To mark the 60th birthday of *Circulation Research* (1953–2013), the editors have commissioned *Circulation Research Classics*, a series of commentaries highlighting seminal articles published in the journal over the past 6 decades that have importantly shaped cardiovascular research. Written by leading experts, *Circulation Research Classics* are intended to describe the impact of these articles on the field by putting them in a historical perspective. The concept of classic is inextricably linked to time—a classic is something that maintains its value regardless of its age. Thus, an important consideration in selecting the articles to be highlighted is that they have stood the test of time, which is the most reliable indicator of the value of scientific work. By looking back at the illustrious past of *Circulation Research*, we hope to promote a deeper appreciation of the contributions of this journal to the advancement of knowledge.

Sarcoplasmic Reticulum Ca-ATPase and Heart Failure 20 Years Later

David Eisner, Jessica Caldwell, Andrew Trafford

Relation Between Myocardial Function and Expression of Sarcoplasmic Reticulum Ca^{2+}-ATPase in Failing and Nonfailing Human Myocardium
Hasenfuss et al
Circ Res. 1994;75:434–442.

This article reflects on the impact of a classic paper identifying the effects of loss of sarcoplasmic reticulum Ca-ATPase activity in heart failure.

The Classic Article

Understanding the effects of heart failure on contraction and Ca signaling in the heart has long been a priority. By the late 1980s, many studies on animal models had shown that heart failure resulted in a slowing of the decay of the systolic Ca transient[1] because of a decrease in the expression of the sarcoplasmic reticulum (SR) Ca-ATPase (SERCA).[2] Work using human tissue found similar changes.[3,4] In 1994, Hasenfuss et al[5] published a now classic article in *Circulation Research* entitled "Relation between myocardial function and expression of sarcoplasmic reticulum Ca-ATPase in failing and non-failing human myocardium." Previous work had shown that SERCA expression and activity were decreased in heart failure. Other work had shown that the increase of force seen on increasing the frequency of stimulation (the positive force–frequency curve) disappeared in heart failure. Hasenfuss et al[5] showed that the degree of change in the force–frequency relationship correlated with the loss of SERCA. Hearts with low levels of SERCA developed maximum force at lower frequencies than those with higher levels. In addition, and suggested as causative of the reduced force–frequency responses, the failing hearts had reduced SERCA-mediated Ca uptake.

The work in this article has influenced 2 areas of research: changes of SR function in heart failure and restoration of SERCA as a therapeutic strategy. We consider these in turn.

SR Function in Heart Failure

Twenty years later, the role of changes of SERCA expression in heart failure is well-established. The majority of studies of heart failure in either humans or experimental animals show that SERCA activity is decreased in heart failure. This decrease of SERCA has 2 immediate effects on Ca signaling. First, it slows the rate of decay of the systolic Ca transient, thereby impairing relaxation. Second, as a consequence of decreasing SR Ca content, it will decrease the amplitude of the systolic Ca transient and thus contraction. However, subsequent work has shown that changes of SERCA are part of a group of changes of Ca signaling that occur in heart failure. As highlighted in Figure, 2 other factors will also decrease the SR Ca content. First, there is an increase of NCX expression or activity.[6–8] This adaptation has the benefit of making up for the reduced SERCA, thereby preserving relaxation. In a subsequent article, Hasenfuss et al[9] showed that patients with increased NCX had better diastolic function than did those with lower NCX. However, although increased NCX improves diastolic function, it will decrease SR Ca content and thereby depress systolic function further. Second, many studies have now shown that the open probability of the RyR is increased in heart failure.[10–12] This may occur as a result of phosphorylation,[10] hyponitrosylation,[13] or oxidation.[14] This increased opening will lead to a diastolic leak of Ca, thereby decreasing the SR Ca content. In addition to decreasing systolic function, the leak may also interfere with relaxation by opposing Ca reuptake into the SR.[15] The relative importance of decreased SERCA activity as opposed to increased NCX and RyR leak may depend on the exact model used. For example, in a canine model of heart failure induced by rapid pacing,[16] it was found that the bulk of the problems of Ca handling could be attributed to increased leak, whereas in a rabbit model, aortic insufficiency and stenosis changes of NCX were most important.[11]

Restoration of SERCA as a Therapeutic Strategy

The first-choice drugs used in the management of heart failure include β-blockers, ACE inhibitors, and aldosterone

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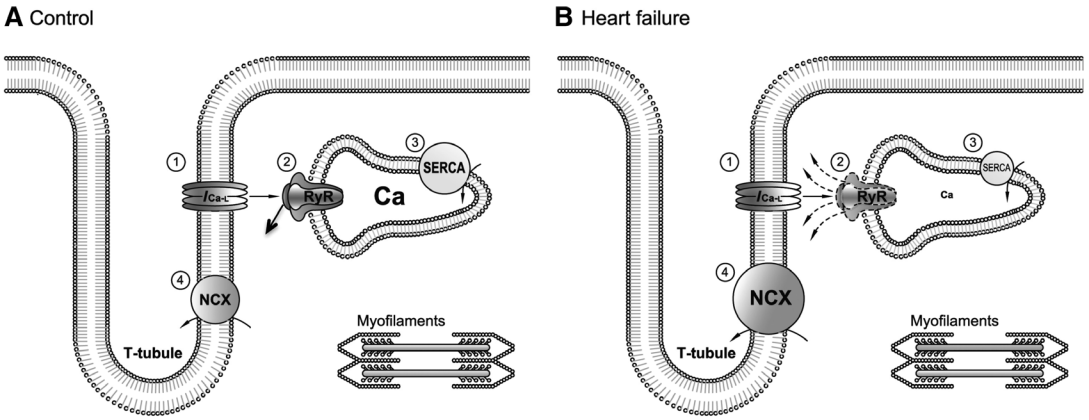

