Integrative Physiology

Reversal of Chronic Molecular and Cellular Abnormalities Due to Heart Failure by Passive Mechanical Ventricular Containment


Abstract—Passive mechanical containment of failing left ventricle (LV) with the Acorn Cardiac Support Device (CSD) was shown to prevent progressive LV dilation in dogs with heart failure (HF) and increase ejection fraction. To examine possible mechanisms for improved LV function with the CSD, we examined the effect of CSD therapy on the expression of cardiac stretch response proteins, myocyte hypertrophy, sarcoplasmic reticulum Ca\(^{2+}\)-ATPase activity and uptake, and mRNA gene expression for myosin heavy chain (MHC) isoforms. HF was produced in 12 dogs by intracoronary microembolization. Six dogs were implanted with the CSD and 6 served as concurrent controls. LV tissue from 6 normal dogs was used for comparison. Compared with normal dogs, untreated HF dogs showed reduced cardiomyocyte contraction and relaxation, upregulation of stretch response proteins (p21ras, c-fos, and p38 alpha/beta mitogen-activated protein kinase), increased myocyte hypertrophy, reduced SERCA2a activity with unchanged affinity for calcium, reduced proportion of mRNA gene expression for alpha-MHC, and increased proportion of beta-MHC. Therapy with the CSD was associated with improved cardiomyocyte contraction and relaxation, downregulation of stretch response proteins, attenuation of cardiomyocyte hypertrophy, increased affinity of the pump for calcium, and restoration of alpha- and beta-MHC isoforms ratio. The results suggest that preventing LV dilation and stretch with the CSD promotes downregulation of stretch response proteins, attenuates myocyte hypertrophy and improves SR calcium cycling. These data offer possible mechanisms for improvement of LV function after CSD therapy. (Circ Res. 2003;93:1095-1101.)

Key Words: heart failure ■ myocyte hypertrophy ■ sarcoplasmic reticulum ■ myosin heavy chain

Heart failure (HF) is a progressive disorder mediated through multiple signaling pathways. Once initiated, HF is characterized by increased neurohumoral activation and ventricular dilation. Although such compensatory changes are initially beneficial, over the long-term they cause adverse structural and functional changes collectively referred to as ventricular remodeling. Ventricular dilation also causes increased mechanical stress and myocardial stretch. Upregulation of stretch response proteins, such as p21ras, c-fos, and p38 alpha/beta mitogen-activated protein kinase (MAPK), have been shown to induce cardiomyocyte hypertrophy.

The Acorn Cardiac Support Device (CSD) has been shown to halt progressive left ventricular (LV) dilation and improve ejection fraction.\(^5\)\(^-\)\(^7\) However, the mechanism(s) underlying the improved cardiac function has not been elucidated. In the present study, we tested the hypothesis that improvement in LV systolic function in dogs with HF after long-term therapy with the CSD results, in part, from downregulation of stretch response proteins, attenuation of cardiomyocyte hypertrophy,\(^1\)\(^-\)\(^4\) and improvement of sarcoplasmic reticulum (SR) calcium cycling. To further understand the mechanisms for the improvement in LV systolic function, we also explored the influence of this form of therapy on the expression of cardiac alpha- and beta-myosin heavy chain (MHC) isoforms.\(^8\)\(^-\)\(^9\)

Materials and Methods

Animal Model

The canine model of chronic HF used in this study was previously described in detail.\(^10\) Chronic LV dysfunction is produced by multiple sequential intracoronary embolization with polystyrene Latex microspheres (70 to 102 \(\mu\)m in diameter), which results in loss of viable myocardium. The model manifests many of the sequelae of HF observed in humans with HF, including marked depression of LV systolic and diastolic function, reduced cardiac output, increased LV filling pressures, and enhanced activity of the sympathetic nervous system.\(^10\) Moreover, this model demonstrates progression of HF long after the cessation of coronary microembolizations. In the present study, 12 healthy mongrel dogs (Marshall Farms, North Rose, NY), weighing between 21 and 31 kg, underwent serial coronary microembolizations to produce HF. Embolizations were performed 1 to 3 weeks apart and were discontinued when LV ejection fraction was between 30% and 40%. Microembolizations were performed during cardiac catheterization under general anesthesia and sterile conditions. The anesthesia regimen consisted of a combination of intra-
venous injection of oxyomophine (0.22 mg/kg), diazepam (0.17 mg/kg), and sodium pentobarbital (150 to 250 mg) to effect.

