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Molecular Basis of Diastolic Dysfunction

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Synopsis

Diastolic dysfunction is characterized by prolonged relaxation, increase filling pressure, decreased contraction velocity, and reduced cardiac output. Phenotypical features of diastolic dysfunction can be observed at the level of the isolated myocyte. A slower relaxation rate is one of the primary underlying causes of the manifestation of this disabling syndrome. We here review the cellular mechanism that control relaxation at the level of the myocyte in the healthy situation, and discuss the alterations that can impact on physiological function during disease. We will specifically focus on the mechanisms that regulate intracellular calcium handling, and the response of the myofilaments to calcium, including the changes in these components that can contribute to diastolic dysfunction.

Keywords

Sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA); $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger (NCX); Phospholamban (PLB); Ryanodine receptor (RyR2); Heart Failure; Calcium transport; Ca^{2+} sensitivity; extracellular matrix; Cytoskeleton; Ca^{2+} leak and contractile proteins

Introduction

Heart failure is a complex syndrome in which both systolic and diastolic functional abnormalities can be identified. In patients with systolic dysfunction the primary abnormality is a reduction in contractile reserve with poor pumping function of the left ventricle; whereas diastolic dysfunction is associated with impaired relaxation and inability of the ventricle to accept adequate volume of blood. Diastolic dysfunction is characterized by prolonged relaxation, increase filling pressure, decreased contraction velocity, decreased responsiveness to β -adrenergic stimulation, and reduced cardiac output. It has become increasingly clear that diastolic dysfunction is predominant in HF patients and to some extent it is present in patients with systolic HF. Often these two conditions co-exist and are not easy to separate as they may contribute to each other. Diastolic dysfunction can cause serious problems including progression into congestive heart failure as a result of fluid retention in the lung, kidney, and

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veins. Currently, other than treating symptoms of these patients there are no effective treatment options. Despite significant advances in our ability to diagnose functional abnormalities, there is only limited progress towards understanding the mechanisms that control muscle relaxation and there is much controversy over to what controls abnormal relaxation/diastolic dysfunction^{1–3}. The focus of this review is to evaluate current knowledge and recent developments in our understanding of mechanisms that regulate cardiac muscle relaxation at the single cell level as well as derangements in these processes.

What causes abnormal muscle relaxation?

It has been long recognized that both structural and biochemical changes within the myocytes are responsible for impaired relaxation of the ventricle. However, it has been very challenging to pinpoint which of these cause diastolic dysfunction and which are actually compensatory factors that are adaptive in failing muscle. The heart is a complex organ both in terms of architecture and geometry. There are multitudes of differences both in architecture and composition between endocardium and epicardium, right vs. left ventricular muscle. Therefore, it is often difficult to state that changes in structure and or function occur in both chambers uniformly during cardiac remodeling. Most often diastolic dysfunction is associated with concentric cardiac hypertrophy and stiffening of ventricular muscle; inability of the muscle fibers to return to normal length and poor ventricular filling under low pressure⁴. Although impaired filling can result from any number of anatomical or organ-level abnormalities that delay filling, this review will focus on intrinsic abnormalities within the myocytes as a result of hypertrophy induced remodeling.

1. The role of sarcoplasmic reticulum Ca^{2+} transport in muscle relaxation

The sarcoplasmic reticulum (SR) Ca^{2+} transport plays a central role in regulating both cardiac muscle contraction and relaxation. The cardiac SR is an intracellular membrane network that surrounds the contractile machinery; it serves not only as a Ca^{2+} store for Ca^{2+} release but also actively maintains cytosolic Ca^{2+} concentration during contraction-relaxation of the muscle^{5–7}. SR function is coordinated by a set of Ca^{2+} handling proteins localized in the T-tubule and SR membrane. These proteins can be classified as involved in 1) Ca^{2+} release, 2) Ca^{2+} uptake, and 3) Ca^{2+} storage^{8–10}. During excitation contraction-coupling, Ca^{2+} entry through the L-type Ca^{2+} channel activates Ca^{2+} release from the SR Ca^{2+} stores via the ryanodine receptor, RYR2 ^{5, 6, 8, 10}. This raises cytosolic Ca^{2+} and initiates muscle contraction, and the free cytosolic Ca^{2+} concentration co-determines the extent of muscle contraction and therefore force development. Subsequent removal of cytosolic Ca^{2+} by the sarco(endo)plasmic reticulum Ca^{2+} ATPase pump (SERCA2a) and $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger (NCX) results in deactivation of the contractile apparatus, allowing for muscle relaxation. The rate of muscle relaxation is influenced by the rate of Ca^{2+} removal and or SERCA2a pump activity^{10–13}. In the heart, SERCA pump activity is regulated by two small molecular weight proteins: Phospholamban^{13–19} and sarcolipin^{14, 16, 19, 20}. In this review we will discuss how alterations in SERCA pump expression and activity affect muscle function in particular relaxation.

