This Review is the final in a thematic series on Endoplasmic Reticulum Stress and Cardiac Diseases, which includes the following articles:

The Role of Endoplasmic Reticulum Stress in the Progression of Atherosclerosis [Circ Res. 2010;107:839–850]
Endoplasmic Reticulum Stress As a Therapeutic Target in Cardiovascular Disease [Circ Res. 2010;107:1071–1082]

Interrelationship Between Cardiac Hypertrophy, Heart Failure, and Chronic Kidney Disease: Endoplasmic Reticulum Stress As a Mediator of Pathogenesis

Masafumi Kitakaze, Guest Editor

Interrelationship Between Cardiac Hypertrophy, Heart Failure, and Chronic Kidney Disease
Endoplasmic Reticulum Stress As a Mediator of Pathogenesis

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Abstract: Synthesis of transmembrane and secretory proteins occurs within the endoplasmic reticulum (ER) and is extremely important in the normal functioning of both the heart and kidney. The dysregulation of protein synthesis/processing within the ER causes the accumulation of unfolded proteins, thereby leading to ER stress and the activation of the unfolded protein response. Sarcoplasmic reticulum/ER Ca2+/H+ disequilibrium can lead to cardiac hypertrophy via cytosolic Ca2+ elevation and stimulation of the Ca2+/calmodulin, calcineurin, NF-AT3 pathway. Although cardiac hypertrophy may be initially adaptive, prolonged or severe ER stress resulting from the increased protein synthesis associated with cardiac hypertrophy can lead to apoptosis of cardiac myocytes and result in reduced cardiac output and chronic heart failure. The failing heart has a dramatic effect on renal function because of inadequate perfusion and stimulates the release of many neurohumoral factors that may lead to further ER stress within the heart, including angiotensin II and arginine–vasopressin. Renal failure attributable to proteinuria and uremia also induces ER stress within the kidney, which contributes to the transformation of tubular epithelial cells to a fibroblast-like phenotype, fibrosis, and tubular cell apoptosis, further diminishing renal function. As a consequence, cardiorenal syndrome may develop into a vicious circle with poor prognosis. New therapeutic modalities to alleviate ER stress through stimulation of the cytoprotective components of the unfolded protein response, including GRP78 upregulation and eukaryotic initiation factor 2α phosphorylation, may hold promise to reduce the high morbidity and mortality associated with cardiorenal syndrome. (Circ Res. 2011;106:629-642.)

Key Words: angiotensin ■ calcium ■ cardiac failure ■ cardiac hypertrophy ■ ER stress

Heart failure can be subdivided into 2 important categories, acute and chronic. Acute heart failure is primarily a manifestation of underlying vascular disease, including atherosclerosis, that leads to a sudden interruption of cardiac blood supply through thrombus formation and consequently myocardial infarction (MI). To a lesser degree, MI may also result from coronary artery vasospasm and hyperreactivity of the underlying vascular smooth muscle layer caused by an imbalance between cellular factors that modulate vasoconstriction and vasodilation. In both cases, cardiac myocyte hypoxia results in cell death through apoptosis or necrosis, an acute inflammatory response and a chronic inflammatory response in the region of cell death. This results in scar tissue formation and the reduction of cardiac contractile capacity through myocyte cell loss.
The ER is the site of folding of transmembrane, secretory, and ER luminal proteins. If cell demand for protein folding exceeds the production capacity of the ER, improperly folded proteins may accumulate. The lack of cellular homeostasis resulting from this accumulation of unfolded proteins is referred to as ER stress. ER stress has been found to be an important mediator of atherosclerosis through a number of pathophysiological mechanisms including hyperhomocysteinemia, free cholesterol accumulation, oxidative stress, and the generation of reactive nitrogen species. These findings offer a new understanding of the molecular mechanisms responsible for atherosclerosis and an opportunity to define new molecular targets for therapeutic intervention into atherosclerosis, with the possibility of preventing MI. These aspects of acute heart failure are the topic of a review in this series by Dr Ira Tabas.

On the other hand, cardiac failure may result from a chronic deterioration of the myocardium because of an imbalance between the demands of the body for blood/oxygen delivery and the capacity of the myocardium to meet this demand. Thus, chronic heart failure results from an insufficiency in cardiac output which in turn affects renal perfusion and may lead to vasomotor nephropathy, as well as release of renal hormones that have direct effects on the myocardium. Cardiac hypertrophy may be adaptive initially, allowing the heart to increase cardiac output and compensate for adverse hemodynamics. This has been demonstrated in animal models where inhibition of cardiac hypertrophy with cyclosporine (Cs)A resulted in increased mortality because of heart failure. However, epidemiological studies have demonstrated that chronic cardiac hypertrophy is an independent risk factor for morbidity and mortality in the general population and in populations of people with hypertension. Cardiac hypertrophy may also exist within populations of otherwise healthy young adults who lack most common risk factors for cardiovascular disease. In competitive athletes, cardiovascular abnormalities represent the most common cause of sudden death. In this population, the most frequent cause of sudden cardiac death was hypertrophic cardiomyopathy (36%) with increased cardiac mass accounting for another 10% of deaths. These facts point to the role of hypertrophic cardiomyopathy plays in chronic heart failure.

In most populations, chronic cardiac failure is associated with a number of important comorbidities. One of the most important comorbidities is renal insufficiency or end stage renal disease. The interrelationship between chronic kidney disease (CKD), cardiac hypertrophy, and chronic heart failure becomes evident on the level of basic integrative physiology. Renal failure–induced hypervolemia can result in chronic heart failure with increased central venous pressure and low systemic arterial pressure. This in turn reduces net renal perfusion pressure, further reducing renal function and stimulating many neurohumoral factors such as the renin–angiotensin system (RAS), the sympathetic nervous system, including the adrenals, and the arginine–vasopressin system, to maintain renal blood supply. These factors may all have effects on the myocardium leading to cardiac hypertrophy and the induction of ER stress.

