Beneficial Effects of Angiotensin-Converting Enzyme Inhibition on Sarcoplasmic Reticulum Function in the Failing Heart of the Dahl Rat

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Inhibition of angiotensin-converting enzyme (ACE) retards the process of myocardial remodeling and contractile dysfunction that leads to heart failure. However, the intracellular mechanisms by which ACE inhibition preserves myocardial contractility are largely unclear. Using a model of heart failure induced by hypertension in Dahl salt-sensitive (DS) rats, the mechanisms by which ACE inhibitors (ACEI) exert a beneficial effect on myocardial contractility were studied. Dahl salt-resistant (DR) rats, DS rats not given temocapril (DS/T–), and DS rats treated with temocapril (10 mg/kg per day from 10 to 17 weeks of age, DS/T+) were fed an 8% NaCl diet from 8 to 17 weeks of age (n=8, each group). Echocardiography, hemodynamic measurement, histology, contraction of isolated skinned papillary muscle, and Western blot analysis were carried out. At an elevated final blood pressure similar to that of the DS/T– rats, DS/T+ rats exhibited (1) a decrease in left ventricular (LV) mass associated with decreases in both cardiomyocyte size and interstitial fibrosis; (2) improvement of both systolic and diastolic LV function; and (3) an increase in caffeine contraction after constant Ca2+-loading with 8-bromo-cAMP into the sarcoplasmic reticulum (SR) associated with an increase in Ser16-phosphorylated phospholamban, as compared with the DS/T– rats. In addition to inhibition of myocardial remodeling, a restoration of the Ca2+-handling ability of the SR by normalized phosphorylated phospholamban may contribute to the improved LV contractile function achieved by chronic treatment with an ACEI. (Circ J 2003; 67: 705–711)

Key Words: Angiotensin-converting enzyme inhibitor (ACEI); Calcium; Heart failure; Hypertrophy; Sarcoplasmic reticulum

EXPERIMENTAL INVESTIGATION

In the diseased myocardium of various etiologies, upregulated neurohormonal factors such as angiotensin II (Ang II), endothelin-1 and β-adrenoreceptor agonists induce myocardial hypertrophy and interstitial fibrosis, both of which promote myocardial remodeling and contractile dysfunction, ultimately leading to heart failure.1–3 In principle, these factors activate the Gq class of GTP-binding protein, which, it has been proposed, plays a central role in inducing myocardial hypertrophy.4 Activated Gq subsequently stimulates several intracellular signaling pathways, such as those of protein kinase C, ERKs, JAK/STAT, p38-MAP kinase and calcineurin, leading to myocardial hypertrophy and fibrous proliferation.4–7

Several large clinical trials have proved that treatment with angiotensin-converting enzyme inhibitors (ACEI) prevents heart failure, resulting in decreased morbidity and mortality.5,9 and a number of experimental studies have been performed to explain how ACE inhibition exerts these beneficial effects on the failing heart. Inhibition of ACE retards the process of myocardial hypertrophy leading to myocardial remodeling, thus preserving cardiac contractility and preventing heart failure caused by hypertension and myocardial infarction.10–13 The mechanism by which ACE inhibition exerts these beneficial effects on the heart can be explained in part by inhibition of the Gq-mediated intracellular signaling pathways. Previous studies have focused mainly on morphological changes; that is, the inhibition of myocardial hypertrophy and remodeling achieved by ACEI. From the functional standpoint, Yamaguchi et al used a rat model of heart failure with myocardial infarction and reported that chronic treatment with trandolapril preserved the function of sarcoplasmic reticulum (SR) because it prevented any reduction in the Ca2+-release channel (ryanodine receptor).14

In the present study, we investigated other intracellular mechanisms by which chronic treatment with an ACEI could preserve myocardial contractility in the failing heart of Dahl salt-sensitive hypertensive rats.15