Study Protocol

Dogs underwent a left and right heart catheterization at baseline, before any coronary microembolizations. At 2 weeks after the last coronary microembolization, dogs underwent another left and right heart catheterization (pretreatment) while anesthetized. The 2-week period was allowed to ensure that all infarctions produced by the last microembolization were completely healed. The CSD was surgically implanted in 6 dogs as previously described.8 The remaining 6 dogs served as concurrent controls. All CSD-treated dogs and controls were followed up for 3 months during which time no cardioactive drugs were used. At the end of the follow-up period, a final left and right heart catheterization was performed. After the final catheterization, and while under general anesthesia, the chest was opened and the heart removed and the tissue prepared for histological and biochemical examination. LV tissue from 6 normal dogs was prepared in an identical manner and used for comparison. The study was approved by the Henry Ford Hospital Care of Experimental Animals Committee and conformed to the “Position of the American Heart Association on Research Animal Use.”

Angiographic Measurements

Single-plane left ventriculograms were obtained during left heart catheterization with the dog placed on its right side. Ventriculograms were recorded on 35-mm cine film at 30 frames per second during the injection of 20 mL of contrast material (Reno-M-60, Squibb). Correction for image magnification was made with a radiopaque calibrated grid placed at the level of the LV. LV end-systolic and end-diastolic volumes (ESV and EDV, respectively) were calculated from LV silhouettes using the area-length method,11 LV EF as previously described.10

Determination of Stretch Response Proteins

Expression of stretch response proteins, specifically p21ras, c-fos, and p38 α/β MAPK, was determined by Western blotting using homogenate of cardiomyocytes isolated from the LV free wall.12,13 In parallel, expression of calsequestrin (CSQ), a protein that is not altered in HF, was also determined and used as internal control. All stretch response proteins were normalized to CSQ. The Western blot membranes were incubated with primary (p21 rabbit polyclonal IgG; p38 α/β MAPK rabbit polyclonal IgG; c-fos rabbit polyclonal IgG; all from Santa Cruz, Inc) and then with secondary (goat-anti-rabbit HRP conjugated; Chemicon) antibody for 2 hours each. The antibody-bound antigen was identified by chemiluminescence (Repicard; Dupont-NEN). In parallel, CSQ was also determined in the LV homogenate. The intensity of the bands was quantified using a densitometer.

Contraction and Relaxation of Isolated Cardiomyocytes

Cardiomyocytes were isolated from the LV free wall as previously described.14 Cardiomyocyte contraction and relaxation were recorded using an edge detection algorithm.15 Contraction was evoked by electrical field stimulation at a frequency of 1.0 Hz. Percent cardiomyocyte shortening, peak velocity of shortening, and peak velocity of relengthening were measured in 5 to 10 cardiomyocytes from each dog selected at random. For each cardiomyocyte, 20 consecutive cycles were averaged to obtain a representative value, which was then used to calculate the average measures for each dog.

Determination of Cardiomyocyte Hypertrophy

Cardiomyocyte hypertrophy was determined by assessing average cardiomyocyte cross-sectional area from frozen LV tissue sections using computer-assisted planimetry.5,16 The length and width of isolated cardiomyocytes were also determined. Isolated cardiomyocytes were visualized using a Labophot-2 Nikon microscope with objective 20. The field was transferred to a computer using a digital video camera and projected on a digital screen. The maximum length and width of approximately 1200 rod shaped cardiomyocytes from each dog were measured using computer-assisted planimetry.