The clinical coincidence of cardiac failure and CKD in patient populations suggests an inter-relationship between these 2 pathologies. In a prospective cohort study of all patients undergoing coronary angiography and having received a diagnosis of chronic heart failure, it was found that the incidence of CKD was 39%. The direct effect on the myocardium of renal insufficiency and of the failing heart on the decline in renal function is a new and emerging concept of increasing importance in the management of a growing patient population experiencing the interaction of these comorbidities. Thus, cardiorenal syndrome significantly affects the morbidity and mortality associated with chronic heart failure in the population. This review focuses on cardiac hypertrophy and chronic heart failure taking into account one of their major precipitating comorbidities, CKD, and the role that ER stress plays as a causal agent in both of these conditions.
Cardiorenal Syndrome: Therapeutic Refractivity

Patients with CKD are frequently excluded from large, multicentered randomized controlled clinical trials of drugs to treat chronic congestive heart failure.20 This makes the evidence of therapeutic efficacy available for the management of patients with cardiorenal syndrome limited. Classic management of chronic heart failure in patients involves many therapeutic approaches that may adversely affect renal function. Intravascular volume expansion is frequently managed in patients with congestive heart failure by the use of diuretics. However, diuretics may further exacerbate renal failure because of induction of renal hyoperfusion and ischemia.19 Although diuretics provide short-term symptomatic relief of edema and ascites, higher doses have been associated with heart failure and sudden death.21 Angiotensin-converting enzyme (ACE) inhibitors and angiotensin (Ang) II receptor blockers (ARBs) are frequently used in the management of both chronic heart failure and chronic kidney disease. ARBs have shown benefit beyond their ability to lower blood pressure in renal disease.22 However, the efficacy of ARBs in chronic heart failure is mixed with some trials showing reductions in cardiovascular morbidity and mortality but not all-cause mortality.22 Excessive diuresis leading to hypovolemia and hypotension may lead to a further decline in renal function resulting in greater stress on the failing heart.19 The contradictions between hemodynamic modification needed to alleviate chronic heart failure and CKD suggest the requirement for new therapeutic approaches that target the molecular mechanisms of both these comorbidities simultaneously. In this context, it should be noted that modification of protein synthesis through the use of histone deacetylase inhibition has been suggested as an approach to reduce the morbidity and mortality associated with cardiorenal syndrome.23 These agents appear to play a role in reducing cardiomyocyte hypertrophy. It has been shown that inhibition of histone deacetylation with valproic acid or trichostatin A was able to inhibit cardiac hypertrophy induced by both Ang II infusion (1.3 mg/kg per day) or pressure overload attributable to aortic banding for 14 days in CD1 mice.24 Valproic acid was also shown to reduce myocardial fibrosis induced by aortic banding with 7 weeks of treatment.24 Further of note is that many histone deacetylase inhibitors such as valproic acid, trichostatin A,25 and 4-phenyl butyric acid26,27 have properties that effect protein folding through modifying the expression of ER stress response genes, including GRP78.

Synthesis of Proteins Through the Endoplasmic Reticulum

The rough (R)ER is the cellular organelle where the synthesis of transmembrane and secretory proteins occur.28 ER stress represents a disturbance in the homeostasis of the ER that interferes with proper protein folding. Protein folding is a complex process by which the nascent polypeptide chain is converted into a thermodynamically stable tertiary structure that corresponds to the proper functional conformation of the protein.29 Quality control of protein folding is an important component of ER function in the secretory pathway so that incorrectly folded proteins are recognized before their movement to the Golgi complex and degraded via the proteasome.29 Protein misfolding may lead to the aggregation of misfolded proteins in the RER, resulting in organelle and cellular dysfunction.

The RER is a membrane bound organelle that creates a distinct environment from the cytosolic space specialized for protein folding. Glutathione in the cytosol acts as a major redox buffer and predominately exists in its reduced form, whereas in the ER reduced glutathione to oxidized glutathione levels are equal, creating a more oxidizing environment that facilitates disulfide bond formation.29 The ER also functions as an important storage site for Ca2+ and as such regulates contractility in both smooth and cardiac muscle. Furthermore, many of the ER-resident molecular chaperones are Ca2+ binding proteins,29 including GRP78, calnexin, and calreticulin.

Agents and/or conditions that cause ER stress induce the unfolded protein response (UPR), an integrated intracellular signaling pathway that consists of 3 resident ER membrane bound transducers; insulin-response element (IRE)1, activating transcription factor (ATF)6, and PERK (Figure 1). GRP78 regulates the activation of these transducers through its interaction with them or its interaction with unfolded proteins within the lumen of the ER. Once GRP78 dissociates from these transducers, the UPR is activated, resulting in IRE1 and PERK autophosphorylation as well as ATF6 release from the ER and cleavage by site 1 (S1P) and site 2 proteases (S2P) in the Golgi. The signaling from PERK leads to the phosphorylation of eIF2α, which reduces the translation of many proteins because of inhibition of GTP, eIF2, tRNA complex formation.31 However, under these conditions some proteins are preferentially translated, including ATF4. ATF4 acts in the nucleus as a transcription factor to induce CHOP/GADD153, which then acts to upregulate GADD34/PP1 complex formation to dephosphorylate the α subunit of eIF2 (Figure 1).32 IRE1 activation leads to the splicing of XBP1 mRNA generating an active XBP1 transcription factor that translocates to the nucleus to upregulate the expression of protein folding chaperones.33 The UPR also plays a role in unstressed cells. Specifically it appears to be important in the differentiation of certain cell types including the plasma cell,29 adipocytes,29 and in epithelial-to-mesenchymal transformation (EMT).34 The topic of ER stress in cardiovascular disease has been reviewed by Minamino and Kitakaze and the reader is directed to this review for a more detailed explanation of UPR activation in the pathophysiology of cardiovascular disease.33

Cardiac Hypertrophy Associated With ER Stress

Cardiac hypertrophy is typically regarded as a compensatory mechanism of the heart in response to increased systemic demand for cardiac output. The development of cardiac hypertrophy involves changes in hemodynamics, neurohormonal activation, growth factors and cytokines, resulting in enlarged cardiomyocytes and increased sarcomere assembly. It has been suggested that the discovery of molecular markers specific to different phenotypes of hypertrophic hearts could lead to effective treatments used to combat specific promoters of cardiac hypertrophy.13 It has been hypothesized that ER
stress is a pathogenic factor responsible for inducing cardiac hypertrophy. ER stress may act directly to induce protein synthesis in cardiac myocytes, and thereby produce cell enlargement and cardiac hypertrophy. Hypertrophied cardiac myopathy is often accompanied by increased fibrillar collagen in the interstitial space of the hypertrophied left ventricle (LV), leading to myocardial stiffness.13

