CHAPTER 20

CALCIUM AND CARDIOMYOPATHIES

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Abstract: Regulation of Calcium (Ca) cycling by the sarcoplasmic reticulum (SR) underlies the control of cardiac contraction during excitation-contraction (E-C) coupling. Moreover, alterations in E-C coupling occurring in cardiac hypertrophy and heart failure are characterized by abnormal Ca-cycling through the SR network. A large body of evidence points to the central role of: a) SERCA and its regulator phospholamban (PLN) in the modulation of cardiac relaxation; b) calsequestrin in the regulation of SR Ca-load; and c) the ryanodine receptor (RyR) Ca-channel in the control of SR Ca-release. The levels or activity of these key Ca-handling proteins are altered in cardiomyopathies, and these changes have been linked to the deteriorated cardiac function and remodeling. Furthermore, genetic variants in these SR Ca-cycling proteins have been identified, which may predispose to heart failure or fatal arrhythmias. This chapter concentrates on the pivotal role of SR Ca-cycling proteins in health and disease with specific emphasis on their recently reported genetic modifiers

Keywords: calcium, sarcoplasmic reticulum, cardiomyopathy, mutations

1. INTRODUCTION

Excitation-contraction coupling in cardiac myocytes is initiated by the cardiac action potential (AP), where depolarization-activates an inward Ca current (I_{Ca}), that is called the Ca-trigger, as it promotes the sarcoplasmic reticulum (SR) Ca release. The combination of I_{Ca} and SR Ca release raises intracellular free [Ca] ($[Ca]_i$), allowing Ca to bind to the myofilament protein troponin C, which activates contraction. For

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relaxation to occur $[Ca]_i$ declines, causing Ca dissociation from troponin C. This $[Ca]_i$ decline is due to transport from the cytosol by four pathways: 1) SR Ca-ATPase; 2) sarcolemmal Na/Ca exchange (NCX); 3) sarcolemmal Ca-ATPase; and 4) mitochondrial Ca uniport. The most prominent of these is the SR Ca-ATPase. For the myocyte to be in a steady state with respect to Ca balance, the amount of Ca extruded from the cell during relaxation must be the same as the amount of Ca entry at each beat. Likewise, the amount of Ca released from the SR must equal that re-accumulated by the action of the SR Ca-ATPase.

During heart failure (HF), functional expression of different proteins involved in E-C coupling is altered, and these changes contribute to altered Ca transients, contractility and arrhythmias in HF. Furthermore, genetic mutations in the key Ca-cycling proteins have been recently identified, which contribute to heart failure and fatal arrhythmias. This chapter will concentrate in recent studies on genetic modifiers of cardiac function at the level of the sarcoplasmic reticulum.

2. SR CALCIUM-CYCLING

During cardiac relaxation, Ca is transported into the SR lumen by the SR Ca-ATPase, which is under reversible regulation by phospholamban (PLN). Dephosphorylated PLN binds to SERCA2a and inhibits the enzyme's apparent Ca-affinity. However, phosphorylation of PLN relieves the Ca-ATPase inhibition and enhances Ca-sequestration, associated with increased relaxation rates and contractility *(Simmerman et al., 1998; MacLennan et al., 2003)*. *In vivo*, PLN is phosphorylated by both cAMP-dependent and Ca-CaM-dependent protein kinases (PKA and CaMK) during β-adrenergic stimulation *(Kranias, et al., 1982; Wegener, et al., 1989; Talosi, et al., 1993; Lindemann, et al., 1983; Garvey, et al., 1988; Mundina-Weilenmann, et al., 1996)*. PLN is the major phosphoprotein mediating the positive inotropic and lusitropic effects of β-adrenergic receptor (β-AR) agonists *(Wegener, et al., 1989; Talosi, et al., 1993; Lindemann, et al., 1983; Garvey, et al., 1988; Mundina-Weilenmann, et al., 1996)*. Reversal of PLN phosphorylation occurs by the SR-associated type 1 phosphatase, which is regulated by an endogenous inhibitor-1 protein *(Kranias, et al., 1988)*.

