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Role of CaMKII for signaling and regulation in the heart

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1. ABSTRACT

The Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is the CaMK isoform predominantly found in the heart. Cardiac myocytes signaling during excitation-contraction coupling (ECC) is described by the increase in intracellular Ca^{2+} concentration. In consequence, CaMKII is activated thereby phosphorylating several important Ca^{2+} handling proteins with multiple functional consequences for cardiac myocytes. Specific CaMKII overexpression in the heart and in isolated myocytes of animals can exert distinct and novel effects on ECC. CaMKII activity and expression are reported to be increased in cardiac hypertrophy, in human heart failure, as well as in animal models thereby contributing to cardiac disease through a regulation process termed excitation-transcription coupling (ETC). In the present review important aspects of the role of CaMKII in ECC and ETC are summarized with an emphasis on recent novel findings.

2. EVOLVING ROLE OF CA^{2+}/CALMODULIN PROTEIN KINASE II (CaMKII) IN THE HEART

Intracellular Ca^{2+} ions translate electrical signals into mechanical activity of the heart leading to the shortening of the single myocytes and the contraction of the whole heart. This highly coordinated process is known as excitation-contraction coupling (ECC). Various transporters, pumps, and ion channels (sarcolemmal, and in the sarcoplasmic reticulum, SR) contribute to these cellular Ca^{2+} fluxes. Several of these Ca^{2+} handling proteins but also additional Ca^{2+}-activated intracellular proteins contribute to fine tuning ECC.

One of the many Ca^{2+}-activated intracellular proteins is the second messenger Ca^{2+}/calmodulin-dependent protein kinase (CaMK) of which CaMKII is the predominant cardiac isoform (1-3). Upon activation, CaMKII phosphorylates a number of Ca^{2+} handling

