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CaMKII Regulation of Cardiac Ion Channels

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Abstract

Calcium/calmodulin-dependent kinase II (CaMKII) is a multifunctional serine/threonine kinase expressed abundantly in the heart. CaMKII targets numerous proteins involved in excitation contraction coupling and excitability and its activation may simultaneously contribute to heart failure and cardiac arrhythmias. In this review, we summarize the modulatory effects of CaMKII on cardiac ion channels function and expression and illustrate potential implications in the onset of arrhythmias via a computer model.

Keywords

CaM kinase; ion channels; arrhythmia; heart failure; computer modeling

Introduction

Excitation contraction coupling (ECC) is a mechanism by which membrane depolarization controls the amount of Ca entering the myoplasm and activating contraction, but it also includes feedbacks, whereby Ca regulates the gating of ion channels that are responsible for cardiac excitation. The Ca binding protein calmodulin (CaM) is a major mediator of this Ca-dependent modulation of ion channels through direct Ca-CaM binding or activation of Ca/CaM dependent kinases (e.g. CaMKII) and phosphatases (e.g. calcineurin). CaMKII is a multifunctional protein kinase expressed abundantly in the heart. CaMKII is known to phosphorylate numerous target proteins involved in Ca mobilization (Fig. 1), e.g. Ca influx, release from and uptake into the sarcoplasmic reticulum (SR). More recent findings are that CaMKII modulates sarcolemmal Na and K channels. This can influence myocyte Ca regulation and also confers further Ca-dependence to electrophysiological effects (and makes a complex feedback system).

CaMKII has also emerged recently as potentially a major mechanism of pathological signaling in heart failure (HF), where its expression and activity are greatly enhanced, and where CaMKII overexpression causes HF (1–3). Upregulation of CaMKII activity and expression seems to be a general feature of cardiomyopathy from diverse causes in patients and animal models, suggesting the hypothesis that CaMKII is a signaling molecule in cardiomyopathy. In this setting CaMKII may also be arrhythmogenic, due to altered cardiac repolarization and Ca handling. Indeed, CaMKII may be a key pharmacological target that acts upstream of ion channels and Ca regulatory pathways. Moreover, CaMKII inhibition may be a viable therapeutic target in the treatment of common forms of cardiac disease.

Mathematical models of cardiomyocytes provide a useful framework to advance our understanding of molecular mechanisms underlying cardiac arrhythmias and to consider

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therapeutic targets. Recent models have incorporated elements of the CaMKII signaling cascade to study the role of CaMKII in regulating cardiomyocyte contractility and excitability (4–8). In this review, we will discuss the functional consequences of CaMKII activity on cardiac ion channels (Fig. 1) and will illustrate using a mathematical model how these effects may underlie arrhythmogenic mechanisms for both triggered activity and reentry.

Ca Channels

The L-type Ca channel opens upon cell depolarization, serving as the primary entry point for Ca into cardiomyocytes. Gating of this channel is directly mediated by Ca/CaM through Ca dependent inactivation (CDI). Ca which either enters the myocyte via Ca current (I_{Ca}) or is released from the sarcoplasmic reticulum (SR) binds to CaM tethered to the channel C-terminus, altering the interaction of CaM with the Ca channel C-terminus, thereby accelerating inactivation (9). CDI is an important negative feedback mechanism. When the myocyte has relatively high Ca load and large Ca transients, enhanced I_{Ca} inactivation limits further Ca influx. Conversely, when myocyte Ca is low and SR Ca release is small, there is less CDI and enhanced Ca entry which increases SR Ca content. The presence of CDI also alters action potential duration (APD) which also controls Ca levels in the myocyte. With large Ca releases there is strong CDI, which limits integrated I_{Ca} influx thereby shortening the APD and further limiting Ca entry, and the earlier repolarization would also favor additional Ca extrusion via Na/Ca exchange (10).

Ca/CaM also indirectly regulates the Ca channel to enhance L-type I_{Ca} (I_{CaL}) via activation of CaMKII. This process is functionally seen as a positive staircase of I_{CaL} , in which amplitude increases and inactivation slows over a series of several pulses after a resting interval (11). CaMKII-mediated enhancement of I_{CaL} may contribute to the increased contraction strength obtained with faster heart rates which improves cardiac performance during exercise (12). In fact, since CaMKII is sensitive to the frequency of Ca transients, CaMKII is ideally suited to respond to changes in cardiac rhythm. Thus, the positive regulation of the channel by CaMKII may partly offset reduced L-type Ca channel availability at high heart rates caused by direct Ca/CaM-dependent inactivation. This gating phenomenon requires Ca influx (it is not seen when Ba is the charge carrier and is more strongly apparent when local Ca influx is amplified by SR Ca release), and it is therefore termed Ca-dependent facilitation (CDF). CDF of I_{CaL} coexist with CDI, where CDI responds rapidly (during the same beat), while CDF occurs more slowly (from beat to beat). Some studies even claimed that progressive decrease in SR Ca release (negative staircase in rat) and CDI is responsible for observed CDF (13,14). However, CDF is quite similar in species which exhibit positive staircases, and even when SR Ca release is blocked (11).

Several groups have demonstrated that CDF is mediated by CaMKII dependent phosphorylation. Early studies showed that pharmacological inhibition of CaMKII abolishes CDF in the heart (15–17). Moreover, addition of activated CaMKII to the cytoplasmic side of the sarcolemma results in phosphorylation of the LTCC complex, inducing high activity (mode 2) gating that is characterized by long, frequent openings and is consistent with CDF (18).

