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Remodeling of Calcium Handling in Human Heart Failure

Qing Lou, Ph.D., **Ajit Janardhan, M.D., Ph.D.**, and **Igor R. Efimov, Ph.D.**
Department of Biomedical Engineering, Washington University in St. Louis, MO 63130, USA. (Q.L., I.R.E.). Department of Medicine, Division of Cardiology, Washington University School of Medicine, St. Louis, MO 63110, USA (A.J., I.R.E.)

Abstract

Heart failure (HF) is an increasing public health problem accelerated by a rapidly aging global population. Despite considerable progress in managing the disease, the development of new therapies for effective treatment of HF remains a challenge. To identify targets for early diagnosis and therapeutic intervention, it is essential to understand the molecular and cellular basis of calcium handling and the signaling pathways governing the functional remodeling associated with HF in humans. Calcium (Ca^{2+}) cycling is an essential mediator of cardiac contractile function, and remodeling of calcium handling is thought to be one of the major factors contributing to the mechanical and electrical dysfunction observed in HF. Active research in this field aims to bridge the gap between basic research and effective clinical treatments of HF. This chapter reviews the most relevant studies of calcium remodeling in failing human hearts and discusses their connections to current and emerging clinical therapies for HF patients.

1. Introduction

HF is a rising public health problem, with a prevalence of over 5.8 million in the USA, over 23 million worldwide, and continues to increase.[1, 2] The contractile dysfunction and arrhythmogenesis associated with HF is closely related to the remodeling of calcium handling,[3] which, in turn, is partially controlled by several signaling pathways in which Ca^{2+} has a prominent role.[4] Deriving a mechanistic understanding of alterations in calcium handling and calcium signaling is a critical step towards the development and improvement of physiology-based treatments for HF.

2. Overview of Cardiac Calcium Signaling

Ca^{2+} plays a central part in regulating excitation-contraction (EC) coupling and in modulating systolic and diastolic function in the heart as shown in Figure 1. Ca^{2+} signal transduction in EC coupling comprises four steps.[4–6] Firstly, the trigger Ca^{2+} current (I_{Ca}) is generated by the L-type Ca^{2+} channels expressed in the transverse tubules (T-tubules) and is initiated by membrane depolarization. Secondly, the Ca^{2+} ions diffuse across the narrow junctional zone to activate ryanodine receptors (RyR) and generate Ca^{2+} sparks, which considerably amplifies the original trigger Ca^{2+} signal. This process is known as Ca^{2+}-induced Ca^{2+} release (CICR). Thirdly, the Ca^{2+} efflux from the sarcoplasmic reticulum (SR) then diffuses in the cytoplasm to activate contraction by Ca^{2+} binding to troponin-C. Lastly, Ca^{2+} is transported back to the SR by SR Ca^{2+}-ATPases (SERCA) and out of cell via Na^{+}/Ca^{2+} exchangers (NCX). Abnormal handling of intracellular Ca^{2+} at any of these steps can cause cardiac dysfunction.[7]

Corresponding author: Igor R. Efimov, Department of Biomedical Engineering, Washington University in Saint Louis, 390E Whitaker Hall, One Brookings Drive, St. Louis, Missouri 63130-4899, Tel: 1-314-935-8612; Fax: 1-314-935-8377; igor@wustl.edu.

Intracellular Ca^{2+} homeostasis of cardiac myocytes is regulated by the phosphorylation and dephosphorylation of several key Ca^{2+}-handling proteins. One important regulatory kinase is cAMP-dependant protein kinase (PKA), which has been shown to regulate L-type Ca^{2+} channels, RyR and phospholamban (PLN). Despite the fact that global PKA activity is not changed in the failing human heart,[8, 9] its activity in the RyR macromolecular signaling complex might be locally increased.[10, 11]

Another important regulatory kinase is the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII).[12] CAMKII is a protein kinase that modulates several intracellular Ca^{2+}-handling proteins such as RyR, PLN, L-type Ca^{2+} channels as well as Na^{+} channels.[12] CAMKII is associated directly with the RyR and modulates the activity of RyR.[13–15] Phosphorylation of PLN via CAMKII or PKA enhances the SR Ca^{2+} uptake via increased SERCA activity. The activity of CAMKII was shown to be significantly increased in the failing human heart and is correlated with the impaired ejection fraction.[9,16] Both PKA and CAMKII can be activated by β-adrenergic stimulation.

Finally, multiple isoforms of protein kinase C (PKC) might also play a role in regulating the Ca^{2+} handling. PKC-α is the dominant isoform of PKC in the human heart[17] and its activity is triggered by the activation of $G_{\alpha q}$ coupled receptors (angiotensin II receptor, endothelin-1 receptor, and the α-adrenergic receptor).[18] PKC-α can phosphorylate protein phosphatase inhibitor 1 (I-1), consequently increasing the activity of protein phosphatase 1 (PP1), and leading to dephosphorylation of PLN and thus decreasing the activity of SERCA.[19] The level of PKC-α is increased in human HF.[20–22] The role of other isoforms of PKC in regulating calcium handling remains to be elucidated.

3. Alteration in Intracellular Ca^{2+} and Functional Abnormalities in Human Heart Failure

3.1 Abnormal Ca2+ Handling and Mechanical Dysfunction in Human Heart Failure

The amount of Ca^{2+} delivered to the cytoplasm and the rate of Ca^{2+} removal from the cytoplasm are the two of the major factors determining the rate, intensity and duration of myocyte contraction.[23] Understanding of alterations in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and their causal roles in contractile dysfunction in the failing human heart has been greatly advanced by the use of fluorescent $[Ca^{2+}]_i$ indicators,[5, 24–32] which reflect changes in the free $[Ca^{2+}]_i$ necessary for the activation of contractile proteins.[33, 34]

In isolated cells and tissues from failing human hearts, decreased amplitude of Ca^{2+} transient measured by the fluorescent intracellular Ca^{2+} indicators implies reduced Ca^{2+} release from SR.[28, 30, 35] This is correlated with decreased peak stretch amplitude, a measure of myocardial contraction.[6, 36] The reduced amplitude of Ca^{2+} transient is associated with decreased EC coupling gain[37] and decreased SR Ca^{2+} content.[30,38–42]

Moreover, the Ca^{2+} transient from failing human hearts exhibits a reduced rate of Ca^{2+} removal.[24, 28, 30] The slowed rate of recovery of Ca^{2+} transient recovery is associated with a marked delay in tension relaxation in the failing human heart.[24] Finally, failing human hearts exhibits increased resting intracellular Ca^{2+} level, leading to diastolic dysfunction.[27, 28]

Altered $[Ca^{2+}]_i$ is frequency-dependent and most obvious at high heart rate.[43] Normally, the amplitude of the $[Ca^{2+}]_i$ transient is larger at higher stimulation frequencies.[33] In human HF, however, the amplitude of Ca^{2+} transient was decreased at faster stimulation rates, leading to reduced tension development at higher frequencies.[27, 29] Increased resting $[Ca^{2+}]_i$ and a fusion of Ca^{2+} transient at fast frequencies may also occur, leading to an increase of end-

diastolic tension and a decrease of active tension generation associated with incomplete relaxation and twitch fusion.[27] The blunted or negative force frequency relationship (FFR) observed in both *in vivo* and *in vitro* studies of failing human hearts is in contrast with the positive FFR in non-failing human hearts,[27, 44–46] and is associated with altered Ca^{2+} and Na^{+} homeostasis as well as an inability to increase the SR Ca^{2+} content at increasing stimulation frequencies.[39, 42]