Methods

Experimental Animals and Protocol

Dahl salt-sensitive (DS, strain SS/Jr) and salt-resistant (DR, strain SR/Jr) rats were fed a 0.3% NaCl diet until 8 weeks of age, after which they were fed an 8% NaCl diet for 9 weeks until 17 weeks of age. The rats were then divided into 3 groups (n=8, each group): (1) DS rats receiving no treatment (DS/T–), (2) DS rats treated with temocapril (DS/T+), and (3) DR rats used as a normotensive control.
Temocapril, kindly provided by Sankyo Pharmaceutical Company, was dissolved in drinking water (10 mg/kg per day) and administered orally to the DS rats from 10 to 17 weeks of age. At 17 weeks of age, rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). After an echocardiographic study, the arterial and left ventricular (LV) pressures were measured by direct cannulation with a fluid-filled catheter (PE-50 tube, Becton Dickinson) inserted into the ascending aorta through the right carotid artery. LV end-diastolic pressure (LVEDP) and the rate of changes in LV pressure during systole (+dp/dt) were measured. Study protocols were approved by the Committee on the Ethics of Animal Experiments, Kyushu University, and were in accordance with the ‘Guidelines for the Care and Use of Laboratory Animals’ published by the US National Institutes of Health (NIH publication No. 85-23, revised in 1996).

Echocardiographic Study

Transthoracic echocardiographic studies were performed on the 8- and 17-week-old rats using an 11-MHz phased-array transducer (LOGIC 400, GE Yokogawa Medical Systems). M-mode tracings were recorded through the anterior and posterior LV walls at the papillary muscle level to measure LV dimensions and LV wall thickness at end-diastole. The LV ejection fraction (EF) and % fractional shortening (%FS) were calculated as follows:

\[
\text{LVEF} \times 100 = \frac{(LVEDV - LVESV)}{LVEDV} \times 100
\]

where LVEDV is the LV end-diastolic volume, equal to [7.0/(2.4 + LVDd)] × (LVDd)²; LVESV is the LV end-systolic volume, equal to [7.0/(2.4 + LVDS)] × (LVDS)³ (according to Teichholz’s method); LVDd is the LV end-diastolic dimension; and LVDS is the LV end-systolic dimension.

Histology

For the histological examination, the LV were first arrested by retrograde perfusion with a high-K⁺ (20 mmol/L) oxygenated PSS through the ascending aorta, then fixed by retrograde perfusion with 4% paraformaldehyde through the ascending aorta; 4-μm-thick transverse tissue slices were excised at the level of the papillary muscle. After being embedded in paraffin, the sections were stained with hematoxylin-eosin and Masson’s trichrome. To measure the cross-sectional area (CSA) of the cardiomyocytes, transverse-sectioned cardiomyocytes with rounded nuclei were selected and their margins were traced using NIH Image 1.61 software.

Evaluation of SR Function

In order to evaluate the function of the SR, we used papillary muscle bundles excised from the right ventricles (RV), because of their convenience for skinned muscle preparation and because previous reports have shown that RV papillary muscle from failing hearts caused by myocardial infarction in the rat and tachypacing-induced heart failure in the dog and spontaneously hypertensive rats have similar contractile dysfunction to the LV. Muscle bundles were skinned with 50 mOsm/dl-escin for 30 min in the absence of A23187 to preserve SR function. The experiment was performed with a slight modification of the method described by Tomita et al. Briefly, skinned fibers were immersed sequentially in: (1) relaxing solution (4 mmol/L EGTA) containing 25 mmol/L caffeine for 4 min to deplete the SR of Ca²⁺, (2) low-EGTA (0.5 mmol/L) EGTA-containing solution with 4 mmol/L EGTA for 3 min to load Ca²⁺ into the SR, (3) low-EGTA (0.5 mmol/L) EGTA relaxing solution for 4 min to wash out Ca²⁺, (4) Ca²⁺-releasing solution (the relaxing solution with 0.2 mmol/L EGTA) for 3 min, and finally (5) Ca²⁺-releasing solution containing 25 mmol/L caffeine for 1 min. To observe the effect of cAMP on Ca²⁺-uptake by the SR, 100 nmol/L 8-bromo (8b)-cAMP was added to the solution in steps (2) and (3) [Ca²⁺-uptake phase] (Fig 2A).