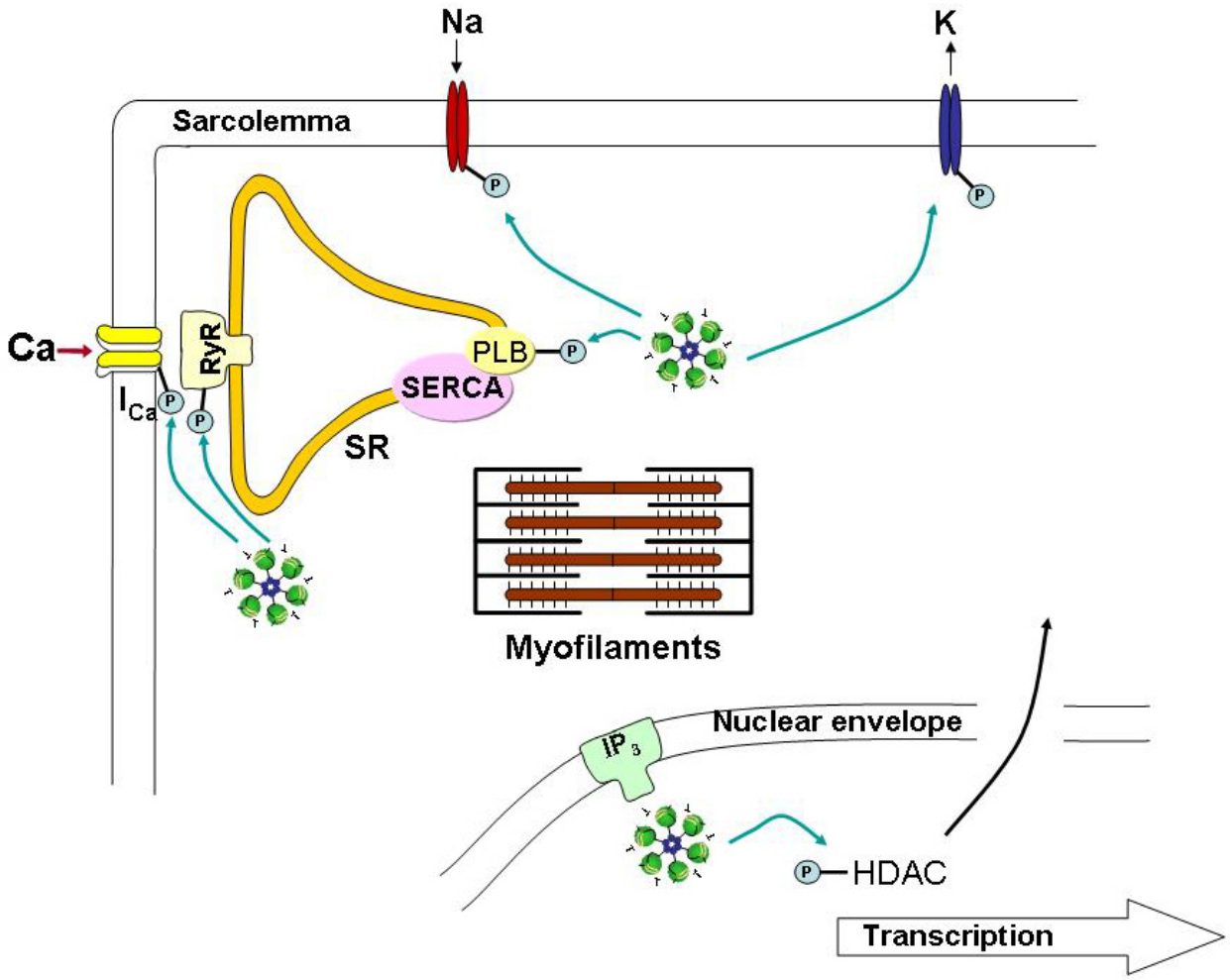

Figure 1. Simple scheme of the effects of CaMKII on excitation-contraction coupling (ECC) and excitation-transcription coupling (ETC). CaMKII phosphorylates several Ca-handling proteins including phospholamban (PLB), SR Ca release channels (RyR), and L-type Ca channels responsible for Ca influx (I_{Ca}). In addition, Na channels and K channels are also regulated by CaMKII. In addition, CaMKII may be activated by local Ca^{2+}-release in the nucleus through IP_3 receptors thereby phosphorylating HDAC leading to nuclear export and transcription.

proteins including SR Ca^{2+} release channels or ryanodine receptors (RyR2), phospholamban (PLB), and L-type Ca^{2+} channels (LTCC) with multiple functional consequences (4,5). Novel data suggest that also non-Ca^{2+} handling proteins such as sarcolemmal Na^+ and K^+ channels are regulated by CaMKII thus influencing ECC via electro-physiological effects.

Almost ten years ago it was described for the first time that CaMKII expression and activity are increased in the myocardium of patients with end-stage heart failure (6-7). Since then, and due to the relevance for pathophysiological conditions such as cardiac hypertrophy and heart failure, a great number of original articles and reviews have been published describing the role of CaMKII for cardiac disease with a recent special series of review articles in *Cardiovascular Research* (8-13). The rising importance in this area can be also appreciated by the increasing number of articles published on CaMKII in the heart which doubled between 2001 and 2006 as compared to the years 1995-2000. Last but not least, an increasing number of symposia on this subject are held such as the joint AHA/ESC morning session about the *"New Evolving Role of CaMK in the Heart"* at the AHA meeting in Chicago, USA in November 2006 or during the session *"CaMKII arrhythmia in heart failure"* of the ISHR World meeting in Bologna, Italy in June 2007 to only name a few. The current review focuses on effects of CaMKII in ECC and excitation-transcription coupling (ETC; see Figure 1) and tries to summarize recent findings in the field.

3. ACTIVATION OF CaMKII

CaMKII is a serine/threonine protein kinase which phosphorylates many intracellular proteins in response to elevated intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) (1-3,5). There are four different CaMKII genes (α, β, γ, δ) with the δ isoform being predominant in the heart (3,4,8). In addition, distinct splice variants have different

subcellular localizations with δ_B being compartmentalized to the nucleus and with δ_C being the cytosolic isoform (14).