2. Dynamic regulation of SERCA pump expression and its role in muscle contractility

SERCA2a is the major isoform expressed in the adult atria and ventricles, while its alternate isoform, SERCA2b, is expressed at low levels in the heart at all stages^{9, 12, 21, 22}. The SERCA pump is localized in the longitudinal SR and represents the most abundant protein in the SR membrane representing about 40% of total SR protein. The expression levels of SERCA2a are not uniform throughout the heart. There are chamber specific differences in the expression levels of SERCA pump. In rodents, SERCA protein level is ~ 2-fold higher in atria compared

to the ventricle²³, and the higher SERCA pump levels may contribute at least in part to the shorter duration of contraction and faster rates of relaxation in atrial versus ventricular tissue²⁴. During cardiac development, the expression of SERCA2a pump gradually increases^{25–27} and this correlates with a shortening of relaxation time in adult versus neonatal ventricle²⁸. It has been well documented that changes in thyroid hormone levels modify SERCA levels, Ca^{2+} transport and cardiac muscle contractility. Induction of hypothyroidism in animals causes a decrease in SERCA2a protein but in an increase in PLB, whereas hyperthyroidism increases SERCA2a levels but reduces PLB expression^{27, 29–31}. The increases in SERCA expression are consistent with increased velocity of Ca^{2+} uptake and enhanced rates of contraction and relaxation observed in hyperthyroidism in adult hearts³², whereas hypothyroidism produced opposite changes. Interestingly, there is a very close correlation between the Ca^{2+} dependence of Ca^{2+} uptake and the ratio of PLB to SERCA in hypothyroid, euthyroid, and hyperthyroid hearts, and this determines the contractile parameters of the heart^{12, 32}. These data indicate that a change in PLB to SERCA ratio can affect 1) SR Ca^{2+} uptake rates, and 2) the affinity of SERCA pump for Ca^{2+} , and thereby influencing to the speed of muscle relaxation. Therefore, an increase in PLB could adversely affect both contractility and relaxation. A decrease in content and activity of SERCA was reported with ageing both in animal models and in senescent human myocardium^{26, 33, 34} and this decrease was associated with a prolonged contraction/relaxation time and depressed myocardial function.

3. Regulation of SERCA pump activity by the phosphoprotein Phospholamban

Phospholamban (PLB) is a 52 amino acid phosphoprotein and interacts with the SERCA pump in a dynamic manner during the contraction relaxation cycle of the heart. Importantly, PLB is responsible for mediating the β -adrenergic responses in the heart. It has been well documented that phosphorylation of PLB at Ser 16 and Thr17 by kinases PKA and CAMKII respectively can increase SERCA pump activity^{13, 14, 17, 35–37}, Ca^{2+} transport, and correlates with improved speed of muscle relaxation. Studies have shown that unphosphorylated PLB acts as an inhibitor (brake) on the SR Ca^{2+} ATPase and that phosphorylation releases inhibition and induces substantial increase in calcium transport to levels up to four fold or greater^{13, 14, 35, 36}. Thus, regulation of the SERCA pump by PLB is considered to be the primary mechanism for β -adrenergic mediated response of the heart, as well for enhanced Ca^{2+} transport by the SR. In addition, the mechanism of PLB action on SERCA and its relevance in cardiac muscle physiology has been studied extensively using genetically altered mouse models. By generating a PLB null mouse model³⁸ Dr. Kranias and colleagues provided the crucial evidence that PLB is an important regulator of the SERCA2a; absence of PLB enhanced SR calcium uptake and increased rates of contraction and relaxation. These studies discovered that PLB affects the pump affinity for Ca^{2+} and loss of PLB resulted in an increase in SERCA2a affinity for calcium³⁸. In contrast, over-expression of PLB in the heart resulted in a decrease in SR Ca^{2+} uptake and depressed cardiac contractile performance *in vivo*³⁹. These studies further demonstrated that an alteration in the PLB-to-SERCA ratio can affect SR Ca^{2+} transport and has profound effects on myocardial contractility.

