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Korean Circulation Journal

A Memory Molecule, Ca^{2+}/Calmodulin–Dependent Protein Kinase II and Redox Stress; Key Factors for Arrhythmias in a Diseased Heart

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Arrhythmias can develop in various cardiac diseases, such as ischemic heart disease, cardiomyopathy and congenital heart disease. It can also contribute to the aggravation of heart failure and sudden cardiac death. Redox stress and Ca^{2+} overload are thought to be the important triggering factors in the generation of arrhythmias in failing myocardium. From recent studies, it appears evident that Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) plays a central role in the arrhythmogenic processes in heart failure by sensing intracellular Ca^{2+} and redox stress, affecting individual ion channels and thereby leading to electrical instability in the heart. CaMKII, a multifunctional serine/threonine kinase, is an abundant molecule in the neuron and the heart. It has a specific property as "a memory molecule" such that the binding of calcified calmodulin (Ca^{2+}/CaM) to the regulatory domain on CaMKII initially activates this enzyme. Further, it allows autophosphorylation of T287 or oxidation of M281/282 in the regulatory domain, resulting in sustained activation of CaMKII even after the dissociation of Ca^{2+}/CaM. This review provides the understanding of both the structural and functional properties of CaMKII, the experimental findings of the interactions between CaMKII, redox stress and individual ion channels, and the evidences proving the potential participation of CaMKII and oxidative stress in the diverse arrhythmogenic processes in a diseased heart. **(Korean Circ J 2013;43:145–151)**

KEY WORDS: Calcium-calmodulin-dependent protein kinase type 2; Oxidative stress; Arrhythmias cardiac; Heart failure.

Introduction

Some pathologic cardiac conditions, including ischemic heart disease, cardiomyopathy and congenital heart disease, are likely associated with arrhythmias, which can lead to sudden cardiac death or may worsen heart failure.

Despite considerable advances in medical treatments for cardiovascular diseases, the development of widely acceptable therapies that can work both for arrhythmia and other associated cardiac diseases remains to be an unsolved issue. The treatment for heart failure may aggravate the associated arrhythmias, or antiarrhythmic drugs, such as ion channel blockers, and are likely to have proarrhythmic side effects in a diseased heart.[1][2] Therefore, new treatments targeting both arrhythmia and heart failure are essential, and the understanding of cellular and molecular mechanisms that underlie the pathogenesis of arrhythmias in a diseased heart is thought to be the first step of this development.

This review provides the understanding of pathogenesis of arrhythmias related to Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and redox stress, which are proven to be associated with a failing heart, both in human and animal studies.

Ca^{2+}/Calmodulin–Dependent Protein Kinase II

The impairment of Ca^{2+} balance and the overproduction of reactive oxygen species (ROS) in diseased cardiac cells are thought be important factors contributing to arrhythmias in a failing heart.[3][4] Even though the underlying mechanisms as to how Ca^{2+} and ROS can produce arrhythmias are still elusive, CaMKII-mediated pathway is a convincing candidate to drive this pathologic process.

Ca^{2+}/calmodulin-dependent protein kinase II is a multifunctional serine/threonine kinase in which the structural states determine the enzyme activities. This kinase is a multimeric protein, which consists of 12 subunits. Each subunit contains three distinct domains: an

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• The author has no financial conflicts of interest.

association domain which directs the assembly of holoenzyme, a regulatory domain which controls the activation of the enzyme, and a catalytic domain which interacts with the substrates and performs kinase functions (Fig. 1).

