A Tale of Two Leaks

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Over the last 2 decades, understanding of the mechanisms that underlie heart failure (HF) has grown enormously. One of the key concepts is that HF is associated with profound alterations in myocardial calcium handling and excitation-contraction coupling.

Myocardial Ca Handling
Most of the calcium that activates contraction comes from the sarcoplasmic reticulum (SR). It leaves the SR through a specialized release channel known as the ryanodine receptor (RyR). The probability that an RyR is open and can therefore allow Ca to leave the SR into the cytosol is increased by an increase in the concentration of either cytosolic or SR (luminal) Ca concentration. During the normal heartbeat, sarcoplasmic Ca channels are closed, and some of the entering Ca binds to the RyRs, making them open, thereby triggering the release of a much greater amount of Ca from the SR into the cytosol. This Ca release causes a rapid rise of cytosolic Ca to levels that activate the myofilaments and initiate contraction. After termination of release of Ca from the SR (because of closure of RyRs), cytosolic Ca levels decline rapidly and relaxation occurs. Ca is rapidly removed from the cytosol by 2 major systems: the sarcoendoplasmic reticulum Ca ATPase and the sarcolemmal sodium/calcium exchanger. Sarcoendoplasmic reticulum Ca ATPase pumps Ca back into the SR, whereas sarcolemmal sodium/calcium exchanger pumps 1 Ca²⁺ out in exchange for the influx of 3 Na⁺ ions into the cell. This rapid cycle of Ca release and reuptake is known as the systolic Ca transient, and it is one of the main factors that control force of contraction in the heart. It is worth emphasizing that the normal Ca transient depends on the RyRs being virtually closed in diastole, opening very briefly to produce the systolic increase of Ca, and then closing to allow Ca to fall to resting levels.

Alterations of Ca Handling in HF
A large body of evidence has demonstrated that, in HF, there is a significant reduction in the amplitude of the systolic Ca transient. This is mainly because of a decrease in the amount of Ca stored in the SR, and 3 mechanisms have been advocated to account for this: (1) decreased levels and activity of sarcoendoplasmic reticulum Ca ATPase resulting in decreased reuptake of Ca into the SR, (2) increased levels and activity of sarcolemmal sodium/calcium exchanger that enhance Ca removal from the cell (see Reference 1 for review), and (3) abnormal RyR function that causes Ca leak from the SR during diastole. Over the last 10 or so years, RyR dysfunction and Ca leak from the SR have been intensively investigated. Several mechanisms have been proposed to explain this abnormal RyR function. The initial suggestion was that RyR dysfunction and SR Ca leak are consequences of increased protein kinase A–dependent phosphorylation (hyperphosphorylation) at serine 2808. An alternative idea was that the culprit is increased Ca²⁺/calmodulin-dependent protein kinase II (CAMKII) kinase phosphorylation at serine 2815. In this issue of Circulation, Fischer et al have investigated this dichotomy. Importantly, rather than using an experimental animal model, this article describes the study of samples from patients with HF. The authors measured levels of phosphorylation at both serine 2808 and serine 2815 in nonfailing myocardium, in hypertrophied myocardium (derived from patients with severe aortic stenosis undergoing valve replacement), and in failing myocardium (from patients undergoing transplant). The levels of serine 2808 were similar in the 3 conditions, whereas those of serine 2815 were increased 3-fold in HF compared with hypertrophy and nonfailing myocardium. Ca transient amplitude and SR Ca content were significantly lower in the HF myocytes compared with the hypertrophy myocytes. An analysis of Ca leak showed that HF elevated Ca leak 2-fold compared with hypertrophy. Finally, in HF, inhibition of CAMKII (but not of protein kinase A) decreased Ca leak and increased SR Ca content. On the basis of these data, the authors concluded that RyR dysfunction and Ca leak observed in human HF are mainly attributed to excessive CAMKII phosphorylation at serine 2815 and that increased activation of CAMKII is one of the main steps in the transition from compensated hypertrophy to HF. This is the first study that comprehensively characterizes Ca leak in human myocardium in the 2 main forms of cardiac pathology, left ventricular hypertrophy and systolic HF. This work builds on a previous article from the same group that clearly demonstrated that CAMKII inhibition increases contractility in HF-derived trabeculae. It further questions a major role for protein kinase A in the genesis of Ca leak in HF. On this point, although the conflicting experimental evidence has been extensively reviewed, it is still unclear why different groups obtain different results. In the remainder of this editorial, we focus on unresolved questions that this important article raises. Although our discussion focuses on the RyR, it should be remembered that there are many other targets of CAMKII involved in calcium cycling (see Reference 5 for review).
Can CAMKII-Induced Ca Leak Produce Enough Ca Leak to Reduce Ca Transient Amplitude?