The multimeric CaMKII holoenzyme consists of homo- or heteromultimers of a few (approximately 6-12) kinase subunits forming a wheel-like structure (3-5). Each CaMKII monomer contains an amino-terminal catalytic domain, a regulatory domain with partially overlapping autoinhibitory and calmodulin (CaM) binding regions, and a carboxy-terminal association domain responsible for oligomerization (3,4). The autoinhibitory region close to the active site of the catalytic domain sterically blocks access to substrates. When $(Ca^{2+})_i$ increases (e.g. during systole), intracellular CaM binds its four Ca^{2+} ions (15). The Ca^{2+}/CaM complex attaches to the regulatory domain of CaMKII and displaces the autoinhibitory domain on CaMKII thereby activating the enzyme (half maximal activation at $(Ca^{2+})_i$ of about 0.5-1 μM). In addition, CaMKII can lock itself into an activated state upon autophosphorylation of Thr-287 on the autoinhibitory segment (3,8). Autophosphorylation is not essential for CaMKII activity but it does have important consequences, i.e. by increasing the affinity of the Ca^{2+}/CaM-kinase complex thereby trapping Ca^{2+}/CaM on the autophosphorylated subunit (16). Even when $(Ca^{2+})_i$ declines to resting levels during diastole (i.e. about 0.1 μM), CaM is still trapped for several seconds. As a result, the kinase retains close to fully active as long as CaM is trapped, regardless of the $(Ca^{2+})_i$ level. Interestingly, autophosphorylation significantly disrupts autoinhibition, such that even after Ca^{2+}/CaM has dissociated from the autonomous state CaMKII remains partially active (about 20-80%) (17-19). For complete inactivation to occur, auto-phosphorylated CaMKII must be dephosphorylated by protein phosphatases (e.g. PP1, PP2A, PP2C) (8).

Various CaMKII inhibitors were used in the past in myocytes, including the organic inhibitors KN-62 and KN-93 which competitively inhibit CaM binding to CaMKII (4). Of note, some of these agents appear to have direct effects on some ion channel which seem independent of CaMKII actions (20,21). In contrast, the peptide inhibitors autocamtide-2 related inhibitory peptide (AIP, a nonphosphorylatable, competitive substrate for autophosphorylation of CaMKII), and autocamtide-2 inhibitory peptide (AC3-I) are not thought to affect ion channels (22,23).

4. ROLE OF CaMKII IN EXCITATION-CONTRACTION COUPLING (ECC)

Without doubts, Ca^{2+} clearly is the central regulator of ECC. During a cardiac action potential, Ca^{2+} enters the cell mainly through voltage-dependent LTCC, triggering subsequent Ca^{2+} release from the SR via RyR2, a process termed Ca^{2+}-induced Ca^{2+}-release by A. Fabiato about thirty years ago (24). The resulting increase in $(Ca^{2+})_i$ causes Ca^{2+} binding to troponin C which activates the myofilaments leading to contraction during systole. Diastolic relaxation occurs when Ca^{2+} dissociates from troponin C and is removed from the cytoplasm. The SR Ca^{2+}-ATPase (SERCA2a) and the Na^+/Ca^{2+}-exchanger (NCX) are the main mechanisms for Ca^{2+} removal in the heart (25).

About 25 LTCC proteins and 100 RyR2 proteins are co-localized forming a local SR Ca^{2+} release unit called junction or couplon (26). This local functional unit can be monitored by confocal microscopy measuring elementary Ca^{2+} release events from the SR (so called Ca^{2+} sparks) occurring spontaneously in resting cardiac myocytes and summating during normal Ca^{2+} transients in ECC. Of pathophysiological relevance is a high frequency of Ca^{2+} sparks responsible for Ca^{2+} leak from the SR and decreased SR Ca^{2+} load (9).

There is convincing evidence that fluctuations in $(Ca^{2+})_i$ modify the activity of ion channels and transporters via CaMKII (4). The integrative responses of these downstream messengers of Ca^{2+} giving a feedback on the ion channels and transporters that regulate $(Ca^{2+})_i$ serve to fine tune ECC. As an example, CaMKII can modulate ECC by phosphorylating several important Ca^{2+} handling proteins in the heart in response to Ca^{2+} signals, including RyR2, PLB, and LTCC with significant functional consequences (4,27-30). These proteins are involved in Ca^{2+} influx, SR Ca^{2+} release, and SR Ca^{2+} uptake with their specific role in ECC being discussed below. In addition, novel findings of CaMKII-dependent regulation of Na^+ and K^+ channels are presented in the subsequent paragraphs.