Determination of SR Ca²⁺ Uptake and Cardiac SR Ca²⁺-ATPase (SERCA2a) Activity

Oxalate-dependent Ca²⁺ uptake was determined in LV homogenate as previously described.17 Briefly, an aliquot of 50 µL of 0.25 mg/mL LV homogenate was incubated at 37°C for 1 minute in 0.4 mL of Ca²⁺ uptake buffer consisting of 50 mmol/L imidazole-HCl (pH 7.0), 100 mmol/L KCl, 6 mmol/L MgCl₂, 10 mmol/L NaN₃ (included to inhibit mitochondrial Ca²⁺ uptake), 10 mmol/L potassium oxalate, 20 µmol/L ruthenium red (included to inhibit SR Ca²⁺ release), 0.5 mmol/L EGTA, and 0.01 to 10 µmol/L free Ca²⁺ (CaCl₂, 10 000 dpmmol/L). The reaction was initiated by adding an aliquot of 50 µL of 50 mmol/L ATP, the assay was terminated 2 minutes later, radioactivity retained on filter paper was counted, and oxalate-dependent Ca²⁺ uptake was calculated as previously described.18 SR Ca²⁺ uptake, expressed as nmol ⁴⁵Ca²⁺ sequestered/min per mg of noncollagen protein, was determined as previously described.17 For thapsigargin-sensitive SERCA2a activity measurements, membrane vesicles were prepared from LV tissue as previously described.18 SERCA2a activity was determined in the absence and presence of thapsigargin at varying calcium concentration (0.1 to 10.0 µmol/L) as previously described18 and the activity expressed as µmol Pi released/min per mg of noncollagen protein.

Determination of Expression of SERCA2a, Phospholamban (PLB), and PLB Phosphorylation

To determine SR protein levels of SERCA2a and PLB, sodium-decyl sulfate (SDS) extract of LV homogenate was prepared as previously described.17,18 To freeze the phosphorylation state of the proteins, LV tissue was homogenized in the presence of the inhibitors of protein kinases (1 mmol/L EDTA, 1 mmol/L EGTA) and protein phosphatases (2 mmol/L sodium pyrophosphate and 10 mmol/L sodium fluoride). Five micrograms or the indicated amount of the SDS-extract was separated on 4% to 20% linear polyacrylamide (BioRad), transferred electrophoretically on nitrocelulose membrane, and the resulting membrane was incubated with primary antibody as previously described.17,18 The accuracy of the electotransfer was confirmed by staining the membrane with 0.1% amido black. Polyclonal antibodies for phosphorylated PLB at threonine-17 (Thr17) and serine-16 (Ser16) or monoclonal antibody for PLB was diluted to 500-fold or 2500-fold, respectively. Primary-antibody binding protein was visualized by incubating the blot with a second antibody, a peroxidase-conjugated anti-mouse in case of monoclonal or anti-rabbit in case of polyclonal antibodies, and the enhanced chemiluminescence assay was used as described by the supplier (Dupont-NEN). In parallel, CSQ was also determined in the LV homogenate. The intensity of the bands was quantified using a Bio-Rad model GS-670 imaging densitometer. The density of the phosphorylated PLB at Thr17 or Ser16 was normalized to CSQ. Before quantifying protein expression levels, the protein dependency of the immunodetectable bands for all proteins was established. In this study, a linear correlation was observed between densitometric units and protein content (<30 µg) for each immunodetectable protein.

Gene Expression of Cardiac α- and β-MHC

Total RNA from LV myocardium was isolated as described previously.19 Tissue samples were homogenized in RNA Stat-60 solution (150 mg tissue/1.5 mL RNA Stat 60) followed by extraction with chloroform, precipitation with isopropanol, and finally washing the precipitated RNA with 75% (v/v) ethanol. The RNA obtained was dissolved in RNase free water. The concentration of RNA was determined by spectrophotometry. Total RNA was diluted to 0.1 mg/mL concentration and denatured at 95°C for 5 minutes followed by rapid cooling in ice bath. Approximately 10 µg of total RNA was primed with 0.5 µg of oligo (dT)15 primer. Total RNA was reversed transcribed by using a cDNA synthesis kit (Promega Inc). After
incubating the samples at 42°C for 1 hour, the reaction was terminated at 95°C for 5 minutes. The mRNA levels of α- and β-MHC were analyzed by amplification of cDNA by reverse transcriptase-polymerase chain reaction followed by restriction enzyme digestion and then identified by agarose gel electrophoresis and ethidium bromide staining. Fluorescent bands corresponding to α- and β-MHC were quantified in densitometric units, each normalized to total MHC (α-MHC+β-MHC) and each reported as percent of total MHC.

