The Role of Calmodulin Kinase II in Myocardial Physiology and Disease

The multifunctional Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) regulates a rich variety of downstream targets in heart. Ca\(^{2+}\) homeostatic proteins are important CaMKII targets that support myocardial excitation-contraction coupling. Under stress conditions, excessive CaMKII activity promotes heart failure and arrhythmias, in part through actions at Ca\(^{2+}\) homeostatic proteins. Here, we briefly review the molecular and cellular physiology of CaMKII in myocardium.

There is a growing interest in the role of protein kinases and phosphatases in cardiovascular disease. At the same time, loss of normal intracellular Ca\(^{2+}\) homeostasis has emerged as a central event initiating and perpetuating myocardial dysfunction, electrical instability, and arrhythmias (41). The multifunctional Ca\(^{2+}\) and calmodulin-dependent protein kinase II (CaMKII) is a serine-threonine kinase that is activated by increases in cellular Ca\(^{2+}\). CaMKII regulates many of the Ca\(^{2+}\) homeostatic proteins in myocardium. CaMKII is now a validated signal for causing heart failure and arrhythmias based on work in cells and animal models and the finding that CaMKII expression is increased in patients with structural heart disease.

CaMKII: Structure Determines Function

Isoforms

The CaMKII monomer consists of an NH\(_2\)-terminal catalytic domain, a centrally located regulatory domain, and a COOH-terminal association domain (56) and a recently identified activating oxidation site (Met\(^{281}\)/Met\(^{282}\)) (24). Phosphorylation of the catalytic and regulatory domains even after dissociation of CaM, thereby converting CaMKII into a Ca\(^{2+}\)/CaM-independent signal. An inhibitory autophosphorylation site at Thr\(^{286}/287\) (the precise numbering is isoform dependent) (24) and a recently identified activating oxidation site (Met\(^{281}\)/Met\(^{282}\)) (24). Phosphorylation of Thr\(^{286}\) or oxidation of Met\(^{281}\) and Met\(^{282}\) prevents re-association of the catalytic and regulatory domains even after dissociation of CaM, thereby converting CaMKII into a Ca\(^{2+}\)/CaM-independent signal. An inhibitory autophosphorylation site at Thr\(^{286}\) (the precise numbering is isoform dependent) (24) and a recently identified activating oxidation site (Met\(^{281}\)/Met\(^{282}\)) (24). Phosphorylation of Thr\(^{286}\) or oxidation of Met\(^{281}\) and Met\(^{282}\) prevents re-association of the catalytic and regulatory domains even after dissociation of CaM, thereby converting CaMKII into a Ca\(^{2+}\)/CaM-independent signal. An inhibitory autophosphorylation site at Thr\(^{286}\) (the precise numbering is isoform dependent) (24) and a recently identified activating oxidation site (Met\(^{281}\)/Met\(^{282}\)) (24). Phosphorylation of Thr\(^{286}\) or oxidation of Met\(^{281}\) and Met\(^{282}\) prevents re-association of the catalytic and regulatory domains even after dissociation of CaM, thereby converting CaMKII into a Ca\(^{2+}\)/CaM-independent signal.

A Ca\(^{2+}\)/CaM-independent signal is dependent on basal/resting intracellular Ca\(^{2+}\) levels (14) and may be involved in intracellular redox state (24). Posttranscriptional mechanisms for regulating CaMKII activity are further discussed below. The association domain is responsible for the assembly of CaMKII monomers into the holoenzyme. A variable/hypervariable region, the product of alternative splicing, is located between the regulatory and association domains and may include a nuclear localization sequence (60).

CaMKII: Structure Determines Function

Isoforms

The multifunctional CaMKII allows its activation to be graded by changes in intracellular calcium. The four known isoforms of CaMKII (α, β, δ, γ) are encoded by separate genes. α and β-CaMKII are the predominant neuronal isoforms, whereas the δ and γ isoforms are expressed in diverse tissues including the heart (62). In heart, the predominant isoform is δ (23).