Initiation of contractions occurs when a Ca-trigger through the outer cell membrane induces SR Ca-release through the RyRs (SR Ca release channels), which are coupled to other proteins at the luminal SR surface (triadin, junctin and calsequestrin) *(Zhang, et al., 1997)*. This quaternary Ca-signaling complex participates in both intra-SR Ca buffering and modulation of the Ca release process. Termination of SR Ca release most likely includes RyR inactivation (or adaptation) and a partial decline in $[Ca]_{SR}$. When the SR Ca load is elevated, it enhances the fraction of SR Ca that is released due to stimulation of RyR open probability *(Bassani, et al., 1995; Shannon, et al., 2000)* . In addition to effects of $[Ca]_{SR}$ on fractional release in response to Ca current during E-C coupling, elevation of $[Ca]_{SR}$ also increases the probability of spontaneous SR Ca release events that can propagate through the

myocyte as Ca waves and activate aftercontractions, transient inward current and delayed afterdepolarizations that are arrhythmogenic *(Bers, DM, 2001)*.

2.1. Regulation of SR Calcium-Cycling by Phospholamban

The functional significance of PLN in cardiac muscle has been elucidated through the generation of mouse models with altered PLN expression levels. Heterozygous (40% of PLN) and homozygous (no PLN) for PLN deficiency mice *(Luo, et al., 1994; Luo, et al., 1996)* indicated that the decreases in PLN levels were associated with a linear increase in the affinity of SERCA2a for Ca, *(Luo, et al., 1996)* and with a linear increase in contractile parameters of isolated cardiomyocytes, perfused hearts and intact mice *(Luo, et al., 1996; Wolska, et al., 1996; Li, et al., 1998; Lorenz, et al., 1997)*. The hyperdynamic cardiac function of PLN null hearts could be minimally stimulated by β-AR agonists. Furthermore, there were no effects of aging on the hyperdynamic cardiac function and there was no compromise of exercise performance *(Desai, et al., 1999)*. On the other hand, cardiac overexpression (two-fold) of PLN was associated with significant inhibition of cardiac function *(Kadambi et a., 1996; Dash et al., 2001)*. The inhibitory effects of PLN overexpression could be reversed by β-AR agonist stimulation, which resulted in phosphorylation of the increased PLN levels. These findings in genetically altered models indicate that PLN is a major regulator of basal cardiac Ca^{2+} cycling and contractile parameters (Figure 1). PLN is also a key determinant of β-AR agonist responses. Furthermore, only a fraction of the SERCA2 molecules in cardiac SR are functionally regulated by PLN *in vivo*.

2.2. Regulation of Cardiac Function by β-Adrenergic Receptor Signalling

β-adrenergic receptor stimulation of the heart increases cardiac contractility through enhanced Ca-cycling. The major substrates for the cAMP-PKA axis include PLN, L-type Ca channels, RyR, troponin I and myosin binding protein C (Figure 2). The relaxant effect of PKA is mediated mainly by phosphorylation of PLN and troponin I. PLN phosphorylation speeds up SR Ca reuptake, while phosphorylation of troponin I speeds up dissociation of Ca from the myofilaments.

Current evidence indicates that PLN phosphorylation appears to be dominant over troponin I phosphorylation *(Li, et al., 2000)*. The faster SR Ca uptake by phosphorylated PLN also contributes to increased SR Ca load, which is available for subsequent release, resulting in an inotropic effect. The increased I_{Ca} by PKA activation also contributes to the inotropic effects of the β-AR agonists. The myofilament effects of PKA appear to be almost entirely attributable to troponin I phosphorylation (vs. myosin binding protein C) because substitution of troponin I with a non-phosphorylatable troponin I abolishes myofilament effects of PKA *(Kentish, et al., 2001; Pi, et al., 2002)*.

