Mind the store: modulating Ca\textsuperscript{2+} reuptake with a leaky sarcoplasmic reticulum

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This editorial refers to ‘Up-regulation of sarcoplasmic reticulum Ca\textsuperscript{2+} uptake leads to cardiac hypertrophy, contractile dysfunction and early mortality in mice deficient in CASQ2’ by A. Kalyanasundaram et al., pp. 297–306, this issue.

Abnormalities in cardiomyocyte intracellular calcium cycling, and specifically Ca\textsuperscript{2+} physiology of the sarcoplasmic reticulum (SR), in cardiac disease states have been an intense focus of research over the past 30 years. It is increasingly apparent that alterations of SR function may contribute to the pathophysiological changes observed in several cardiac diseases including chronic heart failure, acute ischemia-reperfusion injury, atrial fibrillation, and ventricular tachyarrhythmias. Increasingly, experimental evidence suggests that the role of SR Ca\textsuperscript{2+} cycling extends beyond the traditional biophysical control of cardiomyocyte contraction and relaxation, with SR Ca\textsuperscript{2+} cycling contributing to the regulation of hypertrophic signalling, mitochondrial energetics, cell survival pathways, protein folding, autophagy, and gene expression.

Two changes to SR calcium physiology have been proposed to contribute to cardiac disease states: (i) abnormal SR Ca\textsuperscript{2+} uptake and (ii) abnormal SR calcium storage and release. Reported changes in end-stage human failing hearts include reduced expression and activity of the SR Ca\textsuperscript{2+} ATPase 2a (SERCA2a), which slows Ca\textsuperscript{2+} reuptake and cardiomyocyte relaxation. Reduced SERCA2a activity also decreases the Ca\textsuperscript{2+} content of the SR, reducing the magnitude of Ca\textsuperscript{2+} release and contraction. In addition, the SR Ca\textsuperscript{2+} release channel, called the ryanodine receptor (RyR), becomes ‘leaky’ in failing cardiomyocytes. Suggested mechanisms include oxidative modification of the RyR, and greater RyR phosphorylation by Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II (CaMKII) or protein kinase A. The resulting increased RyR open probability further contributes to the release of Sr Ca\textsuperscript{2+} content during heart failure (Figure 1A), and can promote triggered arrhythmias. Increased diastolic RyR opening is also believed to underlie arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia (CPVT), although in this disease the increased opening probability results from a mutation in the RyR or its associated SR calcium storage and RyR regulatory protein, calsequestrin, during phases of physiological or pharmacological stress. Until recently, CPVT has been considered an arrhythogenic condition in the context of a structurally normal heart. However, the potential for increased diastolic SR Ca\textsuperscript{2+} leak alone to cause pathological remodelling, hypertrophy, and heart failure remained to be confirmed. This question is addressed by Kalyanasundaram et al. in the current issue of \textit{Cardiovascular Research}.

The authors elegantly show that an increased RyR leak is not only a key feature of the failing heart, but that increased RyR open probability, when combined with enhanced SR Ca\textsuperscript{2+} uptake, can also trigger disease development. This was achieved by crossing calsequestrin knockout mice, which exhibit CPVT but not hypertrophy or heart failure, with either mice globally overexpressing the skeletal muscle SERCA1a isofrom, or phospholamban knockout mice. The co-existence of enhanced SR Ca\textsuperscript{2+} uptake with a leaky RyR lead to exaggerated spontaneous SR Ca\textsuperscript{2+} release, apoptosis, and the development of hypertrophy, heart failure, and reduced survival.