Although these different isoforms have similar core structures, they differ subtly in their response to Ca\(^{2+}\) oscillations. This may be significant because each isoform has evolved to decode Ca\(^{2+}\) oscillations differently, depending on in which cell system they reside. Different splice variants of these isoforms add further diversity to CaMKII input-output characteristics. One splice variant of the δ isoform (δ\(_{4}\) or δ\(_{8}\)) has a nuclear localization signal in the hypervariable region (between the regulatory and association domains), resulting in nuclear targeting (60). Subcellular targeting of CaMKII is likely to be important for assignment of signal function.
Thr286 allows for the CaMKII to maintain catalytic activity in the absence of calcium, a within the same holoenzyme has been preactivated by CaM. Autophosphorylation of then bind to the regulatory domain. This induces a conformation change that releases binding domain, and Thr287, which becomes autophosphorylated by a neighboring within the regulatory domain is a Ser/Thr kinase binding domain. Within the regulatory analytic domain, a regulatory domain, and a COOH-terminal association domain. Located CaMKII monomer. Met280/281 can undergo oxidation by reactive oxygen species. The process termed calcium-independent activity. Calcium-independent activity allows to the frequency and duration of intracellular Ca2+ transients. However, physiological ramifications of the holoenzyme structure in cardiomyocytes have not been directly tested. Novel approaches are needed to test the role of the holoenzyme structure in CaMKII-mediated integration of Ca2+ signaling responses.

**Molecular Mechanisms**

CaMKII requires Ca2+/CaM for activation (FIGURE 1B). CaM is a bilobed intracellular protein (ubiquitous in vertebrate cells) that contains four Ca2+-binding EF hands [2 EF hands bind Ca2+ to CaM]. The cardiac and brain EF hands are not functionally equivalent (3). CaM binding activates CaMKII preferentially by the COOH-terminal lobe (57). Under basal conditions, CaMKII is inactive, because of intramolecular binding of the catalytic domain to the regulatory domain. Under conditions of low Ca2+/CaM concentration, most CaMKII is inactive, because of intramolecular binding of the catalytic domain to the regulatory domain. However, a fraction of CaMKII is active even under "basal" conditions of Ca2+/CaM, because autophosphorylated Thr286/287 is detectable in quiescent cells (36). This inhibitory interaction between the catalytic and regulatory domains prevents substrate and ATP binding (15, 38, 58). Calculated CaM binds to the regulatory domain, inducing a conformational change that frees the catalytic domain to its pseudosubstrate (43). The allosteric rearrangement of CaMKII on binding Ca2+/CaM allows ATP access to the ATP binding pocket, which in turn allows CaMKII to catalyze the transfer of a phosphate donor group to downstream targets, including itself (i.e., autophosphorylation). Autophosphorylation, which occurs by an intraholoenzyme reaction (41), has several important implications for CaMKII activity. First, Thr306 phosphorylation increases the affinity of CaM-CaMKII binding, a property known as "CaM trapping" (46). Second, autophosphorylation results in the ability of the kinase to maintain catalytic activity even in the absence of CaM binding (56). Phosphorylation at Thr286 provides a negative regulatory mechanism for titrating CaMKII activity. Under resting conditions, phosphorylation occurs preferentially at Thr286, preventing Ca2+/CaM binding, which in turn results in decreased CaMKII activity. This inhibitory autophosphorylation provides a form of feedback regulation of the kinase that is dependent on basal/resting intracellular Ca2+ levels (14).

Recently, a novel form of reactive oxygen species (ROS)-mediated CaMKII activation has been described. Treating Jurkat cells with H2O2 resulted in the activation of CaMKII by a ROS-mediated mechanism, which in turn allows CaMKII to catalyze the transfer of a phosphate donor group to downstream targets, including itself (i.e., autophosphorylation). This inhibition autophosphorylation provides a form of feedback regulation of the kinase that is dependent on basal/resting intracellular Ca2+ levels (14).