4.1 Ca^{2+} influx and I_{Ca} facilitation

LTCC are modulated by CaMKII thereby increasing Ca^{2+} current (I_{Ca}). This is most clearly seen as a positive staircase of I_{Ca} with repeated depolarizations, a process termed Ca^{2+}-dependent I_{Ca} facilitation (31,32). Several groups demonstrated that Ca^{2+}-dependent I_{Ca} facilitation is mediated by CaMKII-dependent processes (33-36). CaMKII is believed to tether to the α_{1C} subunit of the LTCC and to phosphorylate the α_{1C} subunit at both amino and carboxy tails (37). CaMKII also appears to phosphorylate a site on the β_{2a}-subunit (Thr-498), which may be involved in I_{Ca} facilitation (12,38). At the single channel level this CaMKII-dependent I_{Ca} facilitation is manifested as longer single channel openings (39). By overexpressing CaMKIIδ_C in transgenic mouse myocytes as well as in adenovirus-mediated rabbit myocytes I_{Ca} amplitude was increased and inactivation was slowed (40,41). I_{Ca} amplitude could be reduced back to control levels by blocking CaMKII. Enhanced open probability of LTCC due to increased CaMKII activity was thought to contribute to the increased propensity for arrhythmias in CaMKIV transgenic mice that also showed increased CaMKII activity (42).

4.2 SR Ca^{2+} release and SR Ca leak

RyR2 activity is also affected by CaMKII. Initially, it was reported that CaMKII phosphorylates RyR2 at one site (i.e. Ser-2809) activating SR Ca^{2+} release (27). However, others showed that there may be at least four additional CaMKII phosphorylation sites on RyR2 (43). The specific effects of CaMKII on RyR2 remained controversial. CaMKII either increased or decreased RyR2 open probability (27,28,44). In intact cardiac myocytes

endogenous CaMKII increased the amount of SR Ca^{2+} release for a given SR Ca^{2+} content and I_{Ca} trigger in an elegant *in vivo* study (20). This conclusion is also consistent with observations that protein phosphatases (PP1&PP2A) can reduce RyR2 activity for a given I_{Ca} and SR Ca^{2+} load, and conversely that phosphatase inhibitors enhance it (45). However, other studies found opposite results suggesting that CaMKII negatively regulates SR Ca^{2+} release (46,47). Unfortunately, in the previous report no SR Ca^{2+} content was measured whereas in the latter study species differences may have contributed to the divergent results.

New evidence was provided in isolated cardiomyocytes showing that CaMKII indeed is directly associated with RyR2 and transgenic CaMKIIδ_C overexpression increases fractional SR Ca^{2+} release during ECC and spontaneous SR Ca^{2+} release (i.e. Ca^{2+} spark frequency) for a given SR Ca^{2+} load (40,48-50). These results were confirmed by acute CaMKIIδ_C overexpression through adenovirus-mediated gene transfer in rabbit myocytes and direct application of pre-activated CaMKII to permeabilized mouse myocytes (41,51). Similarly, in rabbit hearts the CaMKII peptide inhibitor AIP depresses Ca^{2+} spark frequency and ryanodine binding to RyR2, indicating that CaMKII activates RyR2 (50). It was also shown that CaMKII-dependent RyR2 phosphorylation increases RyR2 open probability using single channel measurements, and that CaMKII-dependent RyR2 phosphorylation may be at Ser-2815, rather than Ser-2809 (49). Whether CaMKII phosphorylates RyR2 at Ser-2809 is now controversial (as is the role of PKA-dependent phosphorylation and subsequent FKBP12.6 dissociation) (52). However, in a series of very recent reports the role of PKA-dependent RyR2 phosphorylation was challenged. Curran *et al.* showed that ß-adrenergic stimulation using isoproterenol dramatically increases SR Ca^{2+} leak whereas CaMKII inhibition (but not PKA inhibition) decreases SR Ca^{2+} leak (53). In addition, bypassing PKA activation using forskolin did not increase SR Ca^{2+} leak showing that ß-adrenergic effects on RyR2 and SR Ca^{2+} leak may be mainly dependent on CaMKII rather than PKA. Two similar studies investigating the effects of ß-adrenergic stimulation on RyR2 phosphorylation (but also PLB phosphorylation) support the prominent role of CaMKII (54,55). Interestingly, the recently described cAMP binding protein Epac, which was shown to phosphorylate the CaMKII site on RyR2 may be a potential link between cAMP and CaMKII-dependent signaling pathways (56).

