SERCA in genesis of arrhythmias: what we already know and what is new?

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ABSTRACT

This review mainly focuses on the structure, function of the sarco(endo)plasmic reticulum calcium pump (SERCA) and its role in genesis of arrhythmias. SERCA is a membrane protein that belongs to the family of P-type ion translocating ATPases and pumps free cytosolic calcium into intracellular stores. Active transport of Ca^{2+} is achieved, according to the E1-E2 model, changing of SERCA structure by Ca^{2+}. The affinity of Ca^{2+} -binding sites varies from high (E1) to low (E2). Three different SERCA genes were identified-SERCA1, SERCA2, and SERCA3. SERCA is mainly represented by the SERCA2a isoform in the heart. In heart muscle, during systole, depolarization triggers the release of Ca^{2+} from the sarcoplasmic reticulum (SR) and starts contraction. During diastole, muscle relaxation occurs as Ca^{2+} is again removed from cytosol, predominantly by accumulation into SR via the action of SERCA2a. The main regulator of SERCA2a is phospholamban and another regulator proteolipid of SERCA is sarcolipin. There are a lot of studies on the effect of decreased and/or increased SERCA activity in genesis of arrhythmia. Actually both decrease and increase of SERCA activity in the heart result in some pathological mechanisms such as heart failure and arrhythmia. *(Anadolu Kardiyol Derg 2007: 7 Suppl 1; 43-6)*

Key words: sarco(endo)plasmic reticulum, SERCA, arrhythmia, calcium channels

Introduction

Cardiac physiology is a major area of research in basic and clinical medicine. Studying the molecular determinants of cardiac disorders becomes more important since a major portion of human ailments comprises cardiac diseases. In particular, disorders of the heart that derive from altered calcium homeostasis, such as, cytoplasmic calcium overload caused by abnormal calcium signaling is thought to be a common mechanism underlying some of these abnormalities (1).

In cardiac muscle, the sarcoplasmic reticulum (SR) plays a central role in the contraction and relaxation cycle by regulating the intracellular Ca^{2+} levels (2, 3). As shown in Figure 1 multitude channels are involved in intracellular Ca^{2+} regulation mechanism. The surface membrane (sarcolemma) is shown invaginating into the transverse tubule system which contains the junctions with the SR and dihydropyridine receptor (DHPR) L-type channels (4). The main role of Ca^{2+} ions entering the myocyte through the DHPR is activation of the ryanodine receptor (RYR) (4). In heart muscle, during systole, depolarization triggers Ca^{2+} entry into the cell via (DHPR) L-type Ca^{2+} channels. And the L-type Ca^{2+} channels triggers the release of Ca^{2+} from the sarcoplasmic reticulum via the ryanodine receptor channels (RYR2). This process, known as Ca^{2+} induced Ca^{2+} release, causes an increase in cytosolic contraction. Subsequently, Ca^{2+} binds to troponin C (TnC), which contains the binding sites for the Ca^{2+} and starts the cross-bridge movement of myofibrils resulting in force development and contraction (5). During diastole, muscle relaxation occurs as Ca^{2+} is again removed from cytosol, predominantly by accumulation into sarcoplasmic reticulum via the action of sarco(endo)plasmic reticulum Ca ATPase (SERCA). The SERCA uses hydrolysis of ATP as a source of energy for Ca transport from the cytosol into the lumen of SR (1, 6). In the SR, Ca^{2+} becomes bound to calsequestrin (CSQ), which is the major calcium binding protein in the sarcoplasmic reticulum (3). The relaxation is facilitated by 1) the SR Ca^{2+} ATPase (SERCA2), which pumps Ca^{2+} back into the SR and is primarily responsible for the myocardial relaxation (2, 5) 2) the Na+/Ca^{2+} exchanger (NCX), which uses the energy of the Na^{+} and Ca^{2+} gradients across the plasma membrane to exchange 3 extracellular Na^{+} ions for 1 intracellular Ca^{2+} ion (28%) and 3) the plasma-membrane Ca^{2+} ATPase (PMCA), which presses out Ca^{2+} from the cell using the energy liberated by ATP hydrolysis (5).