The alteration of Ca^{2+} transient in failing human hearts is also region-dependent. We recently demonstrated the transmural hetergeneous remodeling of Ca^{2+} handing in the coronary-perfused left ventricular wedge preparations from failing and non-failing human hearts.[31] The sequence of Ca^{2+} transient relaxation is from epicardium to endocardium in both failing and non-failing human hearts at a slow heart rate (e.g., 0.67Hz/40 BPM) during endocardial pacing, because the difference of Ca^{2+} transient duration between subendocardium and subepicardium (or duration difference) is larger than the conduction time from subendocardium to subepicardium (Figure 2). Interestingly, this sequence is reversed at a fast heart rate (e.g., 1.67Hz/100 BPM) in the failing human heart due to a significant decrease of this duration difference (Figure 2C and 2D). In contrast, this sequence is not reversed in the non-failing human heart because the duration difference is not significantly changed at faster frequencies (Figure 2B and 2D). We hypothesize that this reversed sequence of relaxation at fast heart rates could contribute to the end-systolic dysfunction[44] observed in the failing human heart. The maintenance of the normal relaxation sequence at slow heart rates in the failing hearts provides another mechanism for the beneficial effects of the heart-rate reduction in the patients with HF.[47]

3.2 Abnormal Ca^{2+} Handling and Arrhythmia in Human Heart Failure

While approximately fifty percent HF patients die from progressive pump failure, the remainder die suddenly, mostly due to tachyarrhythmias.[48] The relevance of arrhythmia to HF is evident from the significant survival benefit conferred by implantable cardioverter-defibrillators (ICD) on patients with advanced HF.[49] Among the multiple mechanisms contributing to the development of arrhythmia in HF patients,[50] changes in Ca^{2+} handling provide both triggers and substrate for the induction of arrhythmia.

Alterated Ca^{2+} handling in HF contributes to triggered activity arising from delayed after-depolarization (DAD) or early after-depolarization (EAD). DADs result from elevated intracellular Ca^{2+} load and spontaneous SR Ca^{2+} release, which leads to activation of transient inward current presumably carried by NCX.[51] In a rabbit model of HF, enhanced NCX in HF increased the frequency of DADs.[51] In a canine model of HF, increased RyR-mediated SR Ca^{2+} leak caused proarrhythmic DADs.[52] EAD could occur in the setting of increasingly labile repolarization observed in HF,[53] possibly the result of synergistic activation of L-type Ca^{2+} current and NCX during phase 2 and phase 3 of the AP.[54] The cellular mechanisms of triggered activity have been reviewed elsewhere.[55]

In contrast to the amount of work in animal models, our knowledge of triggered activity directly from human studies remains rather limited. Three-dimensional intraoperative mapping revealed that arrhythmias were maintained by focal mechanisms in end-stage HF patients with idiopathic dilated cardiomyopathy.[56] However, whether these focal activations were triggered activities was not directly demonstrated in this study. Both DADs and EADs were not observed when the ventricular trabeculae of failing human hearts were perfused with normal Tyrodes' solution. Rather, DADs were only induced upon perfusion with a modified Tyrode's solution that mimicked the extracellular milieu of patients with severe HF.[57] EADs were not observed in the modified solution.[57] In contrast, EADs could be induced in 30% of failing rabbit trabeculae.[57] This species difference signals the caution that

must be used when interpreting animal models and highlights the importance in conducting mechanistic studies directly in failing human hearts.

Abnormal Ca^{2+} handling may also produce the substrate for arrhythmia by facilitating AP duration alternans (APD alternans, or repolarization alternans),[58, 59] the relevance of which was shown in the predictive power of T-wave alternans in predicting arrhythmic events in HF patients.[60] Spatially discordant APD alternans increases dispersion of repolarization and promotes unidirectional block and subsequent induction of arrhythmia.[61] In a dog model of HF, Wilson et al.[59] showed that HF significantly lowered heart-rate threshold for calcium transient alternans. They also demonstrated that enhanced calcium transient alternans enhancement was independent of HF-associated changes in repolarization and appeared to be responsible for the enhanced APD alternans.[59] Finally, improvement of Ca^{2+} cycling through targeted SERCA2a gene expression was shown to retard the development of APD alternans.[62] It remains to be demonstrated how the altered Ca^{2+} cycling leads to cellular alternans in the failing human heart.

4. Molecular and Cellular Basis of Abnormal Calcium Handling and Signaling in Human HF

Alteration in the $[Ca^{2+}]_i$ is attributed to the abnormal calcium handling in the EC coupling process, which is operated by the sarcolemma and SR, including L-type Ca^{2+} channels, RyR, NCX and SERCA2a. Altered EC coupling in HF have been reviewed in detail elsewhere.[3,18,43] Here we mainly focus on reviewing the results regarding the failing human heart.

4.1 Calcium-Induced Calcium Release (CICR)

Triggering of CICR (i.e., I_{Ca}) in the failing human heart is mostly unchanged,[26,28,30,63] though inhibition of I_{Ca} was observed at higher frequencies.[64] Thus, the smaller Ca^{2+} transient observed in HF is mainly due to a reduced capability of I_{Ca} to trigger Ca^{2+} release from the SR (or a reduced EC coupling gain).

The reduced EC coupling gain may result from the hyperphosphorylation of RyRs in the failing human heart.[10] PKA hyperphosphorylation of RyRs leads to the dissociation of the FKBP12.6 regulatory subunit, which inhibits the coupled gating of arrays of RyR channels and thus could result in a loss of EC coupling gain.[10] This is supported by the reduced amplitude and changed properties of Ca^{2+} sparks measured from isolated ventricular myocytes from failing human hearts.[65] More discussion of RyRs can be found in the section 4.2.

Rapid activation of RyRs by I_{Ca} is facilitated by the close proximity of the L-type Ca^{2+} channels and RyRs. Reduced EC coupling gain in HF could thus also originate from the geometric derangement of RyRs and L-type Ca^{2+} channels, as suggested by the spontaneous hypertensive rat with HF (SHR-HF).[37] Disorganization of T-tubules and a decrease in the colocalization of L-type Ca^{2+} channels and RyRs have been demonstrated in fixed ventricular samples from failing human hearts.[66] The actual loss of T-tubules in isolated myocytes from failing human heart was reported in one study [67] but not in another,[68] findings that might be explained by the large spatial variations in T-tubule remodeling in human HF.[66]

The ultrastructural defects in the T-tubule system were demonstrated to cause the dyssynchronous Ca^{2+} release (or defective EC coupling) by confocal line scanning techniques in the isolated ventricular myocytes from SHR-HF, with Ca^{2+} release being delayed in certain regions of a myocyte compared to the other normally coupled areas.[69]

Louch and coworkers showed modest dyssynchrony of Ca^{2+} release in the isolated myocytes from failing human hearts.[70] While the local delayed SR Ca^{2+} release was confirmed in the whole heart level in SHR-HF,[71] no study has been done so far to demonstrate the dyssynchronous Ca^{2+} release at the tissue level from the failing human hearts. However, the morphological changes of Ca^{2+} transient observed in our recent left ventricular wedge preparations from failing human hearts imply the potentially important role of dyssynchronous Ca^{2+}.[31] We observed two components in the rising portion of Ca^{2+} transient, with a slow rising component following an initial fast rising component (Figure 3A and 3B).[31] It is possible that the first fast-rising component corresponds to the normally triggered Ca^{2+} release and the second slow-rising component corresponds to the delayed Ca^{2+} release, which has been shown in SHR-HF.[71] Interestingly, this morphological change of Ca^{2+} was only observed at the subendocardium in 60% of the studied failing human hearts.[31] This regional difference might result from the higher susceptibility to ischemia by the endocardium compared with epicardium,[72,73] and suggests that the extrapolation of results from one region (e.g., epicardium) to another region (e.g., epicardium) in human studies should be done with caution. This delayed Ca^{2+} release might be also underlie the slower recovery of intracellular Ca^{2+} relative to the recovery of the action potential observed in our study (Figure 3A and 3C). That is, the Ca^{2+} transient outlasts the action potential at the subendocardium of the failing human heart, which might lead to phase three early afterdepolarization.[74]

The reduced EC coupling gain could also result from decreased SR Ca^{2+} content, which has been extensively demonstrated in human HF.[30,38,39,41,42] The success of molecular therapies aimed at restoring SR Ca^{2+} content further underscores the importance of SR Ca^{2+} content.[75,76] Reduced SR Ca^{2+} content in the failing human heart could result from leaky RyRs, reduced SR Ca^{2+} uptake via SERCA2a, and increased Ca2+ extrusion via NCX, which are reviewed in the sections below (4.2–4.3).

