Non-voltage gated Ca\textsuperscript{2+} entry pathways in the heart: The untold STOrai?

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Our understanding of cardiomyocyte Ca^{2+} handling is primarily based on the regulation of voltage-gated Ca^{2+} entry, via L-type Ca^{2+} channels (LTCC) and the resulting Ca^{2+} induced Ca^{2+} release from the SR required for excitation-contraction coupling. Ca^{2+} is widely recognized as playing a key signaling role in all cells and in cardiomyocytes the most commonly accepted pathway involves Ca^{2+} release from intracellular stores such as the ER/SR and the nuclear envelope. However, in non-excitable cells agonist-mediated increases in intracellular Ca^{2+} are known to occur as a result of Ca^{2+} entry across the plasma membrane. The most widely studied of these pathways is store-operated Ca^{2+} entry (SOCE) initially proposed by Putney and colleagues where IP_{3}-induced release of Ca^{2+} from ER/SR stores triggered a subsequent influx of extracellular Ca^{2+}, which was required both for subsequent activation of downstream signaling pathways, but also for refilling of ER/SR\textsuperscript{1}. Such Ca^{2+} signals have been shown to play a key role in the regulation of diverse cellular responses, including metabolism, transcription and differentiation. One of the earliest reports demonstrating that SOCE contributed to Ca^{2+} signaling in cardiomyocytes was in 2002 where Marchase and colleagues showed that activation of NFAT by phenylephrine or angiotensin and the resulting cardiomyocyte hypertrophy was SOCE dependent\textsuperscript{2}. At that time the proteins responsible for regulating SOCE were unknown; however, in 2005 with the identification of STIM and Orai protein families, the molecular mediators of SOCE were finally elucidated. It is now generally accepted that the coupling of the ER/SR membrane protein STIM1 with the plasma membrane protein Orai1 is a major mechanism facilitating SOCE. While the most recent studies on non-voltage gated Ca^{2+} signaling and cardiomyocyte hypertrophy have focused on STIM
and Orai mediated pathways, it should be noted that transient receptor potential channels, have also been implicated; however, it has been suggested that this may involve Ca\(^{2+}\) entry via LTCC\(^3\). Clearly further studies are needed to understand the relative importance of non-voltage gated and voltage gated Ca\(^{2+}\) signaling in the heart.

In addition to SOCE there is also growing evidence of another non-voltage gated Ca\(^{2+}\) entry pathway Store Independent Ca\(^{2+}\) entry (SICE), primarily characterized as an endogenous arachidonic acid-regulated Ca\(^{2+}\) (ARC) channel, which has current/voltage characteristics similar to SOCE, but does not require store depletion. Interestingly, ARC channel activation has been reported to involve a heteromeric assembly of Orai1 and Orai3\(^4-6\) as well as coupling with plasma membrane localized STIM1\(^7\). It is of note that while STIM and Orai protein families are highly conserved, with invertebrates having only a single Orai protein and vertebrates two Orai proteins, Orai3 appears to be specific for mammalian cells\(^6\). While there have been a number of studies supporting a role of STIM1 and SOCE in mediating cardiomyocyte hypertrophy, little is known about SICE or ARC activity in the heart. Consequently, the study by Saliba et al\(^8\), which focuses on the interaction between STIM1 with Orai1 and Orai3 and their role in Ca\(^{2+}\) influx in cardiomyocytes, is both highly novel and timely. They demonstrated for the first time the involvement of Orai3 and ARC channels in non-voltage gated Ca\(^{2+}\) entry and hypertrophic signaling in rat cardiomyocytes. Specifically, they found that Orai1 and Orai3 were expressed at similar levels in both normal and hypertrophied rat cardiomyocytes; however, consistent with previous studies\(^9,10\), STIM1 levels were increased in hypertrophic cardiomyocytes. Importantly, they showed that in response to hypertrophy there was increased Orai3 recruitment to the STIM1/Orai1 complex and
that interaction between Orai3 and both Orai1 and STIM1 was increased. Using selective knockdown of Orai1 and Orai3 proteins in vivo, they found that not only was Orai3 rather than Orai1 primarily responsible for interacting with STIM1 but also that Orai3 appeared to regulate SOCE. An earlier study from the same group identified an unknown Ca\textsuperscript{2+} current in hypertrophic cardiomyocytes\textsuperscript{9}; here they have shown that this is an Orai3-STIM1-ARC mediated current. In hypertrophied cardiomyocytes they observed increased rate of Orai3 dependent SOCE as well as an increase in ARC channel current, both of which are consistent with the increased association of Orai3 with Orai1 and STIM1.

