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SERCA2a in Heart Failure: Role and Therapeutic Prospects

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Ca^{2+} is a key molecule controlling several cellular processes, from fertilization to cell death, in all cell types. In excitable and contracting cells, such as cardiac myocytes, Ca^{2+} controls muscle contractility. The spatial and temporal segregation of Ca^{2+} concentrations are central to maintain its concentration gradients across the cells and the cellular compartments for proper function. SERCA2a is a cornerstone molecule for maintaining a balanced concentration of Ca^{2+} during the cardiac cycle, since it controls the transport of Ca^{2+} to the sarcoplasmic reticulum (SR) during relaxation. Alterations of the activity of this pump have been widely investigated, emphasizing its central role in the control of Ca^{2+} homeostasis and consequently in the pathogenesis of the contractile defect seen with heart failure. This review focuses on the molecular characteristics of the pump, its role during the cardiac cycle and the prospects derived from the manipulation of SERCA2a for heart failure treatment.

KEY WORDS: Heart failure; SERCA; gene therapy; sarcoplasmic reticulum.

HEART FAILURE AND Ca^{2+} HOMEOSTASIS

Cardiovascular diseases are currently the most common cause of death in western countries (American Heart Association, Heart Disease and Stroke Statistics—2005 Update). Heart failure (HF), in particular, affects 4.7 million individuals in the United States alone with an age related distribution. The percentage of the population between 65 and 74 years old affected by HF is about 7%, but this number increases to 10% when people over 75 are considered.

Classically, HF is defined as the inability of the heart to meet the metabolic demand of peripheral organs. The normal response of the body to this event is then triggered with the activation of the neuro-hormonal response resulting in cardiac hypertrophy and an increase in left ventricular (LV) volume. Under chronic conditions, the heart starts an irreversible degeneration process that brings about a loss of contractile function, progressively severe disability and death (Francis, 2001; Yano *et al*., 2005).

The etiology of heart failure and the initial insult vary widely and include coronary artery disease, hypertension, infection, inflammatory diseases, genetic, and idiopathic. However, at the end-stage level common features are shared by cardiomyocytes which include impaired Ca^{2+} handling with altered expression and activity of some of the key regulatory proteins for excitation–contraction coupling (Yano *et al*., 2005).

The regulation of cytosolic Ca^{2+} levels in eukaryotic cells is a highly complex process since this ion acts as a second messenger in many important cellular pathways requiring variable concentrations of Ca^{2+}. In the heart, in particular, Ca^{2+} does not only have the role as a second messenger, but it is also the effector of the contractile event. Therefore, several proteins are involved to ensure a finely tuned Ca^{2+} mobilization in the cardiomyocyte, including pumps, channels, and Ca^{2+}-binding proteins.

In the normal heart, the surface of cardiomyocytes is characterized by the presence of tubules that run transversally from the sarcolemma across the cardiac cell

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(T tubules). The excitation–contraction process is triggered by a depolarization event that travels down the membrane of the T-tubules and induces the opening of specialized Ca^{2+} channels, i.e., the L-type voltage dependent Ca^{2+} channels (LTCC). Activation of LTCC allows a small amount of Ca^{2+} to enter the cytosol. Such events, recently described as Ca^{2+} sparks (Cheng *et al*., 1993), are insufficient to induce the activation of the myofilaments. However, since these channels are located in close proximity to the terminal cisternae of the SR, Ca^{2+} can reach the Ryanodine Receptor (RYR2) located on the membrane of the organelle producing a massive release of Ca^{2+} from the SR into the cytosol (Bers, 2002). With this process, the intracellular concentration of Ca^{2+} increases from 0.1 to 1 μM and the released Ca^{2+} ion can initiate the contractile event with cross-bridge cycling when troponin C is exposed to the myosin head and attaches to actin filaments. The termination of this event relies on a rapid and efficient removal of Ca^{2+} from the cytosol. Two molecules are involved in this process, i.e., the SR Ca^{2+}-ATPase pump (SERCA) and the Na^+/Ca^{2+} exchanger (NCX) pump spanning the cell membrane. These molecules are characterized by different affinities for Ca^{2+}, with SERCA binding the ion with a high efficiency, allowing a quick reuptake of Ca^{2+} into the SR followed by the relaxation of the myofilaments (Shannon and Bers, 2004).