4.2 RyR

Most studies showed no change in the protein expression of RyR in human HF.[3, 43] However, the characteristics of Ca^{2+} sparks are altered in isolated myocytes from failing human hearts.[65] Furthermore, RyRs in human HF are “leaky,”[10] contributing to a reduction of SR Ca^{2+} content.[77] The SR Ca^{2+} leak occurred despite reduced SR Ca^{2+} loading in a canine model of HF.[77]

Leaky RyR is thought to result from hyperphosphorylation of RyR by PKA or CAMKII. Increased Ca^{2+} sensitivity and higher probability of open RyR in failing human hearts was first observed by Marx and coworkers.[10] They concluded that the increased local PKA-mediated phosphorylation of RyR in HF leads to the disassociation of FKBP12.6 from RyRs, leading to higher open probability at rest.[10] They also observed decreased association of phosphatases (PP1, protein phosphatase 2A [PP2A]) to RyR, which may exacerbate PKA-mediated hyperphosporylation of RyR.[10] The hyperphosphoryation of RyRs could also result from the deficiency of phosphodiesterase 4D (PDE4D), which resides in the RyR macromolecular signaling complex and regulates the local concentration of cAMP that activates PKA.[11] The impact of PKA phosphorylation in reducing the RyR/FKBP12.6 association remains controversial.[78,79]

Increased SR Ca^{2+} leak in isolated myocytes from failing rabbit hearts was shown to relate to the hyperphosphoryation of RyR by CAMKII.[80] Ca^{2+} leak was reduced by the inhibition of CAMKII but not altered by PKA inhibition,[80] suggesting the potential role of CAMKII inhibition in improving the Ca^{2+} handling in HF. This hypothesis is further supported by a recent study by Sossalla et al.,[16] who showed a significant increase in the expression the CAMKII in both left and right ventricles of the failing human heart, and observed that

inhibition of CAMKII reduced the SR Ca^{2+} leak and increased the Ca^{2+} content. Importantly, they also showed that inhibition of CAMKII improved contractility in isolated ventricular trabeculae.[16] They reported that CAMKII inhibition restored the positive FFR.[16] This is in sharp contrast to the study by Kushiner et al.,[81] which showed that CAMKII inhibition completely abolished the positive FFR in mouse heart. The result from the latter study is consistent with the hypothesis that CAMKII is responsible for sensing the frequency of Ca2+ oscillation[82] as well as for causing the positive FFR via increased phosphorylation of RyR and PLN at increasing frequencies.[13, 83] Kushiner and coworkers also showed that RyR phosphorylation by CAMKII was decreased in failing human hearts in despite of the global increase of CAMKII,[81] and suggested that the impaired RyR phosphorylation by CAMKII plays a role in blunted FFR in human HF. Further studies are needed to resolve these areas of controversy and clarify the molecular mechanism and the promise of CAMKII inhibition in improving the Ca^{2+} handling in human HF.

While much evidence supports altered regulation and function of the RyR leading to abnormal Ca^{2+} handling in failing human heart, there are studies indicating the opposite. Recordings of currents through the RyR from failing human hearts did not reveal any significant alterations at a single channel level.[84] Jiang et al. observed neither structural nor functional change of RyRs from the failing human heart but did report a significant reduction in SERCA2a expression, suggesting that abnormal Ca^{2+} uptake may contribute more to the altered Ca^{2+} handling in human HF.[78]

4.3 SERCA2a, PLN and NCX

SR Ca^{2+} uptake was reduced in the failing human heart.[30, 46, 85, 86] This might be due to depressed protein expression of SERCA2a. Hasenfuss et al. observed downregulation of SERCA2a expression as well as a significant correlation between SERCA protein levels and SR Ca^{2+} uptake in failing human hearts.[46] Overexpression of SERCA2a has been shown to restore the Ca^{2+} handling and the contractile function with positive FFR in isolated failing human myocytes.[87, 88] While some studies observed the downregulation of protein expression of SERCA2a in the failing human heart, others did not find any change in the protein expression of SERCA2a.[89] This inconsistency might be explained by our recent findings.[31] We observed down-regulation of SERCA2a expression in samples from the subendocardium of failing human hearts with ischemic cardiomyopathy but not in samples from epicardium or from failing hearts with dilated cardiomyopathy (Figure 4A), suggesting that the alteration of SERCA2a expression might be region-dependent as well as HF etiology-dependent.[31]

Besides the potential decrease in protein expression, the decreased activity of SERCA2a in HF might also result from altered regulation. This is supported by the findings that decreased SR Ca^{2+} uptake was observed in despite unchanged protein levels of SERCA2a.[85, 86, 90]

SERCA2a is directly regulated by PLN which is mainly phosphorylated by PKA and CAMKII.[89] PLN inhibits SERCA2a activity when it is not phosphorylated, while its phosphorylated form disassociates from SERCA2a. In the failing human heart, majority of the studies indicate no change in the protein expression of PLN,[31, 89] which is consistent with our recent study (Figure 4B).[31] However, the phosphorylation state of PLN was decreased in the failing human heart,[85, 90, 91] suggesting increased inhibition of SERCA2a by PLN in the failing human heart. PLN is mainly phosphorylated by PKA at serine-16 and by CAMKII at threonine-17. Phosphorylation at threonine-17 is decreased due to increased dephosphorylation by calcineurin in failing human hearts with dilated cardiomyopathy.[92] PLN phosphorylation at serine-16 is decreased presumably due to increased level of PP1 in the failing human heart,[90, 93] which might be a result of increased PKC-α.[19, 20]

Interventions to attenuate the inhibitory effect of PLN on SERCA2a have been tested in animal models. Minamisawa et al. found that knockout of PLN significantly increased SR Ca^{2+} content and completely rescued the spectrum of heart-failure phenotype in a mouse model of HF.[94] Decreased PLN expression via adenoviral gene transfer of antisense of PLN was shown to improve both contraction and relaxation in isolated myocytes from failing human hearts.[88] Inhibition of PKC-α was shown to increase the SR Ca^{2+} load and protect the mouse from HF.[19, 95] The importance of PKC-α and other isoforms of PKC in the Ca^{2+} handling in human HF remains to be determined.

While protein expression of NCX was found upregulated in most animal models of HF,[3] it is less consistent in the failing human heart, with most studies finding either increased or unchanged protein expression of NCX.[43] In contrast to reduced SR Ca^{2+} uptake, the NCX current density as a function of $[Ca^{2+}]$ was not changed in the failing human heart.[30] However, the contribution of NCX to the $[Ca^{2+}]_i$ relaxation was increased due to the depressed SR Ca^{2+} uptake.[30] Furthermore, the preference of NCX current direction during the action potential plateau shifted from inward direction (Ca^{2+} efflux) to outward direction (Ca^{2+} influx) due to a reduced submembrane $[Ca^{2+}]_i$ and increased $[Na^+]_i$ in the failing human heart.[96] The reversed-mode NCX during AP plateau could contribute to a slow decay of $[Ca^{2+}]_i$ transient,[41, 96] which may facilitate contraction at slow heart rates but may also lead to diastolic dysfunction at faster heart rates.[42]

4.4 Loss of metabolic capacity

Ca^{2+} handling and energy homeostasis are interdependent.[97] Ca^{2+} homeostasis relies on efficient energy-driven ionic fluxes, i.e., through SERCA2a and Na^+-K^+ ATPase, while $[Ca^{2+}]_i$ in turn determines energy consumption through contraction and Ca^{2+} transport as well as energy production via the regulation of ATP generation in mitochondria.[97, 98] Disturbance of the finely tuned balance between the two could be responsible for the abnormal Ca^{2+} handling and diminished contractility that are hallmarks of HF.