Figure. Cardiac Ca cycling showing the important changes in heart failure. Both parts show a transverse (T) tubule, surface membrane, sarcoplasmic reticulum (SR), and myofilaments. The action potential opens L-type Ca channels (**1**) located in the T-tubules. The Ca entering binds to the RyRs (**2**), causing them to open and release Ca into the cytoplasm. Relaxation occurs as Ca is pumped back into the SR by SR Ca-ATPase (SERCA; **3**) or out of the cell by NCX (**4**). **A**, Control. **B**, Heart failure. Note the leaky RyR, decreased SERCA activity, and increased NCX. All these changes contribute to a decrease in SR Ca content.

antagonists.[17] When used individually or in combination, they may prolong life and relieve symptoms, but they do not correct the underlying causes of contractile dysfunction, such as, for example, decreased SERCA function. Therefore, having identified decreased SERCA expression or function as a hallmark of heart failure, a logical extension of this body of work was to attempt to restore SERCA function to improve contractility. Several strategies have been used to achieve an increase in SERCA function. Of these, pharmacological phosphodiesterase inhibition to increase cAMP availability and hence SERCA activity has been translated from the laboratory setting to clinical trial stages, only to be withdrawn because of an increased incidence of sudden death.[18] In a similar vein, pharmacological stimulation of SERCA with the Na/K ATPase antagonist istaroxime[19–21] has also been pursued in heart failure. However, once again, after initial promise in experimental studies and early clinical trials, substantive clinical trials were suspended.

Other indirect approaches to modulate SERCA activity as a means to improve contractility in heart failure have also targeted various points in the β-adrenergic signaling cascade. Of these, perhaps the most extensively studied has been the G-protein receptor kinase GRK2 (βARK).[22,23] In these studies, GRK inhibition restored β-adrenoceptor signaling and increased SERCA activity in response to catecholamine stimulation. A further approach currently undergoing clinical evaluation includes upregulation of adenylyl cyclase VI through adenoviral-based gene delivery (recruiting but not yet reported). Again, this study is based on extensive preclinical data showing that increasing adenylyl cyclase VI activity would increase cAMP levels and, hence, SERCA activity and contractility in experimental heart failure.[24]

Targeting SERCA Directly—From Bench to Clinical Trial

In 1999, del Monte et al[25] were the first to attempt to directly increase SERCA expression in single failing human ventricular myocytes using an adenoviral expression system. In this study, increasing SERCA protein expression in failing myocytes led to enhanced ATPase activity with increased contractility and accelerated relaxation. In addition, the blunted force–frequency response ordinarily observed in heart failure was converted to a positive force–frequency response as seen in healthy cells. Subsequently, a number of studies have shown beneficial effects on cardiac structure, contractility, Ca cycling (including reduced Ca leak), and myocardial energetics after SERCA gene delivery, either via direct intramyocardial injection or via coronary perfusion, in diverse models of heart failure.[26–29] There are several potential problems that may arise as a consequence of attempts to increase SERCA activity in heart failure. These include an increase in energy/oxygen demand because of the requirement for ATP hydrolysis to drive the increased Ca transport by SERCA.[30] However, improvements in myocardial energetics have been reported[26] and may reflect the improved blood supply to the myocardium and oxygenation as a result of improved contractility. The second major concern is that the increase of SR Ca content might increase Ca-dependent arrhythmias. Interestingly, however, there is a decreased incidence[29] of aftercontractions, the cellular correlate of such arrhythmias, suggesting that the reverse remodeling produced a compensatory decrease of arrhythmogenic potential.

After the promising results from the initial laboratory studies, the Hajjar group has paved the way for in-human clinical trials of SERCA expression. This has involved repackaging the original vectors into an adeno-associated vector to overcome proinflammatory and persistence of target gene expression issues with the original adenoviruses. Results from phase I of the Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) trial were reported in 2009.[31] SERCA gene delivery in patients enrolled with New York Heart Association class III/IV heart failure was associated with improved 6-minute walk tests, New York Heart Association classification, and left ventricular function at 6 months after treatment. Of the surviving patients, 2 did not show any form of improvement, an effect attributed to high neutralizing antibodies at enrollment. Similarly, positive outcomes have more recently been reported after 12-month follow-up in the initial phase II CUPID trial,[32] and an expanded multicentre trial is currently underway.

Although there is a growing body of evidence that targeting SERCA gene expression is a useful therapy in heart failure, inevitably a number of potential problems remain that need to be overcome in the future. Not least of these are issues regarding the choice of adeno-associated vectors as delivery vectors. Although producing a reduced inflammatory response compared with adenoviruses and, with appropriate serotype choices, having reasonable cardiac tropism, 2 major obstacles remain: the presence of neutralizing antibodies against adeno-associated vectors precludes their effective use in a substantial proportion (≈40%) of heart failure patients, and the relatively low transduction efficiency of adeno-associated vectors.

It is hoped that with the demonstration of benefit from current trials targeting SERCA expression in heart failure, alternative approaches to increase the availability of the therapy to all patients and possibly greater extent of gene expression may lead to enhanced longer-term benefit. An alternative approach obviating the need for gene therapy in the future could involve myocardial regeneration using transplantation of induced pluripotent[33] or mesenchymal stem cells,[34] or perhaps driving epicardial progenitor cells to form de novo cardiac mycoytes.[35] However, these therapies are very much in their infancy, and time will tell regarding their effectiveness in the setting of heart failure. Nevertheless, it is clear from the early seminal studies by Hasenfuss et al[5,9] that we have moved truly from bedside to bench and back again with great effect.

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