Altered Inotropic Responsiveness and Gene Expression of Hypertrophied Myocardium With Captopril


Abstract—Inotropic responsiveness to β-adrenergic stimulation is generally found to be impaired in left ventricular (LV) hypertrophy and failure. To investigate the mechanisms by which angiotensin-converting enzyme inhibitor therapy may modulate inotropic responsiveness with long-term pressure overload, we studied the effects of captopril treatment on cardiac gene expression, LV muscle mechanical contraction, and intracellular calcium (Ca\(^{2+}\)) transients from spontaneously hypertensive rats (SHR). LV papillary muscles from untreated SHR, age-matched normotensive Wistar-Kyoto rats (WKY), and SHR treated with captopril (CAPRx, started at 12, 18, and 21 months of age) were studied. All animals were studied at 24 months of age or when heart failure developed. In untreated SHR, α-myosin heavy chain (MHC) gene expression and protein were decreased, the Ca\(^{2+}\) transient (with the bioluminescent indicator aequorin) was prolonged, and abundance of Na\(^+/Ca^{2+}\) exchanger mRNA levels increased in comparison to WKY. Active stress development at L\(_{\text{max}}\) and the maximum rate of stress development were depressed and contractile duration prolonged in SHR relative to WKY. Isoproterenol administration further decreased active stress in untreated SHR despite an increase in intracellular Ca\(^{2+}\) levels. In CAPRx SHR, α-MHC gene expression and protein levels were increased, the Ca\(^{2+}\) transient was not prolonged, Na\(^+/Ca^{2+}\) exchanger expression was downregulated, and papillary muscle function demonstrated increased active stress and maximum rate of stress development in response to isoproterenol. The increased abundance of α-MHC mRNA in conjunction with an increase in V\(_{1}\) myosin isozyme suggests that captopril affects transcriptional regulation of cardiac gene expression. Restored LV inotropic responsiveness to β-adrenergic stimulation in CAPRx SHR appears to be coupled to normalization of Na\(^+/Ca^{2+}\) exchanger mRNA expression, upregulation of V\(_{1}\) myosin isozyme levels, and increased speed of contraction. (Hypertension, 2000;35:1203-1209.)

Key Words: hypertrophy, left ventricular ■ heart failure ■ calcium ■ receptors, adrenergic, β

Decreased myocardial responsiveness to β-adrenergic stimulation with hypertrophy\(^{1,2}\) and failure\(^{3,4}\) has been primarily attributed to changes in the β-adrenergic receptor. However, in the spontaneously hypertensive rat (SHR), a polygenic model of long-term pressure overload–induced hypertrophy and failure, no decrease in β-adrenergic receptor density or affinity was found,\(^5\) suggesting that other factors also may modulate sensitivity. Downregulation of α-myosin heavy chain (α-MHC) with a concomitant upregulation of β-MHC has been observed in murine species during myocardial hypertrophy and failure.\(^6,7\) Until recently, this was not believed to be an important factor in the regulation of myocardial contractility in human myocardium, but recent observations in failing human myocardium indicate that systolic dysfunction was associated with a downregulation of α-MHC\(^8,9\) and upregulation of Na\(^+/Ca^{2+}\) exchanger gene expression.\(^10,11\) A close relation between α-MHC and β-adrenergic receptor gene expression was found, and it has been suggested that these 2 genes may be coregulated.\(^8\) We have recently demonstrated that treatment with the angiotensin-converting enzyme (ACE) inhibitor captopril causes a progressive upregulation of α-MHC gene expression in the SHR and prevents the transition to failure.\(^12\) Although there is generally a parallel relation between expression of mRNA for α-MHC and β-MHC and protein production, a recent report in aortic banded rats\(^13\) indicates a dissociation between gene expression and protein levels. Therefore, it is important to determine the effects of ACE inhibitor on both expression of mRNA coding for α-MHC and on protein production.

