

CaMKII: The molecular villain that aggravates cardiovascular disease (Review)

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Abstract. Pathological remodeling of the myocardium is an integral part of the events that lead to heart failure (HF), which involves altered gene expression, disturbed signaling pathways and altered Ca^{2+} homeostasis and the players involved in this process. Of particular interest is the chronic activation of Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) isoforms in heart, which further aggravate the injury to myocardium. Expression and activity of CaMKII have been found to be elevated in various conditions of stressed myocardium and in different heart diseases in both animal models as well as heart patients. CaMKII is a signaling molecule that regulates many cellular pathways by phosphorylating several proteins involved in excitation-contraction coupling and relaxation events in heart, cardiomyocyte apoptosis, transcriptional activation of genes related to cardiac hypertrophy, inflammation, and arrhythmias. CaMKII is activated by reactive oxygen species (ROS), which are elevated under conditions of ischemia-reperfusion injury and in a cyclical manner, CaMKII in turn elevates ROS production. Both ROS and activated CaMKII increase Ca^{2+} -induced Ca^{2+} release from sarcoplasmic reticulum, which leads to cardiomyocyte membrane depolarization and arrhythmias. These CaMKII-mediated changes in heart ultimately culminate in dysfunctional myocardium and HF. Genetic studies in animal models clearly demonstrated that inactivation of CaMKII is protective against a variety of stress induced cardiac dysfunctions. Despite significant leaps in understanding the structural details of CaMKII, which is a very complicated and multimeric modular protein, currently there is no specific and potent inhibitor of this enzyme, that can be developed for therapeutic purposes.

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1. Introduction

Advances in medicine over the past few decades significantly lowered cardiovascular disease-linked mortality by up to 75% and increased the survival rate of patients with cardiac disease, but at the same time this has led to a great increase in the number of people surviving with injured heart (1). However, the increasing incidence of obesity, and associated hypertension and diabetes coupled with unhealthy lifestyles is causing a significant increase in the number of surviving individuals with heart disease, adding burden on the society in terms of health, economy and productivity (2,3). Several diseases and metabolic disturbances can be contributed to heart failure (HF) and these include myocardial infarction (MI), hypertension, valvular disease, genetic disorders, diabetes and obesity. HF occurs because of the compromised ability of myocardium to exert systolic contraction with enough force to pump blood; with characteristic reduced ejection fraction or it can be due to lowered diastolic filling, but with the preservation of ejection fraction. While acute HF is the sudden appearance of HF symptoms such as congestion and difficulty to breath (4), chronic HF is marked by the inability of heart to function optimally over an extended period of time (4,5).

Pathological remodeling of the myocardium is an integral part of the HF syndromes (6), which involves altered gene expression and disturbed signaling pathways and altered contractile response of myocardium. Of particular significance are the changes in the functionality of proteins that play a central part in intracellular Ca^{2+} handling as well as ion channels involved in Ca^{2+} transport. Cardiac muscle contraction is dependent on the maintenance of Ca^{2+} homeostasis, which is essential for excitation-contraction (E-C) coupling of cardiomyocyte. Thus, electrical depolarization of the cardiomyocyte membrane swiftly moves to the center of

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the cell via the network of transverse tubules (t-tubules), which terminate close to sarcoplasmic reticulum (SR), with a 12 nm gap. Membrane depolarization triggers the rapid diffusion of extracellular Ca^{2+} to the SR, through these gaps, facilitated by the L-type Ca^{2+} channels (LTCC). This influx of Ca^{2+} leads to Ca^{2+} -induced calcium release (CICR) from the SR through the type 2-ryanodine receptor (RyR2). This elevated calcium promotes cross-bridge cycling by relieving actin from troponin C-dependent inhibition, thereby causing cardiomyocyte contraction. Then, Ca^{2+} is taken back into the SR lumen by the SR Ca^{2+} ATPase 2a (7). Considering the significance of Ca^{2+} in heart muscle contraction, it is appreciated that disturbances in the Ca^{2+} handling machinery in cardiomyocyte can potentially lead to HF. Thus HF is characterized by disturbed Ca^{2+} leak from SR, mediated by RyR2, even though the precise mechanisms are not clear (8). Other players such as SERCA are also deranged in HF. Besides membrane depolarization, intracellular Ca^{2+} is important in many other cell processes including oxidative stress, mitochondrial function, apoptosis and autophagy.