Measurement of ACE Activity

The ACE activity was measured by the modified method of Hayakari et al. Briefly, homogenized tissue samples were centrifuged at 5,000 g for 10 min, and the supernatant was dialysed for 24 h at 4°C after which it was used for the assay. The assay for ACE activity was carried out in a 150-ml incubation mixture containing 80 mmol/L potassium phosphate buffer (pH 8.3), 0.6 mol/L NaCl and 3 mmol/L hypuryl-histidyl-L-leucine. The reaction was initiated by addition of the substrates at 37°C for 30 min and was terminated by immersion of the test tubes into a boiling-water bath for 10 min. The enzyme activity in the resulting supernatant fluid was determined from the absorbance at 382 nm using a differential spectrophotometric method. The control run was identical the procedure, but omitted the incubation.

Measurement of Hydroxyproline

The content of myocardial collagen was estimated by measuring the hydroxyproline concentration in the LV as described previously. After LV tissues had been dried for 24 h, the specimens were hydrolysed in a 6 mol/L hydrogen chloride solution at 100°C. After resolution in a buffer at pH 7.0, p-dimethylamino-benzaldehyde was added to form a complex with hydroxyproline. The hydroxyproline concentrations were measured by spectrophotometer at a wavelength of 588 nm.

Western Blot Analysis

Western blot analysis was performed to semi-quantify the expression of SR Ca²⁺-ATPase (SERCA2), PLB, and Ser16-phosphorylated phospholamban (p-PLB), slightly modifying the methods described by Schwinger et al. Briefly, the tissue samples, kept at −80°C, were homogenized in 40 mmol/L HEPES (pH 7.4) containing 1 mmol/L PMSF, 1% Triton-X, and 10% glycerol in the presence of 1 mmol/L cantharidin as an endogenous phosphatase inhibitor. Samples were centrifuged at 5,000 g for 5 min at 4°C to remove unbroken cells and nuclei. The supernatant was subjected to the analysis. Proteins (15 mg each) were separated on SDS-PAGE (10% for SERCA2; 12.5% for PLB and p-PLB) and transferred onto a 0.45-μm polyvinylidene difluoride membrane. The membrane was probed with primary antibodies to SERCA2 (1:100, Santa Cruz Biotechnology Inc), PLB (1:200, Upstate Biotechnology), p-PLB (1:100, Santa Cruz Biotechnology Inc), and α-actin (1:100, Sigma-Aldrich) as an internal control. Horseradish peroxidase-labeled anti-goat IgG (for SERCA2, 1:10,000; p-
PLB, 1:9,000) and anti-mouse IgG (for PLB, 1:9,000) were used as secondary antibodies.

**Statistical Analysis**
Data are expressed as mean±SEM. Comparison of a single parameter among the 3 groups was performed by one-way ANOVA followed by Bonferroni/Dunn’s post-hoc test; p<0.05 was considered significant.

**Results**

**Morphological Changes**

The ACE activity in the LV was significantly elevated in the DS/T− rats after the trial (1.76±0.20 in DR vs 3.62±0.56 in DS/T− [nmol·min−1·mg−1 protein], p<0.01), but was significantly suppressed in the DS/T+ rats (2.16±0.22 nmol·min−1·mg−1, p<0.05 vs DS/T−).

Fig 1 shows representative histological specimens of myocardial sections demonstrating changes in cell size and vascular structure at 17 weeks of age after the rats were fed the high-salt diet. The wall thickness and chamber size increased in the DS/T− rats compared with the DR rats (Fig 1A vs B), and these increments were attenuated in the DS/T+ rats (Fig 1B vs C). The cardiomyocytes were larger in the DS/T− rats compared with the DR rats (Fig 1A vs B), and these increments were attenuated in the DS/T+ rats (Fig 1B vs C).

