Sarcoplasmic Reticulum Ca\textsuperscript{2+} and Heart Failure
Roles of Diastolic Leak and Ca\textsuperscript{2+} Transport

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Heart failure (HF) is a leading cause of death and enormous effort has focused on understanding the molecular and cellular mechanisms of the decreased cardiac contractility. While changes of other components contribute, it is generally agreed that much of the contractile deficit is due to reduced myocyte Ca\textsuperscript{2+} transients.\textsuperscript{1,2} Alterations in Ca\textsuperscript{2+} current (I\textsubscript{Ca}) and action potential characteristics are also seen in HF, but a central factor limiting Ca\textsuperscript{2+} transient amplitude is a decrease of sarcoplasmic reticulum (SR) Ca\textsuperscript{2+} content.\textsuperscript{3–6} HF is extremely complex, but it is easy to appreciate how reduced SR Ca\textsuperscript{2+} content would reduce SR Ca\textsuperscript{2+} release, myofilament activation, and contractility. Despite agreement that SR Ca\textsuperscript{2+} content is reduced in HF, controversy exists about why SR content is low.

How Is SR Ca\textsuperscript{2+} Content Decreased in Heart Failure?
SR Ca\textsuperscript{2+} content reflects the balance between Ca\textsuperscript{2+} uptake (via SERCA) and Ca\textsuperscript{2+} efflux via ryanodine receptor (RyR). Thus, reduced SR content in HF must be due to reduced Ca\textsuperscript{2+} pumping by SERCA or increased SR Ca\textsuperscript{2+} leak via RyRs. Both are supported by experimental data (below). Transsarcolemmal Ca\textsuperscript{2+} fluxes also affect SR Ca\textsuperscript{2+} load. That is, reduced Ca\textsuperscript{2+} influx (eg, via I\textsubscript{Ca}) or enhanced Ca\textsuperscript{2+} extrusion via Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange (NCX) can unload the SR. Results are not unanimous, but most groups find little alteration in peak I\textsubscript{Ca} density in HF, while many find evidence of enhanced NCX expression and function.\textsuperscript{1,2} Increased NCX function can compete with SERCA during [Ca\textsuperscript{2+}] transient uptake and loss of RyR-associated phosphatases (despite inactivation). Indeed, acute NCX overexpression can decrease SR Ca\textsuperscript{2+} content.\textsuperscript{7} While NCX alterations can reduce SR Ca\textsuperscript{2+} load in HF, we focus here on SERCA and RyR function.

SR Ca\textsuperscript{2+} release occurs during both systolic SR Ca\textsuperscript{2+} release triggered by I\textsubscript{Ca} and diastolic SR Ca\textsuperscript{2+} leak due to the finite stochastic RyR open probability (P\textsubscript{o}).\textsuperscript{1} Recent publications have raised controversy as to the importance of increased SR Ca\textsuperscript{2+} leak in HF.\textsuperscript{8–13} While reduced SERCA function, enhanced diastolic SR Ca\textsuperscript{2+} leak, and increased NCX function may all contribute to SR Ca\textsuperscript{2+} unloading in HF (and are not mutually exclusive), recent studies focused on SERCA\textsuperscript{10} or diastolic leak\textsuperscript{12} as crucial. Here, we comment on key arguments and summarize the issue as we see it.

Evidence for Decreased SERCA Pumping
While there is not complete agreement, most laboratories find evidence for depressed SERCA expression and/or function in HF (including human).\textsuperscript{1,2,4–6,10} The level of phospholamban (PLB) phosphorylation may also be decreased (depressing SERCA function further).\textsuperscript{1} There are still questions about how functional downregulation of SERCA comes about during HF and its absolute extent. However, some degree of SERCA dysfunction is generally accepted to contribute to both systolic and diastolic dysfunction in HF.