**CaMKII Activation: Molecular Mechanisms**

CaMKII requires Ca2+/CaM for activation (FIGURE 1B). CaM is a bilobed intracellular protein (ubiquitous in vertebrate cells) that contains four Ca2+-binding EF hands [2 EF hands bind Ca2+ to CaM]. The cardiac and brain EF hands are not functionally equivalent (3). CaM binding activates CaMKII preferentially by the COOH-terminal lobe (57). Under basal conditions, CaMKII is inactive, because of intramolecular binding of the catalytic domain to the regulatory domain. Under conditions of low Ca2+/CaM concentration, most CaMKII is inactive, because of intramolecular binding of the catalytic domain to the regulatory domain. However, a fraction of CaMKII is active even under "basal" conditions of Ca2+/CaM, because autophosphorylated Thr286/287 is detectable in quiescent cells (36). This inhibitory interaction between the catalytic and regulatory domains prevents substrate and ATP binding (15, 38, 58). Calculated CaM binds to the regulatory domain, inducing a conformational change that frees the catalytic domain to its pseudosubstrate (43). The allosteric rearrangement of CaMKII on binding Ca2+/CaM allows ATP access to the ATP binding pocket, which in turn allows CaMKII to catalyze the transfer of a phosphate donor group to downstream targets, including itself (i.e., autophosphorylation). Autophosphorylation, which occurs by an intraholoenzyme reaction (41), has several important implications for CaMKII activity. First, Thr306 phosphorylation increases the affinity of CaM-CaMKII binding, a property known as "CaM trapping" (46). Second, autophosphorylation results in the ability of the kinase to maintain catalytic activity even in the absence of CaM binding (56). Phosphorylation at Thr286 provides a negative regulatory mechanism for titrating CaMKII activity. Under resting conditions, phosphorylation occurs preferentially at Thr286, preventing Ca2+/CaM binding, which in turn results in decreased CaMKII activity. This inhibitory autophosphorylation provides a form of feedback regulation of the kinase that is dependent on basal/resting intracellular Ca2+ levels (14).

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These data suggested that CaMKII activation may be more complex than previously realized, potentially indicating that CaMKII is a nodal signal for integrating cellular Ca\(^{2+}\) and ROS into downstream responses.

Our group recently mapped a pathway for activation and conversion of CaMKII into a Ca\(^{2+}\)/CaM-independent species by oxidation of paired Met residues (Met\(^{281/282}\)) in the regulatory domain (24). These Met residues are oxidized by angiotensin II stimulation and generation of ROS by NADPH oxidase in myocardium. Oxidation prevents inhibitory reassociation of the catalytic and regulatory domains, much like Thr\(^{286/287}\) autophosphorylation, but Met\(^{281/282}\) oxidation and Thr\(^{286/287}\) autophosphorylation are independent events. In contrast to autophosphorylation, Met oxidation does not result in CaM trapping due to simultaneous oxidation of Met\(^{286}\) located near the negative regulatory residue Thr\(^{287}\). A Met\(^{281}/282\) CaMKII mutant was activated by oxidation and exhibited CaM trapping similar to Thr\(^{286/287}\) autophosphorylation. These data suggest that activating and inhibitory Met residues are readily available to oxidation. Met oxidation is specifically reversed by Met sulfoxide reductase A (MsrA) (68). Mice null for MsrA showed higher levels of oxidized CaMKII in myocardium after angiotensin II stimulation or after myocardial infarction compared with controls. MsrA mice had worse myocardial dysfunction and increased mortality after pathological stress. Taken together, these findings show that CaMKII is dually activated by "upstream" Ca\(^{2+}\) and oxidative signals and that both phosphorylation and Met oxidation are dynamically reversible events.