SERCA ISOFORMS

In mammals the SR Ca^{2+}-ATPase family includes several isoforms encoded in three different genes (SERCA1, 2, 3). The SERCA1 gene (ATP2A1) is located on Chromosome 16p12 and gives rise to two subtypes by alternative splicing: SERCA1a and SERCA1b. These proteins are respectively the adult and the neonatal isoforms of SERCA present in the fast-twitching skeletal muscle (Martonosi and Pikula, 2003; Odermatt *et al*., 1996; Otsu *et al*., 1993). The SERCA2 gene (ATP2A2 on Chromosome 12), instead, encodes a transcript that can be alternatively spliced into three different isoforms of the SERCA protein: SERCA2a, 2b and 2c. SERCA2a and b are expressed mainly in the cardiac/slow-twitch muscle and in the smooth muscle respectively, while SERCA2c has been detected in epithelial, mesenchymal, and hematopoietic cell lines, as well as in primary human monocytes (Gelebart *et al*., 2003; Martonosi and Pikula, 2003). SERCA3 is encoded by the ATP2A3 gene (Chromosome 17p13). This pump is more ubiquitous and is present also in a large variety of nonmuscle cell types in at least six different isoforms (Bobe *et al*., 2004; Dode *et al*., 1996; Martonosi and Pikula, 2003).

SERCA STRUCTURE/FUNCTION

From the structural point of view, Ca^{2+}-ATPase members are characterized by three different main domains: a large head facing the cytosol, a stalk and a transmembrane domain with only few loops being present on the luminal side of the SR membrane (Toyoshima *et al*., 2000; Toyoshima *et al*., 1993). The head includes both the ATP-binding, the phosphorylation and the actuator domains (i.e., the region that works as an anchor for the nucleotide-binding domain and that is considered responsible for dephosphorylation) (Abu-Abed *et al*., 2004; Ma *et al*., 2005; MacLennan *et al*., 1985; Martonosi and Pikula, 2003; Toyoshima *et al*., 2000). Site-specific mutagenesis analyses of the molecule conducted on the best-characterized isoform (SERCA1a) locate the Ca^{2+}-binding sites in the transmembrane region (Andersen and Vilsen, 1992; Clarke *et al*., 1989a,b; Zhang *et al*., 2000). Significant new insights into the structure and function of the different domains of the protein have been provided. David MacLennan's group has reported the impact of a number of mutants on the Ca^{2+}-binding affinity, Ca^{2+} transport activity, formation of a phosphoenzyme intermediate, and the capacity of structural modification of the pump (Clarke *et al*., 1990a,b, 1993; Maruyama *et al*., 1989; Odermatt *et al*., 2000; Skerjanc *et al*., 1993; Vilsen and Andersen, 1992; Vilsen *et al*., 1989; 1991).

The mechanism of the Ca^{2+} ATPase follows the E1-E2 model of the Post–Elbers scheme described for Na^+–K^+ ATPase (Fig. 1; MacLennan and Green, 2000). In this model, the molecule in E1 conformation exposes two high-affinity Ca^{2+} sites to the cytosol. Following the binding of Ca^{2+}, the nucleotide domain of the molecule can then accept a molecule of ATP, which is hydrolyzed to ADP+P_i. The ADP is now released from the protein, while the inorganic phosphate (P_i) binds the phosphorylation domain of SERCA producing an E1–P_i intermediate. At this point, SERCA assumes the E2 conformation, in which the two Ca^{2+} ions are exposed to the SR lumen. The pump's Ca^{2+}-binding sites are characterized by a low affinity allowing the ion to be released from the molecule into the lumen of the SR and leaving the pump in the E2–P_i state. The release of Ca^{2+} then induces the hydrolysis of the P_i, linked to the head of SERCA, leaving the pump in E2 conformation. The molecule then returns to the most stable energetic conformation (E1), exposing the high affinity Ca^{2+}-binding sites to the cytosol (Toyoshima and Inesi, 2004; Toyoshima *et al*., 2003).