HF is associated with defects in energy metabolism, with decreased energy production as well as impaired energy transfer and utilization.[97] These impaired cardiac energetics may represent the thermodynamic limit for Ca^{2+} handling.[99] Reduced local ATP/ADP ratio, due to a local lack of creatinine kinase, could affect the kinetic and thermodynamic efficiency of SERCA in HF,[99] providing another mechanism for impaired SR Ca^{2+} uptake. Indeed, ATP was reported to protect SERCA2a from being denatured by hydroxyl radicals,[100] implying that energy starvation might render SERCA2a unprotected from increased oxidative stress in human HF.

Improving the myocardial energetics has been shown to normalize the Ca^{2+} cycling in isolated failing human myocytes.[101] β-blockers, which decrease the energy demand and thus ameliorate the mismatch between energy production and consumption, has been shown to normalize the function and regulation of key Ca^{2+} handling proteins in failing human hearts.[102] Similarly, left ventricular assist devices (LVADs), which unload the heart and support the circulation, impart improved Ca^{2+} handling in human HF.[10, 103] Finally, hemodynamic improvement by cardiac resynchronization therapy (CRT) is correlated with improved Ca^{2+} handling in the subset of HF patients who respond to this therapy.[104] On the other hand, restoration of Ca^{2+} homeostasis may result in improved cardiac energetics.[105]

5. Correcting Abnormal Calcium Handling in HF

While the causes of HF may differ, there is a common theme underlying the progression from normal to failing heart. An initial cardiac insult, which, in the United States is most commonly inadequate myocardial flow (myocardial infarction), prolonged pressure overload

(hypertension), or abnormal flow through the heart valves (valvular stenosis or insufficiency) causes the heart to alter the shape of its principal pumping chamber, the left ventricle, and the surrounding organs to activate multiple hormonal systems in an attempt to maintain cardiac output. These compensatory changes are initially helpful in sustaining adequate cardiac output and blood pressure.[106] Over time, however, these same changes become maladaptive. The remodeled ventricle becomes increasingly dilated and hypertrophied, resulting in suboptimal pump geometry. In parallel, continuous activation of the sympathetic nervous system and the renin-angiotensin-aldosterone (RAA) axis[107, 108] leads to increased oxygen consumption, increased metabolism and molecular changes that ultimately impair the contractile function of myocytes. In this manner, an initial cardiac insult initiates a cascade of events leading to reduced ejection fraction, reduced myocardial contractility and poor cardiac output. Current clinical therapies aim to halt or reverse these maladaptive events. Myocardial revascularization by coronary artery bypass grafting (CABG) and percutaneous coronary intervention (PCI) restores myocardial blood flow to ensure that adequate oxygen and metabolites are supplied to the myocardium. On the other hand, most medical therapies aim to block the deleterious effects of prolonged hormonal stimulation and the subsequent molecular changes that ultimately impair myocardial contractility and promote fatal cardiac arrhythmias. Importantly, reduced cardiac contractility and diminished contractile reserve are hallmarks of HF, and central to these pathologies is defective intracellular Ca^{2+} handling.[39] Current and future medical strategies to restore the function of the key calcium handling molecules are discussed below.

5.1 Molecular Changes Due to Activation of the Sympathetic Nervous System

After an initial cardiac insult, the sympathetic nervous system is activated to maintain adequate cardiac output. This stress response, also known as the “fight or flight” response, is highly conserved evolutionarily and crucial to increasing cardiac stroke volume and heart rate,[109] the product of which is cardiac output. Catecholamines such as epinephrine and norepinephrine (adrenaline and noradrenaline, respectively) are released into the bloodstream where they effect changes on target organs, including the heart.

In response to sympathetic stimulation, both heart rate and myocyte contractility are increased, the latter of which is directly related to changes in Ca^{2+} handling. Epinephrine and norepinephrine bind to the β-adrenoreceptor, leading to activation of several signaling pathways that ultimately increase the amount of intracellular Ca^{2+} released by the RyR per amount of trigger Ca^{2+} entering the cell through L-type Ca^{2+} channels, thereby increasing EC coupling gain.[37] β-adrenoreceptor binding also increases contractile force and allows more rapid release and reuptake of Ca^{2+}, allowing more time for diastolic filling, increasing stroke volume and therefore increasing cardiac output.[109, 110]

Downstream effectors activated through these pathways include PKA via G protein activation of adenylate cyclase[106] and increased CAMKII activity,[13] while activation of both the RAA axis or β_1-adrenoreceptor leads to activation of PKC.[19, 111] PKA and CAMKII phosphorylate L-type Ca^{2+} channels, RyR and PLN. Many of these signaling events inherent to the “fight or flight” response are seen physiologically and in fact, are crucial for survival during isolated periods of stress or exercise. However, the continuous activation of these pathways that occurs in HF ultimately leads to defective Ca^{2+} handling and diminished contractility,[112, 113] and are therefore attractive targets of current and future medical therapies.

Chronic activation of G protein coupled β-adrenoreceptors by sympathetic hormones leads to several maladaptive changes including decreased expression and function of adenylate cyclase, increased expression of inhibitory proteins G protein G_i and β-adrenoreceptor kinase, and even decreased expression and coupling of the β-adrenoreceptor

itself.[106, 114–116] Downstream of the β-adrenoreceptor, additional changes occur. PKA hyperphosphorylates L-type Ca^{2+} channels, RyR and NCX, while simultaneously, the protein levels of L-type Ca^{2+} channels, RyR and SERCA2a are altered.[10, 41, 117, 118] In summary, chronic β-adrenoreceptor activation induces numerous changes that alter the function of proteins critical to Ca^{2+} handling. Determining which of these changes are causal for the diminished cardiac contractility seen in HF has proven far more challenging. Nonetheless, a number of pathways and targets have been elucidated.

5.2 Combating Chronic β-adrenoreceptor Stimulation

Blockade of β-adrenoreceptors using a class of drugs known as β-blockers restores cardiac function and significantly increase the survival of patients with HF.[119–121122, 123] The effectiveness of this therapy is somewhat counterintuitive since β-blockers depress contractility and heart rate, two major determinants of cardiac output. Indeed, physicians treating acute HF often temporarily discontinue β-blockers to improve cardiac output and restore fluid balance. After the acute volume overload has been corrected, however, treatment with β-blockers is reinstituted. The reasoning behind this strategy is that chronic activation of β-adrenoreceptors is ultimately maladaptive to the heart, and leads to the progression of HF. β-blockers combat this chronic sympathetic stimulation. Mechanistically, they decrease intracellular cAMP concentration and thus decrease the activity of PKA, restoring physiologic function and expression levels of downstream effectors.[102, 124, 125, 118, 126–128] Treatment with β-blockers also reverses the hyperphosphorylation of RyR to improve binding of FKBP12.6, thereby restoring physiologic RyR function.[102, 125, 128, 129] Diminished SERCA2a protein levels are also restored by β-blocker therapy, allowing appropriate reuptake of Ca^{2+} to allow adequate diastolic function and filling time.[129] Thus, β-blocker therapy may halt or reverse disease progression and reduce mortality by reversing the diminished Ca^{2+} transient amplitude in systole, while simultaneously reversing increased intracellular Ca^{2+} concentration and slowed rate of Ca^{2+} transient decay in diastole.[28, 130] It is important to point out that despite numerous studies showing improvement in HF with the use of β-blockers, the precise mechanisms responsible for their well established beneficial effects remain controversial. And while effects on Ca^{2+} handling have been demonstrated, improved organ level structural changes have also been observed.[106]

5.3 Inhibiting the Effect of Angiotensin II

In addition to the sympathetic nervous system, another hormonal axis is chronically elevated in patients with HF. The renin-angiotensin-aldosterone (RAA) axis is central to maintaining blood pressure. In response to low blood pressure, renin is released from the kidney and converts the pre-hormone angiotensinogen to angiotensin I. Angiotensin I is converted to Angiotensin II (ATII) in the lungs by angiotensin converting enzyme (ACE). ATII potently increase blood pressure by binding ATI receptors in the vasculature and causing constriction of blood vessels. ATI receptors are also located on myocytes, where they are acted upon by ATII. Two major categories of drugs designed to lower blood pressure by inhibiting this pathway are angiotensin converting enzyme inhibitors (ACEI), which block the conversion of ATI to ATII, and angiotensin receptor blockers (ARB), which inhibit the binding of ATII to ATI-receptors.

