Targeting Sarcoplasmic Reticulum Ca\textsuperscript{2+} Uptake to Improve Heart Failure
Hit or Miss

Karin R. Sipido, Peter Vangheluwe

The association between disturbed Ca\textsuperscript{2+} handling within cardiac myocytes and heart failure has been established in many studies of human myocardium from patients with end-stage heart failure undergoing heart transplantation.\textsuperscript{1–3} The causality is more difficult to prove at this late time point.\textsuperscript{4} Yet, the observed reduction in the Ca\textsuperscript{2+} available for myofilament activation and reduced Ca\textsuperscript{2+} reuptake in the sarcoplasmic reticulum (SR) are obvious targets to improve myocyte contraction and relaxation and, thus, cardiac pump function.

Early pharmacotherapy mimicked adrenergic stimulation, the physiological boost of contraction and relaxation, by raising cAMP. Although this worked well, and still does, for acute treatment, the long-term results showed an increase in mortality. Alternative therapy to enhance myofilament sensitivity seems a safer alternative.\textsuperscript{5,6}

Many animal models have been designed to reproduce heart failure in humans allowing study of myocyte remodeling at different stages of disease. The most extensively studied are genetic predisposition (the cardiomyopathic hamster), pressure overload (hypertensive models, aortic banding), myocardial infarction (MI), and tachycardia pacing (in dog or rabbit). Except for genetic model, obvious discrepancies with human disease are the fast time course of the disease and the relative lack of complexity, as, for example, the absence of atherosclerosis in most models of MI. Nevertheless these models have been very useful to demonstrate that very often in vivo cardiac dysfunction paralleled changes in Ca\textsuperscript{2+} handling, suggesting causality.

It should be pointed out that disturbed Ca\textsuperscript{2+} handling is not always the one or only cause of in vivo heart failure. Loss of myocardium in MI, increased loading on healthy myocardium by the scar tissue, reduced perfusion distal to coronary stenosis, suboptimal preload because of stiffness of the ventricle with hypertrophy, and matrix remodeling are but a few factors that can contribute to global cardiac dysfunction with (more or less) preserved intrinsic function of the myocytes.

The smaller amplitude of Ca\textsuperscript{2+} transients in heart failure results from reduced SR Ca\textsuperscript{2+} release. Underlying mechanisms are reduced efficiency of the triggering process and reduced availability of Ca\textsuperscript{2+} in the SR. Reduced efficiency of triggering was postulated quite early\textsuperscript{7} and could relate to changes in Ca\textsuperscript{2+} channel properties, ryanodine receptor (RyR) or ultrastructure. Loss of T-tubules is one mechanism that has come under study recently,\textsuperscript{8,9} loss of coupled gating of RyR with hyperphosphorylation was proposed earlier.\textsuperscript{10}

The best studied molecular defects are those that cause reduced SR Ca\textsuperscript{2+} availability: reduced expression of the SR Ca\textsuperscript{2+} pump (SERCA), enhanced inhibition of SERCA by phospholamban (PLN) because of low phosphorylation levels, enhanced activity of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX), increased leak and loss of Ca\textsuperscript{2+} from the SR because of hyperphosphorylation of the RyR. Not all of these changes are present in each model of heart failure. Several reviews have highlighted the contribution of each of these, with more or less emphasis,\textsuperscript{11–14} but each can be considered a target to enhance SR Ca\textsuperscript{2+} release and thus improve contractile function. Alternatively, one can target the pathways leading to remodeling, including the changes in Ca\textsuperscript{2+} handling, eg, acting on the G protein–coupled receptor pathways, from receptor to signaling cascade.

Transgenic mice have helped to advance our understanding of heart failure. Some were designed to reproduce molecular defects observed in heart failure and could be considered tests for causality. At the level of Ca\textsuperscript{2+} handling, this approach lent support to the importance of the changes but not to causality. Lowering SERCA expression reduces contractility but does not induce heart failure.\textsuperscript{15} Full knockout (KO) of SERCA eventually leads to heart failure but with a transition phase of compensated function.\textsuperscript{16} Two-fold PLN overexpression\textsuperscript{17} or overexpression of NCX reduce contractile function and tolerance for increased load.\textsuperscript{18} Only severe SERCA inhibition by either a 4-fold overexpression of PLN or by expression of superinhibitory PLN mutants triggers heart failure in the longer term.\textsuperscript{15,20} Frank heart failure is more rapidly induced by tampering with signaling pathways such as overexpression of β-adrenergic receptors,\textsuperscript{21} of tumor necrosis factor-α,\textsuperscript{22} or calcineurin.\textsuperscript{23} Other transgenic mice designed to study specific proteins, such as muscle LIM protein (MLP) KO mice or Ca\textsuperscript{2+}/calmodulin kinase (CaMK) II overexpression, developed heart failure and have been used subsequently as models for heart failure.\textsuperscript{24,25}
Taken together, these data underline the complexity of the changes underlying heart failure and caution for a single-hit approach.

