The contraction-relaxation cycle of the heart is controlled by the sequential rise and fall of the cytosolic calcium concentration. Calcium entry through L-type calcium channels during the action potential serves to trigger calcium release from the sarcoplasmic reticulum (SR) leading to activation of contractile proteins and force generation (for review, see Reference 1). In addition, there is calcium influx by reverse-mode sodium-calcium exchange. The contribution of calcium influx through L-type calcium channels, or sodium calcium exchange, and of SR calcium release to systolic calcium accumulation and availability for systolic activation of contractile proteins has been extensively studied in various animal species (human, rabbit, ferret, cat, and guinea pig) the relevance of calcium extrusion by the sarcolemmal calcium exchanger (Na$^{+}$-Ca$^{2+}$ exchanger), by mitochondrial calcium uptake, and by calcium extrusion via the sarcosomal calcium pump. Again, the contribution of these systems removing calcium from the cytosol to the decay of the calcium transient and to subsequent relaxation varies in a species-dependent manner. Using the rapid-cooling contracture technique, Bers et al suggested that in rat ventricular myocardium, $\approx$92% of calcium removal occurs by SR calcium uptake and only $\approx$7% by Na$^{+}$-Ca$^{2+}$ exchange. In other species (human, rabbit, ferret, cat, and guinea pig) the balance is more in the range of 70% to 75% SR calcium uptake and 25% to 30% Na$^{+}$-Ca$^{2+}$ exchange. According to these studies, calcium uptake by mitochondria and transsarcolemmal calcium extrusion by the sarcosomal calcium pump were suggested to be of minor importance.

The relevance of calcium extrusion by the sarcosomal calcium pump is again evaluated in the study by Hammes et al$^{5}$ in this issue of Circulation Research. The plasma membrane calmodulin-dependent calcium ATPase (PMCA) is a ubiquitous calcium transporting enzyme that plays a dominant role in nonexcitable cell types.$^{5,7}$ So far, 4 different isoforms have been cloned, and 3 different isoforms seem to be expressed in myocardial tissue.$^{6,9}$ PMCA is not homologous with SR Ca$^{2+}$-ATPase and has a different ATP to calcium stoichiometry.$^{10}$ One calcium ion seems to be transported per ATP hydrolyzed.$^{10,11}$ Hammes et al$^{5}$ established transgenic rat lines carrying the human PMCA 4 cDNA under control of the ventricle-specific myosin light chain-2 promoter. Although transgenes exhibited a 2.5-fold PMCA overexpression in neonatal and a 1.6-fold overexpression in adult cardiomyocytes, no significant differences in hemodynamic parameters between control and transgenic animals were observed in anesthetized rats. Accordingly, no relevant differences in fura-2 calcium transients and L-type calcium currents were observed. These data confirm elegantly that PMCA does not significantly contribute to calcium elimination in cardiac myocytes. However, because the rate of protein synthesis was increased in neonatal myocytes from transgenic compared with wild-type animals in the presence of 2% FCS, as well as on incubation with phenylephrine or isoproterenol, the authors suggest that PMCA may influence growth regulation.$^{5}$ Moreover, because the authors were able to show that PMCA is at least partially localized in caveolae, they speculate that PMCA may modulate caveolar signal transduction and thereby influence growth regulation. This is an interesting hypothesis that is in line with previous studies suggesting that PMCA isoform 1a overexpression is involved in regulation of vascular smooth muscle cell growth.$^{12}$ However, additional research is warranted to study the role of PMCA in myocardial growth regulation and the influence of altered expression or functional modulation of this calcium pump on myocardial development, hypertrophy, or failure.

Expression of the SR Ca$^{2+}$-ATPase and other calcium regulatory proteins has been extensively studied in various animal models of heart failure as well as in the failing human heart (for review, see Reference 13). There is accumulating evidence that reduced expression and/or function of the SR Ca$^{2+}$-ATPase as well as increased expression and function of the Na$^{+}$-Ca$^{2+}$ exchanger are key changes contributing to altered calcium homeostasis in the failing human heart.$^{13}$ Altered calcium homeostasis is obvious from studies showing a reduced total amount of calcium cycling in isolated muscle strip preparations,$^{14}$ reduced systolic calcium transients and increased diastolic calcium levels in isolated myocytes,$^{15}$ as well as reduced rate of calcium removal in both muscle strips and myocytes from failing human hearts.$^{14,15}$ Furthermore, disturbed calcium cycling underlies blunting of the frequency potentiation of contractile force, which is an important defect in the failing human heart.$^{16}$ SR Ca$^{2+}$-ATPase and the Na$^{+}$-Ca$^{2+}$ exchanger work in concert to eliminate calcium from the cytosol, facilitating dissociation of calcium from myofilament regulatory proteins and subsequent relaxation.$^{17}$ However, the Na$^{+}$-Ca$^{2+}$ exchanger in its forward mode is a competitor of SR Ca$^{2+}$-ATPase with respect to SR calcium accumulation and availability for systolic activation of contractile proteins. The relevance of calcium influx by the
$\text{Na}^+\text{-Ca}^{2+}$ exchanger to calcium transients is not clear. On the basis of these considerations, inhibition of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger or stimulation of calcium transport by SR Ca$^{2+}$-ATPase should increase SR calcium load and thus improve systolic myocardial function. However, whereas the former possibility would likely result in cytosolic calcium overload, only the latter would improve both systolic and diastolic performance of the myocardium.

