CaMKII Inhibition in Heart Failure Makes Jump to Human

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In this issue of Circulation Research, Sossalla et al1 show that acute inhibition of Ca2+/calmodulin-dependent protein kinase (CaMKII) in failing human myocardium causes functional improvement in contractility by restoring a positive force–frequency relationship (FFR) in cardiac trabeculae. The loss of this normal positive FFR (and often an induction of a negative FFR) is a functional hallmark of failing human myocardium2 and has been seen as an intrinsic limitation of the failing heart to enhance contractility appropriately at higher heart rates. This failure of positive FFR also constitutes a reduction in functional cardiac reserve. So, why might CaMKII inhibition be beneficial in heart failure (HF)?

CaMKII Activity in Heart Failure and Functional Molecular Targets

For several years, evidence has been accumulating that CaMKII is increased in both expression level and activity in both animal models of HF and in failing human hearts.3–5 It has also come to light that CaMKII has many different functional effects in cardiac myocytes, including alterations in ion channels, Ca2+ handling proteins, myofilaments, and transcriptional regulation.10–12 Moreover, many of these CaMKII effects on myocyte properties have been implicated in hypertrophy, HF, and arrhythmias in animal models. Indeed, CaMKII inhibition (or knockdown of the dominant cardiac isoform CaMKIIβ) can improve cardiac function and delay the onset of maladaptive aspects of the hypertrophic/HF phenotype in animal models, resulting in beneficial functional effects.13–15 Sossalla et al1 provide important novel data that acute CaMKII inhibition in human HF myocardium causes functional improvement. This is an important validation step with respect to consideration of cardiac CaMKII inhibition as therapeutic strategy in human HF.

So, what are some key acute functional effects of CaMKII that can alter cardiac Ca2+ handling and excitability? CaMKII phosphorylates Thr17 on phospholamban (PLN), which can relieve the inhibitory effect of PLN on the sarcoplasmic reticulum (SR) Ca-ATPase and thereby enhance Ca2+ uptake into the SR. This PLN effect of CaMKII would be positively inotropic and lusitropic (like PKA-dependent phosphorylation at Ser16 on PLN). These PLN effects could enhance arrhythmogenic events indirectly by enhancing SR Ca2+ content (and enhancing diastolic SR Ca2+ release), but that is not the point here. Moreover, blocking this inotropic effect of CaMKII would be in the wrong direction for the observed effects.

The SR Ca2+ release channel (ryanodine receptor [RyR]) associates with and is phosphorylated by CaMKII at Ser2814 (and possibly Ser2808).10 This phosphorylation has generally been found to activate both diastolic SR Ca2+ leak (sometimes visualized as Ca2+ sparks) and sensitize the RyR to Ca2+-induced Ca2+ release during excitation–contraction coupling. When this CaMKII effect is strong, as in transgenic mice overexpressing CaMKIIβc (the predominant cardiac cytosolic isoform) and in HF models, it can cause dramatic reduction in SR Ca2+ content available for release. This can result in strong depression of contractility, even though CaMKII enhances the fractional SR Ca2+ release during excitation–contraction coupling.5,16,17 This seems to be the key pathway involved in the human HF studies of Sossalla et al,1 where CaMKII inhibition improved contractility at higher heart rates by reducing SR Ca2+ leak and enhancing SR Ca2+ content.

CaMKII also mediates Ca2+-dependent facilitation of Ca2+ current (Ica) in cardiac myocytes, whereby Ica amplitude is increased and inactivation is slowed by repetitive depolarization or at higher heart rates. Ica facilitation would tend to increase both Ca2+ influx and SR Ca2+ content, and thus inhibition would be in the opposite direction to explain the beneficial effects of CaMKII inhibition. On the other hand, the slow [Ca2+]i decline seen in HF would tend to slow recovery of Ica from inactivation (especially at high heart rates),18,19 because of direct effects of Ca2+/calmodulin-dependent inactivation (independent of CaMKII). Therefore, Ica reduction might contribute to the more negative FFR in HF. However, in this case, CaMKII inhibition could only enhance Ica by reducing late diastolic [Ca2+], which could again be mediated by reduced diastolic SR Ca2+ leak via RyR. In addition, enhanced SR Ca2+ uptake could also reduce diastolic [Ca2+], but CaMKII inhibition neither accelerated twitch relaxation rate nor altered PLN phosphorylation in HF in the present study.