The effects of Ca leak on the Ca transient have been studied using caffeine, an agent that sensitizes RyRs to Ca. Previous work has shown that caffeine, at submillimolar concentrations, increases Ca leak and substantially decreases SR Ca content but does not produce any significant reduction in Ca transient amplitude because the reduction in SR Ca content is compensated by increased sensitivity to Ca. At higher concentrations (and, therefore, levels of leak) caffeine decreases the SR Ca content to sufficiently low levels so that even the release of all of the SR Ca results in a decreased Ca transient. This consideration raises the question of whether CAMKII activation can induce enough Ca leak to reduce the Ca transient or, alternatively, whether the observed reduction in Ca transient is because of other mechanisms, such as alterations in sarcoplasmic reticulum Ca ATPase and sarcolemmal sodium/calcium exchanger function. Several lines of evidence suggest that CAMKII activation may not produce enough leak to reduce the amplitude of the Ca transient and that other alterations may be responsible for the reduction in Ca transient. First, in a knock-in mouse model that is homozygous for the RyR S2814D mutation that mimics the effects of CAMKII phosphorylation, there is a substantial increase in Ca leak and reduction in SR Ca content without any reduction in Ca transient amplitude. Second, acute overexpression of CAMKII increases Ca leak and decreases SR Ca content but, also, does not decrease Ca transient amplitude. Third, in a rabbit model of HF, acute inhibition of CAMKII-reduced leak improved contractility but had only minimal effects on the amplitude of the Ca transient, suggesting that the improvement in contractility may be caused by factors other than changes in the amplitude of the Ca transient. A previous article on human tissue from the Goettingen group found that inhibition of CAMKII increased SR Ca content and contractility, but no data on systolic Ca was provided. This underlines the need for future work to obtain data on changes of the Ca transient. Finally, it is worth noting that, in a canine tachycardia model of HF where reduction in Ca transient is caused exclusively by severe Ca leak, this leak results from a combination of CAMKII-dependent phosphorylation and oxidation of RyR. In the initial period of rapid pacing, when there is only increased CAMKII-dependent phosphorylation of RyR, leak is increased but the Ca transient is unaffected. After 4 to 6 months, when oxidation of RyR occurs, there is enough Ca leak to reduce the Ca transient. This makes the point that factors other than phosphorylation must be considered when interpreting changes of Ca leak in HF. Overall, the available evidence suggests that leak induced by calmodulin kinase phosphorylation in isolation may not be sufficient to reduce the amplitude of the Ca transient.

Is Inhibition of CAMKII-Mediated SR Ca Leak a Therapeutic Target in HF?

The argument (above) that CAMKII-related Ca leak may not reduce the amplitude of the Ca transient would suggest that prevention of CAMKII-mediated phosphorylation of RyR should not have any therapeutic effect on HF. However, much evidence suggests that CAMKII-mediated phosphorylation plays an important role in the development of HF and that prevention of CAMKII phosphorylation of RyR delays the development of HF. How can we reconcile these apparent discrepancies? The most likely explanation is that leak related to CAMKII is an essential step that is necessary to initiate and modulate the molecular functional and structural changes that occur in HF. In more concrete terms, one can imagine 2 hypothetical scenarios: high levels of CAMKII phosphorylation and increased RyR opening could facilitate a second modification of the RyR, such as oxidation or nitrosylation, and the combination of the 2 would induce enough leak to decrease the Ca transient amplitude, or the presence of leak increases diastolic Ca levels, thereby activating calcineurin and CAMKII signaling in the nucleus to promote transcriptional changes that result in remodeling. The development of new RyR inhibitors should enable us to elucidate the precise mechanisms that mediate the therapeutic effects of reduction of SR Ca leak in HF.

Is CAMKII Implicated Only in Nonischemic HF?

Another important issue that has recently come to light is whether CAMKII-induced Ca leak plays a prominent role in all forms of HF. A recent article studied levels of phosphorylation of RyR in a small number of human HF samples. Although increased levels of CAMKII-related phosphorylation were observed in nonischemic HF, normal levels were seen in HF after myocardial infarction (ischemic HF). Using a transgenic animal model, it was also shown that preventing calmodulin kinase phosphorylation of RyR had protective effects in HF induced by aortic banding but not in HF after a myocardial infarction. In the study published in this issue of Circulation, Fischer et al did not divide their HF samples on the basis of the etiology of HF or other clinical parameters. However, in a previous article, they demonstrated that levels of CAMKII activity were increased by a similar amount both in ischemic and nonischemic cardiomyopathy. This raises the possibility that the difference in the serine 2815 phosphorylation between the 2 forms of HF is attributed either to different subcellular localization of calmodulin kinase or to reduction in the levels of phosphatases in nonischemic HF. This suggests that the initial steps that lead to the development of HF differ (not surprisingly) on the basis of the etiology of HF. In practical terms, this has important implications for the design of new treatment strategies for various forms of HF. It is, therefore, important to perform larger studies (preferably on human samples) to clearly delineate the involvement of CAMKII in the development of the various forms of HF.

Disclosures

None.

References

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References


**Key Words:** Editorials ■ calcium ■ heart failure ■ ryanodine

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**Note:** The above text appears to be a list of references, possibly from a scientific paper, discussing various studies related to calcium handling in cardiac myocytes and heart failure. The references are numbered sequentially, with each entry providing a citation for a study, typically involving the use of various enzymes and receptors to understand calcium regulation in cardiomyocytes. The text mentions specific proteins and their roles in cardiac function, including calcium/calmodulin-dependent protein kinase II (CaMKII), ryanodine receptors, and sarcoplasmic reticulum (SR) calcium leak. The studies cover a range of topics from basic mechanisms to clinical relevance, including the effects of overexpression of CaMKIIΔC and the role of Ca2+ in heart failure.
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