2. Ca^{2+} /calmodulin-dependent protein kinase II

Several studies have indicated that a Ca^{2+} -regulated protein kinase, Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) plays a critical role in E-C coupling, contractility of cardiomyocyte (9,10), mitochondrial function and cardiomyocyte survival (11,12). Expression and activity of CaMKII have been found to be elevated in various conditions of stressed myocardium and in different heart diseases in both animal models as well as heart patients (9-14). The activation of CaMKII can be either at the level of this enzyme protein itself or at an upstream signaling event involving catecholamines (15) or renin-angiotensin-aldosterone systems (16). Abnormally elevated CaMKII activity can cause dysfunction of several downstream events whose components are regulated by CaMKII, such as E-C coupling, structural remodeling, and transcriptional activation of certain inflammatory proteins and apoptosis (17).

Structure/function features of CaMKII. CaMKII is a serine/threonine kinase with a broad range of protein substrates and wide tissue distribution. There are 4 isoforms of CaMKII (α , β , δ and γ), coded for by 4 separate genes, with heart expressing predominantly the δ -isoform, with some γ -isoform as well. Alternate splicing of mRNA adds further complexity to the CaMKII isoforms and their function and regulation (18). There are three main domains in the CaMKII monomer-N-terminal catalytic domain, regulatory domain and the C-terminal association domain (Fig. 1) (19). The regulatory domain, which interacts with the catalytic site, maintains the catalytic activity low under unstimulated basal conditions, and contains binding sites for Ca^{2+} and calmodulin. The C-terminal association domain participates in the multimerization process, thus forming the mature dodecameric holoenzyme, with two hexameric stacked rings (20). Complex of calmodulin and Ca^{2+} binds with the regulatory domain and this displaces this domain from the catalytic domain thereby restoring the activity of the enzyme (activation) and also exposes certain other regulatory binding sites, which can influence the CaMKII activity.

CaMKII can phosphorylate several proteins involved in Ca^{2+} homeostasis, the well-studied protein targets being LTCC, RyR2, voltage-gated Na^{+} channel and K^{+} channels (21,22) and also ATP-sensitive K^{+} channels (23) and chloride channels (24), which have been shown to be important for cardiac arrhythmias. Regulation and activity level of CaMKII depends upon its holoenzymic state and post-translational modifications including phosphorylation, glycosylation and oxidation. CaMKII is known to autophosphorylate itself at Thr286/287 residue of the calmodulin- Ca^{2+} bound catalytic domain, mediated by another adjacent catalytic domain. This autophosphorylation renders the catalytic domain to maintain its activity even in the absence of calmodulin and Ca^{2+} (25,26).

Organization of CaMKII in cardiomyocytes. Subcellular localization is critical for the maintenance of membrane excitability and CaMKII is found to be distributed in high density near the t-tubules of cardiomyocyte, close to LTCC (Cav1.2) and to RyR2 channels of SR, which regulate the Ca-induced Ca release intracellularly (Fig. 2). Thus, phosphorylation of S2814 of RyR2 by CaMKII leads to dysregulated intracellular Ca^{2+} homeostasis, which in turn cause perturbation of maladaptive stress response and proarrhythmic events, thus further aggravating the HF (Figs. 1 and 2). Thus, mouse models which express RyR2 with S2814A mutation and thus are not phosphorylated by CaMKII, are protected from pressure overload *in vivo* (27). CaMKII is also found in mitochondria, nucleus and near the intercalated disc (17). CaMKII subcellular localization appears to be dependent on the nature of the target and its location and the presence of interacting domains on the target. Thus α - and β -subunits of LTCC, which are phosphorylated by CaMKII, bind with CaMKII, because of the homology between the phosphorylation sites and the auto-inhibitory region of the CaMKII (21,28). A similar homology domain, as seen in the LTCC β -subunit, is also found in the actin-associated protein, β IV-spectrin, to which CaMKII is known to bind. This interaction is a prerequisite for the CaMKII-mediated phosphorylation of the voltage-gated Na^{+} channels at the intercalated disc in cardiomyocytes (29).