Evidence for Increased RyR Leak
Marks’ group spearheaded the hypothesis of enhanced diastolic SR Ca\textsuperscript{2+} leak in HF.\textsuperscript{8,12,13} Their biochemical and single-channel RyR bilayer recording placed this hypothesis in a compelling molecular mechanism. They showed that an RyR macromolecular complex includes cAMP-dependent protein kinase (PKA), phosphatases (PP1 and PP2a), and FKBP12.6. They found that the RyR was “hyperphosphorylated” by PKA in HF and attributed this to hyperadrenergic state and loss of RyR-associated phosphatases (despite increased global myocyte phosphatases). This RyR phosphorylation caused FKBP12.6 dissociation from the RyR and altered RyR gating, analogous to when FKBP12.6 is displaced from RyRs by FK-506 or rapamycin.\textsuperscript{14,15} Single-channel RyR recordings showed increased overall P\textsubscript{o} in HF, with some openings at lower conductance levels, resulting in a net increase in ion flux. In cellular terms, this would translate into increased diastolic SR Ca\textsuperscript{2+} leak and reduced SR Ca\textsuperscript{2+} content. This diastolic leak hypothesis has many attractions and support from another laboratory.\textsuperscript{16,17}

Limitations of the RyR
Hyperphosphorylation Hypothesis
This is an important hypothesis but requires further testing. Some published data are inconsistent with critical aspects of

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the theory and its universality in HF. One limitation is a lack of supporting data in intact myocytes. For example, more frequent Ca2+ sparks might be expected in HF myocytes, but to our knowledge this has not been reported. This contrasts with strong cellular data concerning altered SERCA and/or NCX function in HF myocytes.

PKA-dependent RyR effects have mainly been studied in lipid bilayers, where RyRs are divorced from their natural environment. Even in this simplified system, results are divergent.8,18 One study18 showed that PKA increased initial RyR Po in response to a jump in [Ca2+]i (simulating a cellular Ic, trigger), but then Po relaxed rapidly to a lower steady-state level, such that PKA slightly reduced steady-state Po. In contrast, another study8 (steady-state effects only) found that PKA increased overall Po and caused subconductance states. This difference is unresolved.

It is difficult to study RyR properties in intact cells, because PKA increases both Ic, and SR Ca2+-ATPase activity, which independently alter SR Ca2+ release. Nevertheless, cellular data are essential and were provided by Li et al.8 They used mice lacking phosphorylatable PLB (to avoid altered SR Ca2+-ATPase) and avoided PKA effects on Ic, by studying SR Ca2+ release in both permeabilized and intact quiescent myocytes. PKA-dependent RyR phosphorylation (measured) did not significantly activate diastolic SR Ca2+ leak (assessed by Ca2+ spark frequency, Figure 1B). Nor did PKA alter Ca2+ spark amplitude, duration, or spatial spread. In control myocytes (with PLB present), PKA dramatically increased Ca2+ spark frequency, an effect attributed to the measured increases in SR Ca2+ content.9 This was not a study of HF, but it was concluded that if diastolic SR Ca2+ leak is increased in HF, additional factors to RyR phosphorylation may be involved.

The altered RyR phosphorylation hypothesis8 (Figure 1A) is that RyR activation shifts to lower [Ca2+]i, with physiological sympathetic activation, enhancing SR Ca2+ release during excitation-contraction coupling (without causing diastolic leak). In HF, RyR hyperphosphorylation causes additional shift, resulting in diastolic SR Ca2+ leak and SR Ca2+ depletion. Note that even control phosphorylation should increase diastolic Ca2+ leak (unless leak is irrelevant to load) and would not be selective for hyperphosphorylation. Contrary to concerns,12 the cellular PKA studies of Li et al8 were done precisely where any increase in Ca2+ spark frequency (or Po) should be most readily apparent (several fold increases would be expected, Figure 1). Certainly other factors dramatically alter Ca2+ spark frequency in intact cardiac myocytes (eg, [Ca2+]i, [Ca2+]SR, and CaMKII).9,19 We believe that appropriate data in intact cells (and animals) are the overriding context within which data from isolated systems (eg, bilayers) must be placed in interpreting physiological importance.

The effect of PKA-dependent RyR phosphorylation during excitation-contraction coupling is also controversial, with both increases and decreases reported.20,21 In summary, how PKA modulates RyR in intact cells is not resolved.