In a rabbit heart failure model it was shown recently that there is increased CaMKII expression, more CaMKII is autophosphorylated and more of this CaMKII is associated with RyR2 (57). There was also less phosphatase associated with RyR2, and RyR2 was more heavily phosphorylated (58). Moreover, the enhanced diastolic SR Ca leak could be reversed by CaMKII inhibition but not by PKA inhibition (57,59). This CaMKII-dependent enhancement of SR Ca^{2+} leak in heart failure may contribute to both the diminished SR Ca^{2+} content characteristic of this disease, and also diastolic SR Ca^{2+} release which can activate transient inward Na^+/Ca^{2+} exchange current resulting in arrhythmias. Indeed, CaMKII inhibition increases SR Ca^{2+} content (57). Interestingly, this is associated with only modest inotropy most likely due to the fact that although CaMKII inhibition limits diastolic SR Ca^{2+} leak thus enhancing SR Ca^{2+} content, it also prevents CaMKII-dependent stimulation of ECC at the RyR2, such that there is lower fractional SR Ca^{2+} release. In summary, CaMKII can enhance RyR2 activation during ECC thus influencing fractional SR Ca^{2+} release during systole but also spontaneous SR Ca^{2+} release (i.e. Ca^{2+} leak) during diastole, when it may unload Ca^{2+} from the SR and also contribute to arrhythmias.

4.3 SR Ca^{2+} uptake, FDAR, acidosis

In its unphosphorylated form phospholamban (PLB) is an endogenous inhibitor of SERCA2a (60). Upon phosphorylation of PLB, SERCA2a activity and thus SR Ca^{2+} uptake are enhanced. PLB is phosphorylated by PKA (Ser-16) and CaMKII (Thr-17) (60,61). Bassani *et al.* initially showed that CaMKII enhances SR Ca^{2+} uptake (62). These authors speculated that CaMKII-dependent PLB phosphorylation might be responsible for the frequency-dependent acceleration of relaxation (FDAR) of twitches and SR Ca^{2+} uptake seen typically when increasing stimulation rate. In a different study it was shown that a frequency-dependent increase in PLB Thr-17 phosphorylation occurs in rat myocytes (independent of Ser-16 phosphorylation) and that the level of Thr-17 phosphorylation correlated directly with the rate of relaxation (63).

FDAR is an important intrinsic mechanism to allow faster relaxation (and diastolic filling of the heart) when heart rate is increased. FDAR is also manifest as slowing of twitch relaxation as time between beats is prolonged (i.e. at post-rest contractions) (64). An attractive hypothesis was that FDAR might be due to enhanced SR Ca^{2+} uptake via CaMKII-dependent PLB phosphorylation (with rest leading to PLB dephosphorylation). However, we found that FDAR is still prominent in PLB deficient (PLB-KO) mice (65). Also, the time course of FDAR development is much faster during changes in frequency than that of PLB phosphorylation and in atria of transgenic mice overexpressing the CaMKII inhibition protein AC3-I no difference in FDAR was observed as compared to control mice (66-68). Moreover, in an elegant study by Varian & Janssen it was proposed that troponin I and myosin light chain-2 phosphorylation may be critically involved in FDAR leading to decreased myofilament Ca^{2+} sensitivity at higher frequencies (69). Thus, CaMKII-dependent PLB phosphorylation might contribute to FDAR but is very unlikely to be the sole mechanism for FDAR. These observations are also supported by the fact that FDAR can be suppressed by CaMKII inhibitors in some reports whereas other reports could not detect FDAR inhibition (62,63,65,66,70-73).