3. Evidence for CaMKII as a therapeutic target in heart disease

CaMKII acts as a molecular nexus that connects neurohumoral stimulation to HF and cardiac remodeling (20). There has been a significant development in our understanding of the role of CaMKII in cardiovascular diseases and several reports over the past two decades have suggested such roles, making CaMKII a potential therapeutic target. Thus, it has been noted that cytosolic CaMKII δ C isoform as well as the nuclear CaMKII δ B isoform were found to be elevated in the two ventricles of patients with ischemic cardiomyopathy (30). There is also a significant elevation of autonomous activity of CaMKII and its expression, in patients with advanced and end stage HF (31). As the upregulation of CaMKII is associated with heart disease and failure by promoting apoptosis, inflammation that leads to cardiac dysfunction (32,33), the possibility that inhibition of this enzyme activity can have therapeutic effects has been considered. Experimental transgenic animal models, overexpressing CaMKII have been

Domain structure of CaMKII monomer and potential regulatory sites

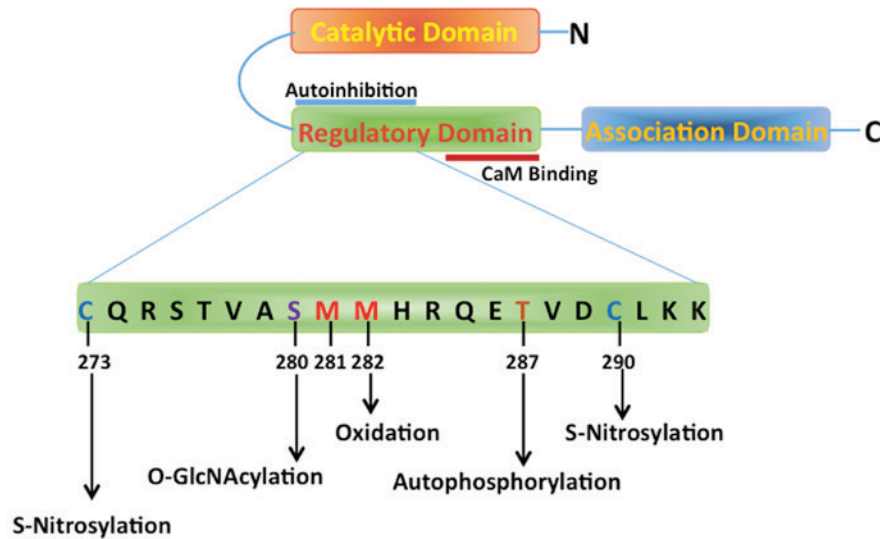


Figure 1. Domain structure of Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) monomer and potential regulatory sites. A schematic showing the CaMKII monomer with catalytic, regulatory and association domains. Catalytic domain function is normally obscured by the regulatory domain. Association domain is instrumental in forming the holoenzyme that consists of 12 monomers. Post-translational modifications by different stress stimuli and neurohormonal signaling at the indicated sites in the regulatory domain lead to sustained activation of CaMKII by relieving the catalytic domain.

