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Pathways for CaMKII activation in disease

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Introduction

The multifunctional Ca^{2+} and calmodulin-dependent protein kinase II (CaMKII) has emerged as an interesting proarrhythmic and procardiomyopathic signaling molecule with therapeutic potential. The importance of this growing body of evidence showing that CaMKII plays a central role in myocardial disease is evidenced, in part, by this thematic series dedicated to CaMKII. The first reports that CaMKII could participate in ion channel biology in heart were in 1994 when separate papers from the Anderson, Bers and Lakatta groups identified CaMKII as a signal that was important for L-type Ca^{2+} current (I_{Ca}) facilitation. CaMKII induces L-type Ca^{2+} channels to enter a highly active gating mode, characterized by long and frequent openings,¹ which can underlie arrhythmia-inducing afterdepolarizations (the topic of xyz Pitt). I became interested in the potential for CaMKII to act as a proarrhythmic, feed-forward, signal to L-type Ca^{2+} channels when I was a trainee in cardiovascular medicine and electrophysiology at Stanford. I was fortunate to develop a collaboration with Howard Schulman, who discovered CaMKII when he was a post-doctoral fellow in the laboratory of Paul Greengard in 1978.² Initial investigations of CaMKII were focused on its role in non-cardiac tissue, particularly by neuroscientists but, in my opinion, the current best understood role of CaMKII as a disease signal is in the cardiovascular system. Our group was the first to identify CaMKII as a proarrhythmic signaling molecule.³ But since the time of these early reports, work from many laboratories represented in this series, and from others, has shown that CaMKII promotes pathological membrane excitability, hypertrophic transcription, activates cell death machinery and disturbs intracellular Ca^{2+} homeostasis. The diversity of these processes sparked the realization that CaMKII acts as a master regulator of cellular processes that are intimately involved in heart failure and arrhythmias. It may be that the apparent vulnerability in the design of the CaMKII pathway is a natural consequence of the multiple downstream CaMKII targets and the core physiological roles of CaMKII in myocardium. For example, by targeting sarcolemmal ion channels and intracellular Ca^{2+} homeostatic proteins, CaMKII is positioned to serve as a physiological interface between membrane excitability and sarcoplasmic reticulum Ca^{2+} release, two processes that are essential for excitation-contraction coupling.⁴ However, this interface is also a hotspot for linking pathological membrane excitability (i.e. arrhythmias) and contractile dysfunction (i.e. heart failure).

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Disclosures

Dr Anderson is a named inventor on issued patents claiming to treat arrhythmias and heart failure by CaMKII inhibition and is a co-founder of Allosteros.

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CaMKII is a molecular integrator of Ca^{2+} and ROS

High frequency Ca^{2+} stimulation promotes CaMKII autophosphorylation

CaMKII is an intracellular serine-threonine kinase that is abundant in myocardium. There are four known, highly homologous, CaMKII gene products, α , β , γ and δ . The δ isoform appears to be most plentiful in myocardium. The importance of the δ isoform for abetting cardiomyopathic phenotypes, in response to transaortic banding surgery, was recently confirmed in separate studies of CaMKII $\delta^{-/-}$ mice by the Olson and Heller-Brown laboratories.⁵ CaMKII monomers consist of three distinct domains (Fig 1). The catalytic domain is the N terminus; the C terminus forms the association domain. The association domain allows for assembly of the CaMKII holoenzyme, a stacked hexamer consisting of 12 monomer subunits. The CaMKII holoenzyme seems optimally configured to amplify the actions of 'upstream' signals, Ca^{2+} and reactive oxygen species (ROS), on CaMKII activity. The CaMKII regulatory domain forms a critical intersection between the catalytic and association domains, and the regulatory domain is essential for the defining identity of CaMKII as a Ca^{2+} activated enzyme. Under resting conditions of intracellular Ca^{2+} CaMKII is mostly inactive, because the catalytic domain is constrained in an inactive conformation by a pseudosubstrate region lying toward the N terminus of the regulatory domain. When intracellular Ca^{2+} rises, it complexes with the Ca^{2+} sensing protein calmodulin (CaM), and calcified CaM ($\text{Ca}^{2+}/\text{CaM}$) associates with the CaM-binding region toward the C terminus of the regulatory domain. $\text{Ca}^{2+}/\text{CaM}$ binding allosterically reorders the relationship between the catalytic domain and the pseudosubstrate region. After $\text{Ca}^{2+}/\text{CaM}$ binding, the basal constraint of the pseudosubstrate region is released, leaving the enzyme in an active conformation. Under low frequency $\text{Ca}^{2+}/\text{CaM}$ stimulation, most CaMKII cycles through active and inactive conformations that are driven by $\text{Ca}^{2+}/\text{CaM}$ binding and unbinding. However, under conditions of high frequency stimulation, CaMKII becomes increasingly likely to catalyze phosphorylation of a particular threonine residue (Thr 286 or 287, the numbering varies slightly with isoform) in the pseudosubstrate region of the regulatory domain.⁶ This so-called autophosphorylation is a consequence of the holoenzyme structure, where adjacent monomers can cooperatively amplify activation. Autophosphorylation has two main consequences: it induces CaMKII to bind $\text{Ca}^{2+}/\text{CaM}$ with ten-thousand fold tighter avidity (a phenomenon referred to as CaM trapping) and to render CaMKII $\text{Ca}^{2+}/\text{CaM}$ autonomous (i.e. CaMKII remains active even after $\text{Ca}^{2+}/\text{CaM}$ unbinding). Thus, autophosphorylation confers broad flexibility on CaMKII to transition from a $\text{Ca}^{2+}/\text{CaM}$ -dependent to a $\text{Ca}^{2+}/\text{CaM}$ -autonomous activity. At this point in time, the in vivo physiological consequences of CaMKII autophosphorylation for the cardiovascular system have not been experimentally tested, but multiple lines of evidence support a view that the proarrhythmic and procardiomyopathic effects of CaMKII derive from autophosphorylated or oxidized CaMKII.

