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Ca^{2+} Signaling Domains Responsible For Cardiac Hypertrophy and Arrhythmias

Steven R. Houser

Ca^{2+} activates and regulates multiple processes in every cell type. In the mammalian heart, cyclic fluctuations in cytosolic [Ca^{2+}] induce and regulate the strength of cardiac contraction (termed "contractile" [Ca^{2+}]). In addition, changes in Ca^{2+} appear to be centrally involved in normal and pathological signaling (termed "signaling" [Ca^{2+}]) that regulates myocyte growth, hypertrophy, apoptosis, and necrosis.[1] Whether or not contractile and signaling [Ca^{2+}] are derived from common or distinct sources and are constrained to unique cellular microdomains is not established.[2] What is clear is that cardiovascular diseases including hypertension and myocardial infarction are associated with alterations in contractile and possibly signaling [Ca^{2+}] that are centrally involved in pathological cardiac hypertrophy, heart failure progression,[1] and lethal cardiac arrhythmias.[3] Defining the sources of signaling Ca^{2+} involved in the induction of pathological hypertrophy and the bases of dysregulated contractile [Ca^{2+}] in cardiovascular disease should identify novel ways to treat heart disease.

In this issue of *Circulation Research*, 2 independent reports address fundamental aspects of alterations in signaling and contractile [Ca^{2+}]. Chiang et al[4] have studied the idea that Ca^{2+} influx through voltage operated α1H ($Ca_V3.2$) T-type Ca^{2+} channels (TTCCs) is the source of the signaling [Ca^{2+}] that activates the calcineurin (Cn)-NFAT (nuclear factor of activated T cells) signaling cascade and induces pathological cardiac hypertrophy in pressure overload. In a separate report, Terentyev et al[5] explore the idea that microRNA (miR)-1, a muscle-specific microRNA that increases in abundance in cardiac disease,[6] causes dysregulated contractile [Ca^{2+}] and induces single cell arrhythmias. These 2 reports are provocative and, if independently confirmed, will have identified novel mechanisms for abnormalities in the signaling and contractile [Ca^{2+}] that cause hypertrophy and sudden death.

Almost 20 years ago, we[7] and others[8] showed that TTCCs are reexpressed in adult ventricular myocytes after pressure overload. TTCCs are expressed in fetal/neonatal heart but are not normally found in the adult ventricular myocyte. We speculated that the Ca^{2+} influx through these channels was involved in the induced cardiac hypertrophy.[7] The report by Chiang et al[4] explores this idea in TTCC knockout (KO) mouse models. There are 3 TTCC genes, and 2 (α1G [$Ca_V3.1$] and α1H [$Ca_V3.2$]) are found in the heart.[9] $Ca_V3.1$[10] and 3.2 KO[11] animals, each of which is viable with modest basal phenotypes,[10,11] were used. The authors make the provocative observation that thoracic aortic constriction (TAC) induces cardiac hypertrophy in the $Ca_V3.1$ KO and control animals, but not in $Ca_V3.2$ KO. $Ca_V3.2$ KO animals had similar degrees of pressure overload after TAC, documenting a similar degree of stress. The inability of TAC to induce hypertrophy in $Ca_V3.2$ KO appeared to be attributable to the fact that Cn-mediated nuclear NFAT translocation, which is known to induce pathological hypertrophy,[12] was not activated in these animals. Surprisingly, the fetal gene program activated with pathological hypertrophy was induced by TAC in $Ca_V3.2$ KO without left ventricular hypertrophy.

These are provocative results that, if confirmed, will change thinking in the field. These results suggest that most if not all of NFAT mediated pathological hypertrophy is induced by a very small influx of Ca^{2+} through reexpressed α1H TTCCs. These new findings also suggest that Cn-NFAT signaling is not influenced by changes in the amplitude and duration of the systolic [Ca^{2+}] transient (contractile [Ca^{2+}]). Contractility in $Ca_V3.2$ KO mice after TAC must be greater than in controls which develop left ventricular hypertrophy, because $Ca_V3.2$ KO hearts are generating high pressures with less cardiac mass. Therefore, the systolic Ca^{2+} must be greater in $Ca_V3.2$ KO TAC myocytes than in control TAC hearts, yet there was no activation of Cn-NFAT signaling. These results are different from those that have linked the activation of Cn-NFAT signaling with increases in either the rate or amplitude of the cytoplasmic (contractile) [Ca^{2+}] transient in skeletal[13] and cardiac muscle.[2,14]

The report by Chiang et al[4] also suggests that Ca^{2+} activated Cn-NFAT signaling does not play a role in the activation of the fetal gene program after TAC. Their studies show no activation of Cn-NFAT signaling in $Ca_V3.2$ KO animals after TAC, but the fetal gene program was induced. In fact, the induction was greater than in controls after TAC. These results suggest that NFAT nuclear translocation has no role in the activation of these well studied fetal genes. Such results are in stark contract to studies that have shown equally convincing data documenting that block of NFAT nuclear translocation eliminates agonist and pressure overload induced hypertrophy and the activation of the fetal gene program.[12] Because these data sets seem mutually exclusive this topic clearly needs additional study.