As for the targets of CaMKII phosphorylation, Hudmon *et al.* showed that CaMKII phosphorylates the α_{1C} subunit and that tethering of CaMKII to the α_{1C} carboxy-terminus is an essential molecular feature of CDF, because mutations to a putative C-terminus binding site prevent CDF. More recently, Grueter *et al.* (19) showed that CaMKII binds to the β_{2a} subunit of the L-type Ca channel and preferentially phosphorylates Thr498 in β_{2a} . In addition, mutation of Thr498 to Ala (T498A) in β_{2a} results in ablation of CaMKII-mediated I_{CaL} facilitation in cardiomyocytes.

By overexpressing CaMKII δ_C in transgenic mouse myocytes as well as acutely in rabbit myocytes I_{CaL} amplitude was increased and inactivation was slowed (20,21), consistently with tonic CDF. I_{CaL} amplitude could be reduced back to control levels by blocking CaMKII with KN-93 (20) or AIP (21).

It is now well accepted that CaMKII modulates I_{CaL} , and that this modulation may be important under pathophysiological conditions (e.g. arrhythmias). Interestingly, a transgenic mouse model of cardiac hypertrophy that overexpresses CaMKIV and also shows increased CaMKII activity, showed an increased propensity for ventricular arrhythmias (22). This was caused by triggered activity due to early afterdepolarizations resulting from an inward current during the late plateau phase of the action potential. The increased propensity for arrhythmias has been attributed to enhanced open probability of L-type Ca channels due to increased activity of CaMKII (22). Additionally, in the presence of the CaMKII-inhibitor KN-93, both the increased Ca channel open probability as well as the increased occurrence of arrhythmias were reversed to control values (22). Enhanced CaMKII activation seems to play a major role in I_{CaL} remodeling in a murine model of pressure overload HF (3). Indeed, in cardiomyocytes from these animals I_{CaL} amplitude was increased and the decay slowed, suggesting a role for CaMKII. In HF myocytes CDF could not be induced, suggesting that I_{CaL} was already maximally activated. In addition, the HF-related alterations were recapitulated by application of exogenous CaMKII on control myocytes, whereas exogenous CaMKII did not produce any effect on failing myocytes. These findings implicate increases in CaMKII activity as a direct mechanism governing HF-related changes in I_{CaL} and foster the emerging importance of CaMKII as an anti-arrhythmic target.

T-type Ca (I_{CaT}) current is present to a variable extent in pacemaking, atrial and conduction cells. In the ventricle T-type channels are expressed during cardiac development until the end of the fetal/neonatal period (23), then their expression decreases and there is little or no I_{CaT} in normal adult ventricular myocytes. Significant I_{CaT} may reappear in ventricular myocytes during the development of cardiac hypertrophy and failure (24–26). T-type Ca channels are phosphorylated by CaMKII, which increases the probability of single channel openings (27, 28) and is also upregulated in hypertrophied ventricles (29). Thus, the enhanced CaMKII activity in hypertrophic and failing hearts could further enhance T-type Ca channel openings. However, the potential role of CaMKII signaling to regulate cardiac I_{CaT} has not yet been fully explored. At this point one might only speculate that the enhanced I_{CaT} activation in myocytes may enhance their automaticity, and thus contribute to arrhythmogenesis.

Na Channels

The sodium current (I_{Na}) is responsible for the rapid depolarization of the membrane potential, which initiates the electrical cascade responsible for cardiac rhythm. Numerous life-threatening arrhythmias arise from Na channel dysfunction, thus illustrating its central role in controlling cardiac excitability. Gating of the Na channel appears to be modulated by Ca. However, the mechanism of this modulation (direct Ca binding and/or indirect regulation via CaM or CaMKII) is not yet fully resolved.

Wingo *et al.* (30) proposed a novel mechanism for modulation of Na channel function where Ca ions bind directly to hH1 via an EF-hand motif in the C-terminal domain. However, more recent and direct experiments have provided evidence against the possibility that Ca binds to this region (31).

Tan *et al.* (32) have shown that CaM enhances I_{Na} slow inactivation by binding to the carboxy-terminal IQ domain of the human cardiac Na channel (hH1) in a Ca-dependent manner. Direct interaction of CaM with the IQ motif of $Na_v1.5$ has been shown in other studies, however, the effects on the Na channels have been discordant: Kim *et al.* (31) showed that CaM modulates

the C-terminal interaction with the III–IV linker (suggested to be necessary to stabilize the inactivation gate), to minimize sustained channel activity during depolarization, and to prevent cardiac arrhythmias that lead to sudden death; Young and Cadwell (33) showed a hyperpolarizing shift in the voltage dependence of activation, whereas no effect was reported by Deschênes *et al.* (34).

Recent reports have shown that also Na channels may be a target of CaMKII (35,36). Wagner *et al.* (35) have shown that CaMKII coimmunoprecipitates with and phosphorylates Na channels. CaMKII δ_C overexpression in rabbit myocytes (acutely) and in transgenic mice (chronically) slows fast I_{Na} inactivation, enhances late I_{Na} (Fig. 2D), but also enhances accumulation of intermediate I_{Na} inactivation (Fig. 2B), slows recovery from inactivation (Fig. 2C), and shifts steady-state inactivation of Na channels to more negative voltages (Fig. 2A) in a Ca-dependent manner (35). Moreover, all of these effects could be reversed with CaMKII inhibition (except impaired inactivation in TG mice). Deschênes *et al.* (34) found that inhibition of a CaM kinase (possibly CaMKIV) modifies cardiac Na channel inactivation, shifting the steady-state inactivation curve in the depolarizing direction, slowing entry into inactivated states, and hastening recovery from inactivation (KN-93 vs. KN-92 effects). They excluded CaMKII because the specific CaMKII inhibitor AIP did not seem to alter the Na channel gating. CaMKIV seems unlikely because it is poorly expressed in the heart and localizes to the nucleus. Maltsev *et al.* (36) tested the inhibition of CaMKII and CaM in failing vs. normal canine myocytes. They found that three of the above molecular mechanisms can be potentially involved in the effects of complex Ca/CaM/CaMKII modulation of the late Na current (I_{NaL}) characteristics: CaMKII slows the decay of I_{NaL} , Ca shifted steady-state inactivation towards more positive potentials (opposite the effect of CaMKII assessed by Wagner (35)). Thus, CaM and CaMKII effects on Na current may coexist.