In the resting state, CaMKII is bent at the area between the regulatory domain and the catalytic domain, leading to a close association between these two domains. This resting state structure prevents the binding of a substrate to the catalytic domain, resulting in the autoinhibition of kinase activity (Fig. 1).[5]

When intracellular calcium concentration rises, calcium-calmodulin binding increases. This calcified calmodulin (Ca^{2+}/CaM) can bind to CaMKII at the regulatory domain and disrupts the association between the regulatory and catalytic domains, leading to a conformational change that exposes a catalytic domain for substrate binding and induces kinase activity (Fig. 1).[6]

The activation of CaMKII by Ca^{2+}/CaM binding is transient and readily reversible. Since calcium-calmodulin binding is very sensitive to intracellular Ca^{2+} concentration, calmodulin becomes immediately decalcified when intracellular Ca^{2+} concentration returns to a baseline. The decalcification of calmodulin causes a dissociation of calmodulin from CaMKII, resulting in a reassociation of the regulatory and catalytic domains as well as the inactivation of the kinase.
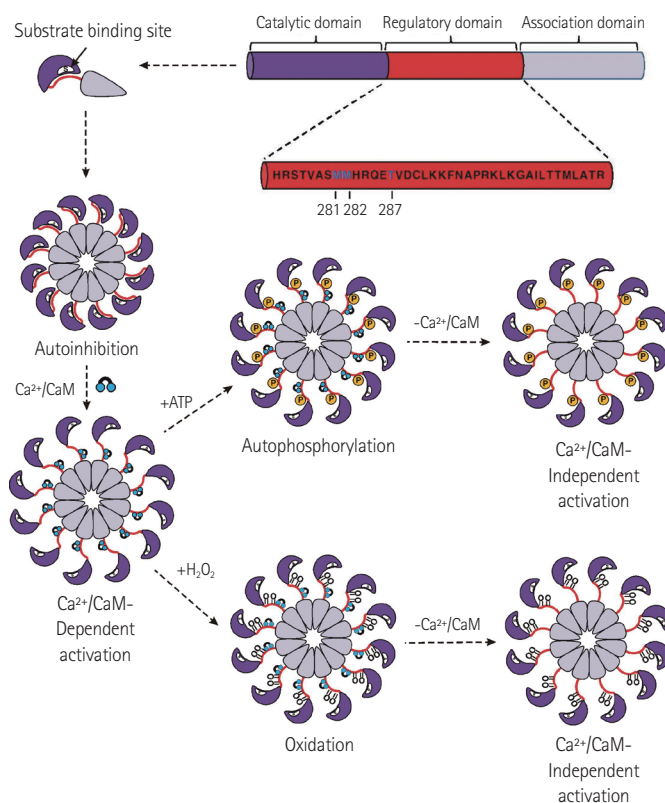

Fig. 1. Binding of Ca^{2+}/CaM initially activates CaMKII, and allows autophosphorylation and oxidation of target amino-acids in the regulatory domain, thereby leading to sustained activation of CaMKII, independently of Ca^{2+}/CaM. CaMKII: Ca^{2+}/calmodulin-dependent protein kinase II.

Neuroscience studies investigating learning and memory have found that CaMKII, which is an abundant and major synaptic protein in the neuron, plays a central role in memory storage in brain via long-term potentiation (LTP) process whereby brief-periods of synaptic activity can produce a long-lasting increase in the strength of a synapse.[7]

Ca^{2+}/calmodulin-dependent protein kinase II can act as a protein switch; once activated (turned-on) by Ca^{2+}/CaM binding, the enzyme can be autophosphorylated at T287 within the regulatory domain in the presence of sufficient amounts of adenosine triphosphate (ATP) in the cell, an event that produces persistent activation (keeping turned-on state) of CaMKII even after the Ca^{2+} concentration falls to baseline levels (Fig. 1).[8] This is the underlying mechanism of LTP, a key process of memory storage in the brain. For this reason, we call the enzyme "a memory molecule".

In the heart, CaMKII can phosphorylate a diverse array of ion channel proteins, thereby affecting the electrical activities of the myocardium.[9] Calcium-dependent facilitation (CDF) of L-type Ca^{2+} channel is a typical example of interaction between CaMKII and ion channels in cardiac myocytes. CDF is a dynamic positive feedback mechanism in physiologic condition, which augments L-type Ca^{2+} currents in response to increased intracellular Ca^{2+} concentrations.[10-12] This reaction contributes to cardiac force-frequency relationship; a physiologic phenomenon in which an increased heart rate leads to the augmentation of cardiac contractility. The activation of CaMKII by Ca^{2+}/CaM binding and then the autophosphorylation at T287 is an essential reaction for CDF. Unlike in a neuron, autophosphorylation-mediated CaMKII activation is not persistent in the heart, seeing that CDF lasts only several seconds. This is due in part to the actions of cardiac protein phosphatases, such as protein phosphatase (PP) 1 and PP2A, whereby autophosphorylated CaMKII and phosphorylated ion channels can be easily dephosphorylated in physiologic conditions.