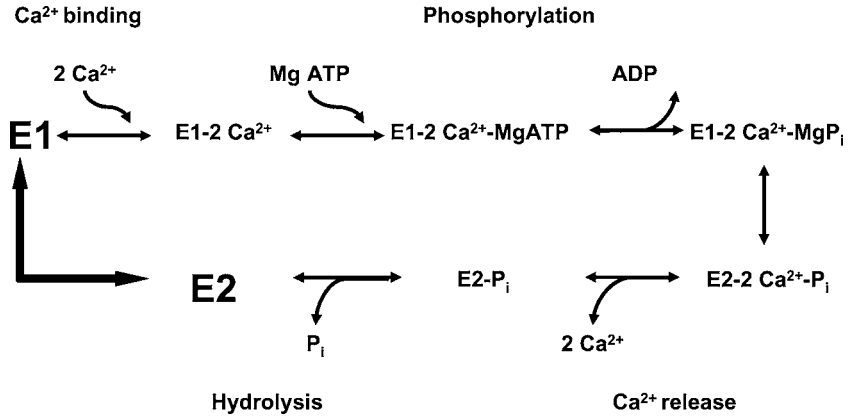

Fig. 1. Scheme of the Post–Elbers model for SERCA. During the reaction cycle SERCA undergoes four interconvertible phosphorylated and unphosphorylated states by which the increase in the $[Ca^{2+}]_i$ saturates the two high affinity Ca^{2+}-binding sites of the cytoplasmic side. The Ca^{2+} ATPase takes up two Ca^{2+} ions from the cytoplasm to form $[Ca^{2+}]E1$. By ATP phosphorylation the two Ca^{2+} ions are retained and occluded within the protein forming an high energy intermediate $[Ca^{2+}]E1P$. This intermediate phosphoenzyme through transient intervening states gets into a low Ca^{2+} affinity and ADP insensitive form *E2P*. The rate limiting transition to *E2P* is accompanied by release of Ca^{2+} into the SR lumen. Water enters the catalytic site and by hydrolysis of the *E2P* and regeneration of the $[Ca^{2+}]E1$ the reversible cycle is completed.

REGULATION OF SERCA2a ACTIVITY

As mentioned, SERCA2a is the most abundant isoform of the Ca^{2+} ATPase within the heart. The activity of the pump is specifically regulated by phospholamban (PLN). PLN is a small homopentamer (25 kDa) present on the SR membrane and characterized by a regulatory hydrophilic domain and a hydrophobic transmembrane region. The physical interaction between PLN and SERCA2a is mediated by specific residues in the transmembrane domain which are thought to directly interact with SERCA2a producing an inhibitory effect due to a modification of the tertiary structure of the molecule. This results in a decrease in Ca^{2+} affinity of the pump (Asahi *et al*., 2003; Chen *et al*., 2005; Koss and Kranias, 1996). The PLN-mediated inhibition of SERCA2a is finely tuned by phosphorylation of the PLN regulatory domain. In particular, the hydrophilic region of PLN contains three distinct phosphorylation sites (Ser^{10}, Ser^{16}, and Thr^{17}) that are subject to the control by three different protein kinases (protein kinase C, cAMP-dependent protein kinase and Ca^{2+}-calmodulin protein kinase). Even though the phosphorylation of each of these residues is related to an increase of SERCA2a activity as a result of detachment of PLN from the SR Ca^{2+} ATPase, only phosphorylation of Ser^{16} is sufficient to induce maximal increases in the activity of the Ca^{2+} transporter. The inhibitory effect of PLN on SERCA2a has been studied in detail by our group and others using antisense sequences or site specific mutations (Chen *et al*., 2005; del Monte *et al*., 2002a,b; Kaprielian *et al*., 2002; Li *et al*., 2005; Zhai *et al*., 2000).