The CaMKII-induced changes in I_{Na} gating reported by Wagner (35) strikingly resemble those in a human genetic mutation in *Nav1.5* (1795InsD) that is linked with simultaneous arrhythmogenic LQT3 and Brugada syndrome (37). In addition, CaMKII expression is upregulated and more active in HF (1,38,39) and arrhythmias are a major cause of death in HF. There are also reports of enhanced late I_{Na} in HF (40). Thus, increased CaMKII-dependent I_{Na} modulation in HF may be an acquired arrhythmogenic Na channel defect that could affect millions of people with HF.

K Channels

The transient outward K current (I_{to}) is known to be critical for initiating cardiac repolarization in the early phase of action potentials. Despite its transient nature I_{to} influences the overall duration of action potential as it profoundly influences phase 1 and the level of plateau, thereby affecting the currents that are active later in the action potential.

Tessier and colleagues (41) provided evidence that CaMKII regulates (slows) inactivation of I_{to} in human atrial myocytes thus contributing to atrial fibrillation. They showed that CaMKII inhibition accelerated $I_{to,fast}$ inactivation, whereas okadaic acid (phosphatase inhibitor) had opposite effects (41). Similar results were obtained from rat ventricular myocytes (42) and in heterologous expression of $K_V4.2/K_V4.3$ (42,43). It was suggested that CaMKII acts on $K_V4.3$ by a direct effect at Ser550, thereby prolonging open-state inactivation and accelerating the rate of recovery from inactivation (43). Recent evidence suggests that chronic inhibition of CaMKII in murine hearts results in APD shortening and prevents remodeling after myocardial infarction and excessive β -adrenergic stimulation (44). That study showed an enhancement of I_{to} during chronic CaMKII inhibition, suggesting that CaMKII may be involved in the regulation of repolarization in HF. However, acute dialysis of a CaMKII-inhibitory peptide into myocytes did not increase I_{to} (44), suggesting that I_{to} modulation is not

caused by the acute effect of CaMKII dependent phosphorylation. Another recent report from Xiao et al. (45) has shown that I_{to} is downregulated in canine ventricular myocytes paced at 3 Hz, and suggested that CaMKII is involved in the I_{to} remodeling during sustained tachycardia via crosstalk with calcineurin/NFAT signaling.

Wagner *et al.* (46) studied how CaMKII alters I_{to} , both acutely by adenoviral CaMKII overexpression in rabbit myocytes and chronically in CaMKII overexpressing transgenic mice with heart failure. CaMKII activation accelerates I_{to} recovery from inactivation for both $I_{to,fast}$ and $I_{to,slow}$, and this appears to be an acute regulatory effect of phosphorylation by CaMKII, as it is acutely reversed by CaMKII inhibitors. There are also longer term alterations in $I_{to,fast}$ and $I_{to,slow}$ which may result from CaMKII-dependent changes in the expression and function of the $K_v1.4$ underlying $I_{to,slow}$, which was enhanced by both acute and transgenic CaMKII δ_C -overexpression in rabbit and mouse myocytes. Conversely, $K_v4.2/4.3$ (the molecular correlates of cardiac $I_{to,fast}$) was decreased during chronic CaMKII δ_C -overexpression in mice.

An interesting finding by Wang and colleagues (47) suggests that the I_{to} inhibitor 4-AP binds to the I_{to} channel and facilitates I_{CaL} via activation of CaMKII. Further studies are needed to test their hypothesis that a significant amount of inactive CaMKII forms molecular complex with $K_v4.3$, and dissociation from $K_v4.3$ facilitates CaMKII activation (48).

Differences in the expression pattern of $I_{to,fast}$ and $I_{to,slow}$ in different regions of the heart contribute to regional variations in AP duration. In addition, downregulation of I_{to} is a central and consistent electrophysiological change in cardiac diseases (e.g., HF). Thus, it is very important to understand how CaMKII modulation of I_{to} influences the electrical heterogeneity of the myocardium in diseased and undiseased states.

I_{K1} not only stabilizes the myocyte resting potential, but also plays an important role in modulating cellular excitability and regulating membrane repolarization, and it is therefore an important determinant of action potential initiation and termination in cardiac cells. In mice with chronic CaMKII inhibition, where the AP is shortened and I_{to} enhanced, I_{K1} density was significantly upregulated (44); however, exposure of ventricular myocytes to acute CaMKII inhibition failed to increase I_{K1} , suggesting that I_{K1} upregulation in these mice is an adaptive response to chronic CaMKII inhibition (increased functional expression of I_{K1}), rather than the acute effect of reduced CaMKII dependent phosphorylation.

Wagner *et al.* (46) have shown that either short term (24 hr) or chronic transgenic CaMKII δ_C overexpression reduces Kir2.1 gene expression and reduces I_{K1} (when acute modulation during measurement is prevented by CaMKII inhibition with AIP). Thus CaMKII δ_C alters expression level of the channel responsible for I_{K1} . This is consistent with several HF studies (49,50) where I_{K1} is downregulated ~40–50% and it raises the novel possibility that CaMKII (which is upregulated and more active in HF) might cause the depression of I_{K1} in HF. Additionally, CaMKII may acutely modulate I_{K1} amplitude because acute CaMKII inhibition with AIP in the patch pipette reduced I_{K1} . This was true in both control and CaMKII overexpressing myocytes suggesting some basal level of CaMKII-dependent I_{K1} activation.