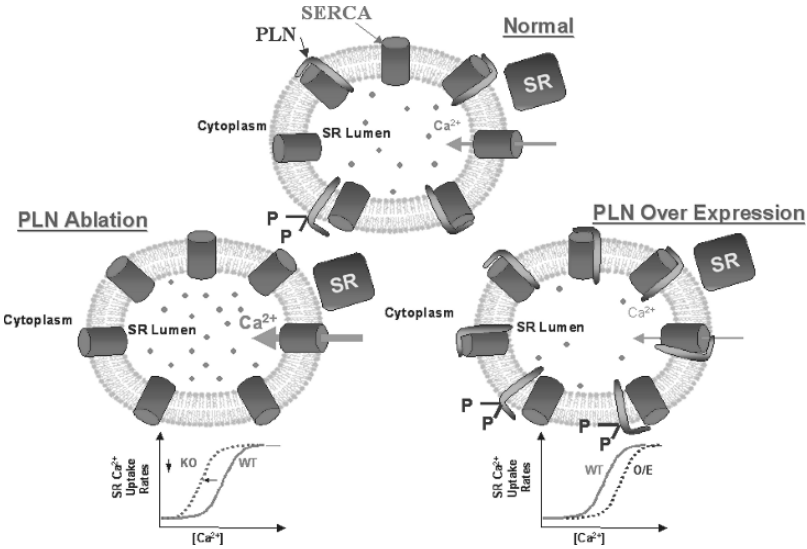

Figure 1. Schematic representation of PLN regulation of the SR Ca-ATPase Ca-affinity, which reflects altered SR Ca-load (green dots in SR) (See Colour Plate 24)

RyR phosphorylation by PKA also alters its open probability. In recordings of single RyR channels in lipid bilayers, PKA treatment enhanced the immediate RyR opening in response to a very rapid $[Ca]_i$ rise (meant to simulate I_{Ca} activation), but it decreased the steady state open probability at a given $[Ca]_I$ *(Valdivia, et al., 1995)*.

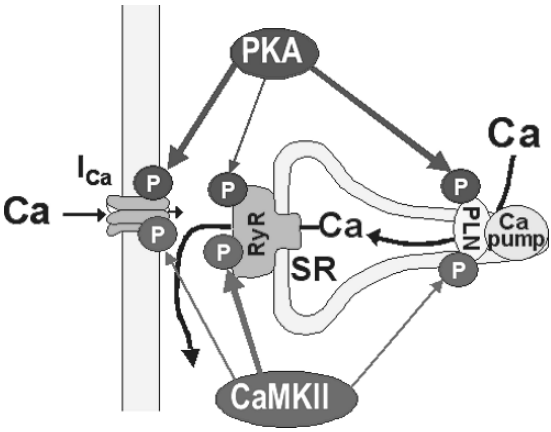

Figure 2. Both PKA and CaMKII have common molecular targets in E-C Coupling (I_{Ca}, RyR and PLN), The specific amino acids that are targets for phosphorylation (P) differ between PKA and CaMKII, as well as the intensity of functional regulation (indicated by arrow thickness). Both kinases may bind directly to the RyR and L-type Ca channel (via an anchoring protein for PKA) (See Colour Plate 25)

In contrast, Marx, *et al.*, *2000,* found that PKA enhanced steady state RyR open probability in bilayers, attributing this to RyR phosphorylation and consequent release of FKBP-12.6 from the RyR. PKA effects on diastolic RyR function remain equivocal because several groups have not found FKBP dissociation from RyR upon PKA-dependent phosphorylation, and Li, *et al.*, *2002*, found no effect of PKA-dependent RyR phosphorylation on Ca spark frequency in intact or permeabilized PLN-knockout myocytes (where SR Ca load was not increased) *(Li*, *et al.*, *2002; Stange*, *et al.*, *2003; Xiao*, *et al.*, *2004)*. During E-C coupling PKA effects on RyR are also somewhat mixed, and are generally complicated by simultaneous enhancement of I_{Ca} and SR Ca-ATPase and SR Ca content upon PKA activation. In a systematic E-C coupling voltage clamp study, where I_{Ca} and SR Ca content were controlled, PKA was found not to alter the amount of SR Ca released, but to increase the initial and maximal rate of Ca release and speed the shut-off of Ca release *(Ginsburg*, *et al.*, *2004)*.