Table 1 summarizes the comparisons among groups by body weight (BW), LV mass and the quantified data of the CSA of cardiomyocytes and the hydroxyproline content of the LV tissue. No significant difference in BW was observed among the 3 groups before the trial. Relative to the DR rats, BW gain was decreased in the DS/T− rats at 17 weeks of age, but this loss in BW gain was recovered in the DS/T+ rats. Both the actual LV weight and LV mass corrected for body weight increased in the DS/T− rats compared with the DR rats, and these parameters were significantly decreased in the DS/T+ rats. In addition, increases in both the CSA of cardiomyocytes and LV hydroxyproline content in the DS/T− rats were significantly suppressed in the DS/T+ rats, consistent with the histological changes shown in Fig 1.

**Hemodynamic Changes**

Table 2 shows the comparison of hemodynamic parameters. There was no significant difference among the groups in blood pressure (BP) during systole before the trial, but BP was elevated to a similar extent in both DS/T− and DS/T+ rats at 17 weeks of age. Compared with the DR rats, DS/T− rats showed an elevated LVEDP, and decreased maximum +dp/dt and −dp/dt, indicating systolic and diastolic LV dysfunction. These parameters were significantly restored to some extent in the DS/T+ rats after the trial.

**Changes in the Echocardiographic Findings**

Table 3 shows the changes in the echocardiographic parameters before and after the trial. Before the trial, there were no significant differences in wall thickness, chamber size, or global LV function among the groups. After the trial, an increase in the thickness of the IVS in the DS/T− rats was significantly suppressed in the DS/T+ rats, but that of LVPW was not. The significant increase of both LVDD and LVDs in the DS/T− rats was attenuated in the DS/T+ rats and as a result, the LVEF and FS, which were de-

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**Table 1  Comparison of the Body Weight and Left Ventricular Mass in the 3 Group of Dahl Rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DR</th>
<th>DS/T−</th>
<th>DS/T+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>24±4</td>
<td>259±7</td>
<td>254±8</td>
</tr>
<tr>
<td>LV weight (g)</td>
<td>402±4</td>
<td>307±11</td>
<td>376±13</td>
</tr>
<tr>
<td>LV mass (mg/g BW)</td>
<td>0.99±0.04</td>
<td>1.62±0.02</td>
<td>1.42±0.04</td>
</tr>
<tr>
<td>CSA (μm²)</td>
<td>5.44±0.23</td>
<td>4.06±0.16</td>
<td></td>
</tr>
<tr>
<td>Hydroxyproline (μg/mg heart)</td>
<td>457.5±2.94</td>
<td>584</td>
<td></td>
</tr>
</tbody>
</table>

§p<0.01 vs DR, †p<0.01 vs DR, ‡p<0.05 vs DS/T−, §p<0.01 vs DS/T−.

**Table 2  Comparison of the Hemodynamic Parameters of the 3 Group of Dahl Rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DR</th>
<th>DS/T−</th>
<th>DS/T+</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>12±2</td>
<td>124±3</td>
<td>126±5</td>
</tr>
<tr>
<td>Post</td>
<td>135±5</td>
<td>207±4</td>
<td>202±9</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>8.0±1.1</td>
<td>26.4±3.1</td>
<td>13.4±2.7</td>
</tr>
<tr>
<td>+dp/dt max (mmHg/s)</td>
<td>5.96±2.80</td>
<td>3.185±334</td>
<td>5.130±310</td>
</tr>
<tr>
<td>-dp/dt max (mmHg/s)</td>
<td>-5.250±408</td>
<td>-2.528±258</td>
<td>-4.685±386</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; †measured by the tail-cuff method, §measured by catheterization; LVEDP, LV end-diastolic pressure; Pre, at 8 weeks of age before the trial; Post, at 17 weeks of age after the trial.

