and intracellular targeting as indicated above with regard to δB and δC isoforms [17,19,26]. Less well documented, but also implicit in their differential localization, is that they may have different substrate specificity, as demonstrated for the multiple CaMKII splice variants found in Drosophila [27] and suggested for the CaMKIIδ and δC subunits in mammals (see below). The different localizations also suggest possible differential regulation of the CaMKII splice variants via different stores of Ca2+, i.e., they may have different modes of activation. Finally, they may have different Ca2+ and/or Ca2+/CaM sensitivity, as demonstrated for the β-CaMKII splice variants, which manifest different sensitivity to Ca2+ oscillations [28]. All of these properties would contribute to specificity in the signal transduction pathways regulated by this ubiquitously expressed enzyme family.

3. CaMK in cardiac hypertrophy and heart failure

There is growing evidence for a pathophysiological role of CaMK in cardiac hypertrophy and heart failure. Studies initiated in in vitro systems, extended to animal models, and reinforced by findings from humans suggest that CaMK is an important sensor of altered Ca2+ signals and a key effector of changes in Ca2+ regulatory proteins and transcriptional responses (see Table 1 for a summary of in vivo studies showing CaMKII involvement in cardiac hypertrophy and heart failure).

3.1. In vitro experiments

In vitro studies using pharmacological inhibitors were among the first to suggest that CaMK was involved in cardiomyocyte hypertrophy induced by hypertrophic agonists. In neonatal rat ventricular myocytes, α-adrenergic receptor activation with phenylephrine (PE) was shown to stimulate expression of atrial natriuretic factor (ANF), a hypertrophic marker, through a Ca2+/CaM-regulated enzyme, as indicated by the inhibitory effect of W-7 on PE-stimulated ANF expression [29]. Subsequently, CaMKII was implicated as the Ca2+/CaM-regulated enzyme in studies using a CaMKII inhibitor, KN-93 [26]. CaMKII activa-
tion has also been suggested to regulate expression of brain natriuretic peptide (BNP) promoter, another hypertrophic marker gene, in response to interleukin-1β (IL-1β) [30]. Endothelin-1 (ET-1) has been shown to increase the activity of CaMKII in cardiomyocytes, and pretreatment with KN-62, a CaMK inhibitor, is able to suppress ET-1-induced cardiomyocyte hypertrophy measured by β-myosin heavy chain (β-MHC) promoter activation, [3H] phenylalanine uptake and cell size [31]. Finally, hypertrophic responses to leukemia inhibitory factor (LIF) in cardiomyocytes have also been shown to be attenuated by KN-62 [32].

Transient overexpression of CaMK in cardiomyocytes provides further evidence for a role of CaMK in cardiomyocyte hypertrophy. Expression of the wild-type δB isoform of CaMKII in neonatal rat ventricular myocytes increased ANF protein expression and led to an enhanced transcriptional response to PE, as assessed by activation of an ANF-luciferase reporter gene [26]. In contrast, expression of the δC isoform of CaMKII did not enhance ANF expression, and co-expression of δC with the δB isoform prevented nuclear CaMKII localization and concomitantly inhibited the hypertrophic response [26]. These data are consistent with other published observations indicating that constitutively activated forms of CaMKI and CaMKIV, which also enter the nucleus, induce hypertrophic response in cardiomyocytes in vitro [10].

### 3.2. Animal models

Cardiac CaMKII expression and activity have been reported to be altered in a variety of animal models of cardiac hypertrophy and heart failure. Studies examining hypertrophied myocardium from spontaneously hypertensive rat (SHR) models showed increased expression of CaMKIIδ [33] and increased CaMKII activity [34]. Notably, these changes in CaMKII could be reversed by angiotensin (Ang)-converting enzyme (ACE) inhibition, which also led to complete regression of the myocardial hypertrophy (presumably AngII-mediated) that developed in this model [35]. Hearts from two transgenic rat models with AngII-dependent hypertension also showed changes in the pattern of CaMKIIδ isoform expression [33]. Additionally, in coronary artery ligated rabbit hearts in which hypertrophy developed, there was increased CaMKII activity and upregulation of CaMKIIδ protein expression in isolated SR fractions [36]. Most recently, pressure overload hypertrophy induced by TAC in mouse hearts or by acute increases in pressure in perfused rat hearts has been shown to be accompanied by increased CaMKII expression and activity [12,37,38]. In some heart failure models (e.g. in a rat myocardial infarction model [39] and in a canine intracoronary microembolization model [40]), reduced CaMKII activity and expression were observed.