**CaMKII Activation: Cellular Mechanisms**

The cardiac action potential plays an important role in mediating CaMKII activation (FIGURE 2). The ventricular cardiomyocyte action potential duration (APD) lasts several hundred milliseconds, approximatively two orders of magnitude longer than neuronal action potentials. The prolonged cardiac APD is due to a long plateau phase (so-called phases 2 and 3) that is a balance of inward currents (mostly L-type Ca\(^{2+}\) current) and repolarizing outward currents (mostly K\(^{+}\)). This prolonged plateau phase of the cardiac action potential is critical for allowing Ca\(^{2+}\) entry, mainly through voltage-gated L-type Ca\(^{2+}\) current (I\(_{\text{Ca}}\)) and limiting cell membrane excitability by imposing a prolonged electrical refractory period. CaMKII activation is enhanced by APD prolongation because the intracellular Ca\(^{2+}\) transient lengthens in step with APD prolongation (74). In cardiomyocytes, CaMKII activity appears to be increased by APD prolongation (5) and faster heart rates (67). Under these conditions that are tested, novel forms of the holoenzyme of CaMKII have been detected (9). The ubiquitin in OPH-terminal residues of CaMKII is inactivating the catalytic domain. However, when the catalytic domain is inactivated, a fraction of CaMKII remains active, indicating that the regulatory domain (FIGURE 1B)

![FIGURE 1B](https://www.physiology.org/physiologyonline/article-5.jpg)
remodeling in heart failure may be initially adaptive by favoring cellular Ca\(^{2+}\) entry and restoration of contractility. However, these changes are ultimately maladaptive and promote dysregulation of Ca\(^{2+}\) homeostasis (11) and worsening of cardiac function (86) that are mediated at least in part by excess CaMKII activation.

Excess CaMKII activity itself can promote APD prolongation in part through a posttranslational mechanism. CaMKII induces L-type Ca\(^{2+}\) channels (LTCCs) to enter a highly active gating mode marked by frequent, long openings (20). CaMKII is responsible for dynamically increasing \(I_{\text{Ca}}\), a phenomenon termed facilitation (4, 77, 82). CaMKII can also increase APD by a phosphorylation-dependent increase in a non-inactivating component of cardiac Na current (\(I_{\text{Na}}\)) (44). Both \(I_{\text{Ca}}\) and \(I_{\text{Na}}\) increase net inward current during the AP plateau leading to APD prolongation. CaMKII overexpression leads to cardiomyopathy and heart failure with APD prolongation that may be at least partially due to reduced expression of repolarizing K\(^+\) currents (44). Mice with CaMKIV overexpression lead to cardiomyopathy and arrhythmias in heart failure (5), early afterdepolarizations (EADs) by increasing K\(^+\) currents (44). Mice with CaMKIV overexpression lead to cardiomyopathy and arrhythmias in heart failure (53).

CaMKII overexpression leads to cardiomyopathy and ventricular arrhythmias in heart failure (53) that are mediated at least in part by excess CaMKII activity. Excess CaMKII activity itself can promote APD prolongation in part through a posttranslational mechanism. CaMKII induces L-type Ca\(^{2+}\) channels (LTCCs) to enter a highly active gating mode marked by frequent, long openings (20). CaMKII is responsible for dynamically increasing \(I_{\text{Ca}}\), a phenomenon termed facilitation (4, 77, 82). CaMKII can also increase APD by a phosphorylation-dependent increase in a non-inactivating component of cardiac Na current (\(I_{\text{Na}}\)) (44). Both \(I_{\text{Ca}}\) and \(I_{\text{Na}}\) increase net inward current during the AP plateau leading to APD prolongation. CaMKII overexpression leads to cardiomyopathy and heart failure with APD prolongation that may be at least partially due to reduced expression of repolarizing K\(^+\) currents (44). Mice with CaMKIV overexpression lead to cardiomyopathy and arrhythmias in heart failure (53). CaMKII overexpression leads to cardiomyopathy and ventricular arrhythmias in heart failure (53).