Stimulation of SR calcium pump activity, therefore, may be an attractive approach to improve systolic and diastolic function of the failing human heart. SR Ca$^{2+}$-ATPase is inhibited by phospholamban, and this inhibition is relieved on phosphorylation of phospholamban by protein kinase A or calcium calmodulin-dependent kinase. Therefore, interventions that specifically result in increased phospholamban phosphorylation, or in decreased phospholamban protein levels, or interventions that inhibit SR Ca$^{2+}$-ATPase to phospholamban interaction may be promising therapeutic possibilities to treat heart failure. In this regard, it was shown that overexpression of phospholamban in transgenic animals decreases cardiac contractility whereas phospholamban knockout increases myocardial performance.

Alternatively, function of the failing myocardium may be improved by overexpression of the SR calcium pump. Sarcoendoplasmic reticulum Ca$^{2+}$-ATPases are encoded by 3 genes, and 5 different isoforms are expressed: the adult fast-twitch skeletal muscle isoform (SERCA1a), its alternatively spliced neonatal isoform (SERCA1b), the cardiac/slow twitch skeletal muscle isoform (SERCA2a), its alternatively spliced smooth muscle/nonmuscle isoform (SERCA2b), and an isoform expressed in a broad variety of muscle and nonmuscle tissues (SERCA3) (for review, see Reference 33). In embryo cardiac myocytes. By using specific antibodies to SERCA1a and SERCA2a, the authors showed that both endogenous and transgenic ATPases are prevalently associated with the microsomal fraction (ie, sarcoplasmic reticulum). Calcium uptake measurements suggested that a 4-fold increase in uptake rates may reflect an upper limit for the ability of these myocytes to express functional protein. Similar to the finding of Loukianov et al, SERCA2a appeared to be reduced by 30% to 60% in transgenic myocytes expressing SERCA1a.

The structural and functional differences of the respective SERCA isoforms are not completely understood. It has been shown that the primary structure of SERCA1a and SERCA2a protein is more than 80% identical. Furthermore, expression of the different pump isoforms in COS-1 cells revealed that SERCA1a and SERCA2a isoforms are similar regarding calcium transport capacity and apparent affinity for calcium. However, those in vitro studies are limited by absence of phospholamban and other native regulators. This may explain why the calcium transporting capacity of cardiac microsomes was found to be much lower than that in fast skeletal muscle SR, although this may partly be explained by differences in SERCA pump density as well.

From the studies by Loukianov et al and Inesi et al, no information on phospholamban regulation of SERCA1a can be derived. However, it was shown that phospholamban can regulate SERCA1a as well as SERCA2a activity. Furthermore, additional studies showing that apparent calcium affinity of SERCA is similar in wild-type and SERCA1a transgenic animals indicate that SERCA1a and SERCA2a are similarly regulated by phospholamban.

It is important to keep in mind that mouse myocardium and rat myocardium are considerably different from human myocardium regarding excitation-contraction coupling and contractile protein isoforms (for review, see Reference 33). In particular, mouse myocardium appears to be much more tolerant of calcium overload than other species. Therefore, what is true in the mouse and rat cannot be readily extrapolated to other animal species and humans.

In summary, the studies presented indicate that overexpression of SERCA2a as well as transgenic expression of SERCA1a and most likely other genetically engineered calcium pump...
proteins can influence myocardial calcium handling and function substantially. Because disturbed calcium handling seems to play a significant role in the pathophysiology of heart failure, the relevance of overexpression of SERCA to rescue disturbed calcium cycling and myocardial function of the failing heart is of particular importance. Moreover, overexpression of SERCA as an inotropic intervention may be attractive from the point of view of energy economy. Although SERCA overexpression may result in increased ATP hydrolysis related to SR calcium transport, unlike cAMP-mediated inotropic interventions, it should not decrease myofilament calcium sensitivity or economy of force generation at the level of the contractile machinery. Therefore, from a pathophysiological as well as an energy economy point of view, overexpression of SERCA seems to be a challenging approach to correct altered calcium cycling in heart failure. SERCA2a but also SERCA1a and other modified SERCA molecules should be evaluated, and the functional differences between those molecules must be clarified. Most importantly, studies in human myocardium or in animal models of human heart failure are needed to support the hypothesis that stimulation of SR calcium transport can rescue altered excitation-contraction coupling in the failing myocardium.

References


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