**Transgenic Mice as Proof-of-Concept: Hit or Miss With PLN KO**

Different transgenic mice with heart failure have been used to test the proof of concept for targeting Ca\(^{2+}\) handling to treat heart failure. Crossing mice with the PLN KO provides a means to boost SR Ca\(^{2+}\) content and improve contraction, as well as Ca\(^{2+}\) removal and relaxation (Figure).\(^{26}\) This has been tried in many “models” of heart failure, as in the study in this issue of *Circulation Research*.\(^{29}\) Initial enthusiasm has been tempered since a number of studies did not result in rescue of the phenotype.\(^{27}\) It is beyond the scope of this editorial to review all of them, but it is important to highlight a few characteristics of both positive and negative studies. Success did not necessarily correlate with an initial phenotype depending on disturbed Ca\(^{2+}\) handling. In the case of the MLP KO mice, changes in cytoarchitecture are a key feature, whereas reduced SR Ca\(^{2+}\) uptake is not essential in the contractile deficit, yet PLN KO rescued the phenotype.\(^{26}\) In the mice with Gq\(\alpha\) overexpression, reduced SR Ca\(^{2+}\) uptake is present and restored with PLN KO, but the heart failure type persists.\(^{27}\) Whether or not the heart failure develops consequent on primary hypertrophy was also not a predictor for success (eg, Song et al\(^{27}\) versus Sato et al.\(^{28}\)).

The study by Zhang et al in this issue of *Circulation Research*\(^{29}\) confirms the possible dichotomy between rescue of global Ca\(^{2+}\) handling by PLN KO and failure to rescue heart failure. The CaMKII\(\delta\)C mice have dilated cardiomyopathy which is not rescued by the crossbreed, but rather exacerbated. The major step forward in the present study is the attempt to understand the mechanisms of this therapeutic failure.

First, the authors show that although SR Ca\(^{2+}\) release and uptake are normalized, the SR Ca\(^{2+}\) content is supranormal. Diastolic release events, sparks, are even more numerous in the TG/KO than in myocytes from the CaMKII\(\delta\)C mice and may be a consequence of the enhanced SR Ca\(^{2+}\) content in the face of a “leaky” RyR. In the intact heart, the count of apoptotic cells is higher in the TG/KO mice compared with WT or CaMKII TG mice. Given that apoptosis can result from mitochondrial damage, the authors examine mitochondrial Ca\(^{2+}\) content and find that it is elevated. CaMKII inhibition reduces diastolic SR Ca\(^{2+}\) release events, as well as mitochondrial Ca\(^{2+}\) content and spontaneous cell death of isolated myocytes. This leads to the hypothesis that increasing SR Ca\(^{2+}\) content under conditions where the RyR is more leaky enhances mitochondrial Ca\(^{2+}\) loading and puts cells at risk for apoptosis. This in turn exacerbates heart failure.

This is an elegant hypothesis, and Ca\(^{2+}\)-related cell death was also put forward to understand heart failure in mice with L-type Ca\(^{2+}\) channel overexpression.\(^{30}\) In that model, however, cell death was through necrosis, whereas apoptosis was unchanged. The role of the Ca\(^{2+}\) overload, which was more pronounced than in the case of the CaMKII TG mice, was further supported by in vivo studies to reverse the phenotype.

In the study by Zhang et al,\(^{29}\) a more modest Ca\(^{2+}\) overload is present and could thus lead to a different phenotype. Nevertheless, several aspects deserve further study. At present, the observed association with apoptosis does not exclude additional or alternative mechanisms of the worsening of heart failure and increased mortality in the TG/KO mice. Other causes of exacerbation could be arrhythmias related to the Ca\(^{2+}\) overload or the lack of reversal of hypertrophy, which, in itself, may impact on pump function. Whether the observed increase in apoptosis has a major impact on pump function also needs to be confirmed. Total mass increased suggesting global hypertrophy, but the wall thinning suggests unfavorable remodeling.

