Cytosolic-free calcium (\(\text{Ca}^{2+}\)) is a multifunctional intracellular messenger that regulates many different cellular processes in cardiac myocytes. \(^1\) A transient rise in intracellular free \(\text{Ca}^{2+}\) concentrations ([\(\text{Ca}^{2+}\)]
) during excitation-contraction (E-C) coupling is required for initiating contraction of cardiac muscle. Membrane depolarization activates voltage-gated L-type \(\text{Ca}^{2+}\) channels within the transverse tubules (invaginations of the sarcolemma), and extracellular \(\text{Ca}^{2+}\) enters cardiac myocytes. The increased [\(\text{Ca}^{2+}\)], in the junctional space between transverse tubules and the sarcoplasmic reticulum (SR) triggers the \(\text{Ca}^{2+}\)-induced \(\text{Ca}^{2+}\) release from the SR via ryanodine receptor (RyR2)/intracellular \(\text{Ca}^{2+}\) release channels. The resultant transient rise in global \([\text{Ca}^{2+}]\), activates the myofilaments to produce cardiac contraction. Myocyte relaxation occurs when \([\text{Ca}^{2+}]\), levels decline quickly through transport by the \(\text{Ca}^{2+}\) recycling proteins, such as SR \(\text{Ca}^{2+}\)-ATPase (SERCA), which pumps \(\text{Ca}^{2+}\) back into SR, and the Na⁺/\(\text{Ca}^{2+}\) exchanger, which extrudes \(\text{Ca}^{2+}\) out of myocytes. \(^1\) In addition to its pivotal role in cardiac E-C coupling, \([\text{Ca}^{2+}]\), is also a critical regulator of multiple signaling transduction pathways, including activation of protein kinases or protein phosphatases and modulation of gene transcription and expression (Figure). \(^2\);

\[\text{SR \(\text{Ca}^{2+}\) content reflects the balance between \(\text{Ca}^{2+}\) uptake (via SERCA and \(\text{Ca}^{2+}\) efflux (via RyR2). A normal SR \(\text{Ca}^{2+}\) content is the key for the maintenance of physiological \([\text{Ca}^{2+}]\), levels and, thus, normal contractile function of cardiac myocytes. Under conditions of persistent pathological stress on the heart, such as pressure overload–induced myocardial hypertrophy and heart failure (HF), SR \(\text{Ca}^{2+}\) content is reduced, and \([\text{Ca}^{2+}]\), is increased. Both reduced \(\text{Ca}^{2+}\) pumping by SERCA and increased SR \(\text{Ca}^{2+}\) leak via RyR2s have been shown to contribute to the reduced SR \(\text{Ca}^{2+}\) content and increased \([\text{Ca}^{2+}]\). Other possible sources of the hypertrophy-associated increase in \([\text{Ca}^{2+}]\) may be from increases in influx of extracellular \(\text{Ca}^{2+}\) through the voltage-gated L-type \(\text{Ca}^{2+}\) channel or store-operated \(\text{Ca}^{2+}\) channels and release of stored \(\text{Ca}^{2+}\) from the nucleus via the inositol 1,4,5-trisphosphate receptors (IP₃Rs; Figure). \(^1,2\) Sustained elevation of \([\text{Ca}^{2+}]\), drives a complex pattern of remodeling of \(\text{Ca}^{2+}\) handling proteins and plays a key role in the activation of several hypertrophic signaling pathways. \(^1,2\) These include the \(\text{Ca}^{2+}/\text{calmodulin (CaM)}\)-calcinulin-nuclear factor of activated T cells (NFAT)\(^+\) and the \(\text{Ca}^{2+}/\text{CaM–dependent kinase II (CaMKII)}\)-histone deacetylase (HDAC) pathways. \(^3\) However, it remains a mystery exactly which subcellular \(\text{Ca}^{2+}\) pools initiate these hypertrophic signaling and how these \(\text{Ca}^{2+}\)-dependent signaling pathways are regulated in contracting cardiac myocytes given the highly specialized manner in which \(\text{Ca}^{2+}\) concentration rhythmically cycles in E-C coupling. \(^6\) It has been demonstrated previously that IP₃R-mediated nuclear envelope \(\text{Ca}^{2+}\) fluxes may activate \(\text{Ca}^{2+}/\text{CaM-CaMKII-HDAC5} \) signaling in ventricular myocytes, and the enhanced store-operated \(\text{Ca}^{2+}\) entry into myocytes with overexpressed transient receptor protein C3 may increase NFAT transcriptional activity. \(^7\) It was suggested that enhanced \(\text{Ca}^{2+}\) release through IP₃Rs may cause sensitization of RyR2s and a further increase in diastolic \([\text{Ca}^{2+}]\). However, it is very controversial at present whether altered patterns of \(\text{Ca}^{2+}\) release and reuptake associated with E-C coupling may affect hypertrophic signaling pathways and whether the diastolic SR \(\text{Ca}^{2+}\) leak might also activate \(\text{Ca}^{2+}\)-dependent hypertrophic signaling pathways under pathological conditions. \(^2,6\)