CaMKII also causes complex effects on Na+ current (Ina) gating,20 which could indirectly influence [Ca2+]i via Na/Ca exchange. CaMKII reduces Ina availability and enhances intermediate inactivation, both of which are loss-of-function effects. This effect would be most prominent at high heart rate, but would not appreciably influence action potential duration.21 On the other hand, CaMKII also increases late Ina and elevates intracellular [Na+]i ([Na+]i). Although enhanced late Ina could contribute to elevated [Na+]i, elevated [Na+]i is seen even at high heart rates, where the late Ina has little impact on action potential duration. Blocking the elevated [Na+]i and consequent diastolic [Ca2+]i by CaMKII inhibition could enhance Ica availability at high heart rate (as
above). However, this would also tend to reduce SR Ca²⁺ content, which is not what was observed. CaMKII also influences potassium currents in ways that can shorten action potential duration. In that setting, CaMKII inhibition could prolong action potential duration and enhance Ca²⁺ loading of the myocyte and SR. The bottom line is that the beneficial effect of CaMKII inhibition on contractility and Ca²⁺ transients in human HF myocytes could include a complicated mix of multiple effects on the above pathways. However, it is quite plausible that the RyR effects are the most important factor, and their measurements of Ca²⁺ sparks and SR Ca²⁺ load are consistent with this interpretation.

There is general agreement that in HF CaMKIIδ is upregulated and is also more autophosphorylated (which leads to autonomous CaMKII activity even after [Ca²⁺], declines and calmodulin dissociates from the kinase). CaMKII can also reach this autonomous activation state by oxidation of 2 methionine residues in the same regulatory domain of CaMKII. However, the detailed mechanism is not yet known for either the upregulation or activation of CaMKII in HF. In addition, the phosphorylation level of each CaMKII target is the net result of kinase and phosphatase action. In HF, there is an increase in myocyte phosphatase activity, although it seems to be lost from the local RyR complex. The enhanced global phosphatase activity in HF might explain why Sossalla et al. did not see reduction in PLN or associated phosphatase in HF (they might have been kept low in HF by the high global phosphatase activity). However, the loss of RyR-associated phosphatase in HF may allow the more active CaMKII bound there to more fully phosphorylate the RyR (and cause enhanced SR Ca²⁺ leak) that is reversed by CaMKII block.

We previously showed in a rabbit HF model that acute CaMKII inhibition could reduce diastolic SR Ca²⁺ leak, enhance SR Ca²⁺ load, and significantly increase twitch Ca²⁺ transient amplitude. However, the systolic Ca²⁺ transient restoration was modest, falling short of the control heart level (despite essentially full restoration of SR Ca²⁺ load). We reasoned that the benefit of the enhanced SR Ca²⁺ load with CaMKII inhibition was significantly limited by the loss of a normal CaMKII-dependent enhancement of fractional SR Ca²⁺ release for a given I_{Ca} trigger and SR Ca²⁺ load. It is possible that the balance of these 2 facets of RyR effects of CaMKII differs in the failing human heart here (eg, versus that rabbit HF model). On the other hand, it is not clear whether SR Ca²⁺ load experiments here were performed in conditions that allow this sort of quantitative analysis. It is also unclear how well the CaMKII inhibition restores function versus nonfailing human tissue, because nonfailing samples were not available for comparison (although the FFR with KN-93 was comparable to prior work from this group with nonfailing human trabeculae). In any event, the functional improvement in the human HF muscles here is quite substantial at higher heart rates, making this an exciting result with CaMKII inhibition, even if the mechanistic explanation is incompletely resolved.