HF is a state of low cardiac output, which results in diminished blood pressure. However, low blood pressure is typically seen only at the last stages of HF. This is likely due to the maintenance of blood pressure by chronically elevated levels of ATII seen in HF patients.[107, 108] Importantly, the use of ACEIs and ARBs has been shown to further significantly reduce mortality and fatal cardiac arrhythmias in patients with HF.[122] Hence, in addition to β-blockers, ACEIs or ARBs are recommended first line agents for the treatment

of HF.[108] ACEIs and ARBs likely reduce mortality by lowering blood pressure and reducing myocardial fibrosis in patients with HF. More recently, however, they have been shown to alter signaling cascades that are involved in Ca^{2+} handling.

ATII binding to ATI receptors on myocytes activates the G protein Gq, which has multiple downstream effectors that impact the progression of HF. One of these effectors is PLC, which, in turn, activates PKCα, the predominant cardiac PKC isoform.[131] PKC-α activates I-1, which increases the activity of PP1 to dephosphorylate PLN.[19] In a mouse model of diastolic HF, ACE was overexpressed to increase levels of ATII, resulting in dephosphorylation of PLN and diastolic HF. [132] Importantly, dephosphorylated PLN inhibits the ability of SERCA2a to pump Ca^{2+} back into the SR, which may impair heart function by increasing the Ca^{2+} transient duration and decreasing the SR Ca^{2+} content. This alteration likely exacerbates the reduced diastolic filling time, stroke volume and contractility that are seen in heart failure.

ATII mediated Gq activation also causes activation of the downstream effectors calcineurin[133, 134] and mitogen-activated protein (MAP) kinases such as extracellular signal regulated kinases 1 and 2 (ERK1/2),[118, 135] leading to pathological hypertrophic ventricular remodeling. Calcineurin, a Ca^{2+}-dependent phosphatase that regulates hypertrophic gene transcription by desphosphorylating transcription factors such as nuclear factor of activated T-cells (NFAT), has been identified as a key enzyme involved in the induction of pathological cardiac hypertrophy. NFAT activity was upregulated in mouse models of pressure overload and HF but not in mice with exercise-induced physiological hypertrophy.[136] Supporting this notion, inhibition of calcineurin prevented cardiac hypertrophy in rodent models of cardiomyopathy and pressure overload.[137] ACEIs and ARBs may thus impart some of their beneficial effects on failing hearts by reversing maladaptive effects of ATII on Ca^{2+} handling, which, in turn, may also ameliorate ventricular remodeling via the Ca^{2+} dependent calcineurin pathway. Interestingly, all of these effects appear to occur through activation of major phosphatase, rather than kinase effectors.

Patients with HF exhibit elevated levels of reactive oxygen species (ROS).[138] ATII has been shown to be a key player in producing destructive ROS that are involved in the progression of HF.[139, 140] ATII induces the production of ROS in the heart,[141] leading to damage to cellular components that are critical to calcium handling in myocytes such as the outer and inner membranes of organelles such as the SR and protein involved in Ca^{2+} signaling and handling.[142–145] ROS alter the function of NCX, decrease L-type Ca^{2+} channel currents and depress the activity of SERCA2a.[146–148] Thus, blockade of ATII through ACEIs and ARBs provides clinicians a tool to reduce oxidative stress in failing myocytes and move closer to the goal of restoring calcium handling.

Interestingly, blocking ATII may also directly inhibit β-adrenoreceptor overstimulation. The ARB valsartan binds presynaptic ATI receptors to inhibit the release of norepinephrine while also stimulating its reuptake,[149] thereby reducing the amount of norepinephrine released into the bloodstream. Whether this holds true for other ARBs is unclear.

5.4 Calcium Channel Blockade

One of the disappointments of clinicians and researchers alike is the lack of benefit and even detrimental effect of drugs that block L-type Ca^{2+} channels (dihydropyridine receptors). These drugs, used primarily to treat blood pressure, have dilatory effects on blood vessels inside and outside the heart. The vasodilatory action of Ca^{2+} channel blockers was theorized by many to improve cardiac performance and reduce myocardial ischemia. However, these

theoretical advantages have not been translated into clinical benefits in controlled clinical HF trials, discussed in more detail below.

Ca^{2+} channel blocking drugs have not improved symptoms of HF or enhanced exercise tolerance, and signficantly, both short- and long-term treatment with these drugs have increased the risks of worsening HF and death.[150–156] L-type Ca^{2+} channel blockade is also ineffective in mild HF,[157] and non-dihydropyridine type Ca^{2+} antagonists, including T-type Ca^{2+} channel blockers, have also proven ineffective.[158, 159] Moreover, Ca^{2+} channel blockers can have life threatening interactions with β-blockers and ACEIs, by inducing severe bradycardia (low heart rate) and hypotension (low blood pressure).[160, 161] As a result, most Ca^{2+} channel blockers are avoided in patients with HF, even when used for the treatment of chest pain or hypertension. Of available agents, only the dihydropyridine drug amlodipine has been shown not to adversely affect survival, although experience with the drug exists largely in patients who are not taking β-blockers.[162] In fact, current clinical HF guidelines state that Ca^{2+} channel blocking drugs are contraindicated in the treatment for patients with current or prior symptoms of HF and reduced left ventricular ejection fraction.[108]

5.5 Experimental Therapies Targeting Abnormal Calcium Handling

The limited medical therapies available for the treatment of HF and the significant abnormalities in Ca^{2+} handling observed in failing hearts has prompted significant interest in developing therapies that directly target and correct these altered molecular pathways. A subset of some promising therapies and strategies are discussed below.

5.5.1. Sensitizing β-adrenoreceptor Function—Restoring sensitization of β-adrenoreceptors via new agents represents an attractive drug therapy. The desensitization of β-adrenoreceptors is mediated in part by G-protein coupled receptor kinase (GRK, also known as β-ARK). Activation of GRK desensitizes β-adrenoreceptors in the heart.[163] In contrast, inhibition of GRK with concomitant β-blocker therapy improves survival in a mouse model of HF.[164, 165] Currently, no drug has been developed to inhibit GRK pharmacologically, but a dominant-negative GRK expressed in a mouse model of HF has been shown to prevent disease progression.[165] In the future, such a multi-pronged approach to combat chronic β-adrenoreceptor stimulation may provide significant further benefit in the treatment of HF.

5.5.2. Improving RyR Function—Hyperphosphorylation of RyR by PKA occurs due to chronic β-adrenergic stimulation and leads to Ca^{2+} leak through the RyR,[10166] which contributes to contractile dysfunction and fatal ventricular arrhythmias.[109, 167, 168] These arrhythmias are a major cause of death in patients with advanced HF.[108] This knowledge has prompted efforts to design a small molecule that will improve RyR function. JTV519 is an experimental drug that enhances the binding of FKBP12.6 to RyR and reduces Ca^{2+} leak from the SR. Encouragingly, this agent has been shown to suppress ventricular arrhythmias in a mouse model[168] and improve contractile function in a canine model of HF.[169] Finally, Inhibition of CAMKII, which also hyperphosphorylates RyR, represents another strategy of reducing the RyR Ca^{2+} leak seen in HF. Chemical or genetic inhibition of CAMKII prevented cardiac remodeling in a murine model of isoproterenol-induced cardiomyopathy.[170]

5.5.3 Enhancing SERCA2a Reuptake of Calcium—As discussed above, diminished Ca^{2+} reuptake in failing hearts is likely due to a combination of decreased levels of SERCA2a expression[4329, 17131] and hypophosphorylation of PLN.[172, 173] Understandably,

methods to increase SERCA2a expression and PLN phosphorylation are being actively pursued by investigators.