In the present study, we examined the relation between the time ACE inhibitor treatment is initiated and its effects on isoproterenol (ISO)-mediated changes in inotropic responsiveness and intracellular calcium ([Ca\(^{2+}\)]) and to quantify changes in α-MHC gene expression and protein and Na\(^+/Ca^{2+}\) exchanger.
exchanger mRNA with long-term ACE inhibition in hypertrophy and failing myocardium from the SHR.

**Methods**

**Animal Model**

Forty-three male SHR and 12 Wistar-Kyoto rats (WKY) were purchased from Taconic. Rats were housed 2 per cage and fed standard rat chow and allowed free access to water. Captopril was added to drinking water at a concentration of 2 g/L (CAPRx), which has been shown to be effective in preventing hemodynamic impairment associated with chronic hypertrophy in the SHR. Groups of SHR had CAPRx, starting at the onset of failure (SHR-F-Rx). All other animals were also observed for evidence of tachypnea and labored respiration; when these findings became evident, animals were killed and not demonstrating symptoms of respiratory distress were studied at 24 months of age. All procedures and animal care were in accordance with institutional guidelines for animal research at the Boston VA Medical Center and Boston University School of Medicine.

**Preparation**

Hearts were quickly removed, and the left ventricular (LV) anterior papillary muscle was dissected free and mounted vertically in a 50-mL glass chamber containing oxygenated Krebs-Henseleit solution at 28°C and stimulated at a rate of 0.2 Hz as previously described. The upper end of the muscle was attached to a low-inertia DC pen motor (300B lever system, Cambridge Technologies Inc). A digital computer with an analog/digital interface allowed control of either tension or length of the preparation; the data were stored on disk for later analysis. After mounting, muscles were allowed to equilibrate and then gradually stretched to the peak of the active force versus length curve (Lmax, defined as the muscle length resulting in the peak active force). At Lmax, isometric contraction parameters of 5 switches were determined and averaged and force normalized for muscle cross-sectional area. There were no significant differences in muscle cross-section area (all muscles averaged 0.94 ± 0.05 mm²).

**Aequorin Studies**

Aequorin was loaded into the muscle preparations by the macroinjection technique as previously described. After loading, muscles were allowed to equilibrate for 90 to 120 minutes until a steady state was achieved. Light and force signals were recorded and analyzed by a digital recording system developed in our laboratory. The fractional luminescence method was used to provide calibration for comparison of light signals among groups.

**Experimental Protocol**

After aequorin loading and equilibration, muscle preparations were exposed to concentrations of Ca²⁺ (0.6, 1.2, 2.5, and 5 mmol/L) for 10 minutes each, a period during which active force stabilized. The muscles were then allowed to reequilibrate at the baseline Ca²⁺ concentration (1.2 mmol/L) for 30 minutes before the addition of ISO (10⁻⁴, 10⁻⁵, and 10⁻⁶ mol/L) to the bath at 10-minute intervals. At 10⁻⁶ mol/L ISO, the Ca²⁺ in the bath was increased to 2.5 and then 5 mmol/L. The muscle bath was then drained and washed with normal Krebs solution and changed to 1.25 mmol/L Ca²⁺ and equilibrated for 30 minutes before calcium calibration.

**Tissue Isolation and Preparation**

After removal of the papillary muscles, the heart was dissected into atria, LV, and right ventricles (RV). Tissues were gently blotted and weighed. LV samples were quickly frozen and stored in liquid nitrogen. LV and RV weight-normalized by body weight were used as indexes of ventricular hypertrophy.

**Myosin Isozyme Studies**

The methods for tissue preparation, myosin extraction, and electrophoretic separation of isomyosins have been previously published. Briefly, myosin was extracted from ~200 mg of the frozen LV. Isomyosins were separated by electrophoresis on polyacrylamide gels, under nondissociating conditions as described by Hoh et al. The gels were fixed, stained with Coomassie blue R 250, then scanned on a Joyce-Lobell scanning densitometer at 520 nm. The relative proportions of the isomyosins present were obtained from the gel scans by measuring the area under each peak to obtain a final estimate of the proportion of V1 (heavy chain-α) and V2 (heavy chain-β) isomyosins present.