I_{Ks} is enhanced by intracellular Ca. This may be a consequence of allosteric interaction of CaM rather than phosphorylation by CaMKII, because a CaM inhibitor (W7), but not a CaMKII inhibitor (KN62) inhibited the Ca-induced I_{Ks} enhancement (51). Interestingly, the α subunit of the I_{Ks} channel, KCNQ1, has two potential binding sites for Ca-free CaM (apo-CaM), an IQ motif and a Φ motif, in its intracellular C-terminal region (52). Yeast two-hybrid assay also suggests that KCNQ1 associates with apo-CaM. Functional and biochemical analysis of LQTS mutations in the KCNQ1 C-terminus showed that disruption of CaM interaction with KCNQ1

interfered with subunit assembly and that the resultant channels generated very small currents. CaM also affects I_{Ks} channel gating, since channels with defective Ca/CaM interaction show prominent inactivation not typical of this current (53). Thus, I_{Ks} is enhanced by increases in $[Ca]_i$ at a physiological range via a calmodulin-dependent pathway, which does not involve phosphorylation.

Ca has been shown to play a role in the regulation of I_f in guinea pig (54) and rabbit (55) sinoatrial node cells. Alterations in intracellular calcium during the cardiac cycle are involved in fine tuning the voltage-dependent properties of I_f and may thus determine its relative contribution to pacemaking. It seems likely that CaM may be involved in this process, since three structurally unrelated CaM antagonists decreased I_f and shifted its voltage-dependence of activation to more negative potentials. However, I_f was not affected by inhibition of CaMKII. CaMKII has been found to regulate the pacemaker activity of the sinus node mainly via modulating I_{CaL} inactivation and reactivation (56), and the local subsarcolemmal SR Ca releases which drive sinoatrial node cell automaticity, seem to involve basal phosphorylation of Ca cycling proteins by both PKA and CaMKII (57).

RyRs

RyR channels directly control SR Ca release in cardiac muscles, activating contraction during EC coupling. CaMKII associates with RyRs, but the molecular targeting is not yet completely resolved. CaMKII has been shown to phosphorylate RyR at Ser2809 (58) (non exclusive site, (59)) and Ser2815 (59). However, additional phosphorylation sites might also exist (60).

The functional consequences of RyR phosphorylation by CaMKII remain somewhat controversial. In early studies in planar bilayer reconstitution experiments CaMKII has been shown to either increase (58,59,61) or decrease (62) RyR open probabilities. In the natural ventricular myocyte environment, Li *et al.* (63) showed that, for a given SR Ca load and I_{CaL} trigger, inhibition of endogenous CaMKII (with KN-93) decreased Ca release. These results were confirmed by Currie *et al.* (39) showing that the CaMKII peptide inhibitor AIP depresses Ca spark frequency in rabbit myocytes due to decreased endogenous CaMKII binding to RyR. Guo *et al.* (64) used saponin-permeabilized myocytes and found that both endogenous and exogenous CaMKII dramatically enhanced spontaneous SR Ca release events (Ca sparks) in a controlled setting where I_{Ca} , phospholamban effects and SR Ca load effects were prevented. A contrasting study (65) showed that adenoviral expression of a constitutively active CaMKII depresses SR Ca release, whereas a dominant negative CaMKII enhances it. Another group found that acute adenoviral overexpression of wild type CaMKII δ_C only enhanced Ca sparks (21), as in the permeabilized myocyte studies noted above. Transgenic overexpression of CaMKII (3-fold increase in CaMKII activity) increased the fractional SR Ca release during ECC, diastolic and spontaneous SR Ca release. Indeed, the frequency of Ca sparks was greatly enhanced, demonstrating increased diastolic SR Ca leak, which could be reversed by CaMKII inhibition (20).

In a rabbit heart failure model of left ventricular pressure and volume overload, Ai *et al.* (1) showed that CaMKII expression is increased and more CaMKII is autophosphorylated and associated with the RyR2. In addition the HF myocyte had enhanced SR Ca leak, which was inhibited by CaMKII inhibition (thereby enhancing SR Ca content). This suggests a CaMKII-dependent phosphorylation of RyR in HF that is involved in the enhancement of diastolic SR Ca leak and decreased SR Ca load. Thus, CaMKII effects on RyR seem to be implicated in the phenotype of HF, both in the diastolic dysfunction (depletion of SR Ca stores), and in arrhythmogenesis (because the diastolic Ca release may activate NCX mediated I_{ti} causing DADs and triggered arrhythmias). On the other hand, the CaMKII effect on RyR may not contribute appreciably to the depressed systolic function in HF. That is because the decreased

SR Ca load is offset by a greater fractional release also attributable to CaMKII-dependent RyR modulation (63). The enhanced diastolic leak and fractional release during ECC may be manifestations of the same RyR2 activating effect of CaMKII.

CaMKII regulation of RyR seems important and most work points to an activating effect of CaMKII on RyR2. However, the fact that some observations differ, may indicate that there are multiple CaMKII effects on RyR2, possibly mediated by different target sites on RyR2 or auxiliary proteins. More work is needed to clarify this issue.

InsP₃ Receptors

Inositol 1,4,5-trisphosphate receptors (InsP₃Rs) are intracellular Ca release channels that are present in cardiac myocytes, at much lower levels than RyRs. The type 2 InsP₃R (InsP₃R2) is the predominant subtype in cardiac myocytes (66), but its role in heart is poorly understood. In atrial myocytes, InsP₃Rs have been shown to exert positive inotropic and arrhythmogenic effects on EC coupling (67). Ventricular myocytes express InsP₃R, but at lower levels than atrial myocytes (68), and InsP₃Rs are mainly in the nuclear envelope where they colocalize with CaMKII (69). CaMKII phosphorylates the InsP₃R2, which may feedback on channel gating. In fact, incorporation of CaMKII-treated InsP₃R2 into planar lipid bilayers revealed that InsP₃-mediated channel open probability is significantly reduced by phosphorylation via CaMKII (69). The phosphorylation of InsP₃R by CaMKII may represent a mechanism through which activated CaMKII feeds back to terminate InsP₃R2-dependent Ca release before exhaustion of the Ca store. Indeed, reports have shown that CaMKII activity is inhibitory with InsP₃-mediated Ca release in *Xenopus* oocytes and HeLa cells (70,71).