Data Analysis
Within group comparisons between baseline, pretreatment, and posttreatment angiographic measures were made using repeated measures analysis of variance (ANOVA) with α set at 0.05. If significance was attained, pairwise comparisons between groups was determined using the Student-Newman-Kuels test with a value of P<0.05 considered significant. Comparisons of biochemical measures between normal, HF controls and CSD-treated HF dogs were based on one-way analysis of variance (ANOVA) with α set at 0.05. If significance was attained, pairwise comparisons between groups were determined using the Student-Newman-Kuels test with a value of P<0.05 considered significant. All data are reported as the mean±SEM.

Results
There were no significant differences at baseline and at pretreatment in EDV, ESV, or EF between dogs that were subsequently treated with the CSD and dogs assigned as concurrent controls (Table 1). After treatment, EF significantly decreased in untreated controls but increased significantly in CSD-treated dogs. This was accompanied by a significant increase in both ESV and EDV in untreated controls and by a significant reduction in both ESV and EDV in CSD-treated dogs (Table 1).

Cardiomyocyte Contraction and Relaxation
Results of cardiomyocyte contraction and relaxation are shown in Table 2. Percent cardiomyocyte shortening, peak velocity of shortening, and peak velocity of relengthening decreased significantly in untreated HF dogs compared with normal dogs. In contrast, in dogs treated with the CSD all three measures were significantly higher than in untreated HF dogs.

Stretch Response Proteins
Western blots depicting changes in p21Ras, c-fos, and p38 α/β MAPK are shown in Figure 1. The summary data for all 6 dogs in each of the three groups are shown in Table 3. All three stretch response proteins, normalized to CSQ, increased significantly in untreated HF dogs compared with normal dogs. In HF dogs treated with the CSD, all three stretch response protein levels were similar to those seen in normal dogs (Figure 1, Table 3).

Cardiomyocyte Hypertrophy
Cardiomyocyte cross-sectional area increased significantly in dogs with HF compared with normal dogs. This increase was significantly attenuated by CSD treatment (Figure 2). Cardiomyocyte length and width were significantly greater in untreated HF dogs compared with normal dogs; whereas treatment with the CSD was associated with a significantly lesser change in length and width of the cardiomyocytes compared with untreated controls (Figure 2).

SERCA2a Activity and Ca2+ Uptake
Maximal velocity (V max ) and the affinity of SERCA2a for calcium (K0.5) are shown in Table 3. V max , but not K0.5, decreased significantly in control HF dogs compared with normal dogs. Therapy with the CSD did not change V max.

![Representative Western blot for the stretch response proteins p21Ras, c-fos, and p38 α/β MAPK. Implantation of the CSD significantly reduced the expression of all 3 proteins (see Table 3). NL indicates normal; HF, heart failure; and CSD, cardiak support device–treated dogs.](image)
TABLE 3. Changes in Stretch Response Protein Levels, Sarcoplasmic Reticulum Ca\(^{2+}\) Uptake, and SERCA2a Activity, and Expression of Other Sarcoplasmic Reticulum Proteins Depicted in Densitometric Units