Mechanisms of CaMKII-dependent cardiac dysfunction

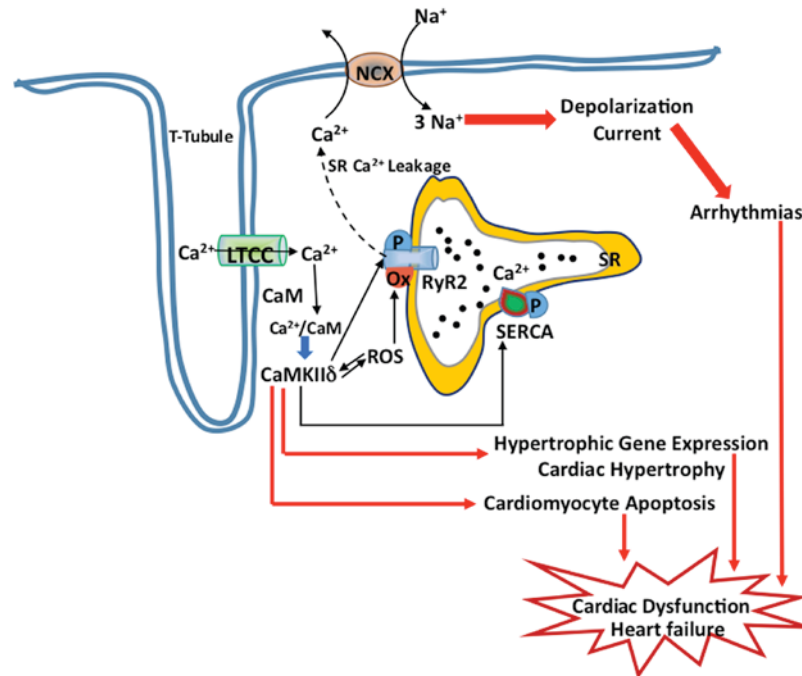


Figure 2. Mechanisms of Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) dependent cardiac dysfunction. Increase in intracellular Ca^{2+} through L-type Ca^{2+} channels, leads to CaMKIIδ activation via Ca^{2+} /Calmodulin (CaM) in cardiomyocyte. Activated CaMKIIδ contributes to elevated reactive oxygen species (ROS), which can also arise from other stress stimuli, themselves can activate CaMKIIδ, resulting in a vicious cycle of CaMKIIδ activation. Both the phosphorylation mediated by CaMKIIδ and ROS-mediated oxidation of the type 2-ryanodine receptor (RyR2) in sarcoplasmic reticulum (SR) lead to enhanced SR Ca^{2+} load and in turn, cause SR Ca^{2+} leak followed by the re-uptake of Ca^{2+} by SERCA into SR. This triggers sodium/calcium exchanger (NCX)-dependent depolarizing current (transient inward current), which contributes to arrhythmia. Activated CaMKIIδ also elevates transcription of cardiac hypertrophy genes, which culminates in cardiac hypertrophy and thus dysfunction of heart. Activated CaMKII is known to trigger cardiomyocyte apoptosis program, leading to loss of cardiomyocytes and thus damaged myocardium. All these events resulting from CaMKII activation, contribute to failure of the heart.

found to suffer from HF (34), whereas CaMKII knockout mice were protected from HF induced by transaortic constriction (35). Additionally, mice expressing a mutant

CaMKII (S2814D), which is constitutively active, suffered exacerbated mortality (36). CaMKIIδγ knockout mice with total deletion of heart specific isoforms CaMKII, are protected

from pressure overload and β -adrenergic stimulation-induced cardiac dysfunction and interstitial fibrosis (32,37). Similarly, the elevated activity of CaMKII is also associated with atrial fibrillation and sinus node disease (38) and several other HF contributory diseases such as inherited arrhythmias (39,40).

Oxidation of 281/282 methionine residue in CaMKII is susceptible to oxidative stress and this oxidation leads to the activation of CaMKII and it has been shown that this oxidation is particularly important in cardiomyocytes as it may relate to conditions of ischemia/reperfusion injury (41). Met281/282 oxidation prevents the re-association of the inhibitory regulatory domain with the catalytic domain of CaMKII (Fig. 1) (42). Angiotensin II and aldosterone are shown to mediate their activation effects on CaMKII via oxidation (43), as cardiomyocytes expressing oxidation-resistant mutant CaMKII were protected from angiotensin II-induced apoptosis (41). Also, diabetic mice expressing an oxidation-resistant CaMKII mutant (MM281/282VV) were found to be protected from MI (44). In fact, it has been noted that increased oxidation status of CaMKII seen after MI in diabetic patients appears to be associated with higher mortality, than in non-diabetic individuals, which again emphasizes the detrimental effects of CaMKII activation, particularly when the heart is stressed.

