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Mini Review

Membrane associated Ca^{2+} buffers in the heart

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Ca^{2+} is a universal signalling molecule that affects a variety of cellular processes including cardiac development. The majority of intracellular Ca^{2+} is stored in the endoplasmic and sarcoplasmic reticulum of muscle and non-muscle cells. Calreticulin is a well studied Ca^{2+}-buffering protein in the endoplasmic reticulum, and calreticulin deficiency is embryonic lethal due to impaired cardiac development. Despite calsequestrin being the most abundant Ca^{2+}-buffering protein in the sarcoplasmic reticulum, viability is maintained in embryos without calsequestrin and normal Ca^{2+} release and contractile function is observed. The Ca^{2+} homeostasis regulated by the endoplasmic and sarcoplasmic reticulum is critical for the development and proper function of the heart. [BMB reports 2010; 43(3): 151-157]

INTRODUCTION

The endoplasmic reticulum (ER) is a multifunctional intracellular organelle that plays a critical role in many cellular processes. The ER is responsible for protein synthesis, folding and post-translational modification; the synthesis of phospholipids and steroids; and is the major storage organelle of intracellular Ca^{2+}. As a universal signalling molecule, Ca^{2+} influences key biological functions including fertilization, development, cardiac contraction and secretion of neurotransmitters and hormones (1, 2). In addition, many molecular chaperones and folding enzymes are located in the lumen of the ER to assist in the proper folding of newly synthesized proteins (3-6). The sarcoplasmic reticulum (SR) of cardiomyocytes is an internal membrane system responsible for the regulation of excitation-contraction coupling. Calsequestrin (CASQ) is a major Ca^{2+}-buffering protein of SR (8). The SR membrane can be classified into two structural and functional domains, the longitudinal SR and the junctional SR (7). The longitudinal SR of cardiac cells consists of many tubules interconnected with each other and forming a network. Enriched in the Ca^{2+}-transport ATPase (SERCA) responsible for rapid removal of Ca^{2+} from the cytoplasm, the junctional SR of cardiac cells causes muscles to relax. In contrast, the ryanodine receptor (RyR) found in the terminal cisternae is responsible for Ca^{2+} release to the cytoplasm and consequently muscle contraction. The cardiac muscle cell is the most physically energetic cell in the body, contracting constantly 3 billion times or more in an average human lifespan. Albeit the cardiomyocyte is a specialized cell type of the heart, it also requires the fundamental ER housekeeping functions to maintain cellular physiology. Protein synthesis and/or secretion in cardiomyocytes is/are influenced by changes in the intracellular Ca^{2+} concentration. The ER in cardiomyocytes is involved in continuous turnover and synthesis of many membrane proteins including ion channel gap junction components, cell surface receptors involved in signal transduction, and cell-cell or cell-extracellular matrix interactions. Some ER-associated proteins have been identified in cardiac muscle, including calreticulin, Grp94, BiP, and protein disulphide isomerase (PDI) (9-11). These proteins affect the Ca^{2+} storage capacity of the ER lumen and are involved in every aspect of the ER function. The purpose of this review is to consider the roles of Ca^{2+} and Ca^{2+} buffer in cardiac function.

Ca^{2+} homeostasis

It is well documented that changes in cytosolic Ca^{2+} concentrations affect many intracellular signalling pathways and influence a diverse range of cellular functions (12). Ca^{2+} directly manipulates gene expression; protein and steroid synthesis; and modification, folding and secretion of proteins (13). Ca^{2+} signalling also influences embryogenesis, membrane excitability, learning and memory (14). Extracellular Ca^{2+} concentration is in excess of 2 mM, free cytoplasmic Ca^{2+} concentration is approximately 100 nM, total ER Ca^{2+} concentration is up to 1 mM and the free ER Ca^{2+} concentration is approximately 200 μM. The large portion of Ca^{2+} in the ER lumen is unbound. Changes in the free Ca^{2+} concentration affects protein synthesis and secretion, the interaction between ER chaperones and their substrates (5, 15), the activation of Ca^{2+} influx via channels in plasma membrane (16), and the unfolded protein response (UPR) during ER stress (17). During agonist stimulation, Ca^{2+} release from and uptake by the ER creates continuous fluctuations in the free Ca^{2+} concentration, from as high as 400 μM to as low as 1 μM (18). Fluctuations of the ER luminal Ca^{2+} concentration also result in impaired ER-Golgi