2.3. Regulation of SR by Ca-Calmodulin Dependent Protein Kinase

Parallel to the long-studied regulation of I_{Ca}, SR Ca-ATPase/PLN and RyR by PKA-dependent phosphorylation (Figure 2), these three key targets are also phosphorylated by Ca-Calmodulin dependent protein kinase (CaMKII), and the phosphorylation occurs at different molecular sites *(Maier*, *et al.*, *2003)*. CaMKII is responsible for Ca-dependent facilitation of Ca current, which may contribute somewhat to the positive force-frequency relationship in heart *(Yuan*, *et al*, *1994; Xiao*, *et al.*, *1994; Anderson*, *et al.*, *1994)*. However, this is a quantitatively small stimulation of I_{Ca} compared to that produced by PKA activation. PLN phosphorylation (at Thr-17) by CaMKII also increases SR Ca-ATPase activity similar to PKA-dependent phosphorylation of Ser-16 on PLN. While sympathetic stimulation enhances phosphorylation at both of these sites, PKA-dependent phosphorylation seems to be functionally predominant *(Luo*, *et al.*, *1998)*.CaMKII also phosphorylates the RyR and appears to strongly activate SR Ca release, both during diastole and during E-C coupling *(Li*, *et al.*, *1997; Guo*, *et al.*, *2006)*. Both PKA and CaMKII are likely to be co-activated during normal sympathetic stimulation, creating synergy between these important regulatory signaling pathways.

3. HEART FAILURE (HF)

A major characteristic of human and experimental HF is depressed Ca-cycling in the cardiac myocyte. The differences in Ca-cycling and contractility between non-failing and failing myocytes are mainly observed at high heart rates and the force-frequency relationship is generally less positive in failing vs. non-failing hearts. This is mainly attributed to depressed SR Ca-transport and SR Ca content, as suggested by findings on alterations of the protein levels or activity of the key Ca-cycling proteins (e.g. I_{Ca}, SR Ca-ATPase, Na/Ca exchange, myofilament Ca sensitivity), *(Hasenfuss*, *et al.*, *1998; Richard*, *et al.*, *1998; Mukherjee*, *et al.*, *1998;*

Wickenden, et al., 1998; Nabauer, et al., 1998; Phillips, et al., 1998; de Tombe, et al., 1998; Houser, et al., 2000) and direct cellular measurements of both SR Ca-ATPase function and SR Ca content *(Pogwizd, et al., 2001; Hobai, et al., 2001; Piacentino, et al., 20003)*.

3.1. SERCA2A and PLN

Most reports indicate that the SR Ca-ATPase is functionally decreased in almost all HF models. However the PLN levels are not altered in HF, indicating decreased Ca-affinity of the SR Ca transport system *(Dash, et al., 2001)*. There are also data to suggest that the phosphorylation state of PLN may be reduced in HF *(Richard, et al., 1998; Huang, et al., 1999; Schwinger, et al., 1999)*. This would further reduce the [Ca]-sensitivity of SR Ca uptake and further slow Ca transport at physiological $[Ca]_i$. Reduced SR Ca-ATPase function fits well with the characteristic slowed relaxation and $[Ca]_i$ decline of HF. Moreover, when SERCA2 expression in myocytes or failing hearts is increased or PLN expression is decreased, by adenoviral gene transfer, relaxation and $[Ca]_i$ decline can be accelerated *(del Monte, et al., 1999; Miyamoto, et al., 2000)*. Thus, it seems clear that reduced SR Ca-transport function is important in the slowed relaxation and $[Ca]_i$ decline characteristic of HF, and correction of this depressed SR Ca-uptake may hold promise as a therapeutic approach in heart failure.

3.2. Ryanodine Receptor

Western blots and ryanodine binding generally indicate that the RyR protein levels are unchanged in heart failure *(Go, et al, 1995; Schillinger, et al., 1996; Sainte Beuve, et al., 1997)*. However, in the pacing-induced dog HF model and a rabbit pressure/volume overload HF model, there seems to be down-regulation of RyR *(Vatner et al., 1994; Yano, et al., 2000; Bossuyt, et al., 2005)*.