Several interesting discussion points arise from this work. The first concerns the role of SR-derived Ca\textsuperscript{2+} in triggering hypertrophy and disease progression. Hypertrophy is known to be triggered via the Ca\textsuperscript{2+}-dependent calcineurin-NFAT signalling pathway (presumably through NFAT c3 and c4 isoforms), although the precise nature of the Ca\textsuperscript{2+} signal required to activate this pathway remains elusive. One possibility is that the system can sense an increase in the integrated Ca\textsuperscript{2+} transient, such as is known to occur during the early stages of heart failure when both Ca\textsuperscript{2+} current and SR Ca\textsuperscript{2+} release are enhanced. However, overexpression of SERCA2a or knockout of phospholamban, its endogenous inhibitor, do not trigger hypertrophy despite elevated Ca\textsuperscript{2+} transients. Alternatively, the NFAT pathway may be activated by an increase in diastolic [Ca\textsuperscript{2+}], perhaps in the dyadic cleft where diffusion is limited. While speculative, support for this view comes from the observation that hypertrophy can be triggered by increasing trans-membrane Ca\textsuperscript{2+} entry via the L-type (but not T-type) Ca\textsuperscript{2+} channel or the Na\textsuperscript{+}–Ca\textsuperscript{2+} exchanger. Under some circumstances, decreasing Ca\textsuperscript{2+} removal by reducing SERCA2a activity has also been associated with the activation of calcineurin signalling. Marked NFAT signalling and hypertrophy were recently reported in mice expressing low...
Figure 1  Differing effects of increasing SERCA activity in heart failure and CPVT. (A) During heart failure, SERCA2a protein levels, activity, and SR Ca\(^{2+}\) uptake are reduced, while Ca\(^{2+}\) leak from the SR is increased due to CaMKII-dependent phosphorylation of the RyR. A resulting increase in dyadic [Ca\(^{2+}\)] is hypothesized to trigger hypertrophy and apoptosis in this condition. (B) Reported beneficial effects of restoring SERCA2a expression levels and activity in failing myocytes may be related to lowered dyadic [Ca\(^{2+}\)], and thus, decreased activity of CaMKII and Ca\(^{2+}\)-dependent signalling pathways. (C) CPVT patients also exhibit a leaky RyR, although in this case the deficit results from a mutation. Thus, rather than reversing leak, Kalyanasundaram et al. show that increasing SERCA-dependent SR Ca\(^{2+}\) uptake actually exacerbates the RyR-mediated SR Ca\(^{2+}\) leak in these cells, leading to hypertrophy and apoptosis (D).
levels of SERCA2b, in the absence of SERCA2a and phospholamban.4 Surprisingly, SERCA2a knockout itself does not trigger hypertrophy, although this finding could be explained by the fact that transmembrane Ca2+ extrusion is markedly up-regulated in these animals that may maintain resting dyadic Ca2+ levels.3 Adding further support to this local Ca2+ hypothesis, Kalyanasundaram et al. show that RyR leak may also promote hypertrophy and heart failure if it is large enough, perhaps elevating dyadic [Ca2+] beyond a threshold necessary for NFAT signalling (Figure 1C and D). In contrast, we can hypothesize that this threshold dyadic Ca2+ level is reached in heart failure due to a less extreme leak in combination with reduced SERCA2a activity (Figure 1A). Thus, accumulating evidence indicates that dyadic [Ca2+] close to the RyR is an important signalling microdomain, and that abnormal SR Ca2+ control can be an underlying cause of heart failure.

Based on this above discussion, how might we best therapeutically target these deficits? CPVT represents a genetic cardiac disease underpinned by a relatively fixed, elevated SR Ca2+ release state, and results from this study by Kalyanasundaram et al., and others, would strongly advise against developing a strategy to enhance SR Ca2+ uptake as a therapeutic strategy for genetic CPVT. Rather initial focus on pharmacological RyR stabilization, including flecainide and designer RyR stabilizer drugs, and ultimately replacement of the mutated or absent protein using gene transfer appear the logical and most promising strategies to target cardiac disease resulting from genetic abnormalities of SR Ca2+ storage and release.

