Effects of the Angiotensin-converting Enzyme Inhibitor Enalapril on Sympathetic Neuronal Function and β-adrenergic Desensitization in Heart Failure after Myocardial Infarction in Rats

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SUMMARY

One of the beneficial effects of angiotensin-converting enzyme (ACE) inhibitors in the treatment of heart failure may derive from sympathoinhibition and the prevention of β-adrenergic desensitization. However, the roles of these properties in the overall effects of ACE inhibitor are not clear. We studied the effects of chronic enalapril treatment (20 mg/L in drinking water for 12 weeks) on left ventricular (LV) function, cardiac norepinephrine (NE), sympathetic neuronal function assessed by 131I-metaiodobenzylguanidine (MIBG), β-receptors, and isometric contraction of papillary muscle in rats with myocardial infarction (MI) induced by coronary artery ligation. Decreased LV function in the MI rats was associated with reduced cardiac NE content and MIBG uptake, and severely blunted responses of non-infarcted papillary muscle to isoproterenol, forskolin, and calcium. Enalapril attenuated LV remodeling in association with a reduction of the ventricular loading condition and restored baseline developed tension of non-infarcted papillary muscle to the level of sham-operated rats. However, enalapril did not improve cardiac NE content, MIBG uptake, or inotropic responsiveness to β-agonists. These results suggest that the major effect of the ACE inhibitor enalapril in the treatment of heart failure is not due to sympathoinhibition or restoration of β-adrenergic pathway in this model of heart failure. (Jpn Heart J 2002; 43: 675-688)

Key words: Autonomic nervous system, β-receptors, Remodeling, Rats

Experimental Studies

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SUMMARY

One of the beneficial effects of angiotensin-converting enzyme (ACE) inhibitors in the treatment of heart failure may derive from sympathoinhibition and the prevention of β-adrenergic desensitization. However, the roles of these properties in the overall effects of ACE inhibitor are not clear. We studied the effects of chronic enalapril treatment (20 mg/L in drinking water for 12 weeks) on left ventricular (LV) function, cardiac norepinephrine (NE), sympathetic neuronal function assessed by 131I-metaiodobenzylguanidine (MIBG), β-receptors, and isometric contraction of papillary muscle in rats with myocardial infarction (MI) induced by coronary artery ligation. Decreased LV function in the MI rats was associated with reduced cardiac NE content and MIBG uptake, and severely blunted responses of non-infarcted papillary muscle to isoproterenol, forskolin, and calcium. Enalapril attenuated LV remodeling in association with a reduction of the ventricular loading condition and restored baseline developed tension of non-infarcted papillary muscle to the level of sham-operated rats. However, enalapril did not improve cardiac NE content, MIBG uptake, or inotropic responsiveness to β-agonists. These results suggest that the major effect of the ACE inhibitor enalapril in the treatment of heart failure is not due to sympathoinhibition or restoration of β-adrenergic pathway in this model of heart failure. (Jpn Heart J 2002; 43: 675-688)

Key words: Autonomic nervous system, β-receptors, Remodeling, Rats

THE initial adaptive hemodynamic, myocardial, and neurohormonal responses to myocardial infarction (MI) may lead to progressive ventricular remodeling and
heart failure. In clinical\textsuperscript{1-3} and experimental studies,\textsuperscript{4-6} chronic treatment with an angiotensin-converting enzyme (ACE) inhibitor attenuates ventricular remodeling, and improves ventricular function and mortality. The efficacy of ACE inhibitor therapy probably results from a combination of hemodynamic, biological, and structural effects. ACE inhibitors decrease ventricular preload and afterload,\textsuperscript{7} and reduce neurohormonal activation including sympathetic activity,\textsuperscript{8,9} and, consequently, attenuate ventricular remodeling.\textsuperscript{10} However, the relative roles of these different properties in the overall beneficial effects of ACE inhibitors are not clear.

Increased sympathetic activity and impaired sympathetic neuronal function seen in heart failure\textsuperscript{11} would increase synaptic norepinephrine (NE) levels at the nerve terminals due to an increase in neuronal release of NE, a decrease in neuronal NE re-uptake, or both. The increased local NE concentration may result in a decrease in $\beta$-receptor density\textsuperscript{8,12,13} and impair cardiac $\beta$-adrenergic signal transduction.\textsuperscript{14} Several reports have shown that angiotensin II enhances sympathetic nerve activity by potentiating the release of NE\textsuperscript{15,16} and by inhibiting NE re-uptake at the nerve terminal.\textsuperscript{17,18} These observations suggest that the beneficial effects of ACE inhibitors in the treatment of heart failure may be, at least in part, due to sympathoinhibiton by blocking angiotensin II and prevention of the desensitization of $\beta$-adrenergic signaling.