The regulation of RyR function may also be altered in HF since some studies have reported enhanced RyR phosphorylation by PKA and/or CaMKII. This increased RyR phosphorylation can enhance diastolic RyR open probability, and increased SR Ca leak has been measured in HF *(Marx, et al., 2000; Schwinger, et al., 1999; Bossuyt, et al., 2005)*. Whether this involves loss of FKBP binding to the RyR, *(Marx, et al., 2000; McCall, et al., 1996)* is controversial. Indeed, some investigators have indicated that PKA-dependent RyR phosphorylation has no effect on Ca sparks *(Li, et al., 2002)* and may not alter FKBP12.6 binding *(Stange, et al., 2003; Xiao, et al., 2004)*.

Buffering of Ca inside the SR is probably unaltered in HF, because calsequestrin (and calreticulin) does not seem to be altered in HF *(Maier, et al., 2003; Richard, et al., 1998)*. This means that if SR Ca content is lower in HF, free $[Ca]_{SR}$ may also be lower. Although there are few measures of SR Ca in HF under relatively physiological conditions, SR Ca content seems to be reduced in human, *(Piacentino, et al., 2003; Lindner, et al., 1998)* rabbit *(Pogwizd, et al., 1999 & 2001)* and dog,

(Hobai, et al., 2001) based on caffeine-induced Ca transients. Reduced SR Ca content is sufficient to largely explain the reduced twitch Ca-peak and contractile function in HF.

3.3. Human SERCA2 Mutations in Heart Failure

There is only one report on naturally occurring mutations in the human SERCA2 gene *(Schmidt, et al., 2003)*. This study concentrated on exons 8, 15, 16, 18, and 19, corresponding to the SERCA2-PLN interaction domains, as well as exons 10, 13, and 14, which covered the phosphorylation and the nucleotide binding/hinge domain of SERCA2, since mutations in these regions may predispose to the development of heart failure.

One hundred and sixty one patients with ischemic or idiopathic dilated cardiomyopathy (New York Heart Association functional class II-IV) were screened for SERCA mutations. Double strand sequencing revealed nucleotide changes in exons 8, 15 and 18. However, none of these naturally occurring genetic variants resulted in amino acid alterations. Furthermore, there were no mutations or single nucleotide changes observed in exons 10, 13, 14, 16, and 19. Thus, although the SERCA2 mRNA and protein levels are altered in human heart failure, the SERCA2 gene is highly conserved in patients with heart failure. There were only four nucleotide changes identified in the SERCA2 gene in three out of eight exons examined. All of these alterations were conservative *(Schmidt, et al., 2003)*. Thus, the SERCA2a gene is tightly regulated to maintain proper intracellular Ca^{2+} cycling. It is interesting to speculate that even minor alterations in the SERCA2a gene cannot be accommodated and result in premature death, which may not allow their discovery in adult heart failure.

3.4. Human PLN Mutations in Dilated Cardiomyopathy

Three PLN mutations in the coding region have been reported to date. Interestingly, all three appeared to be inherited in a familial manner. One of these is the mutation of R9C, which was associated with the inheritance of dilated cardiomyopathy in a large American family *(Schmitt, et al., 2003)*. Carriers of this mutation had a mean age of 25 years. The effects of R9C-PLN appeared to be linked to significant decreases in PLN phosphorylation. Transgenic mice overexpressing human PLN-R9C mutant exhibited dilated cardiomyopathy and early death. To elucidate the mechanisms underlying the detrimental effects of this mutant, the R9C-PLN was expressed in HEK cells. When the mutant-PLN was co-expressed with WT-PLN, it did not relieve the inhibition of SERCA2a by WT-PLN. The R9C mutant appeared to exhibit enhanced affinity for PKA, preventing the PKA-phosphorylation of WT-PLN. These findings suggest that the dominant effects of this mutation in affected individuals may be associated with chronic inhibition of SERCA2a. Thus, inhibition of PLN phosphorylation is sufficient to cause the onset of dilated cardiomyopathy in humans in their teenage years.