Attempts to overexpress SERCA2a in humans with advanced HF are currently underway.[76] In this study, a SERCA2a expression vector packaged in an Adenovirus-Associated Virus (AAV) envelope is administered by direct intracoronary injection.[76] Of the 9 patients treated, 5 demonstrated improvements from baseline to month six across a number of parameters important in HF, including symptoms, 6-minute walk test, oxygen consumption, and ejection fraction.[76] Notably, 2 patients who failed to improve had preexisting anti-AAV neutralizing antibodies, underscoring a limitation of such an approach.[76]

Selectively enhancing PLN phosphorylation is another avenue being actively pursued. One such line of experiments involves the delivery of a pseudo-phosphorylated mutant of PLN into failing hearts using a viral vector. Interestingly, this mutant was demonstrated to suppress HF progression in hamsters[174] and post-myocardial infarction rats,[175] and reversed HF in sheep with chronic pacing induced HF.[176] Decreasing the activity of PP1 (which dephosphorylates PLN) is another possible approach. Inhibitor-2 (INH-2) is an endogenous phosphatase that inhibits PP1 that selectively decreases SR microvesicle-associated PP1 activity. Moreover, gene delivery of INH-2 was shown to increase PLN phosphorylation and increase survival in a hamster model HF.[177] Despite these encouraging studies, no small molecules that can selectively increase PLN phosphorylation have been developed to date.

5.5.4 Retarding ROS Effects—Established experimental studies showing that ROS are detrimental have prompted efforts to combat oxidative damage in patients with HF. The β-blocker carvedilol has been proven effective in reducing the progression of HF, and notably, may exert some of its beneficial effects through anti-oxidant activity.[119] Xanthine oxidase (X0), an enzyme involved in the synthesis of DNA precursors, has been shown to generate ROS, and has attracted the interest of researchers as a molecule to target with inhibitors.[178, 179] Encouragingly, inhibition of XO with the drug allopurinol improved myocardial efficiency in patients with HF,[180] and prompted the initiation of a large-scale clinical trial testing its safety and efficacy in patients with advanced HF.[181] While this study did not show a reduction in morbidity or mortality overall, post-hoc analysis revealed benefit in a subset of patients with elevated levels of the XO product uric acid.[182] And while it will be helpful to determine the specific changes in Ca^{2+} handling pathways that were affected in this subset of patient, this study also suggests that more robust improvements in HF may be elicited in future trials by more specifically targeting the subset of HF patients with elevated ROS activity.

5.6 Calcium Abnormalities in Human Ischemic versus Non-ischemic Cardiomyopathy

End-stage HF that is the result of myocardial infarction is known as ischemic cardiomyopathy (ICM). HF in patients with no history of myocardial infarction is referred to as non-ischemic cardiomyopathy (NICM). Interestingly, distinct abnormalities in calcium handling have been demonstrated depending upon the etiology of HF. For example, mRNA expression of RyR is significantly decreased in the hearts of patients with end-stage ICM, but increased (albeit nonsignificantly) in patients with NICM.[183] Similarly, ICM is associated with a decreased rate of calcium uptake into the SR, while NICM is associated with a decreased rate calcium release from the SR.[184] The authors of the latter study concluded that abnormal SR calcium uptake may explain the contractile dysfunction seen in ICM, while abnormal SR calcium release may be the primary disruption in NICM. Thus, different insults leading to cardiomyopathy may do so by disrupting calcium handling via different mechanisms.

While different etiologies of HF exhibit different profiles of calcium handling abnormalities, there are no therapies to date that have been shown to improve calcium handling in an etiology-specific manner. Recently, Sossalla and colleagues demonstrated that inhibition of CaMKII using two different small molecules significantly increased contractility of isolated preparations of human failing heart tissue.[16] The increase in contractility was equal in HF of ischemic and non-ischemic etiologies. These studies suggest that in addition to the need to use human tissue to analyze signaling pathways and small molecules for improved calcium handling, separating observations with respect to etiology will be important.

6. Conclusion

It has been well recognized that abnormal Ca^{2+} handling is a key pathophysiological mechanism in human HF. However, our understanding of the underlying molecular and cellular mechanisms for the altered calcium handling in the failing human heart remains incomplete.

This is partly due to the complexity of the system, which involves the interplay between a number of signaling pathways that regulates the Ca^{2+} homeostasis at different time scales.[75, 185] That is, while interrupting or augmenting one of pathways in the cascade might lead to expected beneficial therapeutic effects, it might also produce unexpected deleterious effects.[75] Nevertheless, the overall structure of this complex system is continually being revealed by ongoing basic and clinical research, which carries the hope of facilitating the development of effective diagnostic and treatment modalities for HF. The progress is also slowed by limited data from human studies. While many mechanistic hypothesis and potential therapeutic intervention for the abnormal Ca^{2+} handling in HF are being proposed and tested in animal models of HF, the examination of these hypothesis and therapies using functional studies of isolated cells or tissues from the failing human heart are rather limited. Basic understanding and clinical translation can be greatly facilitated by testing these hypotheses in explanted human heart and human heart tissue donated for research by patients.[186]

Gaining a clearer understanding of the causative mechanisms of abnormal Ca^{2+} handling is crucial to developing promising new therapies to treat HF. Despite our best efforts, there are currently only two major medical pharmacological approaches available to the clinician for the treatment of patients with HF: blockade of the β-adrenoreceptor and inhibition of the RAA axis. These therapies are used to treat non-ischemic (the majority of which are idiopathic), ischemic and valvular cardiomyopathies, even though we recognize fundamental differences in the insults that cause these separate conditions. Such blanket approaches demonstrate the limits of our current knowledge, and the need for further observation and testing before new therapies can be delivered to the patient. Moreover, it is clear that many pathways involving Ca^{2+} handling converge on and act through a few key molecules. Thus, the complex biological processes leading to HF must be further dissected with respect to specific isoforms, subcellular locations and etiology of HF. Similarly, it is important to realize that individual drugs effects must be categorized based on the species and type of animal model used. Finally, we must recognize that the road to developing a human therapeutic agent, i.e., going from the bench to the bedside, is a time consuming and expensive one, and littered with failures. These complexities may explain why after years of research, the clinical armamentarium for reversing HF remains rather limited. Despite these drawbacks, it is encouraging that many promising new therapies to ameliorate abnormal calcium handling are visible on the horizon, based on findings in animal models of HF. Increased research on functional human heart tissue would facilitate translation of these findings to the clinic.