Other molecules are also involved in regulating the interaction between SERCA2a and PLN, i.e., protein phosphatase I (PPI) and inhibitor I. PPI dephosphorylates PLN which enables PLN to recover its inhibitory activity on SERCA2a. The Inhibitor I protein regulates PPI by interacting with the latter in its phosphorylated form. The interaction of inhibitor I with PPI blocks PPI activity with the result of enhancing SERCA2a transporter activity. The neuro-hormonal system, in turn, affects SERCA2a function through the β-adrenergic pathway by phosphorylation of inhibitor I via protein kinase A. This maximizes the inotropic effects of cAMP and, therefore, of β-adrenergic stimulation on contractility of cardiomyocytes (Pathak *et al*., 2005; Sulakhe *et al*., 1997).

In addition to PLN, the main direct regulator of SERCA2a activity in the ventricles, other inhibitors have been described as minor regulators of the pump. In particular, in the atria and in the fast-twitch skeletal muscle, sarcolipin (SLN) acts as a regulator of SERCA. SLN is a 31 aa molecule that is considered a structural homologue of PLN (Odermatt *et al*., 1998). Both PLN and SLN are co-expressed in heart tissue, even though the relative concentrations differ between cardiac regions and species (Asahi *et al*., 2003).

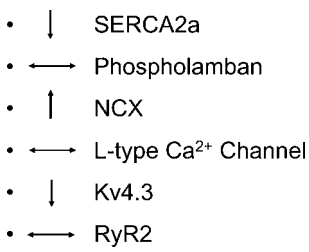

Fig. 2. Changes in Ca^{2+} cycling proteins in failing human hearts.

SERCA IN HF

In failing hearts, the impairment in Ca^{2+} handling affects both the systolic and diastolic phase of the cardiac cycle. As a consequence of alterations in Ca^{2+} homeostasis, diastolic calcium levels often become elevated and systolic Ca^{2+} levels often decrease resulting in a prolonged relaxation phase as first observed in human myocardium (Beuckelmann *et al*., 1992; Gwathmey *et al*., 1993; Gwathmey *et al*., 1987; Gwathmey and Hajjar, 1990; Gwathmey and Morgan, 1985; Monte and Hajjar, 2003).

Several investigators have focused their studies on characterizing the expression and activity of the proteins responsible for controlling excitation–contraction coupling as shown in Fig. 2: L-type Ca^{2+} channel (no change in HF), RYR2 (no change in HF), SERCA2a (decreased in HF), PLN (no change in HF), Kv4.3 (decreased in HF), and the Na^{+}/Ca^{2+} exchanger (decreased in HF)(Gwathmey and Davidoff, 1994). Our group has focused on the role of SERCA2a in the pathogenesis of HF. A decrease in SERCA 2a protein expression has been described by many groups (Beuckelmann *et al*., 1995; Hasenfuss *et al*., 1999; Schmidt *et al*., 1998; Schwinger *et al*., 1995). Subsequently several groups have enhanced SERCA activity in failing myocytes as a means to restore cardiac function in failing hearts. In 1999, we described a successful 7.5-fold increase of SERCA activity in rat neonatal cardiomyocytes obtained by adenovirus-mediated overexpression of SERCA2a (Hajjar *et al*., 1997) as well as on adult rat and human cardiac myocytes isolated from myocardium of end-stage explanted failing hearts (del Monte *et al*., 1999; Miyamoto *et al*., 2000). Interestingly, the improvement in calcium-ATPase activity noted in adult rats was not as high as what was observed in neonatal rat cardiomyocytes. The reason for this discrepancy may be attributed to a different expression pattern of the SERCA2a gene during development. Liu *et al*. reported that the presence of SERCA mRNA is significantly different during different developmental stages and, in particular, it gradually increases from the fetal period through adulthood (Liu *et al*., 2002). During the adult stage, therefore, the SR might be almost totally saturated with SERCA and even an increase of the message induced by gene transfer might not produce effects with the same magnitude observable in rat neonatal cardiomyocytes (Baartscheer, 2001).