Recently, a new mechanism of CaMKII activation by redox stress was reported, which is likely related to the pathologic conditions in the heart.[13] An increased ROS level causes oxidative modification of M281/282 pair within the regulatory domain, blocking the reassociation of the regulatory and catalytic domains and preserving the enzyme activity via a similar but parallel mechanism to T287 autophosphorylation (Fig. 1).

In our previous study, direct perfusion of isolated rat cardiomyocytes with ROS-containing solutions caused large increases in L-type Ca^{2+} currents via the CaMKII-mediated pathway.[14] In contrast to CDF, brief-periods (5 minutes) of ROS exposure produced persistent (>1 hour) rises in L-type Ca^{2+} currents in rat cardiomyocytes, probably through oxidation-mediated CaMKII activation, suggesting that CaMKII senses redox stress and thereby acts as "a memory

molecule" in the heart.[15]

In heart failure, neurohormonal systems, including rennin-angiotensin-aldosterone system (RAAS) and β-adrenergic system, are known to be activated to increase the blood pressure in order to maintain tissue perfusion. Chronic elevation of neurohormonal activities appears to cause diverse pathologic processes in the heart, including arrhythmias.

Rennin-angiotensin-aldosterone system is associated with oxidation- and inflammation-processes in the heart.[16] Angiotensin II is known to stimulate L-type Ca^{2+} currents, Na^+/Ca^{2+} exchanger (NCX), and ROS production, assuming that Angiotensin II may affect Ca^{2+} and ROS homeostases in cardiac cells.[17][18] CaMKII appears to act as a key mediator in the interactions of Angiotensin II with intracellular Ca^{2+} and ROS.

An experimental study revealed that ROS-mediated CaMKII activation via methionine (M281/282) oxidation was a key reaction in Angiotensin II-directed cardiac damages.[13] They suggested that oxidative stress is the main factor for Angiotensin II-induced CaMKII activation, even though Angiotensin II can increase both the intracellular Ca^{2+} and ROS levels.

β-adrenergic receptor agonists increases intracellular Ca^{2+} concentration via facilitation of L-type Ca^{2+} currents, ryanodine receptor (RyR) Ca^{2+} release, and protein kinase A (PKA)-induced Ca^{2+} influx, thereby leading to CaMKII stimulation.[19]

The activation of β-adrenergic receptor can also stimulate CaMKII through Epac (cAMP-dependent exchange protein), which links with CaMKII and β-arrestin at the β-adrenergic receptor.[20] Additionally, the stimulation of PKA and phospholamban (PLN) by β-adrenergic receptor agonist seems to directly activate CaMKII.[21] In contrast to RAAS, there is no evidence that the β-adrenergic receptor affects methionine oxidation-induced CaMKII stimulation, even though the β-adrenergic receptor is responsible for the cardiac redox state.[13]

The expression level of CaMKII is shown to be elevated in cardiac diseases.[22] One possible mechanism for this phenomenon is that calcineurin (a PP) is activated in heart failure and stimulates CaMKII expression.[23] An experimental study revealed that the overexpression of calcineurin increased CaMKII activity in the heart and led to heart failure and sudden cardiac death in mice, implying that calcineurin plays an important role in the activation of CaMKII as well as in the pathologic processes in a failing heart.[24]

Effects of Ca^{2+}/Calmodulin-Dependent Protein Kinase II and Reactive Oxygen Species on Ion Channels

L-type Ca^{2+} channel

L-type Ca^{2+} channel is the main Ca^{2+} influx pathway in cardiac myo-

cytes. L-type Ca^{2+} channel is very closely located to the RyR channel, which is activated by a Ca^{2+} influx through the L-type Ca^{2+} channel and then performs the Ca^{2+} release from sarcoplasmic reticulum (SR), resulting in a large elevation of cytoplasmic Ca^{2+} concentrations, "Ca^{2+} transients".