A second mutation, associated with a termination codon at amino acid 39 (L39stop) was discovered in two large families *(Franz, et al., 2001)*. Truncation of the 52 amino acid protein occurred in transmembrane domain II, which is highly conserved among species *(Mc Tiernan, et al., 1999)* and involved in PLN regulation of SERCA2a affinity for Ca^{2+} *(Brittsan, et al., 2000)*. In the first family, there were two homozygous individuals, which developed severe dilated cardiomyopathy and required cardiac transplantation at young age. Histopathological examination of both explanted hearts revealed fibrosis and myofibrillar disarrangement. The heterozygous individuals exhibited normal left ventricular function but some of them appeared with left ventricular hypertrophy. In the second family, there were two brothers identified, who were heterozygous for the L39stop-PLN mutation. Interestingly, both of them were diagnosed with cardiomyopathy. Their father had also died of dilated cardiomyopathy and their mother was homozygous for wild-type PLN. The rest of the heterozygous subjects in this family had normal left ventricular systolic function but some of them exhibited left ventricular hypertrophy, similar to the first family. These finding indicate incomplete penetrance of the cardiomyopathy phenotype.

The function of PLN-L39stop on SR Ca^{2+} transport was elucidated by expression studies in HEK cells. Co-expression of human wild-type PLN (PLN-WT) with SERCA2a resulted in decreased apparent affinity for Ca^{2+}, but co-expression of SERCA2a with PLN-L39stop had no effect. When the wild type and mutant PLN were co-expressed with SERCA2a, the decrease in the apparent Ca^{2+} affinity was similar to that observed in by WT-PLN, indicating that the PLN-L39stop mutant does not exert any effects on SERCA2a activity.

Furthermore, infection of adult rat myocytes with adenoviral vectors containing either wild-type or L39stop PLN cDNAs indicated that wild type PLN decreased the contractile parameters and calcium kinetics, compared to control cells infected with an adenovirus expression GFP. However, the PLN-L39stop did not alter myocyte mechanics or calcium cycling.

Western blots of microsomal fractions from transfected HEK-293 cells with the PLN-L39stop mutant, indicated that the PLN-L39stop protein could not be detected. In addition, confocal microscopy in HEK-293 cells transfected with PLN-L39stop revealed detectable immunoreactive protein signals in a small percent of cells and the PLN-mutant was mainly localized to the cell membrane, compared with PLN-WT, which localized to the endoplasmic reticulum. Consistent with these findings, human PLN-L39stop homozygous ventricles had no detectable PLN.

Thus, PLN is an important regulator of human SERCA2a due to the large cardiac reserve required for flight or fight situations, allowing 2–3 fold increases in heart rate. In contrast, the mouse exhibits heart rates ranging up to 800 bpm and it is operating close to its maximal rate, with low cardiac reserve. Intuitively, the rapid Ca-cycling in mouse hearts does not depend on PLN, while PLN is essential for normal function in the human heart.

More recently, another human PLN mutation, which deletes Arg 14 in the coding region was identified. This mutation is associated with inherited

human dilated cardiomyopathy and premature death. Some of the heterozygous individuals presented dilated cardiomyopathy with ventricular extra systolic beats and ventricular tachycardia. These symptoms progressed to congestive heart failure by middle age. However, other young heterozygous subjects were asymptomatic with normal echocardiography, indicating that the effects of this mutation may be age-dependent. In accordance, cardiac overexpression of PLN-R14Del in the mouse recapitulated human dialed cardiomyopathy with abnormal histopathology and premature death.

Expression of the heterozygous mutant-PLN in HEK-293 cells resulted in SERCA superinhibition. The dominant effect of the PLN-R14Del mutation could not be reversed, even after β-adrenergic stimulation. Thus, mutant-PLN remains a chronic inhibitor of SERCA and cardiac Ca-cycling. In accordance, cardiac overexpression of PLN-R14Del in the mouse recapitulated human dilated cardiomyopathy with abnormal histopathology and premature death. Increased PLN inhibition over a period of years may lead to cardiac remodeling, which may progress to failure in later years.