An interesting question raised by the study\(^{29}\) is whether the observed increase of mitochondrial Ca\(^{2+}\) and associated apoptosis represent a general mechanism preventing rescue from heart failure with PLN/KO crossbreed. In the CaMKII model for heart failure, the Ca\(^{2+}\) leak may be more central than in more typical models of pressure overload or post-MI remodeling. A first test of the hypothesis is then whether enhanced opening probability of RyR is present in the index cases where PLN KO failed to rescue the phenotype, the Gq\(\alpha\) TG and the MyBP-C TG mouse. Unfortunately, such data are

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**Non-standard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>CaMK</td>
<td>Ca(^{2+})/calmodulin kinase</td>
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<td>KO</td>
<td>knockout</td>
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<td>MI</td>
<td>myocardial infarction</td>
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<td>MLP</td>
<td>muscle LIM protein</td>
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<td>NCX</td>
<td>Na/Ca exchanger</td>
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<td>PLN</td>
<td>phospholamban</td>
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<tr>
<td>RyR</td>
<td>ryanodine receptor</td>
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<tr>
<td>SERCA</td>
<td>sarcoplasmic Ca(^{2+}) pump</td>
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<tr>
<td>SR</td>
<td>sarcoplasmic reticulum</td>
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<td>TG</td>
<td>transgenic</td>
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**Figure.** Potential benefits and risks associated with improving SR Ca\(^{2+}\) handling as a therapy for heart failure. Stimulation of SR Ca\(^{2+}\) uptake activity improves contractile parameters, might reduce adverse Ca\(^{2+}\)-dependent signaling and thereby prevent cardiac remodeling. On the other hand, excessive SERCA activity might be energy demanding to the failing heart. It might also buffer the Ca\(^{2+}\) and limit contraction. Stimulation of spontaneous Ca\(^{2+}\) release might enhance the risk of arrhythmias and induce Ca\(^{2+}\) overload in the mitochondria. This would trigger apoptosis and/or necrosis and lead to significant myocyte loss. A reduced contractile reserve (see text) might also be detrimental for the heart.
not available at present. Conversely, was there no increased RyR opening in models where the rescue was successful? For the MLP KO, again there are no data, but diastolic Ca\(^{2+}\) levels were elevated,\(^3\) whereas for the TG mouse with calsequestrin overexpression, spark frequency was low.\(^3\) Thus, at present, it remains unresolved whether there is such a link.

The Need for Balance

Crossbreeding heart failure mice with PLN KO has not been the only approach to improve SR Ca\(^{2+}\) handling. Many data were obtained in nontransgenic traditional animal models of heart failure. Hajjar, del Monte, Harding, and colleagues have explored in depth the potential of increasing SERCA levels by adenovirus-mediated overexpression of SERCA\(_2\).\(^\text{33,34}\) Gene transfer improved survival in rats with heart failure following aortic banding, whereas arrhythmias during acute I/R were not increased.\(^\text{35,36}\) (Note: Transgenic rats overexpressing SERCA\(_2\) also had less heart failure after MI but at the expense of increased arrhythmias\(^\text{37}\).) These results have led to the ongoing clinical trials in patients with established heart failure which recently reported no untoward effects.\(^\text{38}\)

Besides the risks of increased spontaneous Ca\(^{2+}\) release and mitochondrial Ca\(^{2+}\) overload another potential side effect of increasing SERCA activity is increased Ca\(^{2+}\) binding and buffering.\(^\text{39}\) A shift of the Ca\(^{2+}\) pump toward increased Ca\(^{2+}\) affinity, by isoform switch or by manipulating PLN, could have similar effects.\(^\text{40,41}\) The onset of heart failure in patients carrying a loss of function mutation in the PLN gene also warns for harmful effects of excessive SERCA activity in the human heart.\(^\text{20}\) It was recently proposed that the inability to regulate SERCA activity up and down might on itself act as an independent trigger for heart failure.\(^\text{41,42}\) This could result from reduction of contractile reserve, as can be measured during stress tests such as dobutamine infusion or exercise testing.\(^\text{40}\) Together, these observations suggest that we may have to dose rather precisely our treatment (Figure).

After demonstrating the potential of targeting SR Ca\(^{2+}\) uptake in heart failure, we must now define how and in which conditions it may be useful. We should take into account that some of our transgenic approaches may be too blunt an instrument. The present study by Zhang et al\(^\text{19}\) shows a potential direction for narrowing down the types of heart failure that may benefit from treatment but we should not limit our studies to mice. Several studies point to major differences in Ca\(^{2+}\) handling and, in particular, PLN function in the hearts of larger mammals and humans.\(^\text{34,44}\) We should investigate models of heart failure where we can time and dose interventions that more faithfully resemble clinical testing.\(^\text{40}\) Together, these observations suggest that we may have to dose rather precisely our treatment (Figure).

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Disclosures

None.

References


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