The provocative study by Chiang et al[4] suggests that pressure overload causes hypertrophy by inducing the expression of $Ca_V3.2$ TTCCs. A very small Ca^{2+} influx through these channels would need to enter a specialized subsar-

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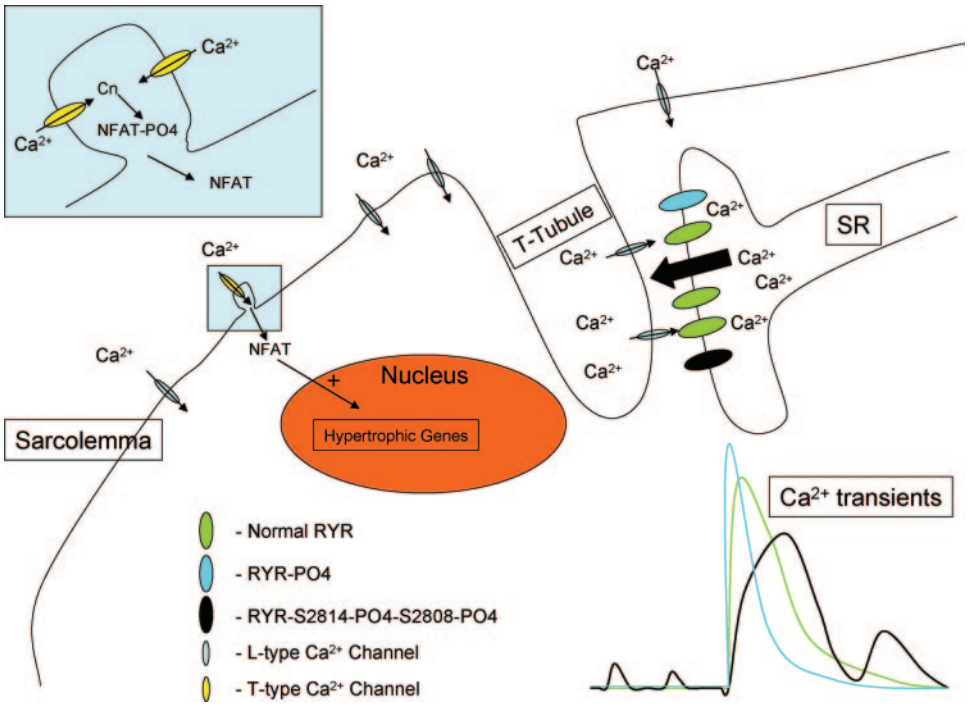

Figure. Illustration depicting the novel signaling pathways in cardiac myocytes reported in this issue of *Circulation Research*. Ca^{2+} primarily enters cardiac myocytes via LTCCs and induces a larger amount of Ca^{2+} release from the SR, by activation of Ca^{2+} release channels (RyR). The report by Chiang et al[4] suggests that the Ca^{2+} required for induction of cardiac hypertrophy is not derived from the Ca^{2+} that induces cardiac contraction. Their data suggest that hypertrophic Ca^{2+} is very small and enters the cell exclusively via TTCCs that are expressed in response to cardiac stress. In this study, changes in the global Ca^{2+} transient did not activate the Cn-NFAT signaling cascade that induces turns on hypertrophic genes. This implies that a very tiny Ca^{2+} influx through TTCCs activates Cn (a phosphatase) within a portion of the cell that is protected from large changes in the global Ca^{2+} transient. The reported data are such that Cn would need to be constrained to this microdomain and dephosphorylate NFAT locally before it can translocate to the nucleus. The report by Terentyev et al[5] suggests that miR-1 influences a phosphatase localized near the junctional SR and this specifically alters CaMKII-mediated phosphorylation of RyR. It appears that modest phosphorylation of RyR at either S2808 (a PKA site) or S2814 (a CaMKII site) produces an increase in SR Ca^{2+} release (from green to blue in the illustration) without arrhythmias. This study also suggests that hyperphosphorylation of RyR at both S2808 and S2814 is needed to induce the abnormal spontaneous and evoked SR Ca^{2+} release associated with arrhythmias (black tracing in the cartoon). These new reports suggest that Ca^{2+} signaling in cardiac myocytes can be controlled locally by constraining downstream mediators to specific microdomains and locally regulating the phosphorylation state of Ca^{2+}-handling proteins.

colemmal signaling domain that is not influenced by large changes in contractile [Ca^{2+}], where it exclusively activates Cn-NFAT signaling cascades. These new results suggest that pathological hypertrophy is induced through a highly specialized signaling [Ca^{2+}] microdomain that protects Cn-NFAT signaling from changes in contractile Ca^{2+} and causes pathological hypertrophy without activation of the fetal gene program. These results also exclude a role for TRPC, IP3R, and L-type Ca^{2+} channels (LTCCs) as a source of Ca^{2+} regulating cardiac hypertrophy and Cn-NFAT activity, in contrast to numerous reports.[2]

The second Ca^{2+} centric report in this issue of *Circulation Research*, by Terentyev et al,[5] identified a novel role for miR-1 in the regulation of contractile Ca^{2+}. Increasing miR-1 in cardiac myocytes caused alterations in the properties of the systolic Ca^{2+} transient, sarcoplasmic reticulum (SR) Ca^{2+} loading, and spontaneous and evoked SR Ca^{2+} release. When myocytes were exposed to catecholamines (isoproterenol [ISO]), only miR-1 myocytes demonstrated arrhythmogenic Ca^{2+} release. These results suggest that when miR-1 is increased in the diseased heart, catecholamine stress could induce life-threatening arrhythmias.