The mechanism by which ER stress induces protein synthesis in cardiac myocytes is unknown. The initiation of the UPR involves the phosphorylation of eIF2α, thereby leading to a general inhibition of protein synthesis in most cell types.29 However, this response is short lived with dephosphorylation of eIF2α via ATF4 induced expression of CHOP/GADD153 and induction of GADD34 reversing this inhibition.35 Many studies have shown that cardiac hypertrophy induced by physiological stress attributable to suboptimal hemodynamics is dependent on cytosolic Ca2+ levels in cardiac myocytes.36 In response to multiple inducers, CsA is known to inhibit the development of cardiac hypertrophy.36 However, CsA has also been shown to induce cardiac failure and increase mortality in a mouse model of pressure overload.13 It has been suggested that calcineurin (CaN) and the NF-AT family of transcription factors are potential targets for new antihypertrophic agents.36 Ca2+ dependent transcriptional activation in cardiac myocytes via the Ca2+, calmodulin, CaN, NF-AT pathway is well reviewed by Bers.37 It has been demonstrated that Ca2+-mobilizing agents, such as Ang II and phenylephrine, which induce increases in cytosolic Ca2+ in cardiac myocytes, stimulate cardiac hypertrophy.38 These effects appear to be mediated by CaN through the dephosphorylation of NF-AT3 and its subsequent translocation to the nucleus to interact with promoters for hypertrophic response genes.38 Indeed, in vivo experimentation has shown that a constitutively active NF-AT3 mutant expressed in the hearts of transgenic mice produced ventricular wall fibrosis and cardiac myocyte enlargement,38 demonstrating the importance of a Ca2+, CaN, NF-AT3 pathway in cardiac hypertrophy.

ER stress may stimulate the Ca2+, CaN pathway in cardiac myocytes by causing increases in cytosolic Ca2+. Ang II, a known mediator of cardiac hypertrophy, has been shown to induce ER stress in rat cardiomyocytes by increased ER chaperone and CHOP/GADD153 expression.39 In cardiac myocytes, thapsigargin, tunicamycin and Ang II significantly increased atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) mRNA, total protein synthesis, and cell surface area, indicating cardiac hypertrophy. Thapsigargin treatment induced ER stress and increased cytosolic Ca2+ levels, indicating decreased sarcoplasmic/endoplasmic reticulum Ca2+ ATPase (SERCA) activity. Thapsigargin-treated cardiac myocytes, pretreated with CsA, showed a decrease in cardiac hypertrophy, ANP and BNP mRNA expression.40 However, another study failed to show 10 nmol/L thapsigargin produced cardiac myocyte hypertrophy or changes in ANP transcript levels. This occurred despite 10 nmol/L thapsigargin increasing resting cytosolic Ca2+ levels.41 However, this study did not examine ER stress induction.41 This dose of thapsigargin (10 nmol/L) was previously shown to produce little cardiac myocyte cell death but significant SERCA2 inhibition after 3 days of treatment.42 When observed, the hypertrophy response to ER stress appears to be dependent on a Ca2+, CaN pathway. Further study is required to examine a broader array of ER stress inducing agents to determine whether they affect cardiac hypertrophy differently depending on their mechanism of action.

Other conditions may also lead to ER stress and cardiac hypertrophy. Heavy, chronic alcohol consumption (7 to 8 drinks per day for greater than 5 years) is a risk factor for the development of cardiac hypertrophy.43 These effects appear to involve multiple cellular pathways, with ER stress playing an important role. It has been demonstrated in albino Friend virus-B type (FVB) mice that chronic alcohol consumption

Figure 1. The unfolded protein response. The ER chaperone GRP78 is typically bound to the UPR activators, ATF6α, PERK, and IRE1. However, GRP78 tends to have a higher affinity for unfolded proteins (UPFs), and when an abundance of UPFs occurs, GRP78 dissociates from these activators and binds to the UPFs. Following the dissociation from GRP78, PERK and IRE1 are auto-phosphorylated, whereas ATF6α is translocated to the Golgi. The auto-phosphorylation of PERK results in the phosphorylation of eIF2α, which in turn attenuates general protein translation, whereas upregulating the translation of the transcription factor ATF4. In the nucleus, ATF4 increases CHOP/GADD153 protein levels, which in turn upregulates the formation of the GADD34-PP1 complex, leading to eIF2α dephosphorylation. When activated, IRE1 results in the splicing of XBP1.
(4% of diet for 12 weeks) resulted in increased heart weight and heart-to-body weight ratio. Class I alcohol dehydrogenase efficiently oxidizes alcohol resulting in increased production of acetaldehyde, an ethanol metabolite.\textsuperscript{44} In the myocardium of FVB mice chronically fed alcohol, GRP78, CHOP/GADD153, and IRE1\textsubscript{α}/H9251 protein expression levels were increased,\textsuperscript{44} indicative of a UPR. Furthermore, in FVB mice overexpressing alcohol dehydrogenase during chronic ethanol treatment resulted in greater UPR upregulation.\textsuperscript{44} These findings indicate that acetaldehyde may induce ER stress. Although the mechanism of ER stress induction in cardiac myocytes by acetaldehyde is in need of further study, it has been shown that acetaldehyde affects ER Ca\textsuperscript{2+} handling in rat ventricular myocytes.\textsuperscript{45}