SERCA is a membrane protein that pumps free cytosolic calcium into intracellular stores, has been implicated in several cardiac disorders (7). SERCA, mainly represented by the SERCA2a isoform in the heart, an facilitates the storage and distribution of Ca^{2+} ions in the SR (8). Decreased sarco(endo)plasmic reticulum (SR) Ca^{2+}-uptake and decreased expression of the SR Ca^{2+}-ATPase, SERCA2a, are key features of cardiac myocyte dysfunction in both experimental and human heart failure (8, 9). In recent years, some authors used a genetic strategy to modify cellular calcium handling by overexpressing sarcoplasmic reticulum ATPase via an adenovirus vector similar to pharmacologic strategies for reducing cytosolic free calcium, such as calcium channel blockers and beta-blockers (10). Excessive Ca^{2+} delivery by SERCA overexpression carries the potential risk of cardiac arrhythmias, as well (7).

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In this brief review, we aimed to give an overview of recent advances in the rapidly growing field of factors modulating SERCA activity and arrhythmia.

The SERCA Pump Molecular structures of SERCA

The SERCA pump is an ~110-kDa transmembrane protein and belongs to the family of P-type ion translocating ATPases, which includes Na^+-K^+-ATPase and gastric H^+-K^+-ATPase among others, and are fundamental in establishing ion gradients by pumping ions across biological membranes. SERCA pumps calcium ion from the cytoplasm into the SR against a large concentration gradient (11-15). The SERCA Ca^{2+} pump protein consists of a single polypeptide chain folded into four major domains. The structure of SR Ca^{2+}-ATPase with two bound Ca^{2+} in the transmembrane (M) region, which consists of ten helices. The cytoplasmic part of Ca^{2+}-ATPase consists of three domains (A, actuator or anchor; N, nucleotide; and P, phosphorylation), well separated in this Ca^{2+}-bound form (13). Molecular cloning analyses identified three different SERCA genes, SERCA1, SERCA2, and SERCA3, which encode at least five Ca^{2+} pump isoforms (11, 15-17). SERCA1 and SERCA2 genes are different in their C-terminate (18). The SERCA1 gene encodes two alternatively spliced transcripts, SERCA1a and SERCA1b, which are exclusively expressed in skeletal muscle. SERCA1a is predominantly expressed in the adult stages; SERCA1b is mainly expressed in the fetal/neonatal stages. The SERCA2 gene encodes SERCA2a, SERCA2b and SERCA2c isoforms. The SERCA2a isoform is identical to SERCA2b except for its carboxyl terminate. The cardiac isoform of sarcoplasmic reticulum calcium-ATPase (SERCA2a) is the main regulator of cytosolic calcium, which is not only determining the electrical, but also the contractile, properties of myocardium (18). SERCA2b isoform is commonly expressed but is found at high levels in smooth muscle tissues (11, 16, 17). Quite recently, a new SERCA2c mRNA was described, and it is mainly expressed in cardiac and skeletal muscle, which exhibits functional similarities, but also functional differences. Relative to SERCA2a and SERCA2b, SERCA2c protein presents a distinct localization in left ventricle of normal hearts (17). The third gene, SERCA3, is expressed in a limited set of non-muscle cells and encoded as SERCA3b-SERCA3f in human, SERCA3b-SERCA3c in mice, and SERCA3b-SERCA3c in rat proteins. The SERCA3 isoforms are differently co-expressed in a variety of cells and tissues including muscle and non-muscle tissues (17).