4. Abnormal calcium homeostasis and diastolic dysfunction in failing hearts

Contractile dysfunction in end-stage human heart failure has been attributed to both depressed myofilament Ca^{2+} sensitivity and as well altered calcium handling. In particular Ca^{2+} transients are decreased^{40, 41}. Smaller amplitude of Ca^{2+} transients could be due to either lower fractional SR Ca^{2+} release or lower SR Ca^{2+} content. A decreased Ca^{2+} store could be due to reduced SR Ca^{2+} uptake, or increased Ca^{2+} leak during diastole⁴² (Figure. 1). Decreases in SR Ca^{2+} -ATPase gene and protein expression levels have been described in animal models of

heart failure and failing human myocardium^{40–49}. In addition, increased activity and gene expression of the sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchanger were reported in failing human myocardium and in animal models of heart failure^{44, 45, 48–50}. A reduction in SERCA and an increase in $\text{Na}^+/\text{Ca}^{2+}$ exchanger are expected to facilitate Ca^{2+} removal from the cell at the expense of its uptake into the SR, resulting in decreased SR Ca^{2+} levels (Figure. 1). Studies document that a decrease in SERCA pump activity and Ca^{2+} uptake are seen in many forms of heart failure regardless of etiology of the disease. In addition, Pieske et al⁵¹ demonstrated a direct correlation between depressed SR Ca^{2+} -ATPase expression and depressed force-frequency response in failing myocardium by studying left ventricular trabeculae isolated from end stage failing heart. They further showed that in failing myocardium the increase in SR Ca^{2+} content at high frequency was only about 10% of that in non-failing myocardium and suggested that a decrease in SR Ca^{2+} transport at high frequency is likely responsible for a blunted or negative force-frequency relation.

Another potential cause of reduced SR Ca^{2+} content is enhanced diastolic leak of Ca^{2+} via the ryanodine receptors (RyRs)^{52, 53}. Recent studies by Marks and coworkers^{54, 55} have suggested that RyR phosphorylation by PKA results in dissociation of FKBP12.6 from RyRs, rendering the channels hyperactive and spontaneous release of calcium in failing hearts. This phenomena has been described as diastolic Ca^{2+} leak (Figure. 1). Other laboratories have contradicted this finding and did not find any evidence for phosphorylation-induced dissociation of FKBP12.6 from RyRs in normal or diseased hearts^{56–58}. Thus, further studies are needed to determine whether the abnormal RyR modulation by luminal Ca^{2+} is caused by an altered interaction with FKBP12.6. There are some recent studies which suggest Ca^{2+} leak might occur in heart failure but through entirely different mechanisms involving luminal Ca^{2+} ion concentration⁵⁹. In a recent study, Guo et al⁶⁰ suggested that CaMKII-dependent phosphorylation of RyR by endogenous CaMKII (but not PKA-dependent phosphorylation) increases resting SR Ca^{2+} release or leak and is likely responsible for enhanced SR diastolic Ca^{2+} leak and certain triggered arrhythmias seen in heart failure. In addition, the quantitative effect of SR Ca^{2+} leak remains incompletely understood. Each heart beat, the SR is triggered to release a substantial amount of Ca^{2+} via the RyR, which gets taken back up via the SERCA pumps. It remains unknown whether a diastolic Ca^{2+} leak that is typically much smaller than a triggered release can actually functionally deplete the SR, and thus a physiological relevance of a diastolic calcium leak has not yet been quantified. In summary, although there is some evidence that diastolic calcium leak can occur, and potentially contribute to impairment of relaxation, the underlying mechanisms responsible for RYR mediated SR Ca^{2+} leak and its functional relevance in heart failure remains to be fully understood.

5. SERCA pump level impacts both muscle contraction and relaxation rates

To understand the significance of alterations in SERCA pump expression on myocardial contractility, transgenic (TG) mouse and rat models that express higher levels of SERCA pump were developed. Transgenic overexpression of SERCA2a or SERCA1a in the heart demonstrated that SERCA pump can be overexpressed in the heart resulting in an increased Ca^{2+} transport and contractility^{11, 12, 61–64}. Overexpression of the skeletal muscle isoform, SERCA1a in the mouse heart results in a net increase of SERCA pump to 2.5 fold compared to non-transgenic (NTG) control hearts. However, the endogenous SERCA2a pump levels in these mice decreased to 50% suggesting that there is a limit how much the SR can accommodate. Mice with higher levels of SERCA pump show normal in vivo heart function, and do not exhibit cardiac pathology. Overexpression of SERCA pump led to increased SR calcium transport which in turn increased the rates of cardiac contraction and relaxation^{11, 12, 61–64}. These studies further demonstrated that increased SERCA expression results in higher rates of contraction and relaxation suggesting that SERCA is a dominant player in SR

Ca²⁺ uptake and could potentially be used to treat conditions such as heart failure where SERCA levels and/or activity is decreased.