Wu *et al.* (72) recently demonstrated an InsP₃R-CaMKII signaling in the nuclear envelope of adult ventricular myocytes which can participate in transcriptional regulation. The physiological hypertrophic agonist endothelin-1, which activates plasmalemmal G protein-coupled receptors and InsP₃ production, elicits local nuclear envelope Ca release via InsP₃R. This local Ca release activates nuclear CaMKII, which triggers phosphorylation of a class II histone deacetylase (HDAC5) and HDAC5 nuclear export (derepressing transcription) (72). Remarkably, this Ca-dependent pathway cannot be activated by the global Ca transients that cause contraction at each heartbeat, suggesting a Ca-dependent reactive signaling circuit that is controlled separately from ECC-based Ca cycling. This is analogous to the very local control involved in Ca-induced Ca release during ECC and to Ca-dependent inactivation of Ca current. These results are relevant since CaMKII activation and its ability to regulate HDAC5 nuclear shuttling represent a critical Ca-dependent signaling circuit for controlling cardiac hypertrophy and heart failure. In fact, InsP₃R2 expression is enhanced in HF (1,73).

Whether InsP₃R in HF contributes to arrhythmogenesis besides transcriptional control in HF is not yet known. Recent work from Domeier *et al.* (68) showed that activation of IP₃ facilitates SR Ca release through RyR release clusters and enhances the Ca transient during ECC, thus the IP₃ signaling pathway may modulate ventricular ECC under conditions in which IP₃/IP₃R signaling is altered, such as HF.

Modeling of CaMKII signaling

CaMKII has numerous effects on transmembrane electrical processes and intracellular Ca fluxes, which can be both acute regulatory effects (phosphorylation), but can also alter the expression levels of ion channels (to alter protein transcription, translation, or trafficking). Transgenic mice overexpressing CaMKII exhibit arrhythmias (22,35), which could have multiple causes via diverse mechanisms. Pharmacologic CaMKII inhibition has also been shown to be protective against arrhythmias (22). We have investigated *in silico* the effects of CaMKII overexpression on cardiac myocyte ionic currents and APs in a comprehensive

computer model of ECC (4). By incorporating the modulatory effects on I_{Na} , I_{CaL} and I_{to} we showed that APs from rabbit myocytes overexpressing CaMKII are shorter compared to control (Figure 3A *left*) as has been shown experimentally (46). However, when I_{to} expression is low (e.g., in HF, or endocardial myocytes), the effects of I_{Na} and I_{CaL} would be predominant, thus potentially leading to a LQT3-like phenotype (Figure 3A *middle and right*) and possibly EAD-induced triggered arrhythmias. In fact, the prolongation of AP plateau may allow for the recovery and reactivation of I_{CaL} that depolarizes the membrane and generates EADs (74). In addition, experimental and modeling data indicate that phosphorylation events promoting mode 2 gating of I_{CaL} contribute to EADs and ventricular arrhythmias (22,75). We have also shown that CaMKII-induced Na channel loss of function effect may reduce AP rate of rise and conduction velocity, especially at fast heart rates (BrS) (4). Heterogeneity of transmural ventricular repolarization has been linked to a variety of arrhythmic manifestations. A certain degree of transmural dispersion of repolarization is normal (Fig 3B *left*), and may be attributed in part to differential expression of I_{to} (where there is higher I_{to} in myocytes at the epicardial side). If CaMKII prolongs APDs in the endocardium (Figure 3A *middle and right*) and shortens APDs in the epicardium (Figure 3A *left*), this could amplify transmural dispersion of repolarization (Fig. 3B, *right*), thus predisposing to the development of reentrant arrhythmias.

When incorporating in the ECC model the full spectrum of HF-induced changes, such as CaMKII mediated RyR-Ca sensitization, enhanced SR Ca leak, decreased I_{K1} and I_{to} (besides the changes in I_{Na} , I_{CaL} and I_{to} gating), coupled with other cellular changes, such as increased I_{NCX} , we observed DAD-induced triggered arrhythmias upon β -AR stimulation. DADs are a consequence of increased $[Ca]_{SR}$ activating SR Ca release and inward I_{NCX} , which depolarizes the membrane toward threshold for a triggered AP. HF myocytes were stimulated to steady state (1 Hz), then stimulation was stopped and DADs and spontaneous APs were monitored. DADs were not seen at baseline in control (not shown) or HF (Fig. 4, *left*). However, when simulating isoproterenol administration in HF myocytes only (i.e., doubling I_{CaL} , I_{Ks} , SR Ca pump Ca affinity, RyR Ca affinity) DADs and triggered APs were seen upon cessation of 1-Hz stimulation (Fig. 4, *right*).

Thus, the computer model shows that there are multiple targets and potential pathways through which CaMKII may be arrhythmogenic. Given the emerging role for CaMKII in cardiac pathogenesis it is important that computer models evolve to capture the dynamic range of CaMKII activity and the functional consequences on cardiomyocyte electrical activity (5–8).