The present study was designed to elucidate the effects of an ACE inhibitor on the coupling between sympathetic neuronal function and $\beta$-adrenergic desensitization in treatment of heart failure after MI. Changes in ventricular function, plasma and tissue catecholamines, sympathetic neuronal function, $\beta$-receptor density, and inotropic responsiveness to $\beta$-agonist stimulation in non-infarcted papillary muscle were studied during long-term treatment with the ACE inhibitor enalapril after MI in rats.

**METHODS**

The present study was undertaken in accordance with the guideline for animal experiments at Toyama Medical and Pharmaceutical University.

**Experimental animals:** Male Wistar rats weighing 300-350 g were used for the induction of MI, that was produced by ligation of the left coronary artery under ether anesthesia as reported previously.\textsuperscript{19} Briefly, the left coronary artery was ligated approximately 2-3 mm from its origin with a suture (6-0 silk). With this method, the 24-hour survival rate was 57\% in the infarcted rats. Control rats were sham-operated using the same procedure without coronary ligation.

All MI rats were allowed to recover for 7 days before being assigned to one of the untreated or enalapril-treated (20 mg/L in drinking water) groups.
The rats were divided into four groups. The first group was used for hemodynamic study and to measure plasma and cardiac tissue catecholamines. The second group was used to assess cardiac sympathetic neuronal function and \( \beta \)-receptor density, the third group for a non-infarcted papillary muscle study, and the fourth group for a \( \beta \)-receptor binding assay using a crude membrane preparation. Data were collected 12 weeks after the operation.

Infarct size was determined using a technique described by Chien, et al.\(^{20}\). Briefly, the right ventricle (RV) and LV including the interventricular septum were dissected, separated, and weighed. Incisions were made in the LV so that the LV tissue could be pressed flat. The LV circumference and the infarction region were outlined on a clear plastic sheet for both the endocardial and epicardial surfaces. Rats with an infarct size \( \geq 30\% \) of LV were included in the data analysis.

**Hemodynamic study and measurement of catecholamines:** Twelve weeks after MI induction or sham operation, transthoracic echocardiography was performed with a 7.5-MHz transducer (SSH140A, Toshiba, Japan) under ether anesthesia as described previously.\(^{19}\) End-diastolic and end-systolic LV internal dimensions and fractional shortening were determined from at least three consecutive cardiac cycles.

After echocardiographic data collection, a 2F micromanometer-tipped catheter was inserted into the right carotid artery and advanced into the LV to determine LV pressure under light anesthesia. The LV pressure signals were digitized on-line at 2-ms intervals and analyzed with a signal processing computer system.\(^{19}\)

After blood sampling for analysis of plasma catecholamines, the chest was opened and the heart quickly removed. The RV and LV were dissected, rinsed in ice-cold saline and weighed, and then the infarct size was determined. Plasma and non-infarcted LV and RV tissue samples were stored at -80°C for later analyses. Tissue and plasma catecholamines were determined by automated high-performance liquid chromatography as described previously.\(^{21}\)

**Sympathetic neuronal function and \( \beta \)-receptor density:** Cardiac sympathetic neuronal function and \( \beta \)-receptor density were assessed by a dual-tracer method using \( ^{131} \)I-metaiodobenzylguanidine (MIBG), an analogue of NE, and \( ^{125} \)I-iodocyanopindolol (ICYP). The method for determining MIBG and ICYP accumulation has been described previously.\(^{21}\) Briefly, 20 \( \mu \)Ci of MIBG was injected via the external jugular vein under anesthesia with pentobarbital sodium (30 mg/kg, IP). Two hours later 10 \( \mu \)Ci of ICYP was given intravenously. The rats were killed at 1 hour after the ICYP injection. After the determination of infarct size, the non-infarcted LV and RV counts of MIBG and ICYP were determined with a gamma
counter (ARC 2000, Aloka, Japan).

Data from the β-receptor binding assay using a membrane preparation were compared with those assessed by cardiac ICYP accumulation. The β-receptor binding method was reported previously. Briefly, a tissue membrane preparation was incubated with [3H]CGP12177 (specific activity 44.5 Ci/mmol, New England Nuclear). The nonspecific binding was defined as radioligand binding in the presence of an excess concentration of dl-isoproterenol. Data from the saturation binding studies were analyzed by Scatchard analysis, giving the Bmax and Kd.