Ca^{2+}/calmodulin-dependent protein kinase II can increase $I_{Ca,L}$ and thereby can engender Ca^{2+} overload in the cardiac cell, which may increase the risk for cardiac diseases, including arrhythmias.[25]

Interestingly, the activation of CaMKII in this reaction is mediated by SR Ca^{2+} release instead of Ca^{2+} influx through L-type Ca^{2+} channel, as evidenced by the fact that the prevention of SR Ca^{2+} release completely blocks CaMKII-mediated $I_{Ca,L}$ facilitation.[26]

L-type Ca^{2+} channel consists of α (pore-forming) and β (regulatory) subunits. Even though CaMKII can interact with various sites on α and β subunits, the phosphorylation of T498 on the β subunit is a key reaction for $I_{Ca,L}$ facilitation by CaMKII.[26][27]

As shown in our previous study, ROS causes $I_{Ca,L}$ facilitation in rat ventricular myocytes, which is prevented by potent Ca^{2+} chelators or CaMKII blockers, implying that this reaction is dependent on the CaMKII pathway.[14] Additionally, endothelin-1 and aldosterone, which can activate NADPH oxidase and thereby increase ROS production, also facilitate $I_{Ca,L}$.[28][29]

These findings are well consistent with the theory that ROS activates CaMKII via oxidation reaction and that this activated CaMKII increases $I_{Ca,L}$ via phosphorylation of the β subunit.

Voltage-gated Na^+ channel

Voltage-gated Na^+ currents (I_{Na}) play many roles in the initiation and duration of the action potential (AP) in the heart. I_{Na} is comprised of a large inward component and a small noninactivating component. The abnormal rise in the noninactivating component of I_{Na} is the main cause of long QT syndrome 3[30] and the potential arrhythmogenic risk factor in a failing heart.[31] The rise in I_{Na} increases AP duration and intracellular Na^+ concentrations, and thereby inhibits the NCX, resulting in intracellular Ca^{2+} increase. These changes by abnormal I_{Na} can produce early and late afterdepolarizations, which are proarrhythmic electrical anomalies.

Voltage-gated Na^+ channel can be affected by CaMKII.[32] A recent study reported that CaMKII increased the noninactivating component of I_{Na} in rabbit ventricular myocytes. These reactions were inhibited by CaMKII blockers.[33]

Another study reported that S571 on the α subunit of Na^+ channel is a phosphorylation site for CaMKII and that $β_{IV}$ spectrin has an important role in the interaction between CaMKII and the Na^+ channel in the heart.[34]

Previous studies reported that ROS had phenotypically similar effects on I_{Na} to CaMKII in dog, pig, and rabbit heart.[35][36] There can be

two possible mechanisms to explain these findings. First, as noted above, ROS can activate CaMKII, and then the activated CaMKII interacts with the voltage-gated Na^+ channel, leading to I_{Na} changes. Second, ROS may directly affect I_{Na} in CaMKII-independent manner, seeing that the oxidation of Met residue of the Na^+ channel was shown to increase the gating of this channel.[37]

Voltage-gated K$^+$ channels

Voltage-gated K^+ currents (I_K) play important roles in membrane repolarization in the heart and have a major influence on the AP shape and duration. Therefore, abnormal I_K can give rise to diverse arrhythmias by changing the AP configuration and duration.