**CaMKII and normal excitation contraction coupling**

A. CaMKII mediates the activity of the LTCC and RyR via phosphorylation-dependent events, which are integral for normal excitation contraction coupling. When a cardiomyocyte is depolarized by a propagating action potential, calcium enters the cell via the LTCC. This initial calcium entry activates the ryanodine receptor, resulting in release of calcium from the SR by a process termed calcium-induced calcium release. Release of calcium from the SR accounts for the majority of intracellular calcium necessary for contractility and other functions of the cardiomyocyte. The majority of cytosolic calcium is removed by SERCA, which is negatively regulated by PLN. The return of cytosolic calcium to basal levels signals the beginning of diastole. In effect, excess CaMKII activity on excitation contraction coupling. Structural heart disease is associated with excess CaMKII activity. This excess CaMKII activity results in hyperphosphorylation of the LTCC, RyR, and SERCA/PLN, which impairs cardiac functions and predisposes to afterdepolarizations. Hyperphosphorylation of the LTCC at the \(\beta\)-subunit results in increased \(I_{\text{Ca}}\), which can predispose to EADs (which occur at phases 2 and 3 of the action potential). Hyperphosphorylation events at the SR results in depletion of SR calcium stores, which results in impaired systolic calcium transients resulting in systolic and diastolic dysfunction. Furthermore, hyperphosphorylation of the RyR results in SR calcium leak that can result in a net inward Na current via NCX that result in delayed afterdepolarizations (that occur after completion of repolarization).

**Cellular Localization**

The structure of CaMKII not only serves to decode Ca\(^{2+}\) frequency, it also serves to target the kinase directly to key regulatory proteins involved in Ca\(^{2+}\) homeostasis. Proper targeting of kinases and phosphatases to protein substrates is essential for maintaining signaling fidelity (51). Other Ser/Thr protein kinases, including PKA and PKC, utilize anchoring proteins for targeting protein substrates (19, 27, 37), but similar adapter proteins suitable for localizing CaMKII to substrate targets have not been identified. CaMKII to substrate targets have not been identified. CaMKII inhibition shortens the AP and cellular Ca\(^{2+}\) transient in surviving myocardium after infarction (85) and leads to APD shortening by augmenting the fast component of the transient outward and the inwardly rectifying K\(^+\) currents by an incompletely characterized nontranscriptional mechanism (42). Taken together, these findings show that CaMKII is selectively enriched along the Z band of the cardiomyocyte. The majority of cytosolic calcium is removed by SERCA, which is negatively regulated by PLN. The return of cytosolic calcium to basal levels signals the beginning of diastole. In effect, excess CaMKII activity on excitation contraction coupling. Structural heart disease is associated with excess CaMKII activity. This excess CaMKII activity results in hyperphosphorylation of the LTCC, RyR, and SERCA/PLN, which impairs cardiac functions and predisposes to afterdepolarizations. Hyperphosphorylation of the LTCC at the \(\beta\)-subunit results in increased \(I_{\text{Ca}}\), which can predispose to EADs (which occur at phases 2 and 3 of the action potential). Hyperphosphorylation events at the SR results in depletion of SR calcium stores, which results in impaired systolic calcium transients resulting in systolic and diastolic dysfunction. Furthermore, hyperphosphorylation of the RyR results in SR calcium leak that can result in a net inward Na current via NCX that result in delayed afterdepolarizations (that occur after completion of repolarization).
ventricular myocytes where it co-localizes with LTCCs and ryanodine receptors (21, 73). CaMKII binds directly to the NMDA receptor NR2B subunit after phosphorylation of Thr498 (81). CaMKII also binds the LTCC (CaV1.2) β subunit at multiple sites, including the COOH terminal. Thus CaMKII selectively adapts to protein targets in a regulated manner in cardiomyocytes.