†p<0.01 vs DR, †p<0.01 vs DS/T−.
increased in the DS/T– rats, were almost normalized in the DS/T+ rats after the trial. The calculated LV mass based on the echocardiographic data was significantly decreased in the DS/T+ rats compared with that in the DS/T– rats.

Changes in the Function and Protein Concentrations of the SR

In order to assess the function of the SR of cardiac muscle, we observed the caffeine-induced contraction after constant Ca2+-loading in skinned papillary muscle fibers. Fig 2A shows a representative tension recording from the muscle, we observed the caffeine-induced contraction after 8br-cAMP during the Ca2+-uptake phase was regarded as the transient contraction that returned to baseline within 1 min. The area under this contraction reflects the amount of Ca2+ released from the SR. The area in the absence of 8br-cAMP, the subsequent caffeine-contraction was augmented (128%, lower trace), suggesting an increased Ca2+-uptake ability of the SR in response to cAMP. Fig 2B shows the changes in the Ca2+-uptake ability of the SR in response to 8br-cAMP in each group. After stimulation with 8br-cAMP, the subsequent caffeine-contraction was augmented in the DR rats (126±1.7%), and to a lesser extent in the DS/T– rats (108±3.2%). This blunted response to 8br-cAMP was restored in the DS/T+ rats (131.7±6.9%).

Finally, we assessed the amounts of the proteins involved in SR function. Fig 3 shows Western blot analysis of SERCA2, PLB, and p-PLB in the LV myocardium. The amounts of protein were evaluated as relative ratios to that of β-actin, an internal control. Whereas the total amounts of SERCA2 (1.41±0.05 in DR, 1.36±0.07 in DS/T–, and 1.33±0.03 in DS/T+) and PLB (0.95±0.05 in DR, 0.96±0.05 in DS/T–, and 0.86±0.04 in DS/T+) did not significantly differ among the groups, p-PLB was decreased in the DS/T– rats (0.80±0.09 in DS/T– vs 1.12±0.05 in DR, p<0.01). This decrement was normalized in the DS/T+ rats (1.04±0.05) and was comparable to the amount in the DR rats (p<0.05).

Discussion

In the present study, which used a model of heart failure in DS rats, chronic treatment with temocapril caused (1) decreases in both cardiomyocyte size and interstitial fibrosis; (2) improvement of both systolic and diastolic function; and (3) an increase in the Ca2+-uptake ability of the SR, associated with increased Ser16-phosphorylated PLB, as compared with the failing heart.

Inhibition of Myocardial Hypertrophy and Remodeling by Temocapril

The ACE activity in the LV, which was elevated in the
Effect of ACEI on SR of Failing Heart

thought to be exerted mainly by both inhibition of intracellular and interstitial fibrosis, thus inhibiting cardiac remodeling. 

hypertension ACE inhibition prevented LV hypertrophy 
confirmed that in a Dahl rat model of heart failure induced by drugs, dosages and duration of administration, we con- 
present results, probably because of differences in the 
minor discrepancies among these previous findings and the 

DS/T– rats. These results suggest that the inhibition of LV hypertrophy by temocapril was caused by the decrease in both cardiomyocyte size and interstitial fibrosis, despite elevated BP. 

Previous studies using the same heart failure model have shown that temocapril (20mg/kg per day from 11 to 17 weeks of age) inhibits LV hypertrophy and interstitial fibrosis which increased in the DS/T– rats, were significantly reduced in the DS/T+ rats, indicating that temocapril inhibits LV hypertrophy. In addition, the increases in both the CSA of cardiomyocytes and LV hydroxyproline content observed in the DS/T– rats were significantly suppressed in the DS/T+ rats. These results suggest that the inhibition of LV hypertrophy by temocapril was caused by the decrease in both cardiomyocyte size and interstitial fibrosis, despite elevated BP. 