Several genetically altered mouse models have confirmed a role for CaMK in the development of hypertrophy. Transgenic mice overexpressing calmodulin were reported more than a decade ago to develop severe cardiac hypertrophy [41]. This phenotype was more recently shown to be accompanied by an increase in the autonomous activity of CaMKII in vivo [42]. Transgenic mice that overexpress CaMKIV were also reported to develop profound hypertrophy associated with specific changes in gene expression.

### Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Models</th>
<th>Phenotype</th>
<th>Changes in CaMKII</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Spontaneously hypertensive (SHR)</td>
<td>Cardiac hypertrophy</td>
<td>↑ CaMKIIδ and δ transcripts</td>
<td>[33,35]</td>
</tr>
<tr>
<td>Rat</td>
<td>Transgenic hypertensive</td>
<td>Cardiac hypertrophy</td>
<td>↑ SR CaMKIIδ expression</td>
<td>[34]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Coronary artery ligation</td>
<td>Cardiac hypertrophy</td>
<td>↑ SR CaMKII activity</td>
<td>[33]</td>
</tr>
<tr>
<td>Mouse</td>
<td>Transverse aortic constriction</td>
<td>Cardiac hypertrophy</td>
<td>↑ SR CaMKII expression</td>
<td>[36]</td>
</tr>
<tr>
<td>Rat</td>
<td>Myocardial infarction</td>
<td>Heart failure</td>
<td>↑ CaMKIIδ expression</td>
<td>[12,37]</td>
</tr>
<tr>
<td>Dog</td>
<td>Intracoronary microembolization</td>
<td>Heart failure</td>
<td>↑ CaMKII activity</td>
<td>[39]</td>
</tr>
<tr>
<td>Mouse</td>
<td>Calmodulin transgenic</td>
<td>Cardiac hypertrophy</td>
<td>↑ CaMKII activity</td>
<td>[40]</td>
</tr>
<tr>
<td>Mouse</td>
<td>CaMKIV transgenic</td>
<td>Cardiac hypertrophy</td>
<td>↑ CaMKIV expression</td>
<td>[35]</td>
</tr>
<tr>
<td>Mouse</td>
<td>CaMKIIδ transgenic</td>
<td>Cardiac hypertrophy</td>
<td>↑ CaMKIIδ expression</td>
<td>[42]</td>
</tr>
<tr>
<td>Mouse</td>
<td>CaMKIIδC transgenic</td>
<td>Heart failure</td>
<td>↑ CaMKIIδ expression</td>
<td>[34]</td>
</tr>
<tr>
<td>Human</td>
<td>Dilated cardiomyopathy</td>
<td>Heart failure</td>
<td>↑ CaMKIIδ expression</td>
<td>[39]</td>
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In this model, transcript levels of CaMKII variants were upregulated [33,35]. The cytoplasmic δC isoform of CaMKII also appear to be differentially regulated by pressure-overload induced hypertrophy [12,37]. While both CaMKIIδB and CaMKIIδC are activated as early as 2 days after TAC [37], as indexed by their autophosphorylation, only the CaMKIIδC isoform shows increased and sustained (for at least 7 days) expression after TAC [12,37]. The early increase in CaMKIIδ activation is consistent with previous work indicating a role for CaMKIIδB in hypertrophy [26,43].