<table>
<thead>
<tr>
<th>Protein</th>
<th>Normal</th>
<th>Untreated HF Controls</th>
<th>CSD-Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>p21ras/CSQ</td>
<td>0.24±0.02</td>
<td>0.92±0.20*</td>
<td>0.35±0.10**</td>
</tr>
<tr>
<td>c-fos/CSQ</td>
<td>0.41±0.04</td>
<td>0.96±0.20*</td>
<td>0.33±0.05**</td>
</tr>
<tr>
<td>p38 α/β MAPK/CSQ</td>
<td>1.18±0.07</td>
<td>2.99±0.37*</td>
<td>1.25±0.10**</td>
</tr>
<tr>
<td>CSQ</td>
<td>4.91±0.35</td>
<td>5.11±0.33</td>
<td>5.22±0.22</td>
</tr>
<tr>
<td>Ca(^{2+}) uptake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_{\text{max}})</td>
<td>22.5±2.0</td>
<td>11.6±1.0*</td>
<td>14.3±1.0*</td>
</tr>
<tr>
<td>(K_a)</td>
<td>0.52±0.03</td>
<td>0.53±0.03</td>
<td>0.28±0.01**</td>
</tr>
<tr>
<td>SERCA2a Activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(V_{\text{max}})</td>
<td>0.36±0.02</td>
<td>0.23±0.02*</td>
<td>0.25±0.02*</td>
</tr>
<tr>
<td>(K_{\text{d}2.5})</td>
<td>0.45±0.03</td>
<td>0.48±0.03</td>
<td>0.32±0.02**</td>
</tr>
<tr>
<td>SERCA2a/CSQ</td>
<td>2.26±0.10</td>
<td>1.74±0.11*</td>
<td>1.76±0.10*</td>
</tr>
<tr>
<td>PLB/CSQ</td>
<td>3.63±0.19</td>
<td>1.57±0.21*</td>
<td>2.23±0.31*</td>
</tr>
<tr>
<td>PLB-Ser16/PLB</td>
<td>0.28±0.01</td>
<td>0.18±0.03*</td>
<td>0.40±0.02**</td>
</tr>
<tr>
<td>PLB-Thr17/PLB</td>
<td>0.54±0.07</td>
<td>0.30±0.03*</td>
<td>0.88±0.02**</td>
</tr>
</tbody>
</table>

CSQ indicates calsequestrin; MAPK, mitogen-activated protein kinase; HF, heart failure; Ca\(^{2+}\), calcium; SERCA2a, Ca\(^{2+}\)-ATPase; PLB, phospholamban; PLB-Ser16, phosphorylated phospholamban at serine-16; and PLB-Thr17, phosphorylated phospholamban at threonine-17.

*P<0.05 Normal vs HF; **P<0.05 CSD-Treated vs Untreated HF Controls.

Compared with control but was associated with a significant decrease in \(K_{\text{d}2.5}\) compared with HF controls, indicating a higher SERCA2a affinity for calcium after CSD therapy (Table 3). \(V_{\text{max}}\) for SR Ca\(^{2+}\) uptake but not affinity (\(K_a\)) decreased in control HF dogs compared with normal dogs. \(V_{\text{max}}\) for Ca\(^{2+}\) uptake did not change after CSD therapy, whereas \(K_a\) decreased indicating an increase in the affinity after CSD therapy (Table 3).

Expression of SERCA2a, PLB, and PLB Phosphorylation
Western blots showing expression of SERCA2a, PLB, PLB at Ser16 and Thr17, and CSQ are shown in Figure 3. All proteins, with the exception of CSQ, decreased significantly in control HF dogs compared with normal dogs. Densitometric analyses in Table 3 show that expression of SERCA2a and PLB was not changed in CSD-treated dogs compared with HF controls, whereas expression of phosphorylated PLB at Ser16 and Thr17 increased with CSD therapy compared with controls.

Expression of \(\alpha-\) and \(\beta-MHC\)
Changes in the proportion of cardiac \(\alpha-\) and \(\beta-MHC\) between normal dogs, untreated HF dogs, and CSD-treated HF dogs are shown in Table 4. In untreated HF dogs, gene expression of LV \(\alpha-MHC\) decreased significantly compared with expression in LV of normal dogs. Three months of chronic treatment with the CSD LV expression of \(\alpha-MHC\) was similar to that seen in normal dogs (Table 4). In untreated HF dogs, expression of LV \(\beta-MHC\) increased significantly compared with normal dogs, whereas treatment with the CSD was associated with LV expression of \(\beta-MHC\) that was similar to that seen in normal dogs (Table 4, Figure 4).