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protein trafficking (19), impeded transport of molecules across the nuclear pore (20) and disrupted chaperone function (21). ER Ca^{2+} homeostasis and signalling are maintained by controlling Ca^{2+} release from the ER by the inositol 1,4,5-triphosphate receptor ($InsP_3R$) and RyR, whereas the ER Ca^{2+} stores are refilled by the SERCA. Ca^{2+} present in the ER stores serve as a source of easily releasable Ca^{2+}, but is also important as a regulator of a number of ER enzymes and proteins, including regulation of the $InsP_3R$, RyR, and SERCA. Release of Ca^{2+} from the ER during Ca^{2+} signalling triggers a distinct event at the plasma membrane, termed SOCI (store-operated Ca^{2+} influx), which is responsible for providing the Ca^{2+} necessary for refilling the ER stores after Ca^{2+} signalling. A protein located at the membrane of the ER, Stim1 (stromal cell-surface molecule 1), has been identified as a sensor of ER luminal Ca^{2+}, which transmits this information to the plasma membrane, Orai 1, a Ca^{2+} transporter that regulates SOCI (22). Interestingly, calreticulin over-expressing fibroblasts demonstrate disrupted SOCI (23-25), owing to a decrease in the ER Ca^{2+} release, demonstrating the involvement of calreticulin in the regulation of SOCI. With over-expression of calreticulin, there may be reduced level of free Ca^{2+} available to bind to the EF-hand of Stim 1.

Ca^{2+} buffering in the ER and SR

A number of Ca^{2+}-buffering proteins that are responsible for binding ER luminal Ca^{2+} and being involved in numerous aspects of ER function reside within the ER lumen. Many of these proteins display high Ca^{2+} binding capacity (10 mol of Ca^{2+} per mol of protein) and low affinity (K_d = 1 μM). A widely known Ca^{2+} binding protein in the ER lumen, calreticulin contains a high affinity (K_d = 1 μM) and low capacity (1 mol of Ca^{2+} per mol of protein) binding site contained in the proline-rich domain with a potential EF-hand like helix-loop-helix motif (26). Interestingly, over-expression of calreticulin leads to an increased amount of Ca^{2+} in the ER intracellular stores (23-25), whereas calreticulin-deficient cells have reduced Ca^{2+}-storage capacity in the ER and delayed agonist-mediated Ca^{2+} release (27, 28). Grp94 is another abundant Ca^{2+}-buffering protein in the ER lumen, constituting 5-10% of total ER luminal proteins (4, 29). Grp94 contains an acidic C-terminal region which comprises its low affinity Ca^{2+} binding site. It contains 19 Ca^{2+} binding sites, 4 of which have high affinity (K_d = 2 μM) and low capacity (1 mol of Ca^{2+} per mol of protein), and 15 of which have low affinity (K_d = 600 nM) but high capacity (10 mol of Ca^{2+} per mol of protein). BiP is a 78-kDa ER luminal Ca^{2+} binding protein (30, 31). BiP has a relatively low capacity for binding Ca^{2+} (1-2 mol of Ca^{2+} per mol of protein), but it may contribute as much as 25% of the total Ca^{2+} storage capacity of the ER (31). The PDI family of oxidoreductase proteins is also involved in buffering a large portion of ER luminal Ca^{2+}. PDI is a 58-kDa ER resident Ca^{2+} buffering protein and has a high capacity Ca^{2+} binding site (20 mol of Ca^{2+} per mol of protein), but only with weak affinity (K_d = 2-5 mM) (32). PDI is highly expressed and widely distributed throughout the ER. ERp57, a 57-kDa protein (33), carries out disulfide bond exchange in complex with calreticulin and calnexin, a 90-kDa ER transmembrane protein (34).

CASQ is the most abundant Ca^{2+}-buffering protein in the SR, but only a minor component of the ER (35). The protein binds approximately 50 mol of Ca^{2+} per mol of protein with low affinity (K_d) = 1 mM). The Ca^{2+} binding site in CASQ is composed of a stretch of 45 acidic negatively charged amino acids located in the C-terminus of the protein (36). Associated with triadin and junctin in the lumen of the SR, CASQ modulates the RyR function (37). CASQ plays a major role in buffering free Ca^{2+} inside the SR and is responsible for the control of excitation-contraction coupling in the cardiac cell. Sarcolumenin, a histidine-rich protein, junctin, junctate, and triadin are unique to the SR membrane and are either specialized in Ca^{2+} buffering or provide a crucial structural support for Ca^{2+} transport and buffering molecules (38).

What is calreticulin?