We recently identified a CaMKII binding sequence in an auxiliary LTCC β subunit (bβ2) (29). CaMKII binding to the β subunit and to NR2B occurs by way of a sequence that resembles the CaMKII regulatory domain. Thr498 autophosphorylation enables CaMKII binding to the β subunit (29), which asso-

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The excitation-contraction coupling (ECC) apparatus is the mechanism for myocardial mechanical function. CaMKII regulates many of the key proteins for ECC. There is an increasing awareness that disorders of ECC are also the basis for many arrhythmias and can lead to cardiac hypertrophy and heart failure. The joint dependence of myocardial mechanical and electrical functions on ECC provides a rationale for understanding why patients with heart failure (i.e., a clinical condition of inadequate mechanical cardiac function) are also at most risk for arrhythmias and sudden death. CaMKII is now known to contribute to cardiac hypertrophy, heart failure, and arrhythmias, and CaMKII activity and expression are increased in failing hearts and animal models of heart failure (53).

CaMKII binding to the β subunit and phosphorylate Thr498, a residue that is conserved in all the major cardiac β subunit isoforms. Mutation of Thr498 to Ala results in ablation of CaMKII-facilitation in cardiomyocytes (29). Interestingly, the CaMKII site (T498) that increases I_{CaL} in close proximity to a protein kinase G (PKG) site (S494) that results in decreased I_{CaL} (79). It is unknown whether these sites interact.

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CaMKII binds the cytoplasmic COOH terminus of CaV1.2 (35, as well as the β subunit (29), which associates with CaV1.2 at the intracellular I1-I2 linker domain (84). Early studies demonstrated ablation of I_{CaL} facilitation using pharmacological and peptide inhibitors of CaMKII (4, 77, 82). Treatment with con-stitutively active CaMKII results in phosphorylation of the LTCC complex, inducing high activity (mode 2) gating that is characterized by long, frequent openings (20). CaMKII anchors to and phosphorylates regions along the CaV1.2 COOH terminus. Mutations to a potas-sive COOH terminus binding site abolish I_{CaL} facilita-
tion (35). Recently, CaMKII was shown to bind the β subunit and phosphorylate Thr498, a residue that is conserved in all the major cardiac β subunit isoforms. Mutation of Thr498 to Ala results in ablation of CaMKII-mediated I_{CaL} facilitation in cardiomyocytes (29). Interestingly, the CaMKII site (T498) that increases I_{CaL} in close proximity to a protein kinase G (PKG) site (S494) that results in decreased I_{CaL} (79). It is unknown whether these sites interact. CaV1.3 is an LTCC that is present in atrium, cardiac pacemaker cells, and the specialized conduction system. CaMKII may increase CaV1.3 by phosphorylating a Ser residue that is present in atrial bundle stalk cells (8). Both CaV1.2 and CaV1.3 share a homologous EF hand domain (26). Both CaV1.2 and CaV1.3 share a homologous EF hand domain (26). Both CaV1.2 and CaV1.3 share a homologous EF hand domain (26). Both CaV1.2 and CaV1.3 share a homologous EF hand domain (26). Both CaV1.2 and CaV1.3 share a homologous EF hand domain (26). Both CaV1.2 and CaV1.3 share a homologous EF hand domain (26). Both CaV1.2 and CaV1.3 share a homologous EF hand domain (26).
(Ca\textsuperscript{2+} sparks), which may contribute to impaired contractility, promote DADs, and activate cardiomyopathic gene programs. The RyR is a 2.5-MDa intracellular Ca\textsuperscript{2+} release channel that consists of four identical subunits. The majority of this channel serves as a cytoplasmic scaffold for myriad accessory proteins, including CaMKII. CaMKII directly binds and phosphorylates RyR at Ser\textsuperscript{16} and Ser\textsuperscript{2815} (16, 67, 70). However, phosphorylation studies suggest CaMKII may phosphorylate additional sites, known and unknown (54).