Previous evidence from HF patients and animal models supports a functional role for enhanced diastolic \(\text{Ca}^{2+}\) leak from SR through RyR2s in the development of contractile dysfunction. \(^8\) In lipid bilayers, RyR2 phosphorylation caused FK506-binding protein 12.6 dissociation from the RyR2 and increased overall open probability of RyR2 channels. A variety of alterations in the subunits of the RyR2 macromolecular complex have been found in HF patients, including decreased levels of FK506-binding protein 12.6 (or calstabin2), protein phosphatases (1A and 2A), and phosphodiesterase 4D3. \(^8\) In addition, changes in RyR2 posttranslational modifications, such as oxidation, S nitrosylation, and phosphorylation, have been shown in HF patients and animal models. \(^8\) It was found that RyR2s were “hyperphosphorylated” by protein kinase A possibly because of the hyperadrenergic state and loss of RyR2-associated phosphatases (despite increased global phosphatases in cardiac myocytes). The combination of these alterations of RyR2 may lead to a decreased ability of the channel to remain closed during diastole, resulting in a net increase in diastolic SR \(\text{Ca}^{2+}\) leak, a reduced SR \(\text{Ca}^{2+}\) content, and an increased \([\text{Ca}^{2+}]\). Therefore, it has been
proposed that enhanced SR Ca\(^{2+}\) leak through “leaky” RyR2 Ca\(^{2+}\) release channels during diastole may underlie contractile dysfunction. However, this attractive hypothesis has been seriously challenged by controversial findings on the effect of protein kinase A–dependent RyR phosphorylation during E-C coupling and the lack of supporting data in intact myocytes or whole-animal models.3

In this issue of Hypertension, van Oort et al9 used knockin (gain-of-function) mice heterozygous for mutation R176Q in RyR2 (R176Q/H11001), which have been shown previously to increase SR Ca\(^{2+}\) release in atrial and ventricular myocytes after catecholaminergic stimulation to specifically address the question of whether enhanced SR Ca\(^{2+}\) leak through RyR2 accelerates the development of cardiac hypertrophy. They found that 8 weeks of transverse aortic constriction decreased systolic and diastolic heart functions and increased ventricular dimensions to a significantly larger extent in R176Q/H11001 mice when compared with wild-type mice. R176Q/H11001 mice displayed an enhanced hypertrophic response compared with wild-type mice as assessed by heart weight:body weight ratios and cardiomyocyte cross-sectional areas after transverse aortic constriction. Transverse aortic constriction pressure overload also resulted in an increased SR Ca\(^{2+}\) leak, associated with higher expression levels of the exon 4 splice form of regulator of calcineurin 1 in R176Q/H11001 mice compared with wild-type mice.

Therefore, the authors concluded that RyR2-dependent SR Ca\(^{2+}\) leak may activate the prohypertrophic CaN/NFAT pathway during pressure overload (Figure). These findings provide convincing in vivo evidence that increased RyR2-mediated Ca\(^{2+}\) release from the SR is able to activate Ca\(^{2+}\)-dependent hypertrophic signaling pathways, preferentially the calcineurin/NFAT signaling pathway, under conditions of pressure overload. These novel findings are consistent with previous reports suggesting that increased diastolic RyR2 Ca\(^{2+}\) leak impairs cardiac contractility because of a secondary decrease in SR Ca\(^{2+}\) loading. Recent clinical studies provided further evidence that genetic defects in the RyR2 gene may predispose patients toward...
the development of hypertrophic cardiomyopathy and HF. Therefore, defective \( \text{Ca}^{2+} \) release from the SR via mutant RyR2 may indeed adversely affect cardiac remodeling.

It should be kept in mind, however, that HF is heterogeneous and complex, and multiple mechanisms may contribute to increased \([\text{Ca}^{2+}]_i\) (reduced SERCA function and enhanced SR \( \text{Ca}^{2+} \) leak) and decreased SR \( \text{Ca}^{2+} \) content (increased \( \text{Na}^+ / \text{Ca}^{2+} \) exchanger function) in HF. However, relative contributions of these mechanisms may vary among species (mouse versus human), pathological stimuli (pressure overload versus volume overload), and disease stages (compensated versus dyscompensated) of HF. It is clear that more studies of SR \( \text{Ca}^{2+} \) leak in the context of signalosome of remodeling in myocardial hypertrophy and HF are warranted to better understand whether this pathway is causative of hypertrophy or HF and whether targeting RyR2 to reduce the diastolic SR \( \text{Ca}^{2+} \) leakage may serve as a novel therapeutic strategy for the treatment of HF.

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None.

**References**

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