Calreticulin is a 46-kDa Ca^{2+}-binding protein and molecular chaperone in the ER lumen. The protein contains an ER retention signal sequence (KDEL) and consists of three functional domains, a globular N-domain, a P-domain, and an acidic C-domain (46). The N-domain contains the carbohydrate binding site (39), the zinc binding site (40), and the disulfide linkage (41). The P-domain is composed of a flexible, extended, finger-like region that interacts with ERp57 (42-45). In conjunction with the N-domain, the P-domain may form a functional protein folding module. The C-domain contains 19 negatively charged acidic amino acid residues that can bind Ca^{2+} with high capacity (25 mol of Ca^{2+} per mol of protein) and a relatively low affinity (K_d = 2 mM) (26). Calreticulin affects Ca^{2+} storage capacity of the ER lumen (24, 47), modulates function of the $InsP_3R$ and SERCA2b (27, 48, 49), and plays a role in the integrin-mediated Ca^{2+} signalling (50). Together with ERp57 and calnexin, a 90-kDa ER integral membrane protein, calreticulin is involved in the chaperoning of nascent polypeptides (51). Cells derived from calreticulin-deficient embryos have impaired Ca^{2+} handling ability as well as compromised protein folding and quality control (27, 45, 52). Ca^{2+} stored in the ER and Ca^{2+}-dependent signalling pathways are significantly affected in the absence of calreticulin. The protein has been implicated to play in other cellular functions including regulation of gene expression, cell-cell adhesion, and wound healing (53).

What is calsequestrin?

CASQ is a well known Ca^{2+}-binding protein in the SR of the skeletal and cardiac muscle. This protein is encoded by the skeletal (CASQ1) and cardiac (CASQ2) calsequestrin genes

(54, 55). The adult fast-twitch skeletal muscle expresses exclusively the Casq1 gene, whereas slow-twitch skeletal muscle expresses mainly the Casq1 gene (>75% of total) and to a minor extent the Casq2 gene (>25% of total). Cardiac muscle expresses exclusively the Casq2 gene. Neither of the CASQ isoforms is present in non-muscle tissues nor in smooth muscle (56). CASQ is highly acidic, containing up to 50 Ca^{2+} binding sites, which are formed by the clustering of two or more acidic residues. Each molecule of CASQ2, a 415 amino acid polypeptide, binds 40-50 Ca^{2+} with low affinity (K_d = 1 mM), allowing cardiac SR Ca^{2+} concentrations to approach 20 mM while free Ca^{2+} concentrations are only 1 mM (57). CASQ2 also participates in regulating SR Ca^{2+} release (58). With junctin and triadin, CASQ2 binds to the RyR to form the SR luminal RyR Ca^{2+} release channel complex, called tetrad (59).

Ca^{2+} and cardiac diseases

Heart failure is a complex pathophysiologic state in which delivery of blood and nutrients is inadequate for tissue requirements. Disturbance of Ca^{2+} homeostasis in the ER and/or SR is one of the causes that leads to heart failure. Mutations in genes encoding the ER proteins including calreticulin and Grp94 affected cardiac development and function, and have a role in congenital heart disease (60). Calreticulin deficiency is embryonic lethal between 12.5 and 14.5 days post-coitum, and the embryos showed significantly decreased ventricular wall thickness and deep intertrabecular recesses in the ventricular wall (Table 1) (27). Electron microscopic analysis revealed wavy and thin myofibrils in the ventricles of calreticulin-null embryos, and there is also deficient intercalated disc formation in the heart of calreticulin-null mice (61). Intercalated discs are adheren-type junctions of cardiac muscle and contain vinculin, N-cadherin, and catenines (62). Interaction between N-cadherin and β-catenin are crucial for cardiomyocyte differentiation and myofibrillogenesis (63). Calreticulin also affects expression of N-cadherin and vinculin and consequently, cell adhesion (64, 65). Changes in expression of adhesion molecules in calreticulin-deficient heart might contribute to the developmental abnormalities caused by the absence of calreticulin. Over-expression of calreticulin in the heart cause bradycardia, complete heart block, and sudden death in mice, characterized by dilated ventricular chamber and atria, thinner ventricular walls and disarray of cardiomyocytes (Table 1) (66). Interestingly, mice over-expressing calreticulin in the heart have a similar phenotype to the complete congenital heart block seen in children, and analysis of transgenic mice results in disruptive cardiac signalling including connexion 43, a component of gap junctions, and Mef2c (66). Grp94, another ER luminal Ca^{2+} binding protein, knockout mice die *in utero* owing to impaired cardiac development. CASQ2 is a critical SR luminal Ca^{2+} storage protein essential for SR Ca^{2+} release in mammalian heart. Surprisingly, Casq2-null mice are viable and display normal Ca^{2+} release and contractile function but in-