**Analysis of Cardiac Na⁺/Ca²⁺ Exchanger and α-MHC mRNA Levels**

Frozen samples of LV tissue were processed and the RNA extracted for Northern blot analysis of Na⁺/Ca²⁺ exchanger and α-MHC mRNA expression as previously published. Briefly, RNA was isolated from cardiac tissue by the method of Chomczynski and Sacchi. Ten micrograms of total RNA was size-fractionated by electrophoresis and transferred to nylon membranes (Genescreen Plus; Dupont NEN). A rat cDNA for Na⁺/Ca²⁺ exchanger was labeled with [³²P]-deCTP by the random hexamer primer by oligo dT method and hybridized to nylon blots for 18 to 24 hours at 42°C. Probes for α-MHC and 18S ribosomal RNA were end-labeled synthetic oligonucleotides previously described. Washed blots were exposed to film, and the signals were quantified by a densitometric system (GS700, BioRad). Blots were probed with a [³²P]-labeled oligonucleotide complementary to 18S ribosomal RNA. Levels of mRNA reported here are normalized to the level of 18S rRNA.

**Statistical Analysis**

Data from the SHR-F, SHR-NF, and WKY groups were compared with the use of 1-way ANOVA with replication. A 2-way ANOVA was used to examine group and treatment effects. The Newman-Keuls multiple-sample comparison test or Tukey's procedure was used to localize differences where appropriate. Differences were considered significant at P<0.05. Data are expressed as mean ± SD.

**Results**

**Animal Weights, LV Weight, Heart Chamber Ratios, and Blood Pressure Changes**

CAPRx decreases cardiac hypertrophy, as indicated by decreases in LV/body weight (BW) and RV/BW in comparison to untreated SHR (Table 1). In CAPRx, SHR RV/BW was not significantly different from the WKY, whereas LV/BW was elevated relative to untreated WKY (P<0.05). Peak systolic arterial pressure was higher in untreated SHR before CAPRx and decreased with CAPRx, but remained higher than WKY.

**Intracellular Calcium Transient and Isometric Stress Recordings**

Examples of light and force signals from untreated WKY, SHR-NF, and SHR-F, and CAPRx SHR (SHR-Rx12) at 1.25 mmol/L Ca²⁺([Ca²⁺]₀) bath, in response to 10⁻⁶ mol/L ISO and the addition of 5 mmol/L Ca²⁺ in the presence of ISO, are presented in Figure 1. Before ISO, peak 10⁻⁶ mol/L Ca²⁺ bath, in response to 10⁻⁶ mol/L ISO and the addition of 5 mmol/L Ca²⁺ in the presence of ISO, are presented in Figure 1. Before ISO, peak σ was depressed in SHR-F relative to WKY and SHR-NF. WKY demonstrated a parallel increase in +dα/dt and [Ca²⁺], in response to ISO. In contrast, muscles from untreated SHR-NF and SHR-F rats demonstrate a decrease in peak σ and +dα/dt in response to ISO despite an increase in peak [Ca²⁺]. In
untreated WKY, superimposed recording of peak $\sigma$ demonstrates a further positive inotropic response to subsequent addition of 5 mmol/L Ca$^{2+}$ to the bath in the presence of ISO (10$^{-6}$ mol/L). The ISO plus 5 mmol/L Ca$^{2+}$ increased peak $\sigma$ and $+\frac{d\sigma}{dt}$ above control (1.2 mmol/L Ca$^{2+}$) levels in the WKY, whereas in the untreated SHR groups, elevated Ca$^{2+}$ bath fails to restore peak $\sigma$ to pre-ISO levels despite a marked increase in the amplitude of the Ca$^{2+}$ signal. In SHR-F, increasing the [Ca$^{2+}$]i bath to 5 mmol/L in the presence of ISO (10$^{-6}$ mol/L) caused a late rises in the diastolic Ca$^{2+}$ signal (Figure 1). CAP$_{Rx}$ SHR demonstrate positive inotropy (peak $\sigma$ and $+\frac{d\sigma}{dt}$) in response to ISO, which is further increased with ISO plus 5 mmol/L Ca$^{2+}$.