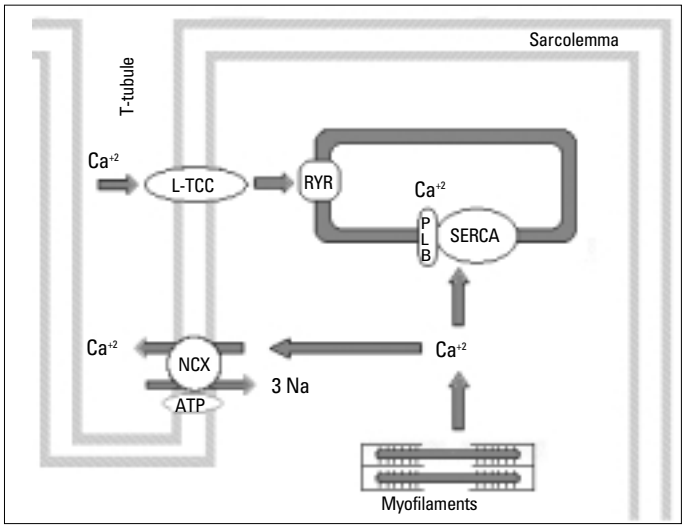

Figure 1. Major control points for calcium in cardiac myocytes. A small amount of calcium enters cells through the (DHPR) the L-type calcium channel (LTCC), triggering release of a much larger amount of calcium from the sarcoplasmic reticulum (SR) through ryanodine receptors (RyR). Most of the calcium is pumped back into the SR by SERCA, and the rest is extruded from the cell by the NCX. Calcium uptake by SERCA is regulated by the inhibitory protein phospholamban (PLB). (Modified from Abdelaziz AI. Untersuchungen zur Funktion der humanen atrialen essentiellen leichten Myosinkette (ALC-1) in einem transgenen Rattenmodell (dissertation). Berlin: Berlin Univ. 2004)

SERCA- sarco(endo)plasmic reticulum calcium pump

SERCA related Ca^{2+} transport cycle

Active transport of Ca^{2+} is achieved, according to the E1-E2 model, by changing the affinity of Ca^{2+} -binding sites from high (E1) to low (E2) (13).

As shown in Figure 2, the conformational change of SERCA by Ca^{2+} provides us a hint for the understanding of how the E2 form changes to the E1-Ca^{2+} form by Ca^{2+}, and the transport of Ca^{2+} into the lumen through the SR membrane (12).

In the E1 conformation, the two Ca^{2+} -binding sites are of high affinity and are facing the cytoplasm. In the E2 state the Ca^{2+} binding sites are of low affinity and are facing the luminal side. Either cytosolic ATP or Ca^{2+} can bind first to the E1 conformation. The $2Ca^{2+}$-E1-ATP form undergoes phosphorylation to form $2Ca^{2+}$-E1-P, the high energy phosphointermediate, in which the bound Ca^{2+} ions become occluded. This intermediate is also called the ADP-sensitive form, because in the presence of ADP the backward reaction occurs with release of the bound Ca^{2+} and synthesis of ATP. Conversion to the low energy intermediate is accompanied by a major conformational change to $2Ca^{2+}$-E2-P (ADP intensive form), whereby the Ca^{2+} binding sites are converted to a low affinity state and reorient towards the luminal face. The cycle ends with the sequential release of Ca^{2+} and phosphate and a major conformational change from the E2 to the E1 state (19).

Regulation of SERCA

The key role played by calcium pumps in controlling of cytoplasmic calcium ions, regulation of calcium pump activity will have profound effects on calcium signaling (12). The need for an accurate regulation of SERCA2's Ca^{2+} affinity is underscored by the existence of two membrane inserted regulator proteins phospholamban (PLB) and sarcolipin (SLN) (5).