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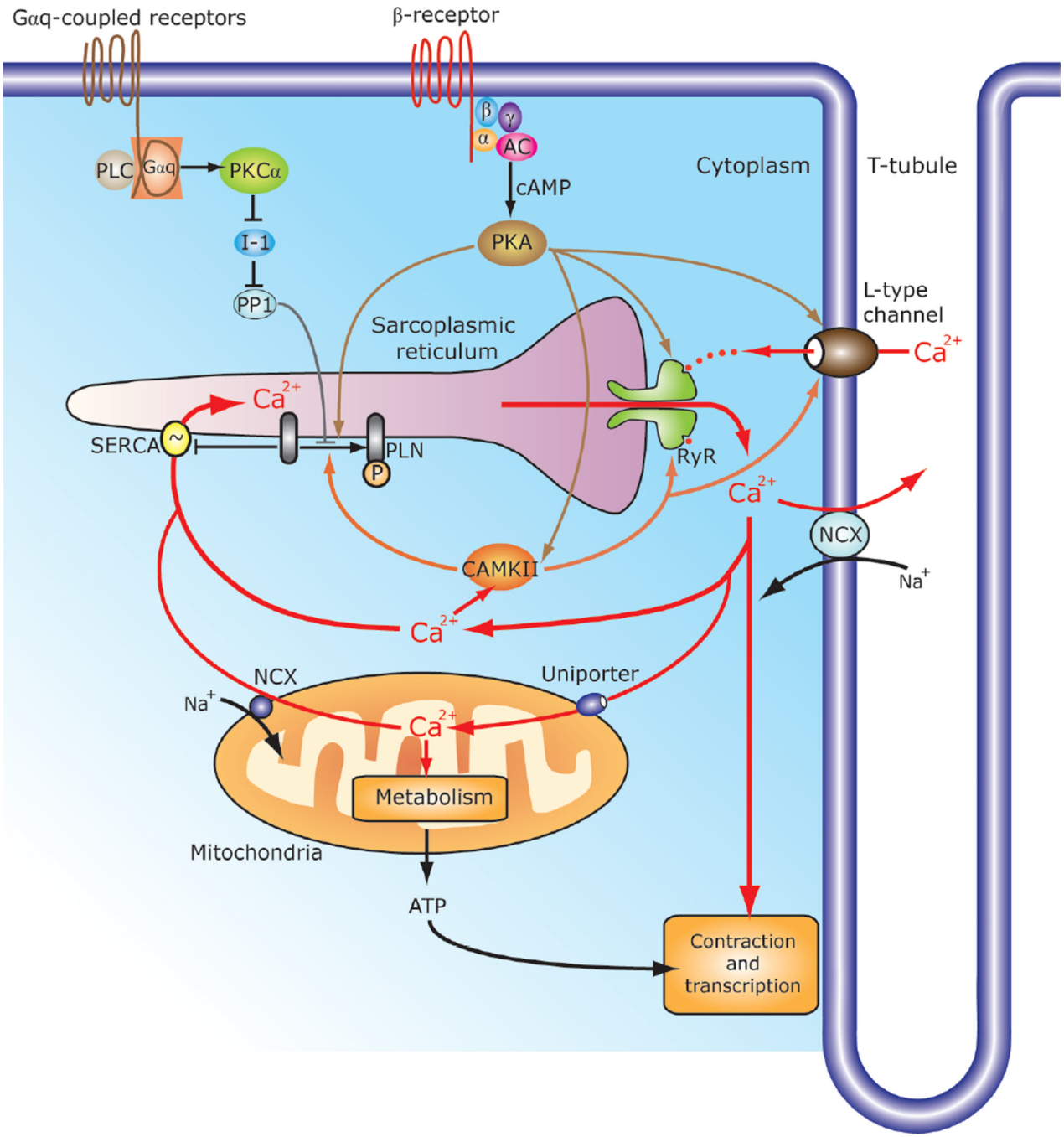

Figure 1.

Intracellular Ca^{2+} cycling and regulation by signaling pathways. After the activation of sarcolemma (including T-tubules), Ca^{2+} enters cytoplasm through L-type Ca^{2+} channel. The entering Ca^{2+} then induces a much larger Ca^{2+} release from the sarcoplasmic reticulum (SR) via the ryanodine receptor (RyR). The released Ca^{2+} binds with Troponin-C to activate contraction. Relaxation starts when Ca^{2+} is returned by sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) to SR and via the Na^{+}/Ca^{2+} exchanger (NCX) to the extracellular domain. Some Ca^{2+} enters mitochondria to stimulate the production of ATP which is utilized for contraction and transcription. SERCA is inhibited by the dephosphorylated phospholamban (PLN). PLN can be phosphorylated by protein kinase A (PKA) and Ca^{2+}/calmodulin-

dependent kinase (CAMKII), both of which can be activated by β-adrenergic stimulation. PLN can be dephosphorylated by phosphotase 1 (PP1), which can be activated through Gαq-coupled receptors (angiotensin II receptor, endothelin 1 receptor, or α-adrenergic receptor). α, G-protein subunit α; β, G-protein subunit β; γ, G-protein subunit γ; AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate.

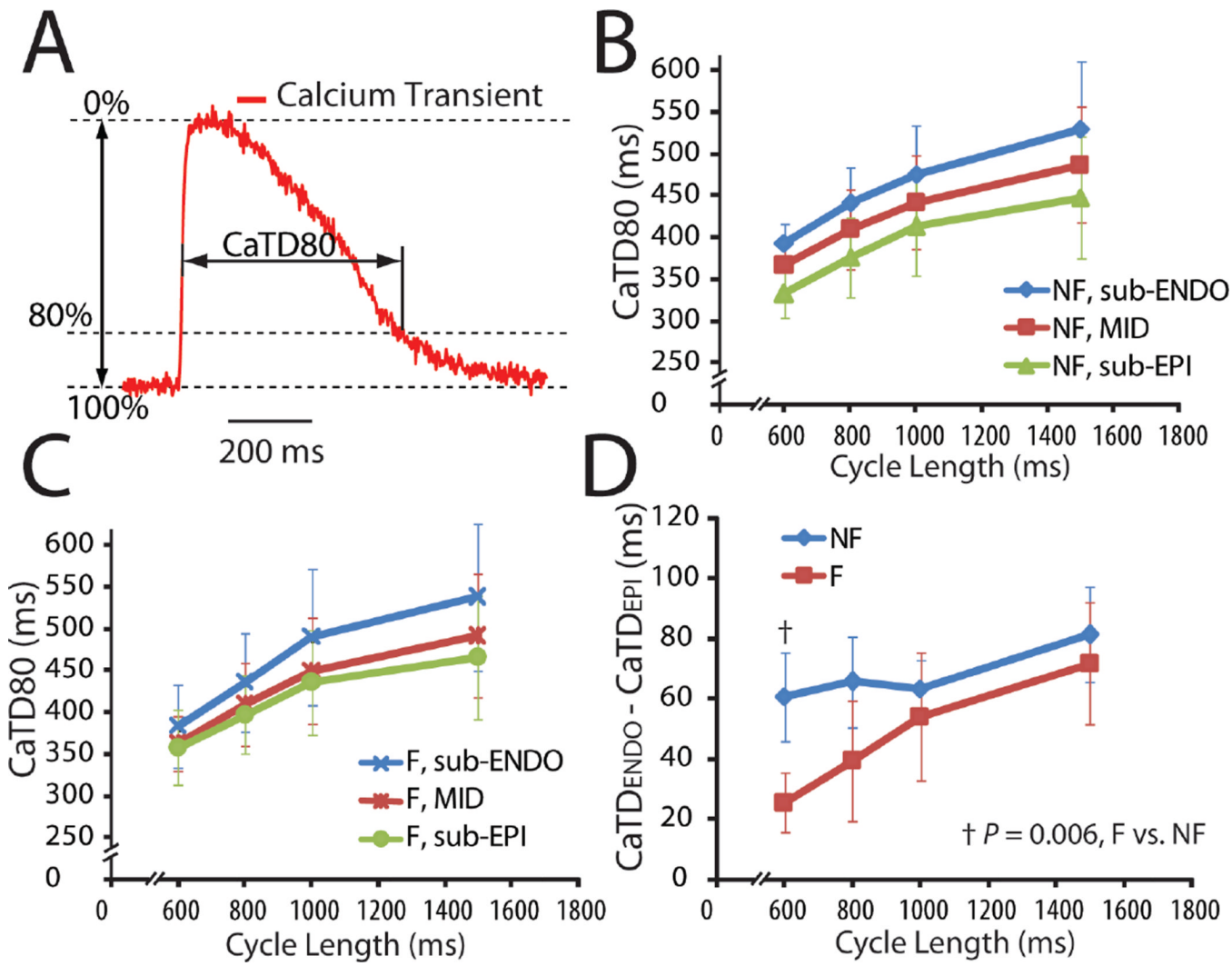

Figure 2.