During acidosis, i.e. when lowering extracellular pH from 7.4 below 7, after an initial depression of Ca^{2+} transients and contractility there is a slow but progressive increase in Ca^{2+} transient amplitudes causing a partial recovery of contractions (74). Interestingly, this recovery

can be prevented by CaMKII inhibition and it was proposed that CaMKII-dependent PLB phosphorylation may be responsible for the faster $(Ca^{2+})_i$ decline and recovery of contractions that partially overcomes the direct inhibitory effect of acidosis (74-76). We previously confirmed that PLB and CaMKII were both required for recovery of Ca^{2+} transients and contraction during acidosis in mouse myocytes (77). Indeed, recovery was prevented in myocytes from PLB-KO vs. wild-type (WT) mice. In line, inhibition of CaMKII completely abolished recovery in WT mice, but was without effect in PLB-KO mice (77). Moreover, recent results show that acute overexpression of CaMKIIδ_C in rabbit (as well as mouse) myocytes for 24 h using adenovirus-mediated gene transfer even improves recovery during late acidosis, with increased twitch shortening, $(Ca^{2+})_i$ transient amplitude and accelerated Ca^{2+} decline as well as relaxation parameters (78). Thus, CaMKII-dependent enhancement of SR Ca^{2+} uptake may be important during acidosis.

4.4 Na^+ channels and K^+ channels

In addition to LTCC CaMKII may also target cardiac Na^+ and K^+ channels (79,80). Tan *et al.* first reported a CaM-dependent regulation of cardiac voltage-gated Na^+ channels (81). The authors showed that Ca^{2+}/CaM binds to an IQ motif at the carboxy-tail of the α subunit. This interaction specifically altered Na^+ channel gating properties. Accumulation of intermediate inactivation was enhanced consistent with a reduced channel function (loss of function). The Ca^{2+}-dependent regulation of Na^+ current (I_{Na}) may thus modulate excitability as a feedback mechanism during ECC.

Moreover, Na^+ channel activity may be also regulated by CaMKII, as these channels are multiprotein regulatory complexes (82,83). The first evidence for CaMK-dependent regulation of cardiac Na^+ channels was by Deschênes *et al.* (84). These authors showed that the CaMK-inhibitor KN-93 slowed current decay, consistent with an inhibition of fast inactivation. Additionally, the steady-state voltage dependence of inactivation was shifted in the depolarizing direction resulting in an increased channel availability. Entry into the intermediate inactivated state was also slowed, while the recovery from inactivation was hastened. This was consistent with a CaMK-dependent loss of function effect similar to the above described CaM-dependent effects on Na^+ channel gating (81). However, KN-92 (the inactive analog of the CaMKII inhibitor KN-93) also had effects on Na^+ channel gating, and the specific CaMKII-inhibitor AIP did not appear to affect Na^+ current. Therefore, the authors concluded that a kinase other than CaMKII might modulate Na^+ channels (suggesting CaMKIV). However, the expression levels of this kinase in the heart are very low (85).

We recently examined Na^+ channel gating in rabbit myocytes overexpressing CaMKIIδ_C. CaMKIIδ_C overexpression resulted in a leftward-shift in the steady-state voltage dependence of inactivation. The development of intermediate inactivation was enhanced and recovery from inactivation was prolonged (loss of function). All effects were reversible with CaMKII-inhibition using either KN-93 or AIP. In addition, increased persistent or late I_{Na} was found (gain of function). These effects argue for specific CaMKII-dependent modulation of Na^+ channels. Moreover, we also found a direct association of CaMKII with the Na^+ channel and phosphorylation of Na^+ channels by CaMKII (79).

This new evidence for additional CaMKII-dependent effects besides other well known effects on Ca^{2+}-handling proteins may be especially of pathophysiological importance since upregulation of CaMKII activity and expression seem to be typical of cardiomyopathy from diverse causes in patients and animal models (6,7,40,57,86). Furthermore, transgenic mice overexpressing CaMKIIδ_C develop heart failure and die early which may be associated with ventricular arrhythmias that can be elicited in these mice (48). Altered Na^+ channel function may therefore be associated with these arrhythmogenic processes. Interestingly, CaMKII inhibition can prevent myocardial remodeling after myocardial infarction or excessive β-adrenergic stimulation (87).