Conclusions

It is increasingly clear that CaMKII has numerous ion channel and Ca regulatory targets in cardiac myocytes, and alters their function. Several of these channels are critically involved in altered electrophysiological and Ca handling properties in pathophysiological settings (e.g. HF), where CaMKII may also have enhanced expression and function. Further studies are needed to clarify details of how CaMKII modulates these and other targets, and especially in the dynamic interactive environment of the cardiac myocyte. Because of the complexity of these interacting systems, computer modeling can be helpful in discerning the relative roles of different pathways and also in terms of testing how well we understand the individual and collective systems. The involvement of CaMKII in the electrical remodeling responsible for pro-arrhythmic signaling and contractile dysfunction (e.g. in HF) suggests that this kinase may be a promising therapeutic target in the treatment of common forms of cardiac disease.

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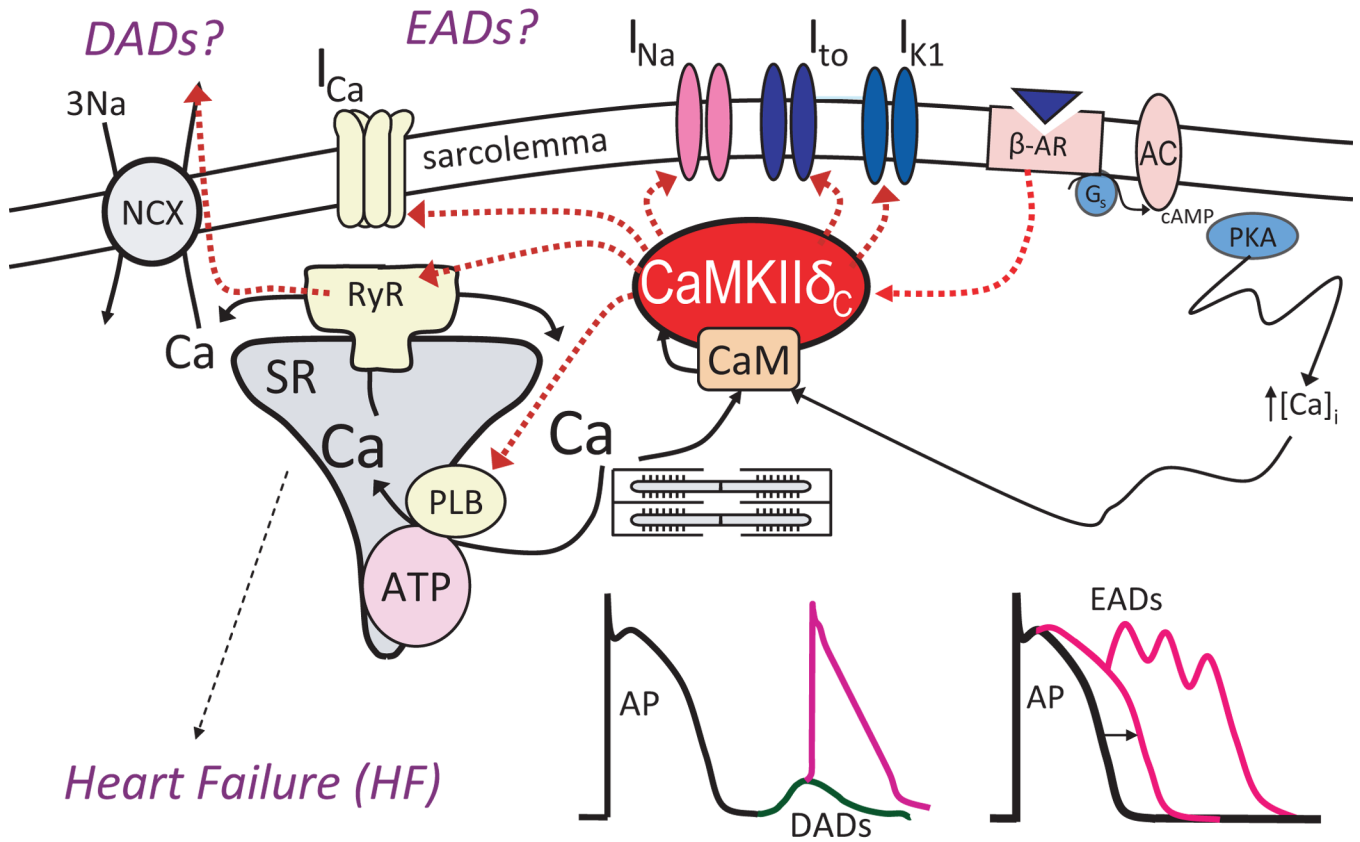


Figure 1. CaMKII influences EC coupling by affecting several electrical and Ca handling proteins, including phospholamban (PLB), SR Ca release channels (RyR), and L-type Ca channels responsible for transsarcolemmal Ca influx (I_{CaL}). In addition, Na channels (responsible for I_{Na}) and K channels (e.g., I_{to} and I_{K1}) have shown to be regulated by CaMKII. By exerting multiple effects on these numerous targets CaMKII can simultaneously favor heart failure and arrhythmias.