While Wolkowicz et al., implicated Orai1 and Orai3 in cardiac arrhythmogenesis\textsuperscript{11}, the in vivo loss of function studies by Saliba et al, are the first to demonstrate a functional role for STIM1-Orai3 in regulating Ca\textsuperscript{2+} signaling in cardiomyocytes\textsuperscript{8}. Indeed, even in non-excitable cells our knowledge on STIM1 function has been primarily in the context of STIM1-Orai1 coupling in the regulation of SOCE; consequently, our knowledge of Orai3 function is fairly limited. However, Orai3 has been shown to be responsible for SOCE in breast cancer cells and also permits SOCE in conditions of increased oxidative stress in leukemia\textsuperscript{12,13}; in light of the report by Saliba and colleagues, further studies clearly are needed to better understand the function of Orai3 in the heart. It should be noted that while present at low levels in the heart, Orai2 could also be involved in voltage-independent Ca\textsuperscript{2+} signaling mechanisms; this is especially important since some of the knockdown strategies used by Saliba et al targeted all Orai proteins\textsuperscript{9}. This is potentially important since Voelkers et al showed that knockdown of Orai1 in neonatal rat ventricular myocytes resulted in an increase in Orai2
rather than Orai3\textsuperscript{10}. Moreover, Saliba \textit{et al}, concluded that SOCE and SICE were independent of voltage-dependent Ca\textsuperscript{2+} entry pathways\textsuperscript{8}; however, given reports that both STIM and Orai proteins interact with LTCC\textsuperscript{14} and SERCA\textsuperscript{15} further studies are clearly required to examine such interactions in the heart.

This study by Saliba and colleagues is part of a growing number of reports demonstrating not only the presence but also showing a clear functional role of STIM and Orai proteins in the heart\textsuperscript{9,10,16-18}. For example, hearts from mice with cardiomyocyte-specific deletion of STIM1 exhibited ER stress, mitochondrial dysfunction and dilated cardiomyopathy\textsuperscript{16}, demonstrating that STIM1 is essential for cardiac homeostasis. In addition, Orai1 knockdown in zebrafish resulted in the development of cardiac contractile abnormalities\textsuperscript{17} and mice lacking Orai1 subjected to trans-aortic constriction develop an accelerated dilated cardiomyopathy\textsuperscript{18}. In addition, Molkentin and colleagues have shown that that the IP\textsubscript{3}R plays a significant role in cardiac hypertrophic signaling via calcineurin through IP\textsubscript{3} mediated Ca\textsuperscript{2+} release\textsuperscript{19} and importantly in cardiomyocytes the IP\textsubscript{3}-Ca\textsuperscript{2+} signaling cascade, activated by agonists such as angiotensin II and phenylephrine, has been linked to SOCE. Moreover, as noted above Ca\textsuperscript{2+} dependent activation of NFAT in cardiomyocytes has also been shown to be SOCE dependent\textsuperscript{2}. Consequently, it is increasingly clear that we need to expand our current models of cardiomyocyte Ca\textsuperscript{2+} signaling and homeostasis to include not only SOCE but also SICE pathways. This will also require a re-evaluation of well-defined Ca\textsuperscript{2+} signaling pathways such as the Calcineurin-NFAT and the MAPK-dependent pathways to identify the downstream components of SOCE and SICE. The development of a more comprehensive and broader based understanding of
cardiomyocyte Ca\(^{2+}\) signaling networks will open up entirely new avenues for understanding the molecular underpinnings of cardiac hypertrophy and other diseases.

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**References**