Ca^{2+}/calmodulin-dependent protein kinase II has complex effects on I_K through transcriptional regulation, gating change via phosphorylation, and control of trafficking to sarcolemmal compartments.[38-40] Chronic overexpression of CaMKII in mice increases the slow transient outward K^+ currents ($I_{to,s}$) and decreases the fast transient outward K^+ currents ($I_{to,f}$) and inward rectifying K^+ currents (I_{k1}), resulting in the prolongation of AP duration.[41][42] In contrast, chronic inhibition of CaMKII was shown to upregulate $I_{to,f}$ and I_{k1}, leading to the shortening of AP.[41][43]

Reactive oxygen species effects on various I_K are well recognized and likely overlap with the effects of CaMKII on I_K in some studies.[39][44-46] However, whether and how ROS is involved in CaMKII signaling to I_K remains uncertain.

Channels involved in sarcoplasmic reticulum Ca^{2+} cycling

Sarcoplasmic reticulum Ca^{2+} uptake and release are the major Ca^{2+} cycling process in the myocardium and the main determinant of cytoplasmic Ca^{2+} concentrations. This process is regulated by RyR, PLN and SR Ca-ATPase (SERCA).

Ryanodine receptor is a SR Ca^{2+} release channel, which is opened by Ca^{2+} influx via $I_{Ca,L}$. RyR is a proven target of CaMKII.[47] CaMKII can phosphorylate S2814 on RyR and thereby induce Ca^{2+} release from SR, leading to increases in cytoplasmic Ca^{2+} concentrations and development of delayed afterdepolarizations, which are potentially arrhythmogenic.[48] ROS are shown to trigger SR Ca^{2+} leak and thereby cause arrhythmia in dogs.[49] However the involvement of CaMKII in the interaction between ROS and RyR is unclear yet.

Phospholamban is an inhibitor for SERCA, which is a SR Ca^{2+} uptake channel.[50] PLN can be phosphorylated by CaMKII; moreover, the phosphorylation of PLN attenuates the PLN activity, leading to the elevation of SR Ca^{2+} uptake by SERCA.[51] In some animal study, the blocking of PLN by CaMKII-mediated phosphorylation caused sudden cardiac death, even though the underlying mechanisms are unclear yet.[52] There is no evidence of a direct interaction between CaMKII and SERCA.

Arrhythmias

Sinus node dysfunction

The cardiac beat is directed by pacemaker cells. These cells have a specific property to create electrical impulses by themselves without electrical triggering. This automatic property is inhibited during excitation and refractory periods. Therefore, the most rapid pacing cell, normally sinoatrial (SA) nodal cell, controls the cardiac rhythm, while the other slower automatic cells act just as conduction systems, since they are already excited by the conducted impulse from the SA node before showing automaticity. The defect in SA node, or abnormal ectopic automatic cell with rapid firing rate, can develop arrhythmias.

Ca^{2+}/calmodulin-dependent protein kinase II and redox stress can affect the SA nodal function. ROS has been shown to raise the depolarizing frequency of SA node in animal studies.[53][54] The activation of CaMKII also leads to the rise of the depolarizing rate in the SA node. Conversely, blocking of CaMKII decreases $I_{Ca,L}$ in the SA node, resulting in a decline of depolarizing frequency.[33][55]

A recent animal study showed that Angiotensin II, which is known to be activated in cardiac diseases, induces oxidative modification of CaMKII, increases apoptosis in the atrioventricular node, and reduces the heart rate in various conditions.[56] Among cardiac disease patients, oxidized-CaMKII level of the atrium was higher in the SA node dysfunction group than in the intact SA node group. Both the inhibition of NADPH oxidase and the suppression of CaMKII were shown to prevent Angiotensin II-mediated SA node damage, suggesting that Angiotensin II activates CaMKII via NADPH oxidase pathway, thereby leading to SA node dysfunction.[56] Interestingly, CaMKII is also involved in the physiological interaction between SA node and β- adrenergic signaling.[57]

Taken together, CaMKII and redox stress play critical roles in the SA node dysfunction in a failing heart.