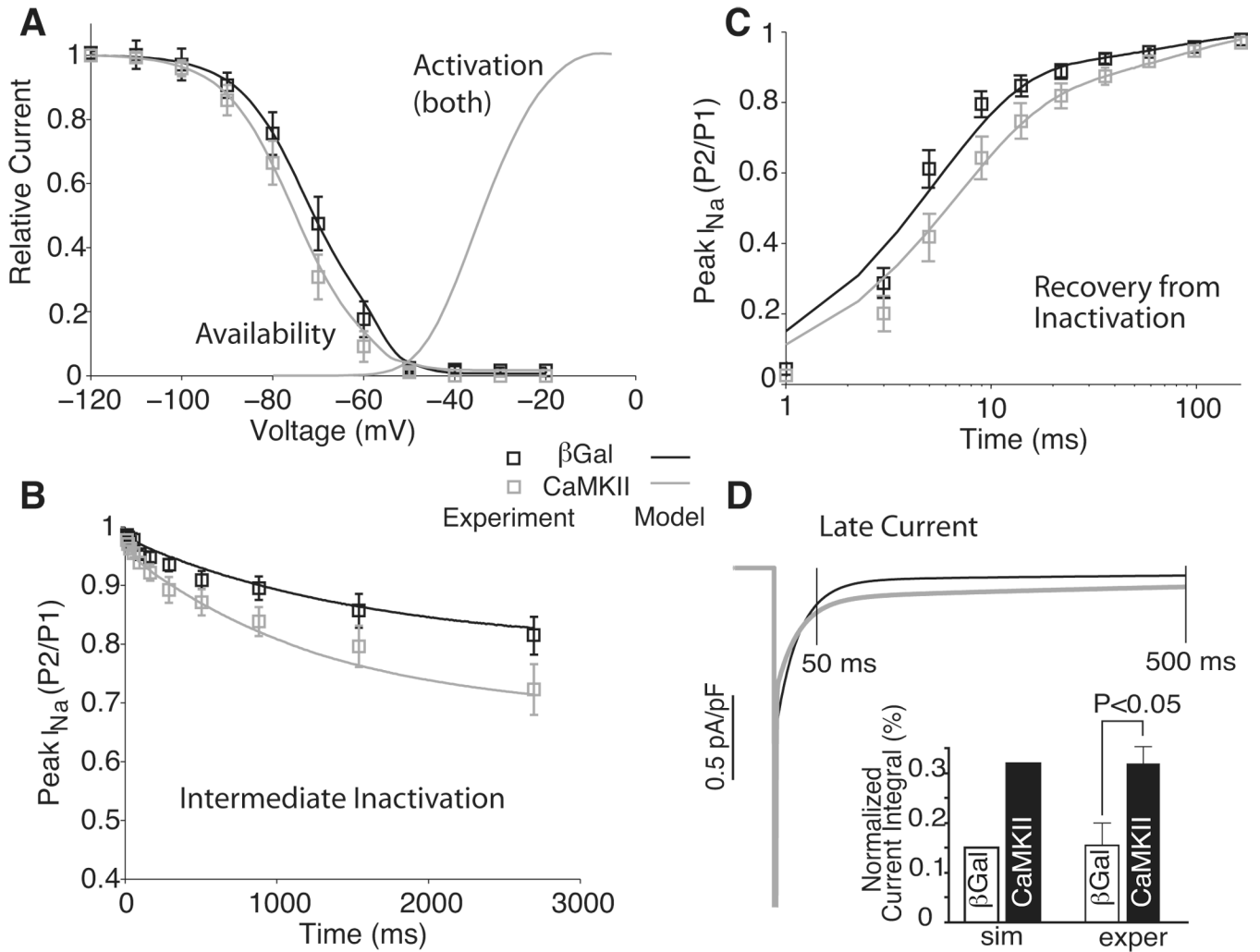


Figure 2. Modulation of Na current gating upon adenoviral CaMKII δ_C overexpression (vs. control β -galactosidase) in adult rabbit ventricular myocytes. Experimental data by Wagner *et al.* (35) and simulated results with our model (4) are reported. With CaMKII I_{Na} availability is shifted towards more negative potentials, whereas activation is unchanged (A), more I_{Na} accumulates in intermediate inactivation (B), I_{Na} recovers more slowly from inactivation (C) and there is more sustained current upon prolonged depolarization (D). Modified from ref. (4).