### Universality of RyR Hyperphosphorylation in HF?

Another issue is the universality of RyR dysfunction (hyperphosphorylation8,12) in HF. Jiang et al10 found in HF unaltered RyR density (measured by [3H]ryanodine binding and immunoblotting) and RyR activity at a wide range of [Ca2+]i (measured with [3H]ryanodine binding and single channel experiments) or RyR phosphorylation (assessed by back-phosphorylation and phospho-specific antibody).22 It was suggested12 that Jiang et al10 could not measure (1) RyR number in samples subjected to back-phosphorylation or (2) RyR activity at diastolic [Ca2+]. However, Figure 4 of Jiang et al10 did show unaltered RyR density in control versus HF in those samples. They also showed that at 100 nmol/L Ca2+, both RyR activity and [3H]ryanodine binding (indicative of RyR Po) were equivalent in control and HF RyRs (Figures 4C and 5A through 5C in Reference 10). Single-channel recordings showed neither altered RyR Po, nor the subconductance states typical of FKBP12.6 dissociation.15 However, traces at 100 nmol/L [Ca2+]i were short, and pooled statistics were not reported. Figure 2B here shows these data, and that RyR Po was not increased in HF. These criticisms thus seem unfounded.

A central tenet of the RyR-PKA/diastolic Ca2+ leak hypothesis is that RyR phosphorylation causes FKBP12.6 dissociation from the RyR. It was suggested12 that Jiang et al10 could not assess FKBP12.6 dissociation because their microsomes contained cytosolic FKBP12. While immunoprecipitated RyRs may provide clearer results, these microsomes should not contain appreciable cytosolic protein. Also, both FKBP12 and FKBP12.6 can associate with cardiac RyRs (although FKBP12 competes poorly in dog).23 Thus, microsomal FKBP12 may still be RyR bound, especially in humans. Since RyR phosphorylation by PKA did not change microsomal FKBP12.6 or FKBP12 levels, phosphorylation did not dissociate either FKBP from RyR. This issue remains controversial.
Impact of Increased SR Ca\(^{2+}\) Leak Versus SERCA and NCX Changes

Most Ca\(^{2+}\) released via Ca\(^{2+}\) sparks returns to the SR due to stronger Ca\(^{2+}\) transport by the SR Ca\(^{2+}\)-ATPase versus NCX. However, the SR unloading effect of enhanced leak would be synergistic with the typical SERCA and NCX changes seen in HF. Local diastolic Ca\(^{2+}\) sparks could also leave RyRs refractory, exacerbating the load-dependent reduction of fractional SR Ca\(^{2+}\) release.

If RyR hyperphosphorylation shifts RyR Ca\(^{2+}\) sensitivity (Figure 1A) during both diastole and systole, then the SR load-lowering effect of diastolic leak would be partly offset by enhanced fractional SR Ca\(^{2+}\) release during systole. This is what RyR sensitization by moderate caffeine concentration does.\(^{24}\) That is, SR Ca\(^{2+}\) load went down due to leak (and enhanced extrusion), but steady-state Ca\(^{2+}\) transients were unaltered. So, the hypothesized SR Ca\(^{2+}\) leak could contribute to SR Ca\(^{2+}\) unloading but might not alter systolic function.

Finally, HF is heterogeneous and complex. Relative contributions of SERCA, NCX, and SR Ca\(^{2+}\) leak probably vary in different origins and stages of HF. While we should seek to understand if there are technical reasons for different re-
sults, we should also embrace the difference for the potential clues it may hold for further understanding fundamental mechanisms. Hasenfuss et al segregated HF patients based on diastolic dysfunction. Forty-four percent of patients with best-preserved diastolic function had large increases in NCX expression but only modest SERCA downregulation, whereas 34% (with more diastolic dysfunction) had more profound SERCA downregulation but modest NCX upregulation. In detailed Ca$^{2+}$ transients analyses, Picentino et al found human HF data most like the latter group. Pogwizd et al found results more like the former group in nonischemic rapid-pacing-induced dog HF.

In summary, three mechanisms may contribute to reduced SR Ca$^{2+}$ load in HF (reduced SERCA function, enhanced NCX function, and enhanced SR Ca$^{2+}$ leak). However, relative contributions may vary among models and disease stages. More quantitative cellular data regarding SR Ca$^{2+}$ leak are required to better understand how this pathway might compare to SERCA and NCX alterations in being causative of, and a therapeutic target in, HF.

References


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