There were no significant differences in peak systolic intracellular [Ca$^{2+}$], or resting [Ca$^{2+}$], (0.2 to 0.3 $\mu$mol/L) range) among groups. The duration of the [Ca$^{2+}$]i signal and TP$\sigma$ was prolonged in the SHR relative to WKY (Table 2). In SHR, CAP$_{Rx}$ decreased TP$\sigma$ and duration of the light signal when treatment was initiated at 12 months of age and to a lesser extent at 18 and 21 months. TPL and RL$_{1/2}$ were significantly abbreviated with CAP$_{Rx}$ (SHR-NF and SHR-F versus SHR-Rx12). There was no significant difference in TP$\sigma$ or TPL+RL$_{1/2}$ between WKY and SHR-Rx12; −dQ/dt/Q was found to be slower in untreated SHR relative to WKY. This index of Ca$^{2+}$ sequestration became progressively higher (faster) in SHR with CAP$_{Rx}$ initiated at 21, 18, and 12 months of age.

Response to $\beta$-Adrenergic Stimulation
The relative changes in peak $\sigma$, $+\frac{d\sigma}{dt}$, and [Ca$^{2+}$], with increasing ISO bath expressed as a percentage of control at 1.2 mmol/L [Ca$^{2+}$], bath from untreated WKY, SHR-NF, and SHR-F are presented in Figure 2 (top; CAP$_{Rx}$, SHR shown at bottom). In WKY, peak [Ca$^{2+}$], and $+\frac{d\sigma}{dt}$ increased with ISO, whereas peak $\sigma$ at $L_{max}$ was only slightly increased and TP$\sigma$ decreased. In SHR-NF and SHR-F, despite an increase in [Ca$^{2+}$], peak $\sigma$ and $+\frac{d\sigma}{dt}$ fell in response to ISO, whereas TP$\sigma$ decreased ($P<0.05$ SHR-NF and SHR-F versus WKY). In contrast, in SHR-Rx12, ISO increased peak $\sigma$ and peak $+\frac{d\sigma}{dt}$, whereas TP$\sigma$ decreased (NS SHR-Rx12 versus WKY). CAP$_{Rx}$ of SHR starting at 21 months of age resulted in a small increase in $+\frac{d\sigma}{dt}$, whereas peak $\sigma$ and TP$\sigma$ deceased slightly.

Response to Calcium
The response of peak $\sigma$, $+\frac{d\sigma}{dt}$, and [Ca$^{2+}$], with increasing [Ca$^{2+}$], bath in untreated WKY, SHR-NF, and SHR-F (top) and CAP$_{Rx}$ SHR groups (bottom) is presented in Figure 3. Peak $\sigma$, $+\frac{d\sigma}{dt}$, and the Ca$^{2+}$ signal peak increased in all groups with and without CAP$_{Rx}$ when the [Ca$^{2+}$], of the bath was increased from 0.6 to 5.0 mmol/L, whereas TP$\sigma$ and TPL+RL$_{1/2}$ did not change.

Na$^+/Ca^{2+}$ Exchanger Expression
Figure 4 (left) shows representative autoradiogram of Na$^+/Ca^{2+}$ exchanger mRNA as detected by Northern hybridization from the LV of WKY, SHR-NF, and SHR-F. There was an increased abundance of Na$^+/Ca^{2+}$ exchanger mRNA of the LV from SHR-NF and marked increase in the SHR-F in comparison to age-matched WKY ($P<0.05$). CAP$_{Rx}$ reduced

### Table 1. Body Weight, Cardiac Chamber Weight, Chamber Weight-to-Body Weight Ratios, and In Vivo Systolic Blood Pressure