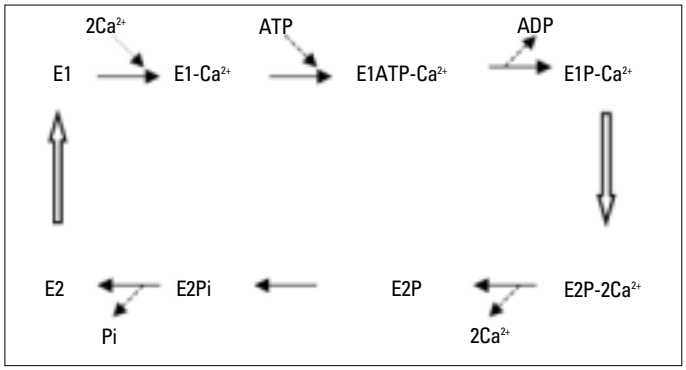

Figure 2. Scheme for the transport of calcium by SERCA (Modified from reference 12)

ADP- adenosin diphosphate, ATP- adenosin triphosphate
SERCA- sarco(endo)plasmic reticulum calcium pump

The main regulator of SERCA2a is phospholamban, which has been accepted as a key regulator of SERCA2a and cardiac contractility. It interacts with SERCA1a, SERCA2a and SERCA2b but not SERCA3. It is a 52-amino acid transmembrane protein and is expressed predominantly in cardiac muscle. Phospholamban monomers interact with SERCA2a and reversibly inhibit the Ca^{2+} transport activity of the pump. It interacts with SERCA molecules to lower the apparent affinity of SERCA2a for Ca^{2+} without altering its maximal pumping rate (12, 15, 20). At non resting Ca^{2+} concentrations, the binding of Ca^{2+} to the pump promotes the dissociation of the PLB/SERCA2a complex (8, 12, 15, 20).

Another proteolipid, which appears to be involved in the regulation of SERCA1 activity is sarcolipin. This 31-amino acid peptide co-localizes with SERCA1 and is most abundant in fast-twitch muscle (12). Indeed SLN and PLB are homologous proteins and members of the same gene family. They appear to bind to the same regulatory site in SERCA. Sarcolipin is superinhibitory compared with co-expression of SERCA1a and PLB (20, 21). Like PLB, SLN interacts with and inhibits SERCA by lowering its apparent Ca^{2+} affinity without pronounced effects on the maximal pumping rate (5). Sarcolipin has the ability to interact not only with SERCA1a but also with SERCA2a affecting their Ca^{2+}affinity to a similar extent and, also its shown that SLN expression may be prominent in the heart. As reported by Vangheluve et al, previously, SLN was considered to be the regulator of SERCA1a and hence the fast skeletal muscle counterpart of the SERCA2a inhibitor PLB in the heart. In humans, SLN mRNA is also found in the heart (5).

In heart failure, PLB and SERCA2a are both down-regulated so that Ca^{2+} stores are less effectively filled through the action of SERCA2a. As the Ca^{2+} store is depleted, the force of contraction is diminished. Since SLN might be involved in such pathological conditions, it is important to understand any potential that exists for the involvement of SLN in heart diseases (20).

The Ca^{2+}/calmodulin dependent protein kinase II (CaMKII) also is accepted as a mediator of SERCA2a (8). It is shown that phosphorylation of SERCA2a by CaMKII modulates the maximal activity of the SERCA2a without changing the apparent affinity of the pump. Hawkins et al (22) and Frank (8) reported that this phosphorylation is selective and comes into being in the cardiac and smooth muscle SR while not in the skeletal muscle.

Phosphorylation of PLB by protein kinase A and/or Ca^{2+}/calmodulin kinase II relieves the inhibition of the pump and stimulates Ca^{2+} uptake activity (5, 15).