The caffeine-induced contraction observed after constant Ca2+-loading into the SR in the presence of 8br-cAMP indicated an increase in the Ca2+-uptake ability of the SR in response to cAMP (Fig 2A) and would result in both an increase in the amount of stored Ca2+ and promotion of the rate of decrease in intracellular calcium ion concentration during diastole, thus enhancing systolic and diastolic function. The caffeine-induced contraction in the presence of 8br-cAMP during Ca2+-uptake phase was only marginally augmented in the failing heart of the DS/T– rats, but this blunted response to 8br-cAMP was restored in the DS/T+ rats (Fig 2B). In the hypertrophied rat heart induced by aortic banding, the increased response in caffeine-induced force after Ca2+-loading with cAMP has been shown to also be diminished21 These findings can be explained by the disabling of the Ca2+-uptake and/or Ca2+-release functions of the SR. 

Two proteins, SERCA2 and PLB, play important roles in regulating Ca2+-uptake ability. PLB is phosphorylated primarily at Ser-16 (p-PLB) by β-adrenergic stimulation, and p-PLB relieves the inhibition of SERCA2 activity by PLB34. There have been conflicting results of the concentration of SERCA2 and PLB in human and rat heart failure; it is either decreased or unchanged30 However, basal p-PLB is reported to be reduced in the failing heart caused by dilated cardiomyopathy in humans25 and by myocardial infarction in the rat32,33. In the Dahl rat model of heart failure, SERCA2 and PLB concentrations are not decreased, but the Ca2+-uptake ability is impaired34 Little information regarding p-PLB in the Dahl rat has been reported and so there are missing links between the expression of these pro-
teins and SR function. Our results offer some clarification. Consistent with the results reported by Yoneda et al., the total amounts of SERCA2 and PLB were unchanged, but we found that the amount of p-PLB was decreased in the DS/T− rats (Fig 3), which can be caused by impaired α-adrenergic signaling and/or increased expression of cardiac type 1 phosphatase in the failing heart of the rat and human. Attenuation of the increase in the caffeine-induced contraction in response to 8brcAMP during Ca2+-uptake phase in the DS/T− rats (Fig 2B) may have been caused by the decreased p-PLB and downregulation of the regulatory subunits of cAMP-dependent protein kinase (PKA) in the failing heart. The present results suggest that chronic treatment with temocapril may be able to restore the Ca2+-uptake ability of the SR in association with normalized p-PLB (Figs 2B and 3B).

However, other reports have shown that the concentration of PKA regulatory subunit II remains unchanged during heart failure, and that PLB can be phosphorylated to a similar level to that in normal heart by stimulation with isoproterenol or forskolin, and PKA. Another possible cause of the blunted response of the caffeine-induced contraction to 8brcAMP is that the Ca2+-release channel (ryanodine receptor) is reportedly hyperphosphorylated by PKA in the failing heart of the Dahl rat, thus resulting in leaky channels that can deplete the SR Ca2+ stores and contribute to impaired Ca2+-release function. This channel dysfunction has been proposed as one of the mechanisms underlying contractile dysfunction in the failing heart and ACE inhibition may be able to correct it. In a rat model of heart failure with myocardial infarction, Yamaguchi et al. have shown that chronic treatment with trandolapril preserved the function of the SR by preventing the downregulation of the ryanodine receptor.

Conclusion

ACE inhibition exerts beneficial effects not only on the morphological deterioration, but also on the functional deterioration that occurs in the hypertension-induced failing heart. ACE inhibition can restore the Ca2+ handling function of the SR in the failing heart of the Dahl rat in association with restored p-PLB. Although the targets of the actions of the ACEI on SR function could not be identified from the present study, the results show a new aspect of the beneficial effect of treatment with an ACEI. The changes in phosphorylation status of other proteins and enzyme activities (protein kinase/phosphatase) related to the SR function remain to be clarified in future studies.

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References


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