Discussion
Heart failure is characterized by progressive LV dysfunction and dilation. Regardless of the type of initiating injury, compensatory mechanisms are evoked to maintain adequate organ perfusion that includes neurohumoral activation, ventricular dilation, and cardiomyocyte hypertrophy. These responses are beneficial initially, but in the long-term cause maladaptive changes in myocardial structural and function recognized as ventricular remodeling. Thus, sustained neurohumoral activation and increased LV mechanical stretch and dilation.

TABLE 4. mRNA Expression of \(\alpha-\) and \(\beta-MHC\) Depicted as Percent of Total Myosin Heavy Chain

<table>
<thead>
<tr>
<th>Protein</th>
<th>Normal</th>
<th>Untreated HF Controls</th>
<th>CSD-Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha-MHC)</td>
<td>23.5±1.0</td>
<td>14.1±1.0*</td>
<td>24.6±0.6**</td>
</tr>
<tr>
<td>(\beta-MHC)</td>
<td>76.5±1.0</td>
<td>85.9±1.0*</td>
<td>75.4±0.6**</td>
</tr>
</tbody>
</table>

MHC indicates myosin heavy chain; HF, heart failure.
*P<0.05 Normal vs Untreated HF Controls; **P<0.05 CSD-Treated vs Untreated HF Controls.
wall stress associated with ventricular dilation represent key mediators that precipitate progression of HF.

In recent years, attenuation of maladaptive ventricular remodeling has become an important goal for the treatment of HF. Numerous pharmacological interventions have been developed to block various neurohumoral factors and, in doing so, attenuate remodeling. Drug therapy with angiotensin-converting enzyme inhibitors and β-adrenergic receptor blockers represent the current standard of care in patients with HF and have been shown to attenuate LV remodeling and, in the case of β-blockers, reverse the maladaptive process, albeit partially. However, the existence of multiple molecular signaling pathways that can trigger HF progression suggest that even the use of multiple pharmacological agents may not completely block all pathways responsible for the progression of LV remodeling. In particular, drugs may not be as effective at blocking the effects of mechanical signals such as wall stress and myocardial stretch. The latter can have direct consequences on biochemical and molecular effector systems that can mediate LV remodeling.

It has long been accepted that certain surgical approaches can be combined with optimal medical therapy to provide better survival and improved quality of life in patients with advanced HF. Functional mitral regurgitation, a common feature of the failing heart, can be eliminated or attenuated by repair or replacement of the mitral valve, a procedure that can improve forward stroke output. Experience with LV assist devices (LVADs) indicates that unloading the heart can promote reduction in LV chamber size, improvement in LV performance, and normalization of gene expression. Cardiomyoplasty is another surgical technique, in which the primary mode of action was originally thought to involve an active assist during contraction. The procedure involved wrapping a skeletal muscle around the heart and electrically stimulating the muscle to squeeze the heart and augment cardiac function. Even though the procedure involved extensive surgery and was plagued with technical difficulties, patients showed symptomatic improvement. However, several experimental and clinical studies have suggested that the improvement was derived primarily from the passive girdling of the heart and not from active contraction of the skeletal muscle. Several studies in various animal models of HF have shown that progressive LV dilation can be prevented or attenuated by wrapping synthetic materials around the cardiac ventricles to elicit containment. These passive mechanical devices and surgical approaches attempted to treat HF by directly preventing progressive LV enlargement and, in doing so, limit the adverse effects of increased wall stress and myocardial stretch.

The CSD is one such device designed to prevent progressive LV dilation and attenuate myocardial stretch and chamber sphericity. Mechanical stretch has been shown to directly and/or indirectly stimulate cardiomyocyte hypertrophy through upregulation of so-called stretch response proteins. The resulting maladaptive hypertrophy is invariably associated with abnormal SR calcium cycling, shifts in myosin isoforms, and other changes associated with ventricular remodeling. Thus, reducing mechanical stress and preventing excessive myocardial stretch may downregulate stretch response proteins and block an important signaling pathway for HF progression.