In the above studies, Li et al and Ren et al attribute alcohol-induced cardiac hypertrophy to acetaldehyde generation and Ca\textsuperscript{2+} handling.\textsuperscript{44,45} To provide further evidence of this effect, Li et al generated FVB mice overexpressing aldehyde dehydrogenase (ALDH)\textsubscript{2}, an enzyme that metabolizes acetaldehyde. When fed an alcohol diet, 4% for 12 weeks, these mice had a blood acetaldehyde level significantly lower than control mice and were less susceptible to ER stress. Protein expression levels of IRE1\textsubscript{α}, GRP78 and CHOP/GADD153 were significantly reduced in alcohol fed mice overexpressing ALDH\textsubscript{2} as well as the phosphorylation of eIF2\textsubscript{α}. However, this was not an inherent effect of elevated levels of ALDH2 because ALDH\textsubscript{2} overexpressing mice not undergoing chronic alcohol consumption showed no difference in ER stress marker expression.\textsuperscript{46} It was also determined that heart weight and heart-to-body-weight ratio were significantly downregulated in ALDH\textsubscript{2} overexpressing mice undergoing chronic alcohol consumption.\textsuperscript{46} Taken together, these studies point to chronic alcohol consumption as an ER stress inducer in the myocardium through acetaldehyde induced-Ca\textsuperscript{2+} disequilibrium and resultant cardiac hypertrophy. However, it needs to be determined if these effects of chronic alcohol consumption on cardiac hypertrophy are also induced through a CaN pathway.

Figure 2 presents a model of the possible effects of ER stress on excitation-contraction coupling in the cardiac myocyte leading to cardiac hypertrophy through a Ca\textsuperscript{2+}/calmodulin, CaN, NF-AT3 pathway. Several studies have shown a relationship between ER stress and changes in Ca\textsuperscript{2+} handling that have been associated with cardiac myocyte hypertrophy,
although these effects have yet to be proven. Increases in cytosolic Ca\(^{2+}\) through changes in excitation-contraction coupling in the cardiomyocyte such as that demonstrated for Ang II may drive this hypertrophic pathway and lead to ER stress induction because of ER Ca\(^{2+}\) depletion. In studies using the ER stress inducer thapsigargin, sarcoplasmic/endoplasmic (SE)R Ca\(^{2+}\) depletion occurs. Other pathophysiological inducers of ER stress may act through a similar pathway including chronic alcohol consumption and arginine vasopressin (AVP). However, it has been recently hypothesized that in smooth, cardiac and skeletal muscle the SR and the ER may be distinct cellular organelles, and as such the ER stress response observed in many of the cited studies may not emanate from the SR. Although this may be the case, ER Ca\(^{2+}\) binding luminal chaperones, such as GRP94 and calreticulin, and the ER protein retention receptor (KDEL receptor) have proved critical in cardiac growth.

**ER Stress Induced by Pathophysiological Modifications of the Circulation: Cardiac Hypertrophy**

Changes in circulatory hemodynamics may result in stress on the heart. For example, elevated total peripheral resistance increases the afterload on the heart and this appears to be the critical determinant of concentric LV hypertrophy. These effects appear to involve mechanical stretch of the cardiomyocytes and Ang II signaling. Furthermore, other physiological modifications of the circulation may have a similar effect. Plasma volume expansion, as typified by electrolyte and fluid retention in chronic kidney disease would involve stretch and so may also result in cardiac hypertrophy. As well, hormones released into the circulation in these pathophysiological states may act directly on certain cell types to induce ER stress.

It has been demonstrated that AVP stimulates the release of ER Ca\(^{2+}\) in a variety of cell types. In intermedullary collecting ducts from Sprague-Dawley rats, AVP was demonstrated to produce increased cytosolic Ca\(^{2+}\), which led to the production of nitric oxide (NO). This AVP stimulated Ca\(^{2+}\) increase and subsequent NO production was abolished by thapsigargin-mediated ER Ca\(^{2+}\) depletion, demonstrating the effect of AVP to release ER Ca\(^{2+}\). In H9c2 cells, a permanent cell line derived from rat cardiac myocytes, it has been observed that 1 \(\mu\)mol/L AVP treatment increased GRP78 expression. Vasopressin treatment (10 \(\mu\)mol/L) also caused a significant increase in the phosphorylation of eIF2\(\alpha\) and a decrease in ER Ca\(^{2+}\) stores. It has been determined in H9c2 cells that ER Ca\(^{2+}\) is released several minutes after vasopressin treatment, and ER Ca\(^{2+}\) stores are slowly refilled over the course of 24 to 48 hours. Using a lucine incorporation assay, vasopressin was shown to produce a large inhibition of protein synthesis (approximately 50%) over the course of 30 minutes, with a full recovery after 100 minutes. The suppression of protein synthesis is modulated by the phosphorylation of eIF2\(\alpha\) and the activation of the UPR. However, prolonged exposure of cardiac myocytes to AVP was invariably accompanied by increased rates of amino acid incorporation into protein. Furthermore, it has been demonstrated that AVP induced hypertrophy in H9c2 cells, as measured by increased protein content in cell culture dishes without increase in cell number. This was accompanied by increased glycolysis that was inhibited by Ca\(^{2+}\) chelation. Although cell culture experiments have provided some evidence that AVP leads to cardiac myocyte hypertrophy through ER stress induced ER Ca\(^{2+}\) disequilibrium, experimentation in animal models is needed to determine whether these effects have relevance to the whole heart under conditions of CKD.

**Cardiac Failure Induced by ER Stress**

**UPR Protection Through Preconditioning**

As discussed above, ER stress leads to the activation of the UPR. However, preactivation of the UPR leading to preconditioning, before the insult of ER stress, may result in short-term protection of cardiac myocytes, thus preserving cardiac function and preventing cardiac failure. In this context, the UPR may protect the myocardium against ischemia or reperfusion-induced injury in the short-term. It has been shown that select molecules induce the expression of ER stress proteins that work in an additive or synergistic manner to attenuate postsischemic myocardial damage. Unresolved ER stress may develop into cardiac remodeling and failure, possibly via the induction of cardiac myocyte apoptosis.

Hearts isolated from control mice and transgenic mice with cardiac-specific overexpression of Na\(^+/H^+\) exchanger isoform (NHE)1 were subjected to global normothermic ischemia and reperfused in the absence or presence of the selective NHE1 inhibitor zoniporide. Results indicate that NHE1 activity was not responsible for the protective effects in the myocardium. It was found that GRP94, GRP78, calreticulin, and CHOP/GADD153 proteins were all significantly upregulated in NHE1 transgenic mice, indicating activation of the UPR producing a short-term protective effect. Long-term ER stress induction attributable to NHE1 cardiac overexpression produced dramatically different results. There was an increase in cardiac myocyte apoptosis and dilatory left ventricular remodelling leading to \(\approx90\%\) mortality in transgenic mice during a 15-month postnatal period, whereas \(>95\%\) of the control mice remained alive. Postmortem examination of the NHE1 transgenic mice showed cardiac dilatation and massive pulmonary congestion, indicative of heart failure. Thus, it appears that short-term UPR activation protects from ischemia/reperfusion injury in the heart; however, chronic ER stress activation likely contributes to cardiac failure.

