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SOCking it to Cardiac Hypertrophy: STIM1 mediated Ca^{2+} entry in the cardiomyocyte

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In the heart, increases in contractile load provoked by hypertension or valvular disease promote increases in cardiac mass through the hypertrophy of cardiomyocytes. Injury or death to cardiomyocytes in a portion of the heart places a greater load on cells within uninjured areas, and stimulates cellular hypertrophy. Similarly, in familial forms of hypertrophic cardiomyopathy, defects in genes encoding proteins of the sarcomere promote growth in the absence of a stimulus. Hypertrophic hearts are susceptible to abnormalities of cardiac rhythm and have impaired relaxation (diastolic dysfunction), though contractile performance can be preserved. However, when hypertrophic stimuli are unrelieved the remodeling phenomenon progresses and increasing numbers of cardiomyocytes are replaced with fibrotic scar, leading to decreased systolic function and circulatory failure.

Neurotransmitters or hormones that bind to cell surface receptors modulate the rate and force of contraction by regulating the flux of calcium across the cell membrane or released from the sarco/endoplasmic reticulum (SR/ER). In addition, Ca^{2+} signaling in the cardiomyocyte regulates the long term remodeling responses of the heart to changing workloads by altering Ca^{2+}-dependent gene expression and metabolism[1]. In this way, Ca^{2+} can sense and respond to changes in muscle contraction through a link with hypertrophic gene expression[2]. It is not intuitively obvious, however, how these Ca^{2+} signals could serve simultaneously as the proximate signal to control cardiac mass, specialized programs of gene expression, and the contractile and metabolic capacities of cardiomyocytes. Ca^{2+}-dependent signaling events activated by increased contractile load somehow must be insensitive to the large fluctuations in cytosolic calcium that occur as a function of ambient contractile activity. This consideration may explain why, although Ca^{2+}-dependent signal transduction pathways that modulate gene expression have been well-defined in other cell types for many years, only recently have similar signaling mechanisms been implicated in excitable cells such as striated muscle.

Hemodynamic and neurohormonal stress activate calcium dependent signaling cascades governed by the serine-threonine phosphatase calcineurin and calmodulin kinase (CamK)[3, 4]. In this way, Ca^{2+}-calmodulin-dependent growth pathways in cardiomyocytes interpret changes in Ca^{2+} transients and activate remodeling events leading to cardiac hypertrophy and, if left unchecked, eventually heart failure. It is likely then that understanding the molecular mechanism which accounts for the changes in Ca^{2+} transients

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to the cardiomyocytes may lead to better therapeutic strategies for cardiac hypertrophy and failure. In fact, research over the last several decades has sought to understand how cardiac stress alters Ca^{2+} transients in cardiomyocytes. Several groups have asserted that defects in SR calcium uptake (SERCA), abnormalities in EC coupling (L-type Ca^{2+} channel and ryanodine receptor), and reverse mode sodium calcium exchange represent just some of the possible lesions that account for the disturbances in Ca^{2+} signaling of hypertrophied cardiomyocytes. It is unlikely, however, that a single culprit channel or transporter induces the complex changes that alter $[Ca^{+2}]_i$ in the stimulated cardiomyocyte. In fact, crosstalk among channels, transporters and pumps integrate calcium cycling in the cardiomyocyte to achieve coordinated excitation contraction coupling (EC coupling). Thus, it is likely that multiple inputs to the crosstalk fail in the myocyte and manifest as SR calcium store depletion, increased arrhythmias and altered contractility. Moreover, augmented neurohormonal activation of the failing heart may modulate this crosstalk and further influence the SR Ca^{2+} store content.

Recently, store operated calcium entry (SOCE) has emerged as a potential mechanism to alter Ca^{2+} in the cardiomyocyte[5–8]. It is likely that Ca^{2+} entry through SOC channels effects changes in gene profiles governed by calcineurin/NFAT signaling, as has been shown for lymphocytes and muscle cells. Recently several groups simultaneously identified stromal interaction molecule 1 (STIM1) and Orai1 channels as the key molecules responsible for SOCE[9–11]. STIM1 is a single pass membrane protein found initially as a surface protein in bone marrow stromal cells where it activated genetic programs required for B-lymphocyte differentiation[12]. Subsequent work has shown that the EF hands of STIM1 bind Ca^{2+} from the lumen of the ER/SR and thereby sense Ca^{2+} store content. Upon depletion of the SR/ER stores and reduced binding of Ca^{2+} to STIM1, STIM1 migrates underneath the plasma membrane and activates Orai1 channels[13]. STIM1 may also have additional cellular functions including ER remodeling that involves a physical interaction with the microtubule protein EB1 as well as a surface membrane protein[14]. In this issue of *Circulation*, Hulot et al tested the hypothesis that hypertrophic agonists activate stromal interaction molecule1 (STIM1) dependent SOCE to promote cardiac hypertrophic signaling (15). They demonstrate that STIM1 is expressed in neonatal cardiomyocytes and to a lesser extent in the adult cardiomyocytes of rat hearts. Genetic manipulation of STIM1 *in vivo* by use of gain and loss of function strategies greatly influenced cardiac size and function. STIM1 overexpression resulted in greater SOCE currents and activation of the hypertrophic program in cardiomyocytes. In contrast, silencing STIM1 expression resulted in less SOCE and protected cardiomyocytes from the deleterious consequences of pressure overload including activation of the calcineurin/NFAT signaling cascade. These studies provide important new molecular insight into how cardiomyocytes effect changes in gene expression in response to the altered Ca^{2+} dynamics known to occur in hypertrophic cardiomyocytes.