### FFR in Human and Animal Myocardium

Human nonfailing myocardium exhibits a robust positive FFR, and this is true for rabbit, guinea pig, dog, and most other larger mammals. Extensive work over the past 50 years has provided the major explanations for this phenomenon. The increase in frequency provides greater Ca²⁺ influx (via I_{Ca}) per unit time and there is less diastolic time between beats for Ca²⁺ extrusion to occur, resulting in increased cellular and SR Ca²⁺ load. In addition, there is greater influx of Na⁺ per unit time both because of more frequent I_{Na} but also because of more Na⁺ entry via Na⁺/Ca²⁺ exchange (driven by the higher Ca influx). This limits the ability of Na⁺/Ca²⁺ exchange to extrude Ca²⁺ during the shortened diastolic interval, again adding to the cellular and SR Ca²⁺ gain. This mechanism has been confirmed by measuring SR Ca²⁺ load, which rises in parallel with the FFR. If SR Ca²⁺ is already near maximum, little further Ca²⁺ loading occurs with increasing frequency, and the FFR is typically flat or negative. A negative inotropic effect of increase frequency is an accumulation of refractoriness of the RyR to activation by Ca²⁺ (and perhaps reduced Ca²⁺ extrusion and keeps the SR nearly fully charged). However, the increase in frequency also occurs in normal human or rabbit myocytes, but it is usually masked by the even stronger positive effects of SR Ca²⁺ loading. It may well be that the inability of the leaky SR in HF to strongly increase SR Ca²⁺ load at higher frequency allows the negative effect to become more dominant in HF. Inhibition of that CaMKII-induced leak would then allow higher SR Ca²⁺ load to be achieved and restoration of the positive FFR.

A cautionary note here is that rat and mouse ventricular myocytes tend to have flat or negative (and only sometimes slightly positive) FFR and little or no gain in SR Ca²⁺ load with increasing frequency. This is probably because rat and mouse myocytes exhibit much higher physiological [Na⁺], which limits diastolic Ca²⁺ extrusion and keeps the SR nearly at capacity even during rest. Thus, FFR in rat and mouse may differ fundamentally from those in human heart. Kushnir et al. prevented CaMKII-dependent RyR phosphorylation in mice, which express only nonphosphorylatable RyR-S2814A. The authors found (opposite to the Sossalla et al. study) that preventing the CaMKII effect on RyR reduced the modest
positive FFR in their wild-type mice. Although they made no measurements of SR Ca\(^{2+}\) leak, load, or fractional SR Ca\(^{2+}\) release, they concluded that CaMKII-dependent RyR phosphorylation was an important part of the FFR (by increasing RyR responsiveness to the Ca\(^{2+}\) trigger).

My personal opinion is that both I\(_{Ca}\), facilitation, and this CaMKII-dependent enhancement of fractional SR Ca\(^{2+}\) release (that we first described in 1997\(^{17}\)) are, at most minor, contributors to the physiological FFR. Moreover, a larger fractional SR Ca\(^{2+}\) release will gradually unload the SR to a new steady state, where a larger fraction of a smaller SR Ca\(^{2+}\) load is released, resulting in little change in the steady-state systolic Ca\(^{2+}\) transient (as shown elegantly by Eisner et al\(^{25}\)). In a pathophysiological state like HF, strong CaMKII-dependent RyR phosphorylation can elevate diastolic SR Ca\(^{2+}\) leak so strongly that SR Ca\(^{2+}\) load is dramatically reduced, effectively creating a limiting SR Ca\(^{2+}\) load and preventing frequency-dependent increases in SR Ca\(^{2+}\) load. More work is clearly needed, but it seems that cardiac CaMKII inhibition in human HF might be of some functional benefit.

**Sources of Funding**

Supported by NIH grants P01-HL080101 and R37-HL30077.

**Disclosures**

None.

**References**


**Key Words:** Ca\(^{2+}\)/calmodulin-dependent protein kinase \(\bigcirc\) heart failure \(\bigcirc\) Ca\(^{2+}\) transport
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Circ Res. 2010;107:1044-1046
doi: 10.1161/CIRCRESAHA.110.231902
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/107/9/1044

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