Several human cardiac Na^+ channel mutations have been linked to either Brugada or long-QT (LQT3) syndromes with life-threatening arrhythmias (82). One such human mutation (Asp insertion at 1795 in the C-terminus, 1795InsD), shows simultaneous LQT3-like and Brugada-like phenotypes in the same individuals (88). Remarkably, Na^+ channels bearing this mutation expressed in mammalian cells exhibit the same phenotype that we found for CaMKII-modified normal Na^+ channel above (79,88,89). At low stimulation frequencies, the impaired inactivation and persistent I_{Na} can cause AP prolongation consistent with LQT3 syndrome. However, at higher heart rates, incomplete I_{Na} recovery and limited I_{Na} availability further shorten action potential duration, slow propagation and increase dispersion of repolarization similarly found for Brugada syndrome. The intriguing thing is that CaMKII-dependent I_{Na} modulation due to upregulated CaMKII could constitute a common acquired form of arrhythmia, in otherwise normal Na^+ channels (without 1795InsD mutation). Such an acquired Na^+ channel dysfunction may contribute to arrhythmias under conditions when CaMKII effects are enhanced, as in heart failure. Interestingly, CaMKII has already been linked causally to arrhythmias in a mouse model of cardiac hypertrophy and failure by Anderson's group (42,87). In addition, recent experimental data and simulated data suggests that CaMKII inhibition may be of relevance as a possible antiarrhythmic intervention (90,91).

CaMKII may also regulate transient outward K^+ current (I_{to}) in human atrial myocytes from hearts with chronic atrial fibrillation (80). These authors showed that CaMKII regulates I_{to} and CaMKII inhibition results in faster I_{to} inactivation. It was even speculated that K^+ channels or associated regulatory proteins must be in a certain state of phosphorylation in order to be available for cumulative inactivation resulting in a CaMKII-dependent "memory effect" (92). Similarly, CaMKII was shown to regulate Kv4.2 and Kv4.3 in neuronal cells, and these two channels are known to contribute to cardiac I_{to} (93,94).

There is even evidence suggesting that CaMKII directly phosphorylates Kv4.3 at Ser-550 (94). CaMKII-dependent regulation of I_{to} is interesting in so far that in heart failure, $I_{to,fast}$ mainly mediated by Kv4.2/3 is functionally reduced and expression of the channel proteins is lower (64). In preliminary studies in myocytes acutely overexpressing CaMKIIδ_C, we find CaMKII-dependent enhancement of I_{to} consistent with increased Kv1.4 function, and consequent APD shortening (95). Further studies need to be performed to elucidate the role of CaMK-dependent K^+ channel regulation.

5. ROLE OF CaMKII IN EXCITATION-TRANSCRIPTION COUPLING (ETC)

In addition to the acute effects of CaMKII on ECC in the heart, CaMKII is also critically involved in long term regulation of gene expression, or excitation-transcription coupling (ETC) (8,9,26). Almost 15 years ago it was shown that cardiac overexpression of CaM in transgenic mice resulted in severe cardiac hypertrophy, associated with higher CaMKII activity, and the expression of the hypertrophic marker atrial natriuretic peptide (ANP) (96,97). These results where recently confirmed for ANP and BNP in isolated perfused atria paced at increasing stimulation rates (98). Interestingly, the CaM antagonist W-7 as well as the CaMKII antagonist KN-62 could prevent hypertrophy in cultured myocytes, further implicating Ca^{2+}/CaM as a mediator of hypertrophic response (although CaM expression was reported to be rather decreased in human heart failure) (99-101). Moreover, the expression of mutant CaM which could bind Ca^{2+} but not activate downstream targets failed to induce hypertrophy. This suggests that CaMKII as a downstream target of Ca^{2+}/CaM may be of major importance for hypertrophic signaling.

In line with this, Ramirez *et al.* showed that activation of nuclear CaMKIIδ_B in cultured neonatal myocytes lead to hypertrophic gene expression (102). In addition, transgenic mice overexpressing nuclear CaMKIIδ_B resulted in cardiac hypertrophy and dilation (103). Activated CaMKIV (nuclear localization) also induced hypertrophic responses in cultured cardiomyocytes and transgenic mice (104). Most importantly, transgenic overexpression of CaMKIIδ_C resulted in massive cardiac dilation and heart failure as described in detail above (40,48).