One example of a select molecule that induces the expression of ER stress proteins and attenuates posts ischemic myocardial damage is dimethylxalylglycine (DMOG). DMOG is an inhibitor of prolyl hydroxylase. ER stress induction was shown by increased ATF4, GRP78, and XBP1s mRNA and protein in the myocardium of male B6,129 wild-type mice after an intraperitoneal injection of DMOG. Furthermore, in mice treated with prolyl-4 hydroxylase small interfering (si)RNA to induce prolyl hydroxylase inhibition, it was determined that the UPR was activated and CHOP/GADD153 mRNA expression was attenuated. These results show DMOG-induced UPR upregulation was associated with a protective effect, though it is unclear whether the mecha-
nism behind this effect is dependent on UPR activation. Further research involving the inhibition of the UPR in cells treated with DMOG is required to prove that prolyl hydroxylase inhibition–induced UPR preconditioning attenuates posts ischemic cardiac injury.

Prostatic androgen repressed message (PARM)-1 is a transmembrane protein expressed abundantly in rat cardiac myocytes, primarily found in the ER. PARM-1 expression was significantly upregulated in cultured cardiac myocytes treated with ER stress inducers. Using siRNA to silence PARM-1 expression, it was determined that PARM-1 plays a protective role against ER stress in cardiac myocytes. ER stress induction with siRNA-mediated PARM-1 repression significantly upregulated the expression of CHOP/GADD153 in cardiac myocytes. This coincided with increased TUNEL staining, showing a significant increase in ER stress–induced apoptotic cell death, indicating an antiapoptotic role for PARM-1 in response to ER stress. Thus, PARM-1 is an ER stress inducible gene in cardiac myocytes that plays a role in the protective effect of UPR induced preconditioning through its antiapoptotic effects.

**UPR Protection: Protein Folding Chaperone Overexpression**

In many cell types, it has been shown that overexpression of GRP78 can reduce apoptosis and cytosolic Ca\(^{2+}\) overload induced by ER stressors. In ventricular myocytes from 2-day old Sprague–Dawley rats, it was determined that proteasome inhibition, by MG132 or epoxomicin, increased the protein expression level of cytosolic unspliced XBP1, as well as the mRNA and protein levels of CHOP/GADD153. Overexpression of GRP78 dose-dependently decreased the protein level of CHOP/GADD153, increased cardiomyocyte viability and inhibited proteasome-induced apoptosis. These findings demonstrate that GRP78 overexpression in cardiac myocytes produced a cytoprotective effect against the induction of apoptosis.

**ER Stress–Induced Cardiac Failure Through Cytosolic Ca\(^{2+}\) Disequilibrium**

Wehrens et al have indicated in their review the role of Ca\(^{2+}\) channels in physiological stress-induced cardiac arrhythmia and contractile dysfunction. Heart failure results from the chronic inability of the heart chambers to sufficiently fill or eject blood because of direct or indirect maladaptive changes. These changes diminish intracellular Ca\(^{2+}\) cycling and ER Ca\(^{2+}\) concentrations, thereby releasing less Ca\(^{2+}\) via calcium-induced calcium response and resulting in a decrease in force production during excitation-contraction coupling. Heart failure can cause protein kinase A hyperphosphorylation of the ryanodine receptor (RyR) Ca\(^{2+}\) release channels and hyperactive channel function, resulting in further depletion of the Ca\(^{2+}\) stores in the SER. Aberrant Ca\(^{2+}\) cycling results in the inability of the heart to adequately pump blood, leading to further disease development in the failing heart. In addition, this drop in cardiac output leads to renal hypoperfusion and the stimulation of neural/hormonal mechanisms to maintain renal perfusion that may have long-term detrimental effects on the failing heart.

When a cardiac myocyte is excited, Ca\(^{2+}\) is released from the SER by the RyR, a Ca\(^{2+}\) release channel, increasing cytosolic Ca\(^{2+}\) levels. Ca\(^{2+}\) is removed from the cytosol via the SERCA-type Ca\(^{2+}\) ATPases. The SERCA3f isoform can be found at a submembrane area in close vicinity to the sarcolemma in human cardiac myocytes and is significantly upregulated in failing human hearts. In cardiac myocytes and human embryonic kidney 293 cells, it was determined that SERCA3f induces ER stress, indicating a possible mechanism of ER stress induction in heart failure.

It has been reported that ten individuals receiving the cancer therapeutic agent imatinib developed LV dysfunction and congestive heart failure. Effects of imatinib on mice were then investigated. In hearts isolated from wild-type mice treated with imatinib (50 mg/kg per day for 3 weeks), the protein level of phosphorylated eIF2\(\alpha\) was significantly increased, indicating an increase in the activation of the PERK pathway. The IRE1 pathway was also upregulated, as characterized by an increase in the mRNA expression of XBP1s, which can cause the activation of the Jun N-terminal kinase (JNK) pathway, leading to mitochondria-dependent cell death. Using salubrinal, an inhibitor of the dephosphorylation of eIF2\(\alpha\), it was determined that JNK activation was inhibited, suggesting that JNK activation was largely responsible for ER stress–induced cell death of cardiac myocytes. Imatinib-mediated ER stress–induced JNK activation leads to the release of cytochrome c from the mitochondria, eventually resulting in cell death, severe LV dysfunction, and heart failure.