However, the SR abnormalities observed in the more common clinical problem of acquired heart failure are very different mechanistically, and therefore caution must be considered before over-interpretation of the results from these genetic models are applied to strategies to target the SR in acquired heart failure. The first and most critical issue is that the abnormalities of SR Ca2+ uptake and release in heart failure are potentially reversible. Changes in gene expression and post-translational modifications of SERCA2a, phospholamban, and RyR can be partially or completely normalized by treatments that effectively correct the remodelling of the failing heart, underpinning the plasticity of the system. In addition to established interventions, there is recent growing interest in targeting the SR abnormalities directly using RyR stabilizers or SERCA2a gene transfer. In contrast to the genetic SR models, which cannot be corrected by up-regulation of SERCA2a activity, and are actually exacerbated as reported by Kalyanasundaram et al. and others, restoring SR calcium uptake in acquired models of heart failure has a strong beneficial effect upon SR Ca2+ physiology and the heart failure phenotype. This is multifaceted, including improvements in contractile function, myocardial energetics and survival, and reversal of the abnormalities of gene expression, microRNAs, and hypertrophy signalling, which promote heart failure progression.8,9 In contrast to increasing SR uptake in the CSQ knockout model, SERCA2a gene transfer or genetic SERCA2a overexpression, improves cardiomyocyte survival and reduces apoptosis in chronic heart failure, including diastematically opposite changes to the aik and apoptosis pathways to those reported by Kalyanasundaram et al. Myocardial SERCA2a gene transfer also reversed the acquired leak SR phenotype, by increasing SR Ca2+ uptake as expected from the intervention, but also reducing RyR-mediated SR leak, and normalizing SR Ca2+ load (Figure 1B).10 This effect translated into improvements in cardiac contraction and relaxation, a reduction in delayed afterdepolarizations in vitro, and most importantly reduced spontaneous and catecholamine-provoked arrhythmias in vivo. At the molecular level, SERCA2a gene transfer reversed CaMKII-dependent phosphorylation of the RyR, which is perhaps indicative of normalized dyadic [Ca2+] (Figure 1B). This finding highlights the contrasting effects of SERCA up-regulation in acquired vs. genetic abnormalities of cardiomyocyte SR function. Beyond triggered activity, Cutler et al.11 recently reported that SERCA2a gene transfer can eliminate Ca2+ and repolarization alternans, and inducible ventricular fibration in a guinea pig model of heart failure. Therefore, in addition to targeting the ‘leaky’ SR substrate for ventricular arrhythmias, SERCA2a gene transfer also appears to modify the substrate of the failing heart at multiple scales, which synergistically impart an antiarrhythmic effect. Key to this is the ability to correct reversible abnormalities of SR physiology in the acquired heart failure which is not possible in the genetic conditions.

Support for therapeutic benefits of increasing SERCA2a activity in acquired heart failure also comes from on-going clinical trials. Thirty-seven patients with advanced heart failure have received a single dose of recombinant adeno-associated adenovirus, expressing SERCA2a across an open-label phase 1 and a double-blinded phase 2 trial.12 Most importantly, this strategy showed no signs of increased toxicity, with no evidence of increasing ventricular arrhythmias in this patient group potentially at risk of sudden cardiac death. The most striking finding was the consistent benefit across a number of relevant clinical parameters of heart failure, including death and hospitalization, in the highest dose arm receiving SERCA2a gene therapy. This has formed the platform for a larger 200 patient phase 2b trial which is currently enrolling (EudraCT:2012-001700-37). The answers from this study, and other trials using SERCA2a gene therapy or RyR stabilizers currently planned or underway, will be the most informative regarding the relevant effects of targeting the SR therapeutically.

Where will all these observations potentially lead the scientific and clinical community? The SR remains a fascinating and intriguing organelle within the cardiomyocyte, with many further facets still worthy of scientific exploration in the laboratory. The increasing knowledge base that SR dysfunction may contribute to the pathophysiology of a number of cardiac diseases, and the emerging ability to selectively target the SR in patients with these diseases, is slowly capturing the imagination and interest of the clinical cardiology community. The work of Kalyanasundaram et al confirms that a genetically determined augmentation of SR Ca2+ leak has the potential to induce pathological remodelling, hypertrophy and heart failure, and in this context increasing SR Ca2+ uptake only serves to exacerbate the problem. This exemplifies the notion that ‘one size does not fit all’ when it comes to designing new treatment strategies for the SR. In the dawning era of personalized medicine, one can perceive a standard of care involving a screen for genetic abnormalities of SR storage and release in patients with cardiac disease. Those cardiomyocyte patients with cardiac disease and a ‘positive’ or ‘high risk’ genetic result (e.g. CPVT) would require stratification to agents aimed at improving SR storage and reducing spontaneous SR release (e.g. RyR stabilizers, CaMKII inhibitors, CSQ gene therapy). Conversely patients without a high genetic risk, and a disease associated with acquired SR abnormalities (e.g. heart failure) would be suitable for a range of treatment options normalizing SR calcium uptake (SERCA2a gene therapy) and/or reducing SR leak (RyR stabilizers, CaMKII inhibitors). On the basis of the above discussion, we anticipate that such patient-specific therapies will optimize Ca2+ physiology, thereby improving triggering of contraction, relaxation and signalling, and reducing arrhythmias.
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