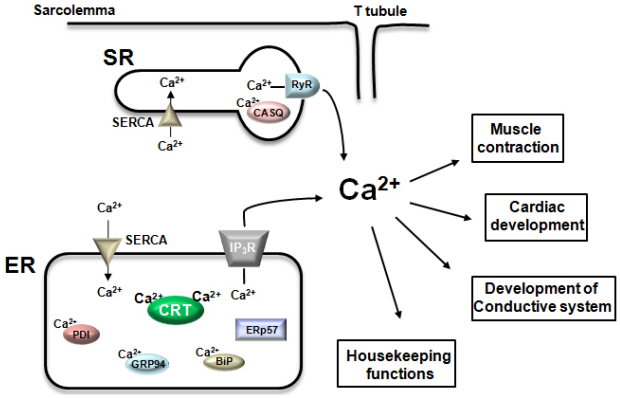

Fig. 1. Cardiac muscle cell contains both sarcoplasmic reticulum (SR) and endoplasmic reticulum (ER). The SR is responsible for excitation-contraction coupling of cardiomyocytes. For contraction, Ca^{2+} is released from the SR through the ryanodine receptor (RyR) Ca^{2+} channel and then Ca^{2+} is taken up by the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) of the longitudinal SR domains causing relaxation. Cardiac muscle cells may also contain ER. Ca^{2+} fluxes from the ER are required for cardiac development and housekeeping functions of cardiomyocytes. In the lumen of the ER, Ca^{2+} is stored bound to Ca^{2+} binding proteins including calreticulin (CRT), protein disulfide isomerase (PDI), Glucose regulated protein 94 (Grp94), Immunoglobulin binding protein (BiP), and ERp57. These proteins play a critical role in folding and posttranslational modification of newly synthesized proteins.

Table 1. Physiological roles of calreticulin and calsequestrin in the heart

Gene	Expression	Ca^{2+} buffering	Mouse model
Calreticulin	Loss-of-function	Reduced free Ca^{2+} concentration in the ER lumen (27)	Embryonic lethal caused by impaired cardiac development (27)
	Gain-of-function	Increased free Ca^{2+} concentration in the ER lumen (22)	Complete heart block and followed sudden death (66)
Calsequestrin	Loss-of-function	Maintains functional SR Ca^{2+} storage and increased diastolic SR Ca^{2+} leak (67)	Viable and display normal Ca^{2+} release and contractile function (67)
	Gain-of-function	Increased SR Ca^{2+} content but reduced the gain of E-C coupling (71)	Cardiac hypertrophy and increase in heart mass (70)

creased SR volume (Table 1) (67). Comprehensive evaluation of cardiac function and structure in the Casq2-null mice generates some new insights. First, CASQ2 is not essential for providing sufficient Ca^{2+} storage for normal function of cardiac muscle. Second, CASQ2 prominently modulates SR Ca^{2+} release but is not required for luminal SR Ca^{2+} sensing (67). Human patients with catecholaminergic polymorphic ventricular tachycardia (CPVT) caused by CASQ2 mutation display interestingly normal cardiac contractile function (68). Transgenic mice that over-expressed CASQ2 survive into adulthood but show severe cardiac hypertrophy with a two-fold increase in heart mass and cell size (69, 70). The CASQ2 over-expressed cardiomyocytes show increased Ca^{2+} content but reduced the gain of excitation-contraction coupling (Table 1) (71).

ER vs. SR in the heart

The heart is the first organ to form in the embryo and all subsequent events in the life of the organism depend on its function. Excitation-contraction coupling is the main task of the cardiac muscle cell and is totally depends on intracellular Ca^{2+} fluctuation. The role of the ER and SR in the regulation of Ca^{2+} homeostasis in cardiomyocytes is presented in Fig. 1. Ca^{2+} derived from the CASQ-containing SR is responsible for the regulation of muscle contraction, and Ca^{2+} released from the calreticulin-containing ER is involved in housekeeping functions, cardiac development, and conductive system formation in the heart. The important message we learn from the calreticulin-null mouse is that the ER and SR may be functionally separate organelles in cardiac cells. Calreticulin- deficient cardiomyocytes develop a functional SR and contract spontaneously, but calreticulin-deficient fibroblasts have impaired Ca^{2+} homeostasis (27). ER resident chaperones are highly expressed in the developing myocardium (60) and genes encoding ER resident proteins are also activated in cardiac hypertrophy (72). Immunostaining for ER resident proteins such as calnexin, BiP, PDI, and ribophorin II revealed their unique localization along the sarcomere I band and junctional SR in cardiac muscle cell (73). However, all these proteins have a localization different from that of SERCA and junctional SR proteins (74, 75), suggesting that the SR and ER might be structurally and functionally unique in the heart. Ca^{2+} homeostasis and regulation have received a lot of attention by basic scientists and clinical cardiologists owing to its association with cardiac pathologies. The biochemical studies on ER and SR proteins interrelated with the heart has revealed that Ca^{2+} binding proteins play a critical role in cardiac development and function. Most importantly the ER compartment is not only involved in protein synthesis, modification and secretion, but in control of intracellular Ca^{2+} homeostasis in cardiac cell. To solve the contribution of ER proteins and the ER-associated signalling pathway to cardiac pathology, a combination of basic studies and clinical trials will be required.

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