<table>
<thead>
<tr>
<th></th>
<th>BW, g</th>
<th>LV/BW, mg/g</th>
<th>RV/BW, mg/g</th>
<th>LV wt, g</th>
<th>RV wt, g</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n=9)</td>
<td>639±132</td>
<td>1.88±0.26</td>
<td>0.48±0.04</td>
<td>1.18±0.16</td>
<td>0.30±0.04</td>
<td>118±9</td>
<td>113±4</td>
</tr>
<tr>
<td>SHR (n=15)</td>
<td>358±55†</td>
<td>3.84±0.44†</td>
<td>0.91±0.13‡</td>
<td>1.35±0.15</td>
<td>0.33±0.06</td>
<td>180±5*</td>
<td>158±7*</td>
</tr>
<tr>
<td>SHR-Rx (n=28)</td>
<td>387±26‡§</td>
<td>2.54±0.38‡§</td>
<td>0.59±0.08§</td>
<td>0.98±0.14</td>
<td>0.23±0.05</td>
<td>190±14</td>
<td>144±10§</td>
</tr>
</tbody>
</table>

WKY indicates normotensive WKY; SHR, all spontaneously hypertensive rats. All CAP$_{Rx}$ SHR combined with treatment initiated at 12, 18, and 21 months of age and all animals studied at 24 months of age (SHR-Rx). Blood Pressure indicates peak systolic blood pressure before study at 12 months of age (Pre) and at time of study 21 to 24 months of age (Post). Values are mean±SD.

*P<0.05, †P<0.01 SHR vs WKY, ‡P<0.05 SHR-Rx vs SHR, §P<0.05, SHR-Rx vs WKY.
LV was greatest in the 12-month CAP Rx group and increased in WKY as well as in SHR (Figure 5).

The present study demonstrates that long-term CAP Rx restores myocardial inotropic responsiveness to ISO, in association with reversal of changes in α-MHC and Na⁺/Ca²⁺ exchanger gene expression, in the SHR during the transition to failure. Impaired responsiveness to β-adrenergic stimulation is one of the earliest changes reported with myocardial hypertrophy and failure. Recent observations in failing human myocardium indicate that systolic dysfunction was associated with a downregulation of α-MHC and upregulation of Na⁺/Ca²⁺ exchanger gene expression.

In acute myocardial infarction and aortic banding models, ISO induces a blunted but nevertheless positive inotropic response, whereas in the SHR with long-standing hypertrophy, ISO decreases isometric force at Lmax (g/mm²); TP, time to peak (ms); peak systolic Ca²⁺ (μmol/L); TPL + RL, time to peak and time to 50% decline in peak [Ca²⁺] signal; and −[dQ/dt]/Q, maximum rate of decline of the Ca²⁺ signal normalized by its peak. Values are mean ± SD.

*P < 0.05 vs WKY; †P < 0.05 vs SHR-NF; ‡P < 0.05 vs SHR-F.