The superinhibitory effects of the PLN-R14Del mutant maybe due to its structure. One charged residue (Arg 14) is missing from the three charged Arg (at positions 9, 13, and 14) in wild-type PLN, which may influence the interaction of PLN with SERCA. Another effect of the Arg 14 deletion is partial disruption of the PLN pentameric structure. Increases in monomeric PLN are expected to gain inhibitory function on the apparent affinity of SERCA2a for Ca^{2+}, which may not be relieved even upon PKA-mediated phosphorylation. A mutation in the PLN promoter region, which increases PLN expression, has been also identified in human hypertrophic cardiomyopathy *(Minamisawa, et al., 2003)*. Consistent with findings in transgenic mice, an increase in the apparent stoichiometry of PLN/SERCA2 is expected to result in depressed Ca-cycling and contractility, which may lead to cardiac remodeling.

Collectively, the human PLN mutant studies indicate that chronic inhibition of either basal SERCA2a activity (PLN-R14Del mutant) or the β-adrenergic stimulation (PLN-R9C mutant) *(Schmitt, et al., 2003)* result in heart failure. On the other hand, absence of PLN inhibition by the PLN-L39stop mutant, associated with the lack of cardiac reserve, also results in heart failure (Figure 3). Thus, the identification of these human PLN mutations point to the paramount importance of PLN and its role in maintaining normal calcium homeostatic mechanisms in the human heart.

3.5. Human Calsequestrin Mutations

Calsequestrin (CSQ) is the most abundant Ca-binding protein in the SR lumen, acting as a low affinity but high capacity Ca buffer (Figure 4) *(Mitchell, et al., 1988)*. CSQ has also been suggested to have a regulatory effect on the activity of the RyR *(Schillinger, et al., 1996)*. Some insight into the physiological function of the protein came from mice overexpressing either canine or murine cardiac CSQ

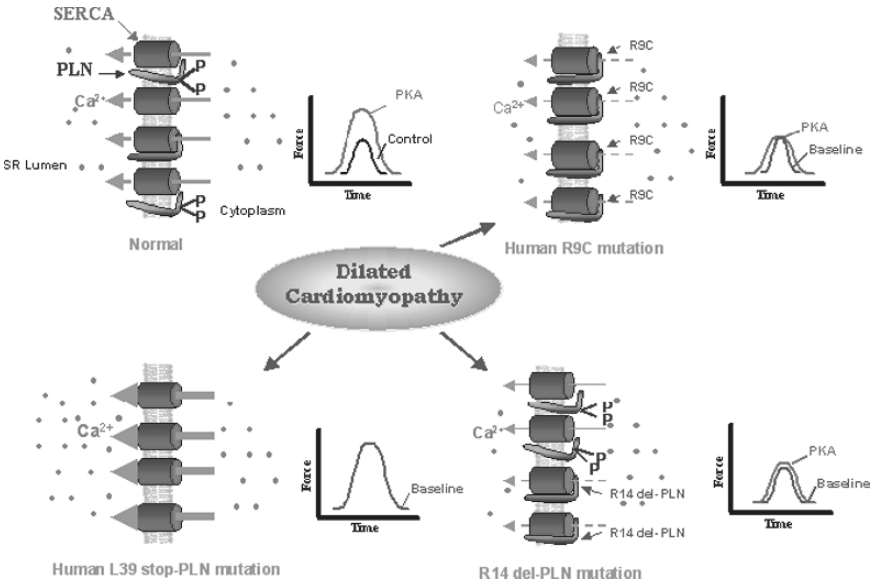

Figure 3. Schematic representation of the effects of Human PLN Mutations on the SR Ca-ATPase Activity and contractility under basal and isoproterenol (PKA)-stimulated conditions (See Colour Plate 26)