Cardiac myocytes isolated from neonatal rat hearts treated with ER stressors displayed significant increases in the mRNA expression of GRP78, CHOP/GADD153, and p53 expression, but not caspase-12 cleavage or JNK phosphorylation, was observed. Four weeks after TAC, GADD153 expression, but not caspase-12 cleavage or JNK phosphorylation, was observed. Three weeks after TAC, GADD153 expression, but not caspase-12 cleavage or JNK phosphorylation, was observed. Using salubrinal, an inhibitor of the dephosphorylation of eIF2\(\alpha\), it was determined that JNK activation was inhibited, suggesting that JNK activation was largely responsible for ER stress–induced cell death of cardiac myocytes. Imatinib-mediated ER stress–induced JNK activation leads to the release of cytochrome c from the mitochondria, eventually resulting in cell death, severe LV dysfunction, and heart failure.

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**ER Stress Induced by Pathophysiological Modifications of the Circulation: Heart Failure**

In male C57BL/6 mice subjected to transverse aortic constriction (TAC), cardiac enlargement was detected after 1 week (without severe lung congestion) and 4 weeks (with lung congestion). LV dilatation and LV systolic dysfunction were also detected after 4 weeks and LV wall thickness was significantly increased after 1 week. In response to TAC, mRNA and protein levels of GRP78, calreticulin and ANP were significantly upregulated in cardiac tissue, as were the number of TUNEL–positive cells. An increase in CHOP/GADD153 expression, but not caspase-12 cleavage or JNK phosphorylation, was observed. Four weeks after TAC, cardiomyocytes expressed increased proapoptotic factors P53 and Bax and decreased antiapoptotic factors Bcl-2 and Bcl-XL, contributing to compensated heart failure. To determine the mechanism of TAC ER stress induction, Ang II
was examined because RAS has an important role in the
development of hypertrophy in failing hearts. Furthermore, Ang II increases protein synthesis and may induce ER stress.

To study Ang II as an ER stress inducer, adult rat cardiomyocytes were treated with Ang II (10⁻⁹ mol/L) for 24 hours resulting in increased protein levels of GRP78 and CHOP/GADD153 and increased protein synthesis. Among TUNEL-positive cells treated with Ang II, it was found that many cells were also CHOP/GADD153-positive or GRP78-positive. From this study, it appears that physiological concentrations of Ang II both increases protein synthesis and induces ER stress in rat cardiomyocytes. Long-term induction of ER stress by Ang II may be associated with chronic heart failure attributable to cardiac myocyte loss through apoptosis.

UPR activation may also play a protective role in chronic heart failure by regulating plasma volume through the cardiovascular axis. It has been determined that the UPR can induce the expression of both ANP and BNP in cardiac myocytes. The UPR appears to be induced in human heart failure, because it was determined that mRNA levels of XBP1s and GRP78 are significantly higher in failing hearts. A positive correlation was found between the protein expression of GRP78 and BNP in the cardiac tissue. Treating rat neonatal cardiomyocytes with tunicamycin resulted in increased nuclear XBP1s and BNP mRNA. When cultured rat cardiac myocytes were transfected with siRNA targeting XBP1 and treated with tunicamycin, results indicated that an XBP1-dependent pathway induced BNP expression. Because of its role in the maintenance of fluid balance and cardiovascular growth, XBP1s-induced BNP may play an indirect role in the recovery from cardiac dysfunction when the UPR is activated. This effect of the UPR to increase BNP synthesis in human hearts may be protective in chronic heart failure by decreasing blood volume through natriuresis.

MI itself may induce ER stress in the heart. Protein and mRNA levels of GRP78, total ATF6, cleaved ATF6, phospho-eIF2α, phospho-PERK, XBP1s, and CHOP/GADD153 were increased after an induced MI in C57BL/6 wild type mice. MI induction in wild type mice, resulted in a lower survival rate in mice treated with the ATF6 inhibitor 4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride, implying that UPR induction, specifically the ATF6 arm of the UPR pathway, offers protection against chronic heart failure. In addition, ATF6 cleavage appeared to prevent cardiac remodelling. Transgenic mouse hearts expressing a dominant negative mutant of ATF6 were found to have lower levels of GRP78 mRNA, a lower survival rate, a thinner LV wall and a higher rate of apoptosis. Mice with ATF6 activation showed an increased survival rate. ATF6 overexpressing mice also show an increase in UPR markers in response to ischemia/reperfusion injury, protecting mouse hearts from dysfunction, necrosis and apoptosis. Murine cardiomyocytes undergoing hypoxia upregulate the UPR markers XBP1s, phospho-eIF2α, ATF4, GADD34, GRP78, and GRP94, decreasing myocyte cell death. After a prolonged period of time, proapoptotic factors (eg, CHOP/GADD153 and caspase-12) are induced, initiating cell death. It appears that activation of ATF6 may have a protective effect in chronic heart failure and may protect against maladaptive cardiac remodelling after MI and ischemia/reperfusion injury.

Some vasodilators may exacerbate heart failure. The anti-hypertensive drug doxazosin is known to lower blood pressure by dilating resistance and capacitance arteries, though it has also been associated with congestive heart failure. A microarray analysis determined the induction of numerous ER stress genes in HL-1 cardiac myocytes treated with doxazosin. Real-time PCR analysis and Western blot quantification confirmed a significant increase in CHOP/GADD153 and CCAAT/enhancer binding protein-β expression in HL-1 cells and primary cultures of cardiac myocytes with similar treatments. Protein levels of phosphorylated p38 MAPK were increased in HL-1 cells treated with doxazosin, and the cells were reported to display a significant upregulation in the cleavage of focal adhesion kinase, resulting in loss of cell adhesion and apoptosis.
inhibitor (Z-VDVAD-FMK) 1 hour before infection with GFP- or MCPiP-GFP adenovirus, it was determined that MCPiP induces IRE1α and the cleavage of caspase 2, indicating a possible mechanism behind MCPiP-induced ER stress resulting in cardiac myocyte apoptosis.

The effect of high blood pressure on induction of ER stress was examined in a genetic model of essential hypertension, the spontaneously hypertensive (SH) rat. SH rats showed a significant elevation of blood pressure over their normotensive control, at both 8 and 32 weeks of age. The mRNA and protein levels of GRP78 and caspase-12 were measured in cardiac myocytes isolated from SH and Wistar–Kyoto rats at 32 weeks of age. It was determined that the SH rats had a significantly higher mRNA and protein level of GRP78 and caspase-12 and also have a significantly higher level of TUNEL staining, with TUNEL staining being positively correlated with GRP78 staining. Based on a significant increase in LV mass index, with a significant increase in the E-wave velocity and a significant decrease in the A-wave velocity, it was demonstrated that the SH rats had some characteristics of diastolic heart failure at 32 weeks of age. Thus, essential hypertension alone may induce prolonged ER stress within the cardiac myocyte and lead to heart failure through apoptotic cell loss.