CaMKII can regulate transcriptional gene expression and Ca^{2+} handling. Thus, under physiological conditions, CaMKII can modulate normal cardiac function. In response to hypertrophic stimuli (such as TAC), both CaMKIIδB and CaMKIIδC are activated. We hypothesize that the nuclear δB isoform serves a compensatory function by initiating hypertrophic gene expression, while the cytoplasmic δC isoform serves a compensatory function by improving Ca^{2+} handling. At early stage of hypertrophy, these changes would be beneficial to the maintenance of normal cardiac function. On the other hand, at longer times, when CaMKIIδC expression is upregulated and alterations in Ca^{2+} handling are sustained, CaMKIIcould contribute to the downward spiral leading to heart failure. This scenario is suggested by studies in our recently generated transgenic mice overexpressing the δC isoform of CaMKII in the heart [37]. These mice develop a dilated cardiomyopathy with markedly decreased contractile function, and die prematurely [37]. Increased RyR2 phosphorylation, which may initially be beneficial, is observed in these animals and appears to be responsible for the development of increased SR Ca^{2+} leak (sparks) and SR Ca^{2+} depletion [44]. Thus, RyR2 phosphorylation resulting from CaMKIIδC overexpression contributes to the development of heart failure in this model. Whether CaMKII mediates both physiological and pathological growth in response to hypertrophic stimuli is not yet established. Studies utilizing CaMKII knockout mouse models should provide more direct evidence for a role for CaMKII isoforms in physiological vs. pathological hypertrophy.

3.3. Human studies

Studies examining cardiac tissue from control and heart failure patients have shown that cardiac CaMKII expression and activity are altered in failing human hearts. CaMKII activity is increased ~3-fold in cardiac tissue from patients with dilated cardiomyopathy [45] and expression of CaMKIIδ is increased ~2-fold in failing human myocardium from patients suffering from dilated cardiomyopathy [22]. More specifically, the expression of δB has been found to be increased at the transcript levels in failing human myocardium [22]. Upregulated CaMKII activity has also been positively correlated with changes in cardiac function in these patients [45], although one cannot ascribe any cause–effect relationship to these events.

4. Signaling pathways for CaMKs in cardiac hypertrophy and heart failure

In response to a variety of intrinsic and extrinsic stimuli, the heart undergoes hypertrophic growth, developing a constellation of phenotypic changes which include increases in cardiomyocyte cell size, sarcomeric reorganization and re-expression of a fetal gene program. Cardiac hypertrophy is initially a beneficial adaptive response required to sustain cardiac function, however, it has also been reported to be an independent risk factor for ischemic heart disease, arrhythmia, and sudden death [46]. As indicated earlier, Ca^{2+} signals have been shown to play a central role in the development of cardiac hypertrophic growth and gene expression, and CaMK acts as an important mediator in this signaling pathway. Prolonged hypertrophy is often followed by the development of dilated cardiomyopathy and eventual heart failure [47]. It is known that many of the same signaling pathways that regulate hypertrophy are involved in the progression to heart failure. On the other hand, heart failure can also develop independently of hypertrophy, i.e. from altered cardiomyocyte Ca^{2+} handling or cardiomyocyte apoptosis. CaMKII activation could thus induce heart failure either through progression of hypertrophy, or by altering Ca^{2+} handling proteins or apoptosis. A schematic of potential mechanisms by which CaMKII signaling contributes to cardiac hypertrophy and heart failure is shown in Fig. 2.

The role of CaMKII in Ca^{2+} signal transduction and regulation of cardiac function appears to be determined by its subcellular localization. We speculate that there are specific functions for the nuclear CaMKIIδB isoform (e.g. in transcriptional responses and gene expression, contributing to hypertrophic growth) and the cytoplasmic CaMKIIδC isoform (e.g. in Ca^{2+} handling and apoptosis, contributing to the development of heart failure). As a corollary to the
hypothesized functional differences in the isoforms, we suggest that their activation may be distinctly regulated at specific sites or by different patterns of Ca^{2+} mobilization.

4.1. Transcriptional activation and gene expression regulated by CaMK

A growing body of evidence has demonstrated that Ca^{2+} signaling can regulate transcription and gene expression [48]. Ca^{2+} signals are thus likely mediators of the changes in gene expression that characterize the hypertrophic response of cardiomyocytes (for review, see Ref. [49]). How Ca^{2+} signals are transmitted to the transcriptional machinery in the nucleus is not yet clear. While Ca^{2+} and/or Ca^{2+}/CaM can translocate into the nucleus to activate their downstream targets [50], there is growing evidence for the existence of mechanisms for generating Ca^{2+} signals within the nucleus (for reviews, see Refs. [51,52]). For example, inositol 1,4,5-trisphosphate (InsP_3) receptors have been identified on the nuclear membrane in ventricular myocytes [2] and in other cell types [53,54], indicating that the nuclear envelope might serve as a pool for releasable Ca^{2+}. Increases in Ca^{2+} in the nucleus of intact cells or in isolated nuclei have been demonstrated in the absence of changes in cytosolic Ca^{2+} [54–56]. Since CaM has also been reported to be associated with nuclear envelope [57], Ca^{2+} that is locally released via nuclear or other nearby InsP_3 receptors would be expected to bind to the nuclear membrane-bound CaM. This could in turn activate the nuclear isoform of CaMKII, resulting in transcriptional regulation. A nuclear Ca^{2+} signaling cascade, including the nuclear δB isoform of CaMKII, would be predicted to play a predominant role in coupling Ca^{2+} signals to transcriptional events in cardiomyocytes. Interestingly, CaMKII has been suggested to phosphorylate InsP_3 receptors [58,59] and also to affect InsP_3 metabolism by regulating both an InsP kinase involved in InsP_4 formation from InsP_3 [60] and a phosphatase involved in InsP_3 breakdown [61].