But how does CaMKII initiate hypertrophic signaling in the nucleus? CaMK has been suggested to modulate gene expression via various transcription factors, including activation protein 1 (AP-1), CAAT-enhancer binding protein, activating transcription factor (ATF-1), serum response factor (SRF), cAMP-response element binding protein (CREB), as well as myocytes enhancer factor 2 (MEF2) (8,26).

CREB is a ubiquitous transcription factor which is phosphorylated at Ser-133 by CaMKII or CaMKIV, as well as at Ser-142 by CaMKIV. However, CREB phosphorylation was unaltered in CaMKIIδ_B or CaMKIV transgenic mice, suggesting that it was not involved in the hypertrophic signaling pathway (103,104). In contrast, studies in crossbred transgenic mice expressing CaMKIV and a MEF2-reporter supported the idea that MEF2 can be a downstream target for CaMK signaling (104). Despite the fact that CaMK can phosphorylate MEF2D isoform in T lymphocytes, it did not phosphorylate other MEF2 isoforms (e.g. MEF2A) (105). Therefore, direct MEF2 phosphorylation cannot be the main mechanism by which CaMK regulates gene transcription. Interestingly, recent studies showed that HDACs, a family of transcriptional repressors, may serve as functional links between CaMK and MEF2 (106,107).

Class II HDACs, including HDAC4, 5, 7, and 9, are expressed in the heart and have a unique MEF2 binding domain. They contain a C-terminal catalytic domain and N-terminal extension that mediates interaction with MEF2 and also contain two conserved serines that when phosphorylated bind to the chaperone protein 14-3-3 (20). The actions of HDACs are opposed by histone acetyltransferases (HATs), which acetylate the N-terminal tails of core histones and result in chromatin relaxation and in consequence transcriptional activation. Therefore, the association of HDACs with MEF2 causes repression of MEF2 targeted genes and may be responsible for the low transcriptional activity of MEF2 in the adult myocardium (104).

In principle, Ca^{2+}/CaM can compete with the MEF2-binding domain in class II HDACs resulting in the dissociation of MEF2-HDAC complex (108). In addition, the MEF2-HDAC interaction can also be disrupted by phosphorylation of the two conserved HDAC serine residues, resulting in nuclear export of the HDAC/14-3-3 complex, freeing MEF2 to drive downstream gene transcription. CaMK may be such a class II HDAC kinase (109). In neurons CaMK inhibition blocked nuclear export of HDAC5 (110). However, little is known about how CaMKIIδ (especially nuclear CaMKIIδ_B) regulates HDAC in the heart. Also, a CaMK-independent mechanism to control class II HDAC function was suggested to exist in heart, in that the cardiac HDAC kinase (s) was resistant to pharmacological inhibitors of CaMK which may very well be due to a possible role of PKD for hypertrophic signaling (13,111).

Recent data from Bers lab are showing that CaMKII may be part of a local signaling complex at the nuclear envelope that is involved in activating HDAC nuclear export. CaMKII associates with inositol 1,4,5-trisphosphate (IP_3) type 2 receptor in the nuclear envelope (112). By this mechanism, hypertrophic neurohumoral agents (e.g. endothelin-1) can initiate the production of IP_3 at the sarcolemma, which diffuses to the nuclear envelope to cause local Ca^{2+} release from IP_3 receptor type 2, and the activation of local CaMKII (namely CaMKIIδ_B) which phosphorylates HDAC triggering its nuclear export. In line with this it was shown that HDAC5 indeed is exported from the nucleus upon endothelin-1 stimulation by local signaling independent of global changes in Ca^{2+} (113). Therefore, it may be very well possible that myocytes can distinguish between Ca^{2+} signaling for ECC and ETC.

6. SUMMARY

CaMKII in the heart has gained tremendous attention over the last few years. Its involvement at multiple levels in ECC and ETC indicate that it is an important protein for cellular signaling and regulation in the heart. Moreover, since CaMKII expression and activation may be elevated in important pathophysiological situations (e.g. heart failure and hypertrophy), investigating CaMKII regulation in the heart will help to understand the pathophysiology of the heart and may identify new modalities of treatment.

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