Many substances or conditions that induce ER stress within cardiac myocytes and lead to hypertrophy or result in heart failure are listed in the Table. Severe CKD may bring about hemodynamic changes in the circulation through plasma volume expansion that result in cardiac hypertrophy. The process of cardiac myocyte hypertrophy appears to necessitate an increase in protein synthesis. This increase in protein synthesis may directly result in ER stress. ER stress may also result from the reaction of the myocardium to hormones induced by CKD or renal hypoperfusion caused by reduced cardiac output from the failing heart. This includes the release of renin and subsequent generation of Ang II and AVP resulting from the reaction of the myocardium to hormones.

Table. Pathological Inducers of ER Stress in Cardiac Hypertrophy and Heart Failure

<table>
<thead>
<tr>
<th>ER Stress Inducer</th>
<th>Evidence Cited</th>
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<tbody>
<tr>
<td>Angiotensin II</td>
<td>■ Ang II induces ER stress, potentially through increased protein synthesis, leading to cardiac hypertrophy, in Wistar-Kyoto rats.</td>
</tr>
<tr>
<td></td>
<td>■ Ang II–induced ER stress triggers cardiac myocyte apoptosis, leading to heart failure in Wistar–Kyoto rats.</td>
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<tr>
<td></td>
<td>■ Ang II–induced maladaptive excitation–contraction coupling and hypertrophy in mouse cardiac myocytes.</td>
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<tr>
<td>Arginine vasopressin (AVP)</td>
<td>■ AVP causes extensive depletion of intracellular Ca2+ stores, leading to cardiac myocyte hypertrophy in the H9c2 cell model system.</td>
</tr>
<tr>
<td></td>
<td>■ Protein synthesis is initially inhibited by AVP through eIF2α phosphorylation, then recovers and increases, leading to cardiac myocyte hypertrophy in H9c2 cells.</td>
</tr>
<tr>
<td></td>
<td>■ AVP activates glycosylation and hypertrophy by a Ca2+-dependent mechanism in H9c2 cells.</td>
</tr>
<tr>
<td>Alcohol/acetalddehyde</td>
<td>■ Chronic alcohol consumption (4% of diet for 12 weeks) leads to cardiac hypertrophy, in FVB- and ADH-overexpressing mice.</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>■ Hypertension upregulates ER stress, leading to apoptosis in spontaneously hypertensive rat hearts.</td>
</tr>
<tr>
<td></td>
<td>■ Prolonged ER stress is induced by pressure overload, leading to cardiac failure in transgenic mice that express monocytic chemotactic protein-1 in the heart.</td>
</tr>
<tr>
<td>Heart failure/BNP response</td>
<td>■ Failing human hearts showed UPR activation. In neonatal rat cardiac myocytes, tunicamycin dose-dependently increased the protein expression levels of BNP, indicating the upregulation of BNP to ER stress.</td>
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<tr>
<td></td>
<td>■ In response to ER stress, XBP1s upregulates BNP expression in neonatal rat cardiac myocytes.</td>
</tr>
<tr>
<td>LPS</td>
<td>■ LPS treatment results in decreased concentrations of intracellular Ca2+, indicating a role for intracellular Ca2+ homeostasis in endotoxemia-induced cardiac failure in FVB mice.</td>
</tr>
<tr>
<td></td>
<td>■ LPS-induced endotoxemia results in ER stress and ER stress-induced apoptosis, directly producing cardiac myocyte contractile dysfunction in FVB mice.</td>
</tr>
<tr>
<td></td>
<td>■ Inducible NOS expression is upregulated in the LPS model of sepsis, indicating inflammation in the heart in FVB and metallothionein overexpressing mice.</td>
</tr>
<tr>
<td>ER stress/Ca2+ disequilibrium/Ca2+/NF-AT3 pathway</td>
<td>■ In primary cultures from neonatal Sprague–Dawley rats, thapsigargin (50 nmol/L) treatment induced cardiac myocyte hypertrophy, characterized by increased protein synthesis rate and larger cell surface area.</td>
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<tr>
<td></td>
<td>■ An alternative study, however, failed to show thapsigargin (10 nmol/L) resulted in cardiac myocyte hypertrophy.</td>
</tr>
</tbody>
</table>

Pathological factors that induce ER stress are associated with cardiac hypertrophy by alteration of excitation–contraction coupling and Ca2+ handling in cardiac myocytes. Prolonged ER stress may also contribute to heart failure through apoptosis in cardiac myocytes. Multiple pathological factors leading to these effects are listed with their references.

**ER Stress in Chronic Kidney Disease**

ER stress in the kidney may result in renal pathology involving tubular epithelial cell differentiation to a fibroblast-like phenotype through EMT, renal tubular epithelial cell loss through apoptosis, and eventually nephron loss that
Figure 3. Model of interaction between chronic heart failure and chronic kidney disease induced by ER stress. A characteristic of the failing heart is a reduction of cardiac output. This reduction in cardiac output leads to reduced net renal perfusion pressure. Renal hypoperfusion results in the stimulation of neural hormonal mechanisms to maintain adequate renal blood flow including the release of renin leading to the generation of angiotensin II (Ang II), arginine vasopressin (AVP) release, aldosterone, and sympathetic nervous system activation. This results in compensatory hypervolemia and cardiac hypertrophy through myocardial wall stretch. Cardiac hypertrophy may induce ER stress through the demand for increased protein synthesis. The mediators of compensatory hypervolemia including Ang II and AVP have also been shown to induce ER stress in cardiac myocytes, and these effects may be attributable to changes in SR/ER calcium handling, as well as direct stimulation of protein synthesis through a Ca\(^{2+}\)/calmodulin-mediated mechanism. Furthermore, ROS generation in the failing heart may also augment the ER stress response. Prolonged ER stress under these conditions may result in cardiac myocyte apoptosis and chronic heart failure.