CaMKII has been shown to be involved in the regulated expression of several hypertrophic marker genes, including ANF [26], BNP [30], β-MHC [31] and α-skeletal actin [10]. The nuclear localization signal of CaMKIIδB was shown to be required for this hypertrophic response as transfection of CaMKIIδC did not result in enhanced ANF expression [26]. It has also been reported that CaMK is involved in regulating various transcription factors and other DNA-binding proteins including cAMP-response element binding protein (CREB) [62], CAAT-enhancer bind-
ing protein (C/EBP) [63], activating transcription factor (ATF-1) [9], activator protein 1 (AP-1) [64], serum response factor (SRF) [65], and myocyte enhancer factor-2 (MEF2) [10]. CREB is a ubiquitous transcription factor and has been shown to be activated by both CaMKII and CaMKIV via phosphorylation at Ser133 [66]. CaMKII can also phosphorylate CREB at Ser142, an event that negatively regulates CREB-dependent transcription [67]. Surprisingly, increased CREB phosphorylation is not detected in transgenic mice expressing CaMKIIδB [43] or CaMKIV [10], although these enzymes localize to the cardiomyocyte nucleus. However, MEF2 has been suggested to act as a common endpoint for hypertrophic signaling pathways in the myocardium [68,69] and studies using CaMKIV transgenic mice crossed with MEF2 indicator mice suggest that MEF2 is a downstream target for CaMKIV [10]. CaMKIV has been reported to be capable of phosphorylating MEF2D [70]. However, this is unlikely to be the predominant regulatory mechanism since there is no evidence that CaMK can phosphorylate other MEF2 family members, such as MEF2A or MEF2C [69,70]. Recent studies have demonstrated that MEF2 can interact with class II histone deacetylases (HDACs), a family of transcriptional repressors, as well as with other repressors that limit MEF2-dependent gene expression. Notably, constitutively activated CaMKII and CaMKIV have been shown to activate MEF2 by phosphorylating and dissociating HDACs, leading to its subsequent nuclear export [69]. SRF has been shown to be activated by CaMKIV in a similar manner, i.e. by increased dissociation of HDACs [71], as well as by direct CaMKII- or CaMKIV-mediated SRF phosphorylation [65,72]. Regulation of MEF2 and SRF association with HDACs has been examined with CaMKII and IV isoforms. The ability of the predominant cardiac CaMKIIδ isoform to control HDAC phosphorylation, and in particular the selectivity of the nuclear CaMKIIδB isoform (vs. cytoplasmic CaMKIIδC isoform) for regulating HDAC and thereby activating cardiac MEF2 and SRF transcriptional activity, has not been explored.

4.2. Ca2+ handling proteins as targets for CaMKII

Altered intracellular Ca2+ handling plays an important role in the pathogenesis of heart failure and changes in Ca2+ cycling can precede cardiac dysfunction. Considerable attention has focused on changes in the function of key Ca2+ regulatory proteins, secondary to their altered expression and/or phosphorylation. CaMKII appears to have significant acute effects on Ca2+ regulation and E–C coupling in cardiomyocytes, mediated through phosphorylation of key Ca2+ regulatory proteins, including ryanoamine receptors (RyR2) [73,74], phospholamban (PLB) [75,76], the SR Ca2+-ATPase (SERCA2a) [77,78] and L-type Ca2+ channel [79]. Phosphorylation of RyR2 has been suggested to alter the channel open probability [2,80] while phosphorylation of PLB has been suggested to regulate SR Ca2+ uptake [2]. The functional consequence of phosphorylation of SERCA2a by CaMKII remains controversial [77,81]. There is also evidence that CaMKII can phosphorylate the L-type Ca2+ channel complex or an associated regulatory protein and thus mediate Ca2+ current (Ica) facilitation [82–84], the development of early after-depolarizations (EADs) and arrhythmias [85–88].