The results obtained with reconstitution of SERCA2a in vitro have been reproduced in vivo and have demonstrated a significant improvement of cardiac contractility in several animal models of heart failure. In 2000, Miyamoto *et al*. reported improvements in the ventricular function in failing rat hearts 4 weeks after adenovirus-mediated SERCA2a gene transfer (Miyamoto *et al*., 2000).

Increasing inotropic function of the heart using pharmacological approaches failed and has been associated with an increase in patient mortality and an increased risk of sudden death. Reconstituting SERCA2a to near normal levels in our rat model of HF normalized left ventricle volume and cardiac function without the side effects that occur with conventional pharmacological inotropes. At least the short term survival in a rat model of HF increased significantly following SERCA2a restoration and the metabolic markers such as the phosphocreatine/ATP ratio were also improved (del Monte *et al*., 2001).

As an alternative approach, SERCA1 has been expressed in the rat heart. As previously mentioned, this isotype is normally not expressed in the cardiomyocytes. The rationale for SERCA1 overexpression was based upon the higher catalytic rate of the fast-twitching muscle form rather than the cardiac one. Problems were shown to be associated with this approach. Some reports suggested that cardiomyocytes derived from transgenic rats overexpressing SERCA1a show a lack of neuro-hormonal response probably due to a decreased expression and phosphorylation of PLN (Huke *et al*., 2002). Also, a high level of expression of the fast-twitching skeletal muscle pump can lead to cytotoxic effects and impairment of the contractile activity of myocardial cells (O'Donnell *et al*., 2001; Teucher *et al*., 2004).

PLN is considered as another attractive target for improving SERCA2a activity in HF. Several approaches have been explored including the use of PLN-mimicking antibodies, antisense sequences, and mutated forms of the molecule (del Monte *et al*., 2002a,b; Dieterle *et al*., 2005; Hoshijima *et al*., 2002; Ziolo *et al*., 2005). These studies emphasized the importance of maintaining the ratio of PLN/SERCA to sustain cardiac function both in animal models of HF and in isolated cells from end-stage cardiomyopathic patients. The importance of the combined regulation of SERCA-PLN in HF has recently been further

supported by a report from Kranias's group showing the action of a constitutively active form of the inhibitor I that can partially reverse the remodeling process and restore cardiac function by efficiently increasing the level of the phosphorylation of PLN (Pathak *et al.,* 2005).

To facilitate the extrusion of Ca^{2+} at the end of the systolic phase another approach would be to manipulate the activity of the Na^{+}/Ca^{2+} exchanger. This molecule has been found to be up-regulated in some HF patients, suggesting this as a possible adaptive mechanism to bypass the deficient activity of SERCA (Seth *et al*., 2004). Unfortunately, recent reports showed that the overexpression of this molecule can significantly reduce intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in cardiomyocytes leading to loss of contractile capacity (Bolck *et al*., 2004; del Monte *et al*., 2002a,b).

Since the first report of abnormal Ca^{2+} handling in animal and human myocardium (Gwathmey *et al*., 1987), impairment in Ca^{2+} handling is considered to be a major defect in HF. Several molecular targets to control SERCA pathway have been proposed by different investigators, some of which have been tested both in vitro and in vivo in animal models of HF. The next frontier will be translating this knowledge to clinical applications, where also more advances in the understanding of the molecular causes of HF and of the possible long-term effects of the proposed strategies are needed to improve the treatment of this disease.

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