Atrial fibrillation

Atrial fibrillation is the major rhythm disturbance in heart failure.[58] In the presence of atrial fibrillation, ROS levels in atrial tissues were elevated both in animal and human studies.[59][60] The overexpression of CaMKII in the atrium was observed in atrial fibrillation patients.[61]

One potential hypothesis to explain atrial fibrillation is that the dysfunction of junctional conduction system may lead to disturbances in electrical impulse spread, likely resulting in atrial fibrillation.[62] A recent study showed that ROS interrupted the action of microtubule in the cell and thereby prevented the migration of connexon43 (an important junctional channel) to the junction of the cell, which is essential for the intercellular impulse conduction.[63] This finding provides a model showing how the oxidative stress

can cause atrial fibrillation.

Another theory for atrial fibrillation is that the dysfunction in RyR may cause abnormal Ca^{2+} release and give rise to spontaneous Ca^{2+} sparks, which is proarrhythmic and thus, potentially contributes to atrial fibrillation. As noted above, RyR is a well-known target of both CaMKII and ROS. Therefore, abnormal SR Ca^{2+} release mediated by CaMKII and ROS can be another possible model for atrial fibrillation in a diseased heart.[64)65)]

Ventricular arrhythmias

Both congenital and acquired heart diseases, such as cardiac hypertrophy, cardiomyopathy, and ischemic heart disease, can be associated with ventricular arrhythmias. In an animal study, the overexpression of CaMKII was shown to cause ventricular hypertrophy and ventricular tachycardia.[66)] CaMKII blockers had antiarrhythmic effects in the animal model with cardiomyopathy and ventricular tachycardia caused by the overexpression of PP.[24)] An experimental animal model of ischemic heart disease with ventricular arrhythmias showed increases in autophosphorylated- and oxidized-CaMKII; further, the blocking of CaMKII reduced arrhythmias in this model.[67)] These results indicate that CaMKII and redox stress likely mediate, at least in part, the generation of ventricular arrhythmias in cardiac diseases.

The above-reviewed interactions of CaMKII and redox stress with ion channels can be the underlying mechanisms for the genesis of ventricular arrhythmias in a diseased heart.

Ca^{2+}/calmodulin-dependent protein kinase II overexpressing animal with cardiomyopathy and ventricular tachycardia had increased-$I_{Ca,L}$, afterdepolarizations, and AP prolongation, which were reversed by CaMKII blocking.[66)] These results implied that the interaction between CaMKII and L-type Ca^{2+} channel plays an important role in the occurrence of ventricular tachycardia in a diseased heart. A heart failure model with ventricular arrhythmias showed an overexpression of CaMKII, hyperphosphorylation of RyR, and rises in SR Ca^{2+} release, which were prevented by CaMKII inhibition.[48)68)] Hence, rises in SR Ca^{2+} release by CaMKII can engender ventricular arrhythmias in cardiac disease. In addition, phosphorylation of the Na^+ channel by CaMKII leads to AP prolongation and afterdepolarizations, likely leading to ventricular arrhythmias.[33)] This finding indicates that the interaction of CaMKII and the Na^+ channel is another proposed mechanism for the development of ventricular arrhythmias.

Redox stress can increases noninactivating I_{Na}, $I_{Ca,L}$ and SR Ca^{2+} release, and thereby causing AP prolongation and afterdepolarizations in the ventricular cells, predisposing to ventricular arrhythmias.[36)69)] CaMKII blockers can inhibit these proarrhythmic changes induced by redox stress, suggesting that CaMKII activation by redox stress plays important roles in the genesis of ventricular arrhythmias

in cardiac disease.

Conclusion

In addition to the dramatic advances in the understanding of interactions between CaMKII and ion channels and the abundant evidences proving involvements of CaMKII and ROS in the genesis of arrhythmias in a failing heart, the discovery of a new mechanism showing how redox stress activates CaMKII gives us one more piece of the puzzle in order to figure out how arrhythmias can be developed in a diseased heart.

However, much remains to be investigated as to how redox stress and Ca^{2+} loading can cause CaMKII to target the specific ion channels and whether and how other signaling- and redox regulating-systems interact with CaMKII in the development of arrhythmias.

Future investigation on CaMKII and redox stress in the heart will help us to develop a more targeted therapy for arrhythmias in a failing heart.

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