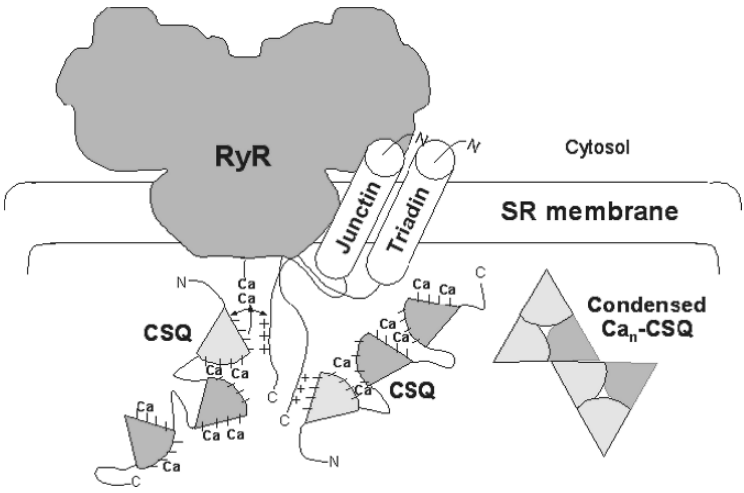

Figure 4. Calsequestrin (CSQ) is the main intra-SR Ca buffering protein, and its structure and interaction with other CSQ and partner proteins (triadin and junctin) is influenced by intra-SR [Ca]. In addition to its role as a low affinity Ca buffer, CSQ may also regulate RyR gating via its interaction with triadin and junctin (See Colour Plate 27)

in the heart. Overexpression of the heterologous protein resulted in hypertrophy which progressed to heart failure *(Solaro, et al., 1974)*. Overexpression of the homologous protein was also associated with cardiac hypertrophy and induction of a fetal gene expression program *(Sato, et al., 2001)*. These mice also exhibited depressed contractility and Ca transients even though the Ca storage capacity of the SR was enhanced, which may be due to increased SR Ca buffering.

There are currently two different CSQ genes, a skeletal isoform and a cardiac isoform *(Lehnart, et al., 2004)*. The cardiac isoform is highly conserved between species and is the only isoform expressed in the heart. The levels of CSQ are not altered in the developing heart and in many pathological disease states such as hypertrophic, ischemic or dilated cardiomyopathy *(Gyorke, et al., 2004; Wang, et al., 2001)*, suggesting that CSQ expression is under rigid genetic regulation. Interestingly, recent studies have indicated that human mutations in the CSQ gene may be responsible for catecholaminergic polymorphic ventricular tachycardia (CPVT). More specifically, a missense mutation was discovered that replaced aspartic acid (negatively charged residue) with histidine, (a positively charged residue), at position 307 *(Farrell, et al., 2003; Wehrens, et al., 2003)*. This residue is localized in a highly conserved Ca binding region and CPVT may therefore be triggered by disrupted Ca binding. However, it is possible that this mutation may also disrupt the interaction of CSQ with the RyR. Also, a nonsense mutation was described, which results in a truncated protein, associated with CSQ ablation *(Leenhardt, et al., 1995)*. In both situations, the patients present with recurrent syncope, seizures or sudden death following physical activity or emotional stress. The seizures associated with the disease are often misdiagnosed as epilepsy, especially since the patients may recover spontaneously without the need of any resuscitation. These patients appear to have structurally normal hearts and marked bradycardia under resting conditions. In general, these patients exhibit arrhythmogenic activity, when a threshold heart rate of 120 beats per minute (bpm) is exceeded. Although the mean age at which the first syncope occurs is around 7 years old, cases have been reported, where the patients were as young as 3 years old. Some insight into the cellular mechanism underlying this disease were obtained from experiments on reduction of CSQ levels, using antisense methodology or expression of the D307H mutant in myocytes. Both of these resulted in disturbances in rhythmic Ca transients with signs of delayed afterdepolarizations (DADs), when undergoing periodic electrical stimulation and exposure to isoproterenol *(Hoit, et al., 1995; Bassani, et al., 1995)*. Thus, it was suggested that the development of this arrhythmogenic disorder is due to impaired SR Ca storage as well as impaired release through the RyR, resulting in an increase in diastolic leak, which may cause delayed afterdepolarizations.

4. CONCLUSION

Many proteins contribute centrally to the delicate balance of Ca in cardiac myocytes that controls cardiac contractility and influence electrical activity. These include voltage-gated Ca channels, RyR, SERCA2, PLN, calsequestrin and regulatory

kinases (and phosphatases), Both genetic and acquired alterations in these Ca handling proteins in cardiac myocytes contribute to pathophysiological cardiovascular disease. Altered cellular Ca^{2+} handling can cause reduced systolic or diastolic cardiac function and also contribute to cardiac arrhythmias.

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