Under different experimental conditions, CaMKII has been shown to either increase or decrease Ca\textsuperscript{2+} release via the RyR (39, 44, 67, 71, 78). Isolated RyRs placed in lipid bilayers demonstrate increased CaMKII-mediated phosphorylation (Ser\textsuperscript{16}) and channel activity at increasing heart rates, which are associated with increased CaMKII autophosphorylation (67). In transgenic mice overexpressing CaMKII, RyRs show increased Ca\textsuperscript{2+} spark frequency, which can be reduced by CaMKII inhibition (44). In a rabbit heart failure model of left ventricular pressure and volume overload, there is increased CaMKII phosphorylation of RyR. Inhibiting CaMKII in this model reduced SR leak and increased SR Ca\textsuperscript{2+} content (2). Adenovirus-mediated overexpression of CaMKII in cultured adult ventricular myocytes increases (39) or inhibits (78) RyR function. Overall, these data support an important regulatory role of CaMKII in RyR function but leave open many questions about how CaMKII acts on RyR and why CaMKII appears to have both inhibitory and stimulatory actions in various models. In heart disease, it appears that CaMKII effects on RyR contribute to the phenotype of heart failure, which includes depressed diastolic Ca\textsuperscript{2+} stores as well as increased arrhythmia-initiating DADs.

**PLN/SERCA**

CaMKII regulates Ca\textsuperscript{2+} transport from the cytoplasm by the sarcoplasmic/endoplasmic reticulum Ca\textsuperscript{2+} ATPase (SERCA), exclusively or in large part, via its effects on phospholamban (PLN). SERCA is located on the membrane of the longitudinal SR where it functions to remove intracellular Ca\textsuperscript{2+}, resulting in cardiomyocyte relaxation. In humans and rabbits, SERCA accounts for 70% of cytoplasmic Ca\textsuperscript{2+} removal, whereas in rats and mice it accounts for ~90% (5). Most of the remaining cytoplasmic Ca\textsuperscript{2+} is removed via the sodium-potassium exchanger (NCX), SERCA is tonically inhibited by PLN, which is a 52-amino acid transmembrane protein. Association of PLN with SERCA lowers the affinity for Ca\textsuperscript{2+} ions. Phosphorylation of PLN by either PKA (at Ser\textsuperscript{16}) or CaMKII (at Thr\textsuperscript{17}) relieves SERCA of PLN's inhibitory effects, accelerating intracellular Ca\textsuperscript{2+} removal and enhancing SR Ca\textsuperscript{2+} content (30, 63).

Frequency-dependent acceleration of relaxation (FDR) is a property of cardiomyocytes caused by the acceleration of intracellular Ca\textsuperscript{2+} removal by SERCA at increasing heart rates. From a physiological perspective, FDR promotes acceleration of myocardial relaxation (diastolic function) for the maintenance of efficient ventricular filling at higher heart rates, despite a decreased diastolic time interval. The ability of CaMKII to detect changes in intracellular Ca\textsuperscript{2+} as well as to promote SERCA activity by phosphorylating PLN makes it a strong candidate for mediating FDR.

In isolated ventricular myocytes, it has been shown that Thr\textsuperscript{17} phosphorylation and FDR increase with increasing stimulation frequency, independent of Ser\textsuperscript{16} phosphorylation (30). It also has been demonstrated that Thr\textsuperscript{17} mutation attenuates FDR at higher pacing frequencies compared with wild-type and Ser\textsuperscript{16} mutation mouse cardiomyocytes (89). CaMKII inhibition suppresses FDR (7, 18). In contrast to these findings, PLN knockout mice still exhibit FDR, which can be ablated with CaMKII inhibition (18). Furthermore, other studies have demonstrated that Ser\textsuperscript{16} is sufficient for mediating β-adrenergic receptor-mediated FDR (13). These data support CaMKII as an important mediator of FDR but do not resolve the controversy about the identity of the key proteins that underlie FDR. CaMKII and PLN appear to wane at high (physiological) steady-state frequencies (49), suggesting that modulatory effects of CaMKII underlie FDAR. The effects of CaMKII and PLN appear to be involved in cardiac remodeling and arrhythmia initiation and prevention. CaMKII appears to have both inhibitory and stimulatory actions in various models. In heart disease, it appears that CaMKII effects on RyR contribute to the phenotype of heart failure, which includes depressed diastolic Ca\textsuperscript{2+} stores as well as increased arrhythmia-initiating DADs.

