A Tale of Two Leaks

Running title: Venetucci et al.; A tale of two leaks

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Journal Subject Code: Heart failure:[110] Congestive

Key words: Editorial, heart failure, calcium, ryanodine receptor
Over the last two decades, understanding of the mechanisms that underlie heart failure has grown enormously. One of the key concepts is that heart failure 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 a RyR is open and can therefore allow Ca to leave the SR into the cytoplasm is increased by an increase in the concentration of either cytosolic or SR (luminal) Ca concentration. During the normal heartbeat sarcolemmal Ca channels open 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 two major Ca removal systems: the sarcoendoplasmic reticulum Ca ATPase (SERCA) and the sarcolemmal sodium/calcium exchanger (NCX). SERCA pumps Ca back into the SR while NCX pumps 1 Ca$^{2+}$ ion 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 heart failure**

A large body of evidence has demonstrated that in heart failure (HF) there is a significant
reduction in the amplitude of the systolic Ca transient. This is mainly due to a decrease in the amount of Ca stored in the SR and three mechanisms have been advocated to account for this. 1) Decreased levels and activity of SERCA resulting in decreased re-uptake of Ca into the SR; 2) Increased levels and activity of NCX that enhance Ca removal from the cell (see 1 for review); 3) Abnormal RyR function that causes Ca leak from the SR during diastole. Over the last ten 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 a consequence of increased protein kinase A (PKA) dependent phosphorylation (hyper phosphorylation) 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 and colleagues have investigated this dichotomy. Importantly, rather than using an experimental animal model, this paper studies samples from patients with heart failure. The authors measured levels of phosphorylation at both serine 2808 and serine 2815 in non failing myocardium, in hypertrophied myocardium (derived from patients with severe aortic stenosis undergoing valve replacement) and failing myocardium (from patients undergoing transplant). The levels of serine 2808 were similar in the three conditions while those of serine 2815 were increased threefold in HF compared to hypertrophy and non failing myocardium. Ca transient amplitude and SR Ca content were significantly lower in the heart failure myocytes compared to hypertrophy myocytes. An analysis of Ca leak showed that HF elevated Ca leak two fold compared to hypertrophy. Finally, in heart failure, inhibition of CAMKII (but not of PKA) 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 heart failure are mainly due 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 heart failure. This is the first study that comprehensively characterizes Ca leak in human myocardium in the two main forms of cardiac pathology - left ventricular hypertrophy and systolic heart failure. This work builds on a previous paper from the same group that clearly demonstrated that CAM kinase inhibition increases contractility in heart failure derived trabeculae. It further questions a major role for PKA in the genesis of Ca leak in heart failure. 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 will focus on unresolved questions that this important paper 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 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 6 has shown that caffeine, at sub-millimolar 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 that even the release of all the SR Ca results in a decreased Ca transient7, 8. 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 due to other mechanisms such as alterations in SERCA and NCX 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. 1) 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. 2) Acute overexpression of CAMKII increases Ca leak and decreases SR Ca content but, also, does not decrease Ca transient amplitude. 3) In a rabbit model of heart failure, 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 paper 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 heart failure where reduction in Ca transient is caused exclusively by severe Ca leak, this leak is due to a combination of CAMKII-dependent phosphorylation and oxidation of RyR. In the initial period of rapid pacing, when there is only increased CAM kinase 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 heart failure. Overall the available evidence suggests that leak induced by CAM 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 Heart Failure?

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 heart failure. However much evidence suggests that CAMKII mediated phosphorylation plays an important role in the development of heart failure and that prevention of CAMKII phosphorylation of RyR delays the development of heart failure. 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 heart failure. In more concrete terms one can imagine two hypothetical scenarios: 1) 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 two would induce enough leak to decrease the Ca transient amplitude. 2) 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 heart failure.

**Is CAMKII implicated only in non ischaemic HF?**

Another important issue that has recently come to light is whether CAMKII induced Ca leak plays a prominent role in all forms of heart failure. A recent paper studied levels of phosphorylation of RyR in a small number of human heart failure samples. While increased levels of CAMKII related phosphorylation were observed in non ischaemic heart failure, normal levels were seen in heart failure following myocardial infarction (ischaemic heart failure). Using a transgenic animal model, it was also shown that preventing CAM kinase phosphorylation of RyR had protective effects in heart failure induced by aortic banding but not
in heart failure following a myocardial infarction. In the study published in this issue of *Circulation*, Fisher et al did not divide their heart failure samples on the basis of the etiology of heart failure or other clinical parameters. However in a previous paper they demonstrated that levels of CAMKII activity were increased by a similar amount both in ischaemic and non ischaemic cardiomyopathy. This raises the possibility that the difference in the Serine 2815 phosphorylation between the two forms of heart failure are due either to different subcellular localization of CAM kinase or to reduction in the levels of phosphatases in non ischaemic heart failure. This suggests that the initial steps that lead to the development of heart failure differ (not surprisingly) on the basis of the etiology of heart failure. In practical terms this has important implications for the design of new treatment strategies for various forms of heart failure. 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 heart failure.

**Conflict of Interest Disclosures:** None.

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Circulation. published online July 19, 2013;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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