Does Ca\(^{2+}\)/Calmodulin-Dependent Protein Kinase \(\delta\)c Activate or Inhibit the Cardiac Ryanodine Receptor Ion Channel?

Naohiro Yamaguchi, Gerhard Meissner

The multifunctional Ca\(^{2+}\)/calmodulin-dependent protein kinase II\(\delta\) (CaMKII\(\delta\)) modulates cardiac muscle function by regulating Ca\(^{2+}\) transport proteins and nuclear signaling molecules. Aberrant activity of CaMKII\(\delta\) is implicated in heart disease. In this issue, Yang et al\(^1\) report that acute overexpression of constitutively active splice variant CaMKII\(\delta\)C phosphorylates the cardiac ryanodine receptor ion channel (RyR2) to decrease the rate of occurrence of local Ca\(^{2+}\) release events (Ca\(^{2+}\) sparks) and Ca\(^{2+}\) waves in cultured rat cardiomyocytes. A dominant negative form of CaMKII\(\delta\)C was shown to have opposite effects.

The cardiac ryanodine receptors are cation selective channels that release Ca\(^{2+}\) from an intracellular Ca\(^{2+}\) storing compartment, the sarcoplasmic reticulum (SR), during a cardiac muscle action potential, in a process known as excitation-contraction coupling.\(^2\) Released Ca\(^{2+}\) cause cardiac muscle to contract. Sequestration of released Ca\(^{2+}\) by the SR Ca\(^{2+}\)-transporting ATPase and extrusion by the Na\(^+\)/Ca\(^{2+}\) exchanger restore the myofibrillar Ca\(^{2+}\) concentration from \(10^{-6}\) to \(10^{-3}\), causing muscle to relax. The RyR2s are regulated by a variety of effectors.\(^3\) During a cardiac action potential, closely apposed dihydropyridine-sensitive L-type Ca\(^{2+}\) channels in the surface membrane and T-tubule mediate influx of Ca\(^{2+}\), which triggers massive release of Ca\(^{2+}\) from SR by opening RyR2s. In addition to Ca\(^{2+}\), endogenous effectors such as Mg\(^{2+}\), ATP, reactive oxygen and nitrogen molecules regulate RyR2. RyR2 is also regulated by calmodulin, cAMP-dependent protein kinase A (PKA), calmodulin-dependent kinase II (CaMKII), protein kinase C, and protein phosphatases 1 and 2A. Phosphorylation of RyR2-Ser2030 by CaMKII, PKA, and CaMKII is described. Marks and colleagues\(^4\) report that PKA-mediated phosphorylation of RyR2-Ser2030 by PKA and Ser2809 by PKA\(^5,6\) and CaMKII has been described. Mutagenesis suggests that CaMKII uniquely phosphorylates Ser2815 near S2809 on recombinant RyR2 expressed in human embryonic kidney 293 cells. However, incorporation of more than one \(\beta\)P per monomer into the native, immunoprecipitated receptor indicates the presence of another CaMKII site in RyR2, in partial agreement with Rodriguez et al\(^7\) that there are 4 CaMKII phosphorylation sites per PKA site or 8 sites based on 2 PKA sites per RyR2 monomer.\(^8\)

In the presence of CaM and elevated local Ca\(^{2+}\) concentrations, the multimeric CaMKIIs are autophosphorylated to become constitutively active. The function of 2 CaMKII splice molecules has been extensively studied in cardiomyocytes. The CaMKII\(\delta\) variant has a nuclear localization signal and transcriptionally regulates signaling pathways that contribute to cardiac myopathies.\(^9,10\) The cytosolic variant CaMKII\(\delta\)C phosphorylates, not only RyR2, but also the voltage-dependent L-type Ca\(^{2+}\) channel and Thr17 of the SR Ca\(^{2+}\) pump regulatory protein phospholamban.\(^11,12\) These phosphorylation events indirectly influence SR Ca\(^{2+}\) release by increasing Ca\(^{2+}\) entry and SR Ca\(^{2+}\) content, and thereby RyR2 activity.