Table 2. Characteristics of Papillary Muscle Function and Intracellular Calcium

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. PM</th>
<th>α Active</th>
<th>TPₐ</th>
<th>Peak [Ca²⁺]</th>
<th>TPL + RL</th>
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<tr>
<td>WKY</td>
<td>8</td>
<td>3.2 ± 0.7</td>
<td>146 ± 10</td>
<td>0.87 ± 0.19</td>
<td>80 ± 14</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>SHR-NF</td>
<td>6</td>
<td>3.0 ± 1.0</td>
<td>179 ± 7*</td>
<td>1.04 ± 0.23</td>
<td>114 ± 16*</td>
<td>36 ± 9</td>
</tr>
<tr>
<td>SHR-F</td>
<td>4</td>
<td>1.7 ± 0.8*</td>
<td>171 ± 13*</td>
<td>0.92 ± 0.15</td>
<td>117 ± 14*</td>
<td>35 ± 4*</td>
</tr>
<tr>
<td>SHR-Rx12</td>
<td>9</td>
<td>3.9 ± 2.3</td>
<td>169 ± 18</td>
<td>0.84 ± 0.19</td>
<td>93 ± 12</td>
<td>41 ± 3</td>
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<tr>
<td>SHR-Rx18</td>
<td>8</td>
<td>3.2 ± 1.5</td>
<td>159 ± 11</td>
<td>0.75 ± 0.11</td>
<td>80 ± 18</td>
<td>47 ± 10</td>
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<tr>
<td>SHR-Rx21</td>
<td>9</td>
<td>4.1 ± 3.0†‡</td>
<td>144 ± 16†‡</td>
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WKY indicates normotensive Wistar-Kyoto rats; SHR-NF, SHR without failure; SHR-F, SHR with failure; SHR-Rx, all CAP Rx. SHR, SHR groups with treatment initiated at 12, 18 and 21 months of age and all animals studied at 24 months of age; PM, papillary muscle preparations; α Active, active stress at Lmax (g/mm²); TP, time to peak (ms); peak systolic Ca²⁺ (μmol/L); TPL + RL, time to peak and time to 50% decline in peak [Ca²⁺] signal; and −[dQ/dt]/Q, maximum rate of decline of the Ca²⁺ signal normalized by its peak. Values are mean ± SD.

*P < 0.05 vs WKY; †P < 0.05 vs SHR-NF; ‡P < 0.05 vs SHR-F.

Discussion

The present study demonstrates that long-term CAP Rx restores myocardial inotropic responsiveness to ISO, in association with reversal of changes in α-MHC and Na⁺/Ca²⁺ exchanger gene expression, in the SHR during the transition to failure. Impaired responsiveness to β-adrenergic stimulation is one of the earliest changes reported with myocardial hypertrophy and failure. Recent observations in failing human myocardium indicate that systolic dysfunction was associated with a downregulation of α-MHC and upregulation of Na⁺/Ca²⁺ exchanger gene expression.

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Discussion

The present study demonstrates that long-term CAP Rx restores myocardial inotropic responsiveness to ISO, in association
β-adrenergic receptor density with failure and an increase in the peak transient intracellular Ca\(^{2+}\). These findings suggest that depressed LV inotropic responsiveness is unlikely to be mediated by downregulation of the β-receptors and may implicate postreceptor mechanisms, such as altered Ca\(^{2+}\) dynamics or myofilament responsiveness.

Responsiveness of cardiac myofilaments to Ca\(^{2+}\) may be modulated by a change in kinetics of the actin-myosin cross-bridge turnover or may be influenced in vivo by a number of intracellular effectors, including pH and cAMP; the effects of these factors on V\(_1\) and V\(_3\) myosin may differ. In CAP\(_{Rx}\) SHR, the relative proportion of V\(_1\) myosin isozyme present was associated with improved inotropic responsiveness to ISO. Epinephrine has been shown to increase cross-bridge cycling rate in V\(_1\) to a greater extent than in V\(_3\) myosin, since cAMP does not increase myosin ATPase activity in myocardium containing V\(_3\) myosin. Depressed myocardial contractile sensitivity to catecholamines has been related to the concentration of V\(_3\) myosin isozyme. Dibutyryl cAMP, which exerts a positive inotropic effect without stimulation of the β-receptor, has also been shown to have a smaller inotropic response in LV papillary muscles with higher V\(_3\) levels from aortic-constricted rats and from rats with large myocardial infarctions relative to controls. Thus, in the SHR, improved inotropic responsiveness with captopril may be mediated by the captopril-induced increase in V\(_3\). It is interesting to note that CAP\(_{Rx}\) increased the expression of α-MHC mRNA by ≈2-fold not only in the SHR but also in the normotensive WKY, in which there was no significant lowering of arterial blood pressure. Thus, captopril affects pretranslational regulation of MHC composition; the finding that increased expression of V\(_1\) myosin in the WKY, in which blood pressure was not altered, suggests that this is not directly related to its blood-pressure–lowering effects. Thus, CAP\(_{Rx}\) appears to reverse genetic factors responsible for hypertrophy and age-associated changes in α-MHC expression.