Whether cardiomyocytes have a specialized mechanism for store repletion, beyond the resequestration by pumping activity of SERCA2a, remains an unresolved issue. SOCE was long considered as necessary only in non-excitable cells such as lymphocytes where SOCE provides a sustained Ca^{2+} entry to regulate Ca^{2+} dependent gene expression. Ca^{2+} entry through SOC channels requires an antecedent depletion of the Ca^{2+} stores from the sarcoplasmic/endoplasmic reticulum (SR/ER). In the laboratory, SOCE is measured in cells by employing strategies that include exposing cells to pharmacologic agents known to inhibit the SERCA pump, or inclusion of strong Ca^{2+} chelators to buffer cytosolic Ca^{2+}. When these maneuvers are performed in the absence of extracellular Ca^{2+}, the changes in cytosolic Ca^{2+} recorded reflect the depletion of stores, and the increase in Ca^{2+} following the readditon of Ca^{2+} to the cell bath reflects the extent of Ca^{2+} entry by SOCE. Cardiomyocytes *in vivo* are never exposed to these harsh conditions and it is therefore important to determine what physiologic conditions activate SOCE in cardiomyocytes. Is

SOCE required to refill internal Ca^{2+} stores in the working cardiomyocyte? If so, for which internal stores (SR/ER, mitochondrial and/or nuclear) is it required? Moreover, how does Ca^{2+} entry by SOCE channels influence the Ca^{2+} transients in an actively contracting cardiac myocte? Does SOCE act as a leak current during cardiac diastole and therefore influence diastolic function? Answers to these questions represent the critical next steps to understanding how STIM1-dependent SOCE influences cardiac growth.

Ca^{2+} entry into cardiomyocytes was once considered the sole domain of the L-type Ca^{2+} channel (LTCC) where LTCC is activated by an action potential to release Ca^{2+} stores for cardiac contraction (Ca^{2+} induced Ca^{2+} release). It is important to point out that STIM1 may also inhibit LTCC as recently indicated by two independent groups in smooth muscle and neurons(16, 17). Here, STIM1 limits the surface expression of the LTCC in order to promote Ca^{2+} entry through Orai1 channels. Future studies will have to address whether STIM1 similarly inhibits the cardiac LTCC in cardiomyocytes since alterations in LTCC are believed to have an important role in the Ca^{2+} signaling of hypertrophic cardiomyocytes. In the present study, the authors characterize two separate STIM1-dependent currents recorded from adult cardiomyocytes isolated from rats subjected to pressure overload. The features of the first current include the inward rectification and resemble Orai1-activated currents in pharmacologic profile. However this current is fully active even when Ca^{2+} stores are replete. This work therefore joins a growing number of studies, including those from my own lab, that show the kinetics of I_{SOCE} activation in skeletal and cardiac myocytes are more rapid than that from non-excitable cells.(18, 19). This more rapid activation and store independent current may be explained by the constitutive association of STIM1 and Orai1 at the membrane as seen in the more immature myotubes. The second STIM1-dependent current is a large non-selective cation current activated by the depletion of Ca^{2+} stores using thapsigargin and may represent a series of Ca^{2+} activated currents by the thapsigargin-induced increase in $[Ca^{2+}]_c$. In fact, STIM1 is known to activate several transient receptor potential (TRP) channels, many of which have been implicated in cardiac hypertrophy (20). The difficulty in separating these currents points out that a major obstacle in this field is the lack of adequate pharmacological tools to activate and inhibit SOCE channels. As a result, genetic modification of STIM1 in mice may provide the best way to understand the contributions of STIM1 to cardiac growth and hypertrophy.

The importance of STIM1 in striated muscle is highlighted by my own group's recent description of mice lacking STIM1 ($STIM1^{-/-}$) as well as the discovery of humans who bear mutations in STIM1 or (21)Orai1(22). $STIM1^{-/-}$ mice manifest a profound reduction in skeletal muscle mass and die from a skeletal myopathy. In fact, we have now validated our findings using an isolated skeletal muscle knockout approach where the deletion of STIM1 only in skeletal muscle recapitulates the defects in growth and myopathy reported in the $STIM1^{-/-}$ mice (unpublished results). Similarly, patients bearing loss of function mutations in the Orai1 and STIM1 genes display a clinical syndrome that includes severe combined immunodeficiency and a skeletal myopathy. In neither the human or murine model of a STIM1 deficient state has a cardiac defect been established and the cellular hypertrophy required for development seems to be intact in these models. In fact, as reported in the present work by Hulot et al. $STIM1^{-/-}$ mice exhibit no differences in the cardiac mass or cardiac function from their WT counterparts as determined by echocardiography. However, the present study also examines the effect of STIM1 knockdown by cardiotrophic adeno-associated virus on adult rat hearts subjected to pressure overload. Even with a modest reduction in STIM1 expression, the hearts of shSTIM1 treated rats were protected from the consequences of pressure overload. Here, shSTIM1 treated hearts displayed reduced LV mass and thickness that corresponded to reduced collagen deposition and reduced calcineurin/NFAT signaling. Importantly, this study demonstrates that reducing STIM1 in the adult heart blunts the hypertrophic response and is the first to suggest a role

for STIM1 in the adult heart. Therefore, while STIM1 may not be important for heart development or for developmental hypertrophy, it may have an increased role in pathologic hypertrophy.

It is clear from a growing number of studies that defects in Ca^{2+} signaling underlying cardiac hypertrophy effect not only cardiac contraction, but also the signaling events associated with cardiac growth and metabolism. Understanding the molecular components of this second Ca^{2+} signaling pathway associated with the hypertrophic program will yield new and innovative therapeutic strategies to address this important clinical problem. STIM1-dependent Ca^{2+} entry has emerged a potential target and more research will be required to best understand how STIM1-Ca^{2+} entry contributes to the cardiac action potential, the modulation of EC coupling, and the hypertrophic program.

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