Progression of Chronic Kidney Disease Through ER Stress

CKD is often progressive and can result in a steady decline in the filtration capacity of the kidney. One result of severe CKD is uremia, which can be defined as the accumulation of waste products in the blood when CKD has reduced renal filtration capacity or estimated glomerular filtration rate by at least 50%. One nitrogenous waste product that has been found to accumulate in the blood of both human patients experiencing CKD and animal models of the disease is indoxyl sulfate (IS). IS is a nitrogen-containing organic anion and has been found to interfere with organic anion transporters 1 and 3 in proximal and distal tubules, accumulate in the tubular epithelium, and hasten the progression of CKD in the 5/6-nephrectomized rat model. Further study into the mechanism by which IS accumulation in proximal tubules leads to the progression of CKD has shown that IS induces the ER stress markers CHOP/GADD153 and ATF4 in the HK-2 proximal tubular cell model system leading to a reduction in the proliferation of these cells. Although the authors recognize that the doses of IS (2 to 5 mmol/L) are high in comparison with what would be expected in the plasma of uremic patients (250 μmol/L), they also demonstrate that IS treatment in HK-2 human proximal tubular cells had additive effects when combined with another uremic toxin, indoleacetic acid, to induce ER stress. Thus, the effects of an array of uremic toxins may act additively to induce ER stress and reduce the ability of the proximal tubule to regenerate leading to the progressive decline found in
severe CKD associated with uremia. Proteinuria is also a frequent complication of CKD and may result from a breakdown of the glomerular filtration barrier. Proteinuria itself or in combination with high glucose as a model of diabetic nephropathy induced ER stress in the HK-2 cell line. In an animal model of streptozotocin-induced diabetes in C57BL/6 mice, ER stress developed and was associated with severe nephropathy at 22 months with CHOP/GADD153 upregulation.92 Furthermore, in patients with nephrotic syndrome, including those with a diagnosis of minimal change disease, IgA nephropathy, primary mesangial proliferative glomerulonephritis, and membranous nephropathy, immunohistochemical staining showed an increase in staining for the molecular chaperones GRP78 and ORP150 in comparison to controls.93 CHOP/GADD153 expression was also increased and showed nuclear localization in the proximal tubule epithelium of nephrotic patients. Human kidney cells exposed to human serum albumin overload showed ER stress induction and CHOP/GADD153-mediated apoptosis.93 Further details of the role of ER stress in proteinuric kidney disease are presented in the review by Cybulsky.94 These findings show the importance of ER stress in CKD generated from a number of primary pathologies.

Therapeutic Approaches to Alleviate ER Stress in Cardiac Failure and CKD

Approaches to alleviate ER stress in cardiac failure and in CKD have been proposed; however, they are at an early stage of therapeutic development. As discussed above, these approaches involve manipulations that upregulate a protective UPR to prevent the pathophysiological consequences of prolonged or severe ER stress in both the kidney and the failing heart. In the context of cardiorenal syndrome, these approaches hold particular promise because ER stress–induced pathogenesis appears to play an important role in both of these comorbidities. Standard therapeutic modalities aimed at alleviating congestive heart failure have the disadvantage, in cardiorenal syndrome, of potentially worsening renal function. This may explain the poor patient prognosis associated with cardiorenal syndrome.19,21 It should be noted, however, that UPR preconditioning as demonstrated in animal models of both cardiac failure and CKD60,95,96 have serious limitations in patient populations because patients present with established disease and preconditioning requires a therapeutic intervention before disease onset. Furthermore, induction of a full UPR response via subtoxic levels of ER stress induction may worsen outcome because the UPR response involves both prosurvival and proapoptotic gene expression at the cellular level.97 Noting these limitations, UPR preconditioning studies, in both kidney and heart cell culture model systems61,98 and the whole animal, suggest a protective UPR may reduce the morbidity and mortality associated with cardiorenal syndrome. Of further promise are approaches that upregulate specific prosurvival aspects of the UPR that increase protein folding capacity within the ER, thereby preventing the induction of cytotoxic aspects of the UPR. The review by Toth et al has summarized specific elements of this strategy in cardiac myocyte apoptosis.99 Furthermore, the use of low-molecular-weight chemical chaperones may also augment the protein folding capacity of the ER, thereby preventing harmful UPR induction. Substances such as 4-phenyl butyric acid26,27 have potential therapeutic applications to inhibit ER stress and preserve cardiac and renal function in cardiorenal syndrome. The eIF2α dephosphorylation inhibitor salubrinal was protective in cardiomyocytes.69 Salubrinal also protected against CsA-induced nephrotoxicity of rat kidney.34 Experimentation in animal models to demonstrate these effects would be the next

Figure 5. ER stress induced epithelial-to-mesenchymal transformation in human proximal tubule cells. A, Human proximal tubule epithelial cells treated with drug vehicle (DMSO). Cells were stained with rhodamine phalloidin for F-actin (arrowheads) (i), GRP78 (arrows) (ii), DAPI (nuclear staining) (iii), and all 3 combined (iv). Staining in the perinuclear region of the cells indicated GRP78 fibrils (arrowheads) (iv). Staining around the periphery of the cells indicated F-actin fibrils (arrowheads) (iv). Bar, 50 μm. B, Human proximal tubule epithelial cells treated with 200 nmol/L thapsigargin for 18 hours, stained and imaged, as in A, for F-actin (arrowheads) (i), GRP78 (arrows) (ii), DAPI (iii), and all 3 combined (iv). GRP78 in the perinuclear region appeared denser after thapsigargin treatment (arrows) (ii). F-actin staining no longer defined the periphery of the cell (arrowheads) (iv). Bar, 50 μm.
step in drug development. Because ER stress appears to be a process driving both chronic cardiac failure and CKD, potential therapeutic approaches to inhibit ER stress may have the benefit of preserving both cardiac and renal function simultaneously. Importantly, this strategy may avoid some of the problems associated with cardiorenal syndrome management.

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Disclosures
None.

References
38. Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, Grant SR, Olson EN. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy induced by enhancer factor 2c pathway mediates cardiac hypertrophy induced by...