SERCA and cardiac arrhythmias

It is reported that, 50% and 80% of deaths in patients suffering from various kinds of heart diseases are caused by cardiac arrhythmias (23). Cardiac arrhythmias mostly occur in diseased hearts as a result of an abnormality in ion channels. Over the past three decades, the researchers have been focused on the role of abnormal calcium signaling in the genesis of cardiac arrhythmias (24). Insufficient calcium delivery to the myofilaments causes a weak contraction, while excessive calcium delivery carries the risk of activation of proteases and other maladaptive calcium-sensitive pathways that lead to cell death, and can result with the generation of pathological membrane currents. In many studies conducted on human and animal models, altered Ca^{2+} homeostasis in cardiac cells has been evaluated as a common finding in heart diseases.

Decreased SERCA uptake and decreased expression of the SERCA2a, cause cardiac myocyte dysfunction (9). Among the most documented alterations in Ca^{2+} homeostasis is a decrease in SERCA function, caused by a decrease in SERCA protein and/or activity resulting from a relative increase of phospholamban. These changes bring the alterations in intracellular Ca^{2+} cycling and damaged cardiac function (7).

On the other hand, Chen et al. (9) had shown that transgenic SERCA2a overexpression increased the risk of acute arrhythmias and sudden death in rats (Fig.3). Some of suggested consequences during overexpression of SERCA are as following: i) SERCA2a overexpression may improve Ca^{2+} handling but also induce arrhythmias due to immediate Ca^{2+} reuptake before troponin C binding can occur (25). ii) Overexpressing SERCA dramatically alters the balance between the major calcium- handling proteins. Like digoxin, SERCA overexpression favors sequestration of calcium by the SR instead of being extruded by the NCX (1). A larger SR store will initially lead to an increase in the calcium transient, autoregulation is ensured by (a) more rapid inactivation of subsequent calcium currents and, therefore, (b) reduced calcium entry through L-type calcium channels. The net effect is to reduce transsarcolemmal calcium flux while maintaining a normal systolic transient (26). iii) Increases in SERCA protein abundance result in an increased SERCA2a load. The SERCA Ca^{2+} overload may produce spontaneous Ca^{2+} releases and thereby lead to ectopic activity. iv) Elevated intracellular Ca^{2+} may also close gap junctions, decreasing cell-to-cell coupling, and thereby decreasing action potential conduction directly provoking arrhythmias (27).

Conclusion

Actually both decrease and increase of SERCA activity in the heart raised many interesting questions resulting in a variety of pathological manifestations including contractile dysfunction and electrical instability. Nevertheless, we can view this as yet another motivation for us to return to the fundamental mechanism of heart failure and arrhythmia in search for better therapeutic approaches.

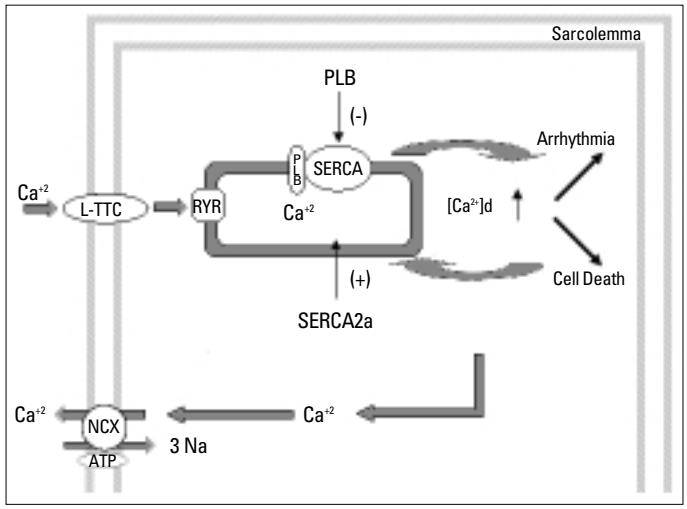

Figure 3. Overexpression of SERCA2a may increased the risk of acute arrhythmias (Modified from reference 1)

ATP- adenosin triphosphate, NCX- $Na+/Ca^{2+}$ exchanger, PLB- phospholamban, RYR- ryanodine receptor, SERCA- sarco(endo)plasmic reticulum calcium pump

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