A novel aspect of this study was that the authors identified that miR-1 targets a regulatory subunit (B56α) of protein phosphatase (PP)2A, leading to reduced PP2A activity and increased phosphorylation of PP2A target proteins. Interestingly, only the phosphorylation state of specific Ca^{2+}/calmodulin kinase (CaMK)II phosphorylation sites were increased in miR-1 myocytes, and inhibition of CaMKII with KN93 reversed dysregulated Ca^{2+} handling. These results add to the growing body of work linking persistent activation of CaMKII to cardiac dysfunction.[15] The authors concluded that hyperphosphorylation of the SR Ca^{2+} release channel (ryanodine receptor [RyR]) at a known CaMKII site (S2814) alters RyR function and is responsible for arrhythmogenic SR Ca^{2+} release in the presence of catecholamines (Figure).

The idea that either protein kinase (PK)A or CaMKII mediated phosphorylation of RyR can induce SR Ca^{2+} leak and cardiac arrhythmias is a contentious topic[16] and, in my view, this new study does not resolve critical issues. Although the authors have shown dysregulated Ca^{2+} in miR-1 myocytes as well as alterations in RyR phosphorylation at RyR S2814, a cause and effect relationship between these 2 miR-1 effects was not proven.

The effects of miR-1 on myocyte Ca^{2+} handling were complex and varied with conditions. In quiescent miR-1 myocytes, RyR S2814 and LTCC phosphorylation were increased, spark activity (an index of RyR activity) was enhanced and SR Ca^{2+} loading was reduced. The authors conclude that RyR phosphorylation at S2814 enhances RyR opening to cause diastolic SR Ca^{2+} "leak," which reduces SR Ca^{2+} loading. In voltage-clamped myocytes L-type Ca^{2+} current and Ca^{2+} transient amplitude were increased at positive potentials, suggestive of increased excitation–contraction coupling gain. ISO failed to further increase L-type Ca^{2+} current and the amplitude of the Ca^{2+} transient in

miR-1 myocytes did not increase and was smaller than in controls. miR-1 myocytes field stimulated at 1 Hz had systolic Ca^{2+} transients that were much larger than in controls and SR Ca^{2+} loading was now normalized. Why increases in RyR phosphorylation at S2814 would unload the SR in voltage-clamped and quiescent myocytes and maintain SR Ca^{2+} load when these myocytes are paced is unclear, and suggest other unmeasured factors contribute to miR-1 effects on myocyte contractile Ca^{2+}. Like most new findings, there are many issues to be resolved in future studies.

ISO induced arrhythmogenic Ca^{2+} release only in miR-1 myocytes. The authors conclude that this resulted from hyperphosphorylation of RyR at S2814. To me, this conclusion is not fully justified. RyR S2814 phosphorylation is increased in miR-1 myocytes under control conditions and arrhythmogenic Ca^{2+} release is not present. This suggests that CaMKII-mediated phosphorylation of RyR at S2814 is not sufficient to induce single cell arrhythmias. Adding ISO to miR-1 cells induced arrhythmias but did not cause further increases in RyR S2814 phosphorylation or the phosphorylation of the LTCCs, so it is unclear how phosphorylation at S2814 alone can be responsible for the induction of arrhythmias. ISO increased PKA-mediated phosphorylation of RyR at S2808, suggesting that hyperphosphorylation of RyR at this site could be the factor that precipitates arrhythmogenic Ca^{2+} signaling. However, after inhibition of CaMKII with Kn93, RyR S2814 phosphorylation was reduced, RyR S2808 remained hyperphosphorylated, and arrhythmogenic Ca^{2+} transients were eliminated. These observations suggest that hyperphosphorylation of RyR at S2808 also is not sufficient to induce Ca^{2+} release mediated arrhythmias in miR-1 myocytes. Therefore, neither CaMKII phosphorylation of RyR S2814 nor PKA phosphorylation of RyR S2808 alone appear to be sufficient to produce the alterations in RyR behavior that underlie arrhythmogenic SR Ca^{2+} release. Hyperphosphorylation of both RyR S2814 and S2808 appear to be necessary for this process. Fortunately the model systems to test these ideas are available and hopefully these issues can be resolved.

In summary, 2 new articles in this issue of *Circulation Research* have identified novel mechanisms for inducing pathological hypertrophy and arrhythmias in cardiac myocytes by altering signaling and contractile Ca^{2+}. New studies will need to confirm these results and determine whether the responsible molecules are good targets for novel therapies for pathological cardiac hypertrophy and arrhythmias.

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None.

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