An emerging body of evidence has demonstrated that functional alterations of the RyR2 can contribute to cardiac dysfunction [80,89]. Hyperphosphorylation of RyR2 has been suggested to play a major role in the development of heart failure [90]. PKA hyperphosphorylation of RyR2 at Ser2809 results in dissociation of FKBP12.6 from RyR2 and enhancement of SR Ca2+ leakage during diastole [90], although there is conflicting evidence in this regard [91]. CaMKII was previously reported to phosphorylate the same site (Ser2809) on the cardiac RyR (RyR2) as PKA [73], however a recent publication suggests that CaMKII can phosphorylate at least four additional sites on RyR2 in vitro [92]. Most recently, studies from Marks' laboratory used site-directed mutagenesis to identify Ser2815 as the CaMKII phosphorylation site on RyR2 [93]. The cytoplasmic CaMKIIδC rather than the nuclear δB isoform would be predicted to have specificity for phosphorylating this substrate in vivo.

Inhibition of CaMKII by KN-93 has been reported to diminish the Ca2+-dependent increase in SR Ca2+ release [94], indicating that activation of CaMKII by Ca2+ transients enhances the efficacy of E–C coupling in cardiomyocytes via phosphorylation of RyR2. Phosphorylation of RyR2 by CaMKII in vitro has also been shown to increase RyR2 open probability [73,74]. Most recently, Ser2815 phosphorylation, elicited by pacing induced CaMKII activation in the heart, has been associated with increased RyR2 Ca2+ sensitivity and activity [93]. Interestingly, there is no increase in FKBP12.6 dissociation in response to RyR2 phosphorylation at the CaMKII site [93].

The functional consequences of RyR2 phosphorylation in vivo have been further assessed through studies with our recently generated lines of transgenic (TG) mice overexpressing the cytoplasmic δC isofrom of CaMKII. These mice develop a gene dose-dependent dilated cardiomyopathy with markedly decreased fractional shortening, and die prematurely [37]. Increased phosphorylation of RyR2 at a CaMKII site is observed prior to development of heart failure, as assessed by several independent measures including back phosphorylation [37] and use of phospho-specific antibodies (unpublished data). In ventricular myocytes isolated from the CaMKIIδB TG mice, SR Ca2+ content is decreased despite significant increases in PLB phosphorylation at the CaMKII site [37,44]. Additionally, diastolic Ca2+ spark frequency is markedly increased and the sparks have increased width and prolonged duration even in the face of the decreased SR Ca2+ load [44], suggesting altered RyR2 function. Acute inhibition of CaMKII by KN-93 treatment of the isolated myocytes normalizes Ca2+ spark
frequency, consistent with the hypothesis that the increase in 
Ca\(^{2+}\) sparks is a direct result of CaMKII-mediated RyR2 
phosphorylation. These results are further supported by 
more recently published work showing that autocradate-2- 
related inhibitory peptide (AIP), a highly specific inhibitor 
of CaMKII, can significantly decrease Ca\(^{2+}\) spark frequen-
duration and width in rabbit ventricular myocytes [95]. 
Importantly, CaMKII\(\beta\) is found to be physically associated 
with RyR2 in immunoprecipitates [37,93,95] and there is an 
increased amount of CaMKII\(\beta\) associated with the RyR2 in 
CaMKII\(\beta\)C TG mice [37]. Since there is no accompanying 
change in RyR2-associated PKA or phosphatases [37], the 
increased phosphorylation of the RyR2 in CaMKII\(\beta\)C TG 
mice cannot be ascribed to alterations in these activities. 
Taken together, these data indicate that CaMKII can mediate 
RyR2 phosphorylation in vivo, that this results in increases 
diastolic SR Ca\(^{2+}\) leakage, and that these changes precede 
and could therefore be causal in the development of heart 
failure [37,44].