Findings from our laboratory and others have demonstrated that long-term monotherapy with the CSD in animals with experimentally induced HF can prevent progressive LV dilation and improve LV ejection fraction. Although one would expect that a passive mechanical device such as the CSD can prevent progressive LV dilation, the mechanism by which the CSD leads to improved LV systolic function is not as clear. The present study addressed this issue by exploring the potential biochemical and molecular alterations that occurred as a consequence of CSD therapy.

In the present study, improvement of global LV function with CSD therapy was associated with lesser extent of intrinsic contractile dysfunction of cardiomyocytes compared with no treatment at all. Therapy with the CSD was also associated with lower tissue levels of stretch response proteins specifically p21ras, c-fos, and p38 α/β MAPK compared with no treatment at all. Expression of these proteins has been shown to increase in HF. These proteins are known to be direct stimuli for cardiomyocyte hypertrophy. Maladaptive cardiomyocyte hypertrophy plays a key role in the progression of HF. In this study, long-term CSD therapy resulted in attenuation of cardiomyocyte hypertrophy as evidenced by decreased cardiomyocyte cross-sectional area, length, and width compared with control.

Findings of this study also showed that CSD therapy was associated with increased affinity of SERCA2a for calcium. This increase in affinity may have been due to increased phosphorylation of PLB. Increased affinity of SERCA2a for calcium can lead to improved calcium cycling within the SR particularly at low cytosolic calcium concentrations. Given that abnormalities in Ca2+ handling may, in part, underlie the decrease in contractile function in HF, we propose that increased affinity of the pump for calcium as seen with CSD therapy may have contributed to the observed improvement of LV function.

Marked differences in the phosphorylation of PLB were observed in the present study and warrant discussion. Phosphorylation of PLB was decreased in HF controls compared with normal dogs. It would be expected that phosphorylation of PLB would be greater in HF dogs due to the increase in plasma norepinephrine associated with the HF state. However, this increase in circulating plasma norepinephrine is
accompanied by downregulation of β₁ adrenoreceptors in the heart and uncoupling between the receptors and their G proteins. In addition, phosphorylation of PLB was increased in the CSD group compared with the HF group despite the improvement of LV function, which is normally associated with decreased plasma norepinephrine. One possible explanation is that in addition to augmented plasma norepinephrine, an increase in phosphorylation activity has also been documented in HF.32–36 A decrease in PLB phosphorylation has been previously noted in our canine model of HF.32,34 It is possible, albeit unproven, that the balance of phosphorylation/phosphatase activation may have favored dephosphorylation in the HF dogs, while reverting to phosphorylation in the CSD-treated animals.

Cardiomyocytes express both α- and β-MHC isoforms. In the rat heart, these two isoforms differ on the basis of ATPase activity, with α-MHC being more active than β-MHC.37,38 Compared with cardiac β-MHC, α-MHC is associated with faster velocity of shortening.37,38 Studies in LV tissue obtained form explanted failed human hearts showed loss of α-MHC expression with increased expression of β-MHC, a condition that can argue in favor of diminished contractile function. Other studies have shown that this maladaptation in the proportion of cardiac α-MHC and β-MHC isoforms can be reversed in animal models of HF after drug or surgical therapy.39–41 In the present study, the proportion of cardiac α-MHC was significantly reduced in HF dogs that were untreated, and the proportion β-MHC was increased. Long-term treatment with the CSD was associated with expression of both MHC isoforms that was close to normal levels, a condition that may have also contributed to the improvement of LV function seen with CSD therapy.

In conclusion, results of this study suggest that the observed improvement in LV function after long-term therapy with the CSD may be due, in part, to the effects of the CSD on limiting LV wall stress and myocardial stretch. These changes were associated with attenuation of muscle cell hypertrophy and improvement of SR calcium cycling. The improvement of LV function with CSD therapy may have also been due, in part, on its effects on the expression of cardiac MHC isoforms.

Acknowledgments

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