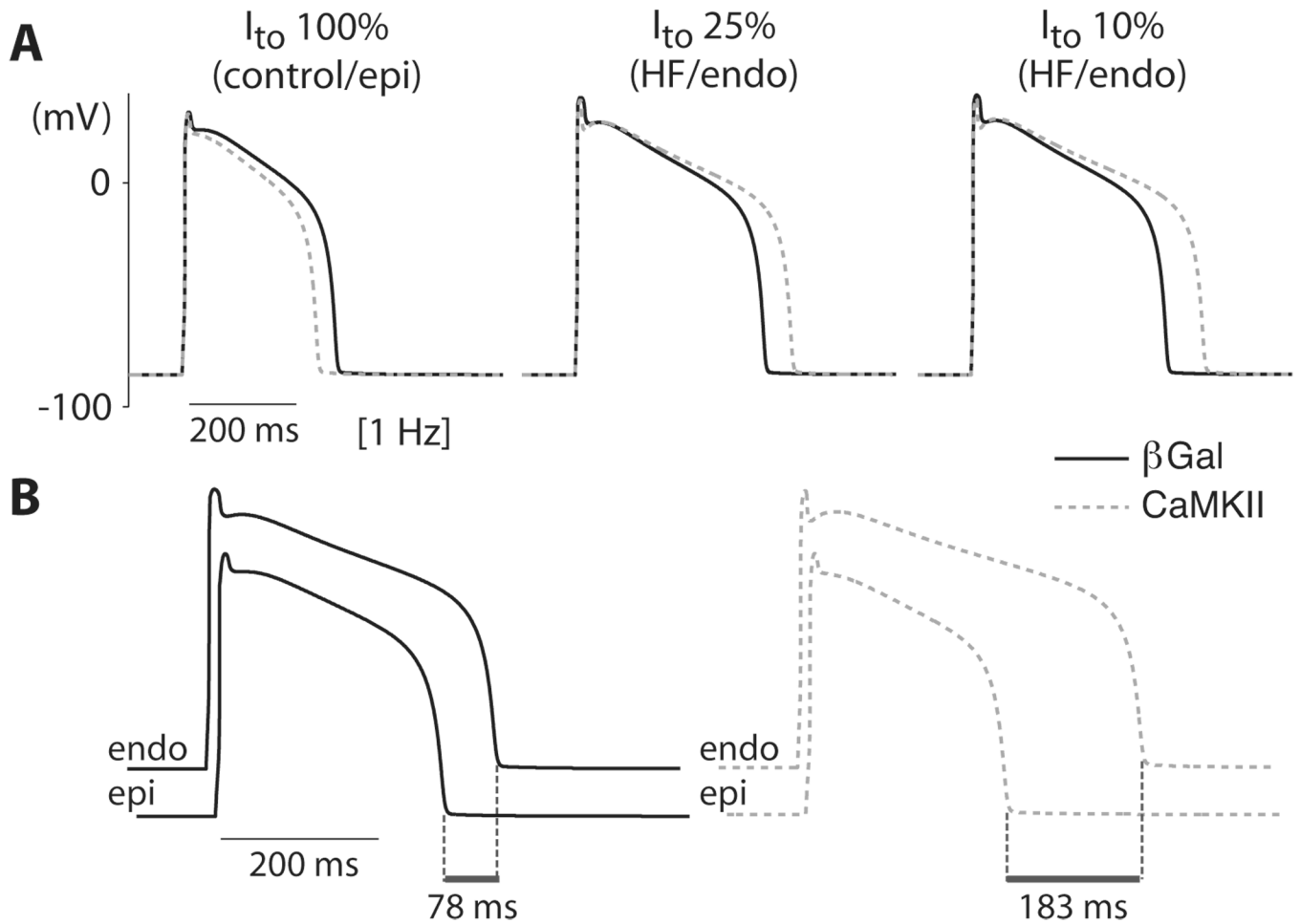


Figure 3.

Simulated APs at 1 Hz in β Gal and CaMKII δ_C -overexpressing cardiac myocytes in the presence of 100% (A, left) 25% (A, middle), and 10% (A, right) I_{to} . When I_{to} is fully expressed (A, epi or control), CaMKII shortens the AP, whereas the AP is prolonged due to I_{to} downregulation (e.g., endo or HF). This could amplify transmural dispersion of repolarization (B, endo-epi: 78 ms for β Gal vs. 183 ms for CaMKII), which may predispose cells to reentry phenomena. Modified from ref. (4).

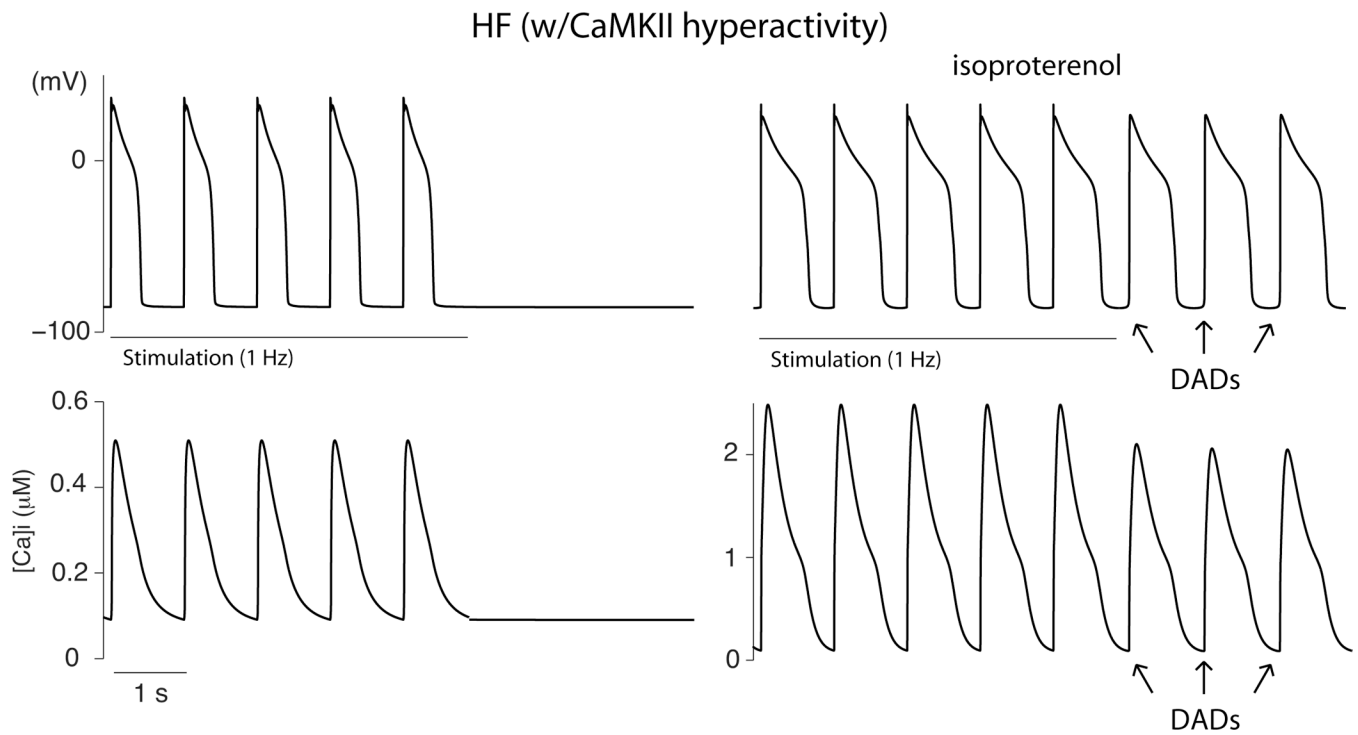


Figure 4.

(A) Stimulation of steady-state AP and Ca transients followed by a period of rest in digital HF cell (*left*) and with isoproterenol (*right*) at 1 Hz. In isoproterenol cessation of 1-Hz stimulation, leads to spontaneous SR Ca release (*right, lower panel*) activating inward I_{NCX} , which depolarizes the membrane generating DADs (*right, upper panel*). DADs were not seen in HF (*left*).