To examine the effect of decreased SERCA expression a SERCA2 gene knockout mouse model was developed^{65–67}. Disruption of both alleles is lethal and the homozygous null (SERCA2^{–/–}) mice die early in development; there was no compensatory upregulation of SERCA1a. The heterozygous (SERCA2^{+/-}) mice did not show any evidence of cardiac pathology, albeit they had reduced SERCA2 levels (65% of WT hearts). These mice did not show any deficit in cardiac function and the hearts responded well to β -adrenergic stimulation^{65, 66}. It is likely that SERCA2^{+/-} mice have adapted to this new state since they were born with reduced SERCA level. However, when stressed with pressure overload, SERCA2^{+/-} mice developed heart failure much more rapidly than WT controls⁶⁸. Thus, these studies employing transgenic and gene knock out animal models proved that SERCA pump level is a critical determinant of SR Ca²⁺ uptake function and decreased SERCA expression can lead to cardiac dysfunction when stress is imposed¹¹.

6. Role of myofilaments proteins in diastolic dysfunction

Myofilament properties play a central role in the governing of cardiac relaxation. Although it is undisputed that the intracellular calcium concentration needs to decline to facilitate and initiate relaxation, the actual rate at which healthy myocardium relaxes is predominantly regulated by myofilament properties. The peak of the calcium transient amplitude is generally reached long before the peak of force development, and once force development starts to decline, the calcium concentration is already near or below the concentration where myofilament can be activated. The exact governing mechanisms of myocardial relaxation are however still not well understood. This incomplete grasp of the normal physiological relaxation process likely has significantly hampered the understanding of impaired relaxation. Insight into impaired relaxation that, at its basis, stems from a myofilament malfunction has come from specific mutations that have been found in various myofilament proteins in familial cardiomyopathies. Specific single amino-acid mutations in the myosin heavy chain⁶⁹, myosin binding protein C⁷⁰, troponinT⁷¹ TroponinC⁷², Tropomyosin⁷³ and other proteins have been implicated to ultimately cause or contribute to impairment of myocardial relaxation. Still, the mechanism via which these myofilament mutations lead to the various cardiomyopathies is largely unknown, and is subject to ongoing studies in many labs. Not only genetic alterations, but also post-translational modification of myofilament proteins can impair cardiac relaxation. One of the most investigated post-translational modifications of the myofilaments is TnI phosphorylation. Protein Kinase A mediated phosphorylation of Troponin-I (TnI) is the major determinant of the acceleration of relaxation that occurs under β -adrenergic stimulation⁷⁴. In addition, when heart rate increases, TnI appears also to become increasingly phosphorylated, via yet unknown mechanisms⁷⁵. Phosphorylation of TnI desensitizes the myofilaments for activator calcium⁷⁶ and thereby facilitates accelerates relaxation. This acceleration of relaxation is essential, especially at higher heart rates. However, if the mechanisms responsible for phosphorylation or are impaired, or fail, the lack of acceleration of relaxation in combination with the high heart rate will cause incomplete relaxation, and likely manifest as diastolic dysfunction. When these sites are no longer available, for instance in mice expressing slow skeletal TnI in the heart⁷⁷, relaxation is hampered. Specifically under conditions where PKA-mediated phosphorylation of TnI is needed to desensitize the myocardium to allow for the prevailing higher heart rates that occur under β -adrenergic stimulation, lack of these PKA sites hampers relaxation. These findings are confirmed in murine model/experiments where the PKA-sites of TnI are mutated to prevent phosphorylation, impairment of relaxation occurs⁷⁸. Besides TnI, several other myofilaments can undergo post-translational modifications. MyBPC, TnT, and MLC-2 can be phosphorylated which then changes their interaction with other proteins, resulting in altered relaxation kinetics. Thus, given the multitude of pathways

that can result in myofilament protein phosphorylation or other post-translational modification, when the processes that either induce or prevent these modifications are impaired, the resulting altered function of these proteins can contribute to impaired contractile performance.