Oxidation directly and indirectly activates CaMKII

CaMKII was also known to respond to increased reactive oxygen species (ROS) with increased activation. Increased ROS likely has two effects that increase CaMKII activity. ROS can increase CaMKII activity indirectly by inactivating phosphatases, enzymes that reduce the energy barrier to dephosphorylating autophosphorylated CaMKII. However, direct effects of ROS are sufficient to activate CaMKII.⁷ We identified a pair of Met residues (Met 281/282) that are conserved in β , δ and γ isoforms (the largely neuronal α isoform substitutes a Cys in the 281 position and is also activated by oxidation of the Cys/Met pair). Met oxidation converts CaMKII into a $\text{Ca}^{2+}/\text{CaM}$ independent enzyme, but without CaM trapping. Thus, conditions of high ROS are anticipated to increase CaMKII autonomy (and pathological cellular signaling) by Met oxidation and increased autophosphorylation, due to reduced phosphatase activity. The lack of CaM trapping appears

to be because oxidation of a Met residue (Met 308) in the CaM binding region prevents the increase in Ca^{2+} /CaM binding that occurs with autophosphorylation. Met 281/282 resides in a hinge region between the catalytic and regulatory domains, near the Thr 287 autophosphorylation site. Met 281/282 oxidation and Thr 287 autophosphorylation seem to operate by distinct but parallel mechanisms that convert CaMKII into a Ca^{2+} /CaM autonomous enzyme. In contrast to autophosphorylation that is reversed by phosphatase activity, the first step of Met oxidation is reversed by Met-specific reductases. One such reductase, methionine sulfoxide reductase A (MsrA), appears to be critical for basic biological functions, as MsrA knock out reduces lifespan in mice, while MsrA over-expression leads to enhanced longevity in *Drosophila*. We found that MsrA^{-/-} mice showed increased oxidized CaMKII and heightened susceptibility to injury from myocardial infarction or angiotensin II stimulation, suggesting the possibility that CaMKII was dynamically regulated as a target of redox signaling in heart.