The functional consequences of CaMKII-mediated RyR2 phosphorylation are less clear. Single channel experiments indicate that phosphorylation by CaMKII increases WT-RyR2 activity\(^5,9\) and Ca\(^{2+}\) sensitivity but not of the mutant RyR2-S2815A that lacks the RyR2 CaMKII phosphorylation site.\(^9\) Other groups report more complex regulation by protein kinases. Valdivia et al\(^13\) suggest PKA regulates RyR2 by increasing its responsiveness to photo-released Ca\(^{2+}\) that results in reduced levels of the steady state open channel. Hain et al\(^14\) speculate that phosphorylation of one subunit of the tetrameric RyR2 by endogenous CaMKII results in channel blockade by Mg\(^{2+}\), whereas phosphorylation of all 4 subunits by exogenous CaMKII opens the channel. Transgenic mice that overexpress CaMKII\(\delta\)C exhibit reduced contractility and altered cardiomyocyte Ca\(^{2+}\) signaling. Increased phosphorylation of RyR2, coimmunoprecipitation of CaMKII and RyR2, and enhanced Ca\(^{2+}\) spark activity despite reduced SR Ca\(^{2+}\) content taken together imply that CaMKII\(\delta\)C RyR2 phosphorylation results in the formation of a leaky SR channel.\(^15,16\)

It is perplexing that some laboratories report that CaMKII RyR2 phosphorylation inhibits the RyR2 ion channel. The Table compares the results by Kohlhaas,\(^17\) Guo,\(^18\) Wu\(^19\) and Yang\(^20\) and colleagues, using intact, permeabilized or patch-clamped adult rabbit, mouse or rat cardiomyocytes. Isolated cardiomyocytes were used to minimize the effects of overexpressing CaMKII for prolonged times in an animal model. The effects of acute overexpression or perfusion of wild-type, constitutively active or dominant negative CaMKII\(\alpha\) or CaMKII\(\delta\)C are summarized in the Table. Conflicting results

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Effects of CaMKII

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<tr>
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N.D., not determined; PLB, phospholamban; CA, constitutively active; DN, dominant negative

were obtained with regard to SR Ca\textsuperscript{2+} content, SR Ca\textsuperscript{2+} release and RyR2 phosphorylation. How can then these differences be explained? Yang et al\textsuperscript{1} suggest species-dependent differences between rat and rabbit or use of intact versus perfused myocytes. Indeed, overexpression of wild-type-CaMKII\textsubscript{CA} increased RyR2 phosphorylation and activity (measured as Ca\textsuperscript{2+} sparks) in rabbit\textsuperscript{19} but not rat cardiomyocytes.\textsuperscript{1} The constitutively activated CaM kinase was required for increased RyR2 phosphorylation; however, this correlated with a decrease in Ca\textsuperscript{2+} spark frequency, a result opposite to that obtained with rabbit cardiomyocytes. A second plausible explanation is that phospholamban Thr17 phosphorylation is responsible for the differences by causing de-inhibition of the SR Ca\textsuperscript{2+} transport ATPase and increased SR Ca\textsuperscript{2+} content. However against this possibility argues that phospholamban KO cardiomyocytes exhibit increased Ca\textsuperscript{2+} spark frequency and duration despite unchanged SR Ca\textsuperscript{2+} content.\textsuperscript{20} Moreover, intact cardiomyocytes display increased Ca\textsuperscript{2+} spark frequency despite a decreased SR Ca\textsuperscript{2+} content.\textsuperscript{19}

A third explanation we favor is that RyR2 phosphorylation (as a measurement of CaMKII activity) does not correlate with RyR2 activity. Most studies report relative RyR phosphorylation changes that depending on the control RyR2 phosphorylation level can represent a small or large increase in RyR2 phosphorylation status. As noted above, the extent of RyR2 phosphorylation may affect its activity.\textsuperscript{16}

In this issue in a related study, Curran et al\textsuperscript{16\textsuperscript{a}} use a pharmacological approach to show in accordance with their previous work that CaMKII increases RyR2 activity. A new finding is that the β-adrenergic receptor agonist isoproterenol results in a CaMKII-dependent but cAMP- and PKA-independent increase in diastolic SR Ca\textsuperscript{2+} leak by a signaling mechanism that remains to be determined. The functional role of CaMKII\textsubscript{CA} in normal and diseased heart remains to be determined. Yang et al\textsuperscript{1} suggest that a CaMKII\textsubscript{CA}-dependent decrease in RyR2 Ca\textsuperscript{2+} sensitivity in the normal heart provides a mechanism that compensates the effects of increased Ca\textsuperscript{2+} influx (I\textsubscript{Ca}, Table). An opposing view is that an increased heart rate enhances CaMKII\textsubscript{CA} autophosphorylation and RyR2 phosphorylation and activity, and thereby contractile function.\textsuperscript{9} In failing heart, CaMKII\textsubscript{CA}-dependent RyR2 phosphorylation may have no major role\textsuperscript{1} or result in a leaky SR Ca\textsuperscript{2+} channel and contractile dysfunction.\textsuperscript{22} The role of CaMKII\textsubscript{CA} in failing hearts is likely more complex because its cytosolic variant not only modulates the activity of key Ca\textsuperscript{2+} transport proteins in excitation-contraction but also has a role in gene regulation.\textsuperscript{23}

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None.

**References**


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