7. The role of cytoskeleton and extracellular matrix

In addition to alterations in calcium handling and myofilament properties, there are multitude of changes occurring in the failing myocyte, it is often difficult to distinguish whether these changes are causative or compensatory adaptations of the failing heart. Among structural alterations, changes in cardiac myocyte cytoskeleton and extracellular matrix have been implicated as potential underlying causes of diastolic dysfunction^{79–82}. Cooper and colleagues^{3, 83, 84} have observed significant changes in microtubule architecture and advanced the hypothesis that changes in the microtubule network (increased network density in hypertrophied/failing myocytes) could contribute to altered viscosity and increased stiffness seen in hypertrophied myocardium; he argues that in these hypertrophied myocytes normal microtubule transport function is disturbed due to alterations in microtubule architecture and could potentially cause heart failure by limiting transport of key macromolecules including RNA and protein. Others have pointed out that changes in collagen expression (ratio of Collagen type I vs. Type III) and cross-linking may potentially play a role in relaxation impairment^{81, 82}. Similarly, changes in titin (responsible for elasticity of muscle stiffness) expression can be correlated with diastolic stiffening^{85–89}. However, currently there is limited data in human samples with regard to its relevance.

8. Therapeutic strategies to treat diastolic dysfunction

There are only limited therapeutic approaches to treat diastolic dysfunction and most of these are already in use to treat heart failure patients, albeit with limited success. Here we will discuss non pharmacological approaches to restore contractility of the heart using gene transfer methods. Recent studies were focused on restoring SERCA pump activity, either by adenoviral mediated gene transfer of SERCA2a or inhibiting PLB. Adenoviral gene transfer into cardiac myocytes showed that overexpression of SERCA2a in failing human ventricular myocytes increased Ca^{2+} transport and restored contraction and relaxation velocity⁹⁰. The negative frequency-response was normalized in cardiomyocytes overexpressing SERCA2a⁹⁰. Encouraged by these in vitro studies, Hajjar and colleagues developed a catheter-based technique to introduce genes into the myocardium^{91–94}. SERCA2a gene transfer in a rat model of pressure-overload hypertrophy (where SERCA2a levels were decreased and severe contractile dysfunction was evident) restored both systolic and diastolic dysfunction to normal levels. SERCA2a overexpression decreased left ventricular size and restored the slope of the end-diastolic pressure-dimension relationship to control levels. Interestingly, overexpression of SERCA2a in failing heart restored and normalized the levels of PCr and ATP and suggested that normalizing Ca^{2+} transport would improve energetics^{91, 93, 94}. PLB ablation was shown to prevent structural and functional abnormalities in mouse models^{14, 95}. Therefore, strategies to suppress PLB inhibition and enhance SERCA activity have been tested. One possible approach to increase SERCA pump activity is to decrease the levels of phospholamban. Del monte et al⁹⁶ showed that adenoviral gene transfer of antisense phospholamban in failing human cardiomyocytes restored contractility, Ca^{2+} handling, and the force frequency response. In addition, a pseudo-phosphorylated mutant PLB peptide (S16EPLN) was tested in BIO14.6 CM hamsters (showing a progressive stage of dilated cardiomyopathy and heart failure). Expression of mutant PLB enhanced myocardial SR Ca^{2+} uptake and suppressed progressive impairment of left ventricular (LV) systolic function and contractility up to 28–30 weeks. In addition, parvalbumin gene transfer was shown to improve diastolic function in senescent and diseased myocytes^{97, 98}.

9. Summary and Conclusions

In conclusion, diastolic dysfunction is a complex disease and it is difficult to pinpoint single etiology or a causative mechanism to be targeted. Diastolic dysfunction is also a progressive disease and could be compounded by other pathological conditions. Despite significant advances in our understanding of basic cellular mechanisms regulating relaxation, there are still many hurdles with regard to the development of efficient therapeutic approaches to combat diastolic dysfunction.

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Dysregulation of Calcium Transport in Heart Failure

Figure 1.

Potential contributors to Ca^{2+} dysregulation in failing heart muscle. A decreased expression and activity of SERCA pump could explain slowed rate of Ca^{2+} uptake and prolongation of muscle relaxation. Enhanced NCX expression and activity could increase Ca^{2+} efflux and compete with SERCA pump, reducing SR Ca^{2+} stores. RyR2 phosphorylation by PKA or CaMKII causes increased opening and Ca^{2+} leak, further contributing to loss of Ca^{2+} . A net loss of Ca^{2+} could contribute to both systolic and diastolic dysfunction.