The Na\(^{+}/Ca\(^{2+}\) exchanger also may affect myocardial responsiveness, and Na\(^{+}\) has been shown to be required for the positive inotropic actions of ISO. In atrial tissue, changing from a normal extracellular Na\(^{+}\) concentration to a Na\(^{+}\)-free medium not only resulted in a negative inotropic effect of ISO but also increased T\(\text{P}_r\) and relaxation time of the isometric contraction, and physiological responses that are similar to those seen in hypertrophied SHR myocardium. Recent studies in failing human myocardium indicate that systolic dysfunction is associated with upregulation of Na\(^{+}/Ca\(^{2+}\) exchanger gene expression. This may be in response to depressed sarcoplasmic reticulum function and impaired regulation of diastolic Ca\(^{2+}\) levels. It is possible that upregulation of this pathway in hypertrophied myocardium may be a compensatory response providing an alternative pathway for mobilizing intracellular Ca\(^{2+}\) (eg, reverse Na\(^{+}/Ca\(^{2+}\) exchange). In isolated sarcolemmal vesicle preparation from rat heart, angiotensin II has been shown to directly stimulate activity of the Na\(^{+}/Ca\(^{2+}\) exchanger; captopril may therefore reduce angiotensin-mediated effects on the exchanger. In the present studies, expression of Na\(^{+}/Ca\(^{2+}\) exchanger was up-regulated during the transition to failure and decreased with CAP\(_{Rx}\). A late rise in the diastolic Ca\(^{2+}\) signal in the SHR-F was induced by ISO at 5.0 mmol/L [Ca\(^{2+}\)]\(_o\). The prolongation of the Ca\(^{2+}\) transient in untreated SHR and normalization with treatment suggests that there may be abnormalities of Ca\(^{2+}\) dynamics in both chronic hypertrophy and failure, which are reversed by long-term ACE inhibition.

In summary, the present study demonstrates that chronic CAP\(_{Rx}\) can restore inotropic responsiveness to β-adrenergic stimulation in the SHR with long-standing hypertrophy that was associated with increased α-MHC gene expression and protein and decreased Na\(^{+}/Ca\(^{2+}\) exchanger mRNA. The findings of depression of contractile function, prolongation of the calcium transient, increased abundance of Na\(^{+}/Ca\(^{2+}\) exchanger expression, depressed inotropic responsiveness to

### TABLE 3. Percent V\(_3\), Myosin Isozyme Composition of LV

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of LV Samples</th>
<th>% V(_3)</th>
</tr>
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<tbody>
<tr>
<td>Untreated</td>
<td>8</td>
<td>24±11</td>
</tr>
<tr>
<td>SHR</td>
<td>8</td>
<td>24±11</td>
</tr>
<tr>
<td>Captopril-treated</td>
<td>5</td>
<td>53±26</td>
</tr>
<tr>
<td>SHR-RX(_{12})</td>
<td>6</td>
<td>64±26*</td>
</tr>
<tr>
<td>SHR-RX(_{18})</td>
<td>5</td>
<td>77±21*</td>
</tr>
</tbody>
</table>

SHR indicates all untreated SHR; SHR-RX\(_{9}\), SHR-RX\(_{12}\), SHR-RX\(_{18}\), captopril-treated SHR with treatment initiated at 12, 18, and 21 months of age; all treated SHR studied at 24 months of age, % V\(_3\), % of myosin isozyme composition. Values are mean±SD. *P<0.05 vs untreated SHR.
cAMP, and myosin shifts, findings not seen in compensated SHR treated with captopril, suggest that several factors may be required for the positive inotropic response to catecholamines and may contribute to contractile depression seen with failure. Because both calcium handling and downstream myofilament responsiveness are affected, the present results demonstrate that captopril can modulate transcriptional regulation of expression of multiple cardiac genes to reverse hypertrophic changes that effect contractility and enhance myocardial β-adrenergic responsiveness.

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With Captopril

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