Of note, CaMKII oxidation and activity is found to be much less following MI in mice with deletion of the *MyD88* gene, an important mediator of inflammatory signaling. These *MyD88*-knockout mice also show lower post MI inflammatory cell infiltration, cardiomyocyte death and fibrosis. Oxidized CaMKII can in turn enhance the transcription of proinflammatory genes by enhancing NF- κ B activity (45). Other post-translational modifications of CaMKII that cause its activation and are involved in the pathology of HF include nitrosylation and O-GlcNAcylation, which are important under hyperglycemic conditions seen in diabetes (46).

4. Therapeutic measures against CaMKII

Inasmuch as the activation of CaMKII is involved with heart disease, several studies have focused on developing CaMKII inhibitors that have the potential to have therapeutic effects in HF and heart diseases. Most of the currently available inhibitors are for research purposes and lack specificity and/or potency. For example, KN-93, which is a commonly used CaMKII inhibitor, also directly affects many other ion channel including LTCC (47). Administration of KN-93 to mice with structural heart disease, for 3 weeks led to chronic inhibition of CaMKII δ and resulted in a dose-dependent improvement in left ventricular function (48). Similarly, peptide molecules (AIP and AC3-I) that mimic the autoinhibitory-regulating domain of CaMKII, also have several limitations regarding their specificity and delivery. Among the several inhibitors tested, the most promising is the endogenous inhibitor, known as CaMKIIN and its derivatives, which bind to the active kinase at the B/C sites, which also prevent protein-protein interactions of CaMKII with other targeting proteins (47). It has been recently shown that targeting CaMKII/ ERK interaction in heart muscle using selective CaMKII peptide inhibitor AntCaNtide was able to prevent hypertrophy in spontaneously hypertensive rats (49). The first generation CaMKII inhibitors based on targeting the ATP binding to

catalytic site and the recent availability of crystal structures of CaMKII holoenzyme both in its autoinhibited as well as active states may be useful in the development of more specific and potent inhibitors for this enzyme. Furthermore, blockade of activating pathways such as O-GlcNAc modification were also found to be effective in preventing arrhythmogenesis in diabetic animals by inhibiting the hexosamine biosynthetic pathway using the inhibitor DON (50). Thus, there are formidable difficulties in achieving the required specificity for developing CaMKII inhibitors that can be developed for therapeutic applications (47).

In addition to pharmacological inhibitors, exercise, which has proven beneficial cardiovascular effects, seems to have the ability to antagonize the negative effects of CaMKII δ in failing heart. Thus, aerobic training caused a reduction in CaMKII δ activity and improved heart function in diabetic mice compared to non-exercising diabetic mice (51). Notably, it has been demonstrated that swimming exercise may obliterate the O-GlcNAcylation-mediated activation of CaMKII in type I diabetic mice with the resultant improvement in heart condition (52). Thus, of note is along with pharmacological approaches, lifestyle changes can be beneficial in protecting from the CaMKII-mediated aggravation of injured or stressed heart. As such, the development of specific drugs that target heart isoforms of CaMKII seems a far-reaching goal and further work is needed in understanding structure-activity relationships of these isoenzymes to accomplish this task.

5. Conclusions

HF involves altered gene expression, disturbed signaling pathways and altered Ca^{2+} homeostasis. Chronic activation of CaMKII isoforms in heart further aggravates the injury to myocardium and the expression and activity of CaMKII is elevated in myocardium in different heart diseases and stress conditions. CaMKII regulates many cellular pathways such as E-C coupling and relaxation events in heart, cardiomyocyte apoptosis, transcriptional activation of genes related to cardiac hypertrophy, inflammation, and arrhythmias. CaMKII and reactive oxygen species, which mutually activate each other, increase CICR from SR, which leads to cardiomyocyte membrane depolarization and arrhythmias. All these CaMKII-mediated changes in heart ultimately culminate in dysfunctional myocardium and HF. Despite significant leaps in understanding the structural details of CaMKII, which is a very complicated and multimeric modular protein, and genetic studies implicating CaMKII in the pathogenesis of HF, currently there is no specific and potent inhibitor of this enzyme, that can be developed for therapeutic purposes and further study is needed in this direction.

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