**CaMKII Inhibition as a Therapeutic Target**

In the United States, 280,000 patients die of heart failure annually (55). With a risk of sudden cardiac death six to nine times that of the general population, about half of heart failure patients die of ventricular arrhythmias. Although drugs that inhibit the adrenergic and renin-angiotensin aldosterone systems have improved survival (1, 49, 52), deaths from heart failure increased 28% from 1994 to 2004. Treating arrhythmias in patients with structural heart disease by ion channel antagonist drugs does not reduce mortality (22, 65). These data support the importance of finding suitable agents for treating both heart failure and arrhythmias.

Pharmacological approaches that inhibit G protein-coupled receptors (GPCR) have produced impressive therapeutic effects, but these methods do not reduce unwanted side effects in diverse intracellular Ca\textsuperscript{2+} release pathways, considered as an important drug target. Pharmacologically selective mechanisms for cardiac GPCRs (6). CaMKII functionally couples to multiple intracellular signaling pathways and several ion channels, including CaMKII. CaMKII directly binds and phosphorylates RyR at Ser\textsuperscript{2809} and Ser\textsuperscript{2815} (16, 67, 70). Furthermore, other studies have demonstrated that Ser\textsuperscript{16} is sufficient for mediating β-adrenergic receptor-mediated FDR (13). These data support CaMKII as an important mediator of FDR but do not resolve the controversy about the identity of the key proteins that underlie FDR. CaMKII and PLN appear to wane at high (physiological) steady-state frequencies (49), suggesting that modulatory effects of CaMKII underlie FDAR. The effects of CaMKII and PLN appear to be involved in cardiac remodeling and arrhythmia initiation and prevention. CaMKII appears to have both inhibitory and stimulatory actions in various models. In heart disease, it appears that CaMKII effects on RyR contribute to the phenotype of heart failure, which includes depressed diastolic Ca\textsuperscript{2+} stores as well as increased arrhythmia-initiating DADs.

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oscardial relaxation of heart rates, despite the ability of CaMKII to inhibit G protein-coupled receptor (GPCR) signaling. ADP and phospholamban (PLN) appear to inhibit G protein-coupled receptor (GPCR) signaling, which is activated by CaMKII (6). CaMKII is an appealing potential target for pharmacological inhibition. CaMKII activity is upregulated in hypertrophy and heart failure (32, 88). CaMKII overexpression in transgenic mice results in impaired cardiac function (66), suggesting the possibility that CaMKII inhibition could reduce mechanical function under some conditions.

There are many potential methods for inhibiting CaMKII function including developing drugs that prevent ATP binding to the catalytic domain, drugs that result in allosteric inhibitory conformational changes to CaMKII, as well as drugs that specifically prevent CaMKII binding to protein targets. Therapies that affect the CaMKII holozyme architecture or prevent the conformation changes necessary for autophosphorylation and Ca^2+—dependent activity may prevent excessive CaMKII activity that results in heart failure and arrhythmias.

Conclusion

CaMKII is a remarkable signaling molecule that is abundant in myocardium and other excitable tissues. CaMKII targets a wide range of proteins in heart but is especially important for regulating proteins necessary for ECC. The CaMKII holozyme is uniquely adapted to integrate and transduce cellular Ca^2+ signals into physiological responses in heart. Under pathological stresses, CaMKII appears to be an important disease signal. The actions of CaMKII on electrical and Ca^2+ handling proteins help to explain how CaMKII can simultaneously favor heart failure and arrhythmias. Recent findings that CaMKII inhibition reduces heart failure and suppresses arrhythmias suggest that developing CaMKII inhibitory drugs may be a new therapeutic approach to these diseases.

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Mark Anderson is a named inventor on patents claiming treatment heart failure and arrhythmias by CaMKII inhibition.

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