Region-dependent and cycle-length-dependent calcium transient duration (CaTD) in failing human heart. (A) CaTD was quantified at 80% relaxation (CaTD80). (B) CaTD80 at nonfailing human hearts (n=6) at subendocardium (sub-ENDO), midmyocardium (MID), and subepicardium (sub-EPI). (C) CaTD80 at failing human hearts (n=5). (D) The difference of CaTD80 between sub-ENDO and sub-EPI. It can be seen that this difference is significantly reduced in failing heart at faster heart rate (cycle length at 600ms).[31] These data are obtained from Ca^{2+} transient measured using Rhod-2AM from the coronary-perfused wedge preparations from both failing and non-failing human hearts.

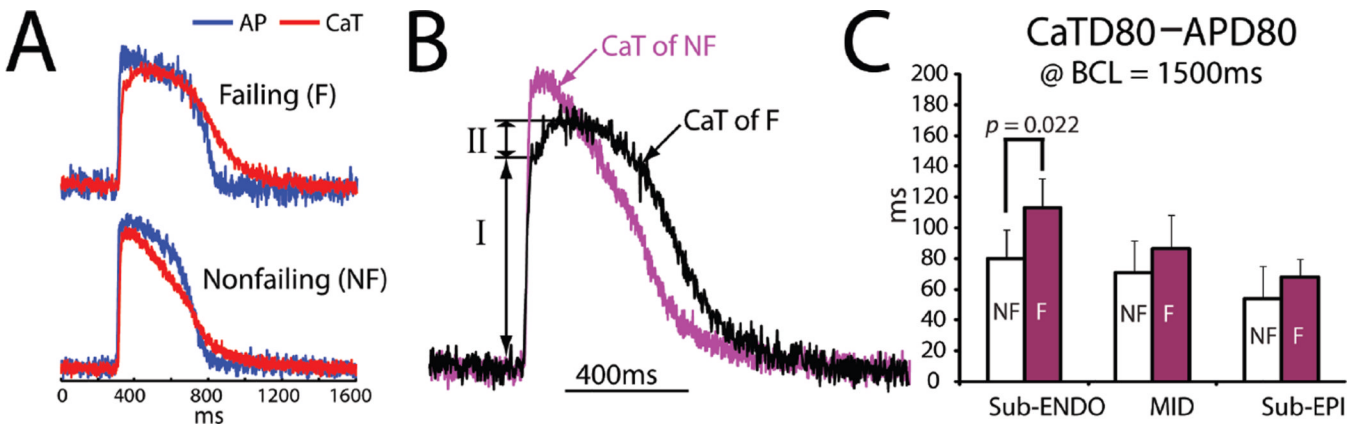

Figure 3.

Morphological changes of calcium transient (CaT) and its relation to action potential (AP). (A) Simultaneous recordings of AP and CaT at one site at subendocardium from a failing human heart (F, top) and a nonfailing human heart (NF, bottom). (B) The two CaTs from panel A are overlapped for easy comparison. Compared to the CaT from NF, there is a distinct second rising component (labeled by “II”) in the CaT from failing human heart. Note that this second component was only observed at the sub-endocardium in 60% of the studied failing human hearts. (C) CaT duration at 80% relaxation (CaTD80) minus AP duration at 80% (APD80), summarized from five nonfailing hearts and five failing hearts (two ischemic cardiomyopathy and three idiopathic cardiomyopathy). It can be seen that this duration difference is significantly longer at the subendocardium in the failing human heart compared with the non-failing human heart, which is reflected in the example shown in panel A.[31]

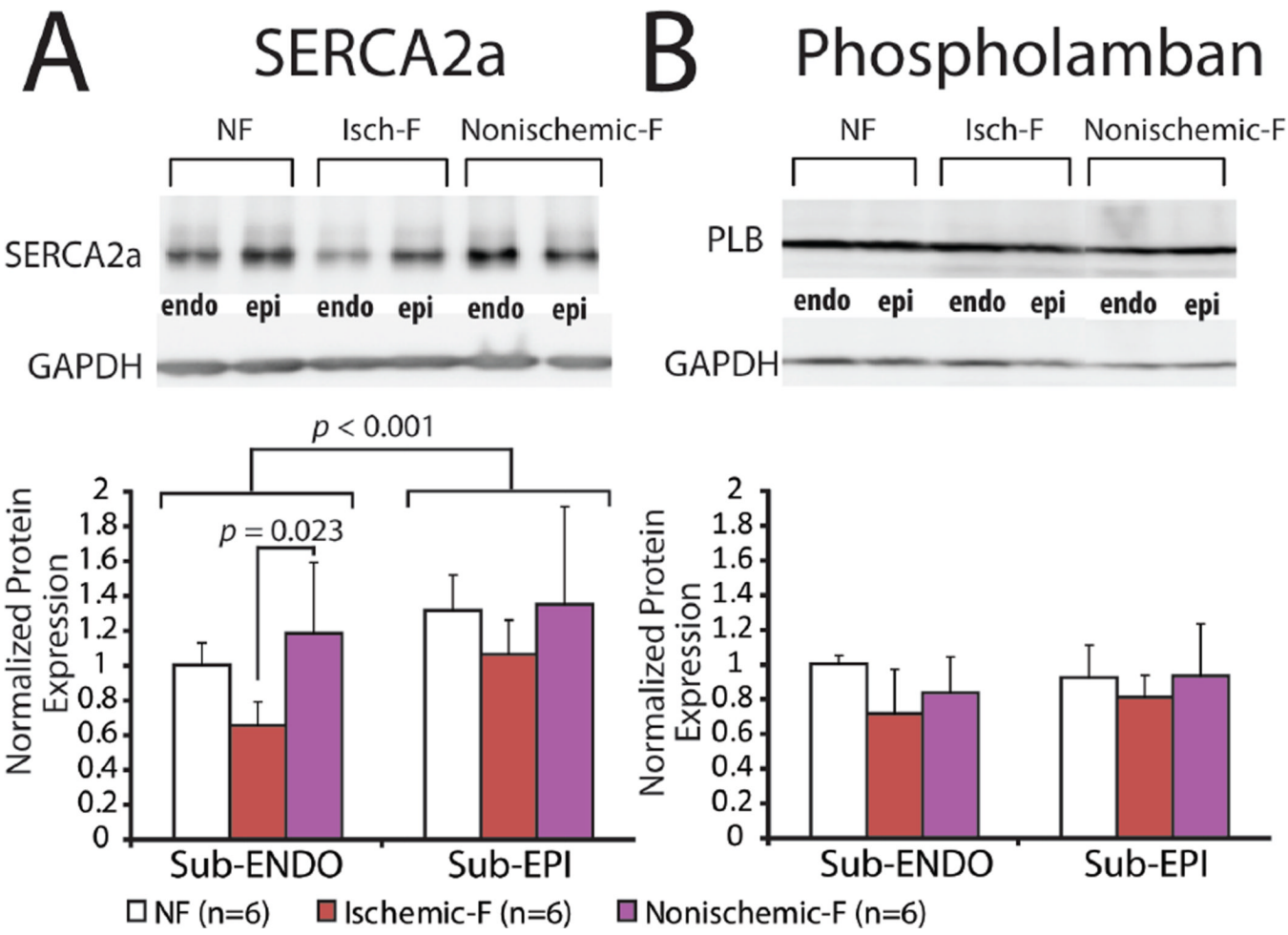

Figure 4.

Protein expressions of sarcoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a) and phospholamban (PLN). Representative examples of Western blots (top) and normalized protein expression (bottom) are shown for SERCA2a (A) and PLN (B). NF (n=6) indicates the group of non-failing hearts; Isch-F (n=6), the group of failing hearts with ischemic cardiomyopathy; and Nonischemic-F (n=6), the group of failing hearts with nonischemic/idiopathic cardiomyopathy.[31] ENDO indicates endocardium; EPI, epicardium.