4.3. CaMKII, apoptosis and heart failure link

Cardiomyocyte apoptosis is now considered to be a 
hallmark and likely causal mechanism for development of 
heart failure. Some evidence suggests that CaMKII can 
transduce signals leading to apoptosis. For example, selective 
inhibitors of CaMKII have been reported to significantly 
inhibit apoptotic responses induced by TNF\(\alpha\), UV- 
irradiation, and the natural toxin microcystin, in noncardiac 
cells [96,97]. CaMKII has also been shown to regulate 
expression and phosphorylation of c-FliP, thus modulating 
Fas-mediated signaling in glioma cells [98]. In our recent 
collaborative studies [99], \(\beta_1\) adrenergic stimulation was 
shown to induce apoptosis in adult cardiomyocytes through 
CaMKII and overexpression of the cytoplasmic CaMKII\(\beta\) 
(but not the nuclear \(\delta_\beta\)) was found to exaggerate the \(\beta_1\) 
adrenergic apoptotic effects. Our studies showing that 
CaMKII\(\beta\)C TG mice develop heart failure associated with 
pronounced ventricular thinning also suggests that apoptosis 
may have developed in these hearts in response to pro-
longed cytoplasmic CaMKII expression [37]. Further work 
is needed to determine whether activation of CaMKII is 
necessary or sufficient to induce cardiomyocyte apoptosis 
and whether this contributes to development of heart failure. 
Identifying an apoptotic pathway activated by CaMKII, and 
the site and isoform specification of this response, will 
provide a better understanding of CaMKII involvement in 
heart failure.

5. CaMKII as a therapeutic target for cardiac 
hypertrophy and heart failure

The apparent involvement of CaMKII in many aspects of 
cardiac hypertrophy and heart failure suggests that CaMKII 
would have potential as a therapeutic target for hypertrophy 
and failure. Selective pharmacological and cell-permeable 
peptide inhibitors of CaMKII have been useful in delineat-
ing CaMKII signaling pathways involved in cardiac func-
tion, and the results of such studies support the need for 
future discovery of new treatment strategies targeting CaM-
KII. Also, because the newly described CaMK phosphatases 
appear to be highly specific for multifunctional CaMKS, 
specific activators or inhibitors of these phosphatases could 
provide new therapeutic approaches for treatment of cardiac 
hypertrophy and heart failure. 

As discussed above, activation of CaMKII regulates Ca\(^{2+}\) 
handling and transcriptional responses in cardiomyocytes, 
both of which have physiological and pathological roles in 
cardiac function. Moreover, the nuclear CaMKII\(\delta\) and 
the cytoplasmic CaMKII\(\beta\) isoforms appear to play distinct 
roles in these processes. Understanding the temporal se-
quence and isoform specificity of these processes will be 
critical for the development of treatment strategies since 
CaMKII activation may subserve physiological or patho-
logical functions depending on the time and location of its 
activation.

6. Concluding remarks

Recent work from our laboratory and others has impli-
cated CaMKII in the development of cardiac hypertrophy 
and heart failure. The role of the CaMKII isoforms in the 
regulation of cardiac function appears to be determined by 
their subcellular localization. We suggest that CaMKII 
signaling pathways serve normal homeostatic functions 
which may be subverted to contribute to the alterations in 
cardiomyocyte function that lead to cardiac hypertrophy and 
heart failure. Our studies and others utilizing transgenic 
CaMK overexpression indicate that increased nuclear 
CaMK activity is sufficient to induce hypertrophy while 
cytoplasmic CaMK can alter Ca\(^{2+}\) homeostasis and contrib-
ute to development of heart failure. Conclusions about the 
requirement for CaMKII activity in control of cardiac 
function are, on the other hand, largely based on experi-
ments with pharmacological inhibitors that may affect other 
kineses and are not specific for the \(\delta\) isoform of CaMKII 
which is predominant in the heart. Thus, genetic ablation 
(knockout) of CaMKII and specifically of the \(\delta_\beta\) vs. \(\delta_C\) 
isoms in the myocardium is currently underway. This 
approach will afford us the opportunity to more directly 
assess the contribution of CaMKII isoforms to cardiomyo-
cyte transcriptional regulation, Ca\(^{2+}\) handling and apoptosis, 
their roles in development of hypertrophy or heart failure, 
and their utility as therapeutic targets.

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