CaMKII is activated by catecholamines and angiotensin II

Multiple processes can increase intracellular Ca^{2+} and/or ROS, and I believe that CaMKII oxidation may be a mechanism for resetting the Ca^{2+} dependence of CaMKII activation for physiological processes, such as excitation-contraction coupling and fight-or-flight increases in heart rate. However, more is known about the participation of CaMKII in myocardial disease. Circulating levels of neurohumoral agonists, such as norepinephrine and angiotensin II, are present in the blood of patients with heart failure, and the degree of increase over normal levels for these molecules correlates with morbidity and mortality in heart failure patients. CaMKII appears to be positioned downstream to β adrenergic receptors, particularly type 1 β adrenergic receptors, and angiotensin II receptors. Isoproterenol stimulation leads to increased CaMKII autophosphorylation⁷ and myocardial CaMKII inhibition, by transgenic expression of a CaMKII inhibitory peptide (AC3-I) that mimics the pseudosubstrate region of the CaMKII regulatory domain, is protective against isoproterenol-induced cardiomyopathy.⁸ The Rockman laboratory recently found that CaMKII was part of a molecular complex with type 1 (but not type 2) β adrenergic receptors and EPAC (exchange protein directly activated by cAMP), providing a rationale for the connection between β adrenergic receptor activation and the pathological downstream effects by CaMKII. However, β adrenergic receptor agonist stimulation also increases the activity of protein kinase A (PKA) and PKA can indirectly activate CaMKII by enhancing intracellular Ca^{2+} entry through voltage-gated Ca^{2+} channels, increasing cytoplasmic Ca^{2+} uptake into the sarcoplasmic reticulum (SR) and augmenting SR Ca^{2+} release through type 2 ryanodine receptors. CaMKII also increases Ca^{2+} flux by enhancing the activity of these same Ca^{2+} homeostatic steps. Ventricular myocytes isolated from mice with CaMKII inhibition had preserved inotropic responses to isoproterenol, suggesting that activation of the CaMKII pathway by β adrenergic receptor agonist stimulation was most important for pathological processes. Isoproterenol can lead to increased ROS, but in our hands isoproterenol selectively activated CaMKII by enhancing autophosphorylation, presumably as a consequence of increasing intracellular Ca^{2+} . Isoproterenol induces cardiomyopathy, at least in part, by increasing myocardial apoptosis by a CaMKII-dependent mechanism. Angiotensin II binds to angiotensin receptors, leading to increased myocardial ROS by activation of the NADPH oxidase complex. Although angiotensin II may increase myocardial intracellular Ca^{2+} , we found that angiotensin II stimulation selectively activated CaMKII by oxidation of Met 281/282. Mice lacking p47 (p47^{-/-}), a critical component of NADPH oxidases prominent in myocardium, and mice with myocardial AC3-I expression were protected from angiotensin II-induced myocardial apoptosis.⁷ Taken together, these studies show that CaMKII can selectively sense Ca^{2+} and ROS and that G protein coupled receptor pathways can also engage CaMKII by autophosphorylation or oxidation.

There are many causes of structural heart disease, but myocardial infarction is the greatest public health problem in terms of its contribution of heart failure and sudden death. Catecholamines and angiotensin II are both increased after myocardial infarction, but we found that myocardial infarction in mice prominently increased oxidized CaMKII. In support of the view that ROS actions on CaMKII were particularly important for increased myocardial apoptosis and heart failure after myocardial infarction, we found that $p47^{-/-}$ mice showed reduced levels of oxidized CaMKII while $MsrA^{-/-}$ mice showed more oxidized CaMKII after myocardial infarction. The $p47^{-/-}$ mice were protected against post-myocardial infarction related apoptosis and dysfunction, while $MsrA^{-/-}$ mice were more susceptible to myocardial infarction associated apoptosis and dysfunction. Taken together, these findings suggest that elevated ROS is pathological to myocardium because it oxidizes and activates CaMKII.

What does CaMKII teach us about rhythm control?

In my opinion, the full meaning of CaMKII for causing human arrhythmias will be unknown until CaMKII inhibitory drugs, safe for human use, become available. In the meantime, it is worth reflecting that our field has only begun to emerge from an epoch of targeting ion channels with highly selective drugs as our core paradigm for rhythm control. Although we have learned a great deal about ion channel physiology and pharmacology, these drugs have failed to reduce mortality in patients. However, drugs designed to inhibit activity of G-protein receptor activated pathways, β blockers, angiotensin converting enzyme inhibitors and angiotensin receptor blockers, have proven highly effective at reducing the risk of sudden death and restoring failing myocardium. Thus, drugs that work indirectly on ion channels through kinase-dependent phosphorylation are more clinically effective than drugs that directly inhibit ion channel currents. The highly promising results in animal models showing CaMKII inhibition can restore intracellular Ca^{2+} homeostasis and reduce arrhythmias in failing myocardium suggest that studying CaMKII may provide critical insights into proarrhythmic signaling that will spawn the next generation of antiarrhythmic drugs.

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Figure 1. CaMKII domain structure

A. A schematic depiction of a CaMKII monomer. The N terminus catalytic domain and the C terminus association domain are labeled. The regulatory domain is between the catalytic and association domains and is functionally divided into pseudosubstrate and CaM-binding regions. Amino acids important for oxidative (Met 281/282) and autophosphorylation (Thr 287) mediated CaMKII activation are highlighted. Met 308 is susceptible to ROS and when oxidized may interfere with $\text{Ca}^{2+}/\text{CaM}$ binding. **B.** A depiction of the CaMKII holoenzyme illustrating the on state occurs when the pseudosubstrate region of the regulatory domain is removed from the active catalytic site. **C.** Dynamic regulation of CaMKII by $\text{Ca}^{2+}/\text{CaM}$ binding and the transition to constitutive activity by reactive oxygen species (ROS) or autophosphorylation.