Isometric papillary muscle function: After pentobarbital sodium (70 mg/kg) was injected intraperitoneally, the heart was rapidly excised. The non-infarcted posterior papillary muscle was rapidly dissected free from the LV wall in an oxygenated calcium-free Tyrode's solution with the following composition (mM): NaCl 135, KCl 4, MgCl2 1, Na2HPO4 0.33, HEPES 10, glucose 10. The muscle was mounted horizontally in a muscle bath suspended in Tyrode's solution with the calcium concentration adjusted to 1.0 mM, at pH 7.4 and 30°C, and equilibrated with 95%O2-5%CO2. The muscle end was attached by a 5-0 nylon thread to a force-displacement transducer (AM20, Uniplus, Japan). The muscle was stimulated by a stimulator (SEN7103, Nihon Kohden, Tokyo) at 0.33 Hz using a 2.5-ms impulse at a voltage of 1.5 times threshold. The muscle was stretched to a peak developed tension level (Lmax) and allowed to stabilize for 1 hour. After the baseline data were collected at a Ca2+ concentration of 1.0 mM, isometric tensions were recorded at Ca2+ concentrations of 0.5, 1.0, 2.0, and 3.0 mM. Data were collected at least 10 minutes after the stabilization of developed tension at each concentration of Ca2+. The bath solution was then replaced with Tyrode's solution containing 1.0 mM Ca2+ and the muscle was stabilized for 15 minutes. Isoproterenol (10^-8 to 10^-6 M) was added to the bath to obtain the dose-response curves. After a 15-minute washout of the isoproterenol, the forskolin study (10^-4 to 10^-2 M) was performed. The signals were digitized and analyzed with a signal processing computer system (7T-18, NEC San-Ei) to obtain developed tension (DT), peak positive rate of tension development (+dT/dt), peak negative rate of tension decline (-dT/dt), time to the peak tension (TPT), and time to half relaxation (T1/2R). Data were normalized to cross-sectional area (CSA) of the papillary muscle determined at Lmax. The papillary muscle CSA was calculated as CSA = muscle mass/1.05/Lmax.

Statistical analysis: Results are expressed as mean ± SD. Statistical significance was estimated with the analysis of variance, followed by the Bonferroni test to identify differences among groups. A P value <0.05 was considered significant.
Results

Hemodynamics: The baseline characteristics of the sham-operated and MI rats are given in Table I. Infarct size ranged from 30% to 49% of LV and did not differ between the untreated and enalapril-treated groups. LV, RV, and lung weights were greater in the untreated MI rats than those in the sham-operated rats. Treatment with enalapril significantly reduced LV and RV hypertrophy and lowered lung weight.

The hemodynamic data are shown in Table II. Compared with the sham-operated rats, MI rats had increased LV internal dimension, elevated LV end-diastolic pressure, and reduced LV systolic function. Treatment with enalapril

Table I. Body Weight (BW), Ventricular Weight, Lung Weight, and Infarct Size of Sham-Operated Rats, and Untreated (MI) and Enalapril-Treated (MI+Enalapril) Rats with Myocardial Infarction

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=8)</th>
<th>MI (n=8)</th>
<th>MI+Enalapril (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g)</td>
<td>480±21</td>
<td>471±37</td>
<td>444±25</td>
</tr>
<tr>
<td>LV (mg)</td>
<td>862±38</td>
<td>993±83*</td>
<td>882±68†</td>
</tr>
<tr>
<td>LV/BW (mg/g)</td>
<td>1.80±0.05</td>
<td>2.11±0.18*</td>
<td>1.99±0.14</td>
</tr>
<tr>
<td>RV (mg)</td>
<td>176±14</td>
<td>442±67*</td>
<td>294±52*†</td>
</tr>
<tr>
<td>RV/BW (mg/g)</td>
<td>0.37±0.03</td>
<td>0.95±0.17*</td>
<td>0.67±0.12*†</td>
</tr>
<tr>
<td>Lung (mg)</td>
<td>1571±231</td>
<td>2910±506*</td>
<td>2167±627†</td>
</tr>
<tr>
<td>Lung/BW (mg/g)</td>
<td>3.15±0.41</td>
<td>6.00±1.17*</td>
<td>4.91±1.49*</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>39±6</td>
<td>39±5</td>
<td></td>
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</tbody>
</table>

LV=left ventricular weight; RV=right ventricular weight. Values are mean ± SD, *P<0.05 vs Sham-operated rats, †P<0.05 vs MI rats.

Table II. Hemodynamic and Echocardiographic Data of Sham-Operated Rats, and Untreated (MI) and Enalapril-Treated (MI+Enalapril) Rats with Myocardial Infarction

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=8)</th>
<th>MI (n=8)</th>
<th>MI+Enalapril (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>384±39</td>
<td>376±22</td>
<td>348±30</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>101±9</td>
<td>93±12</td>
<td>70±12*†</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>118±6</td>
<td>109±8</td>
<td>94±9*†</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3±1</td>
<td>21±5*</td>
<td>11±5*†</td>
</tr>
<tr>
<td>+dP/dt (×10³ mmHg/s)</td>
<td>11.8±1.9</td>
<td>6.5±0.9*</td>
<td>7.0±1.6*</td>
</tr>
<tr>
<td>-dp/dt (×10³ mmHg/s)</td>
<td>8.8±0.6</td>
<td>4.3±0.7*</td>
<td>4.7±0.9*</td>
</tr>
<tr>
<td>(dP/dt)/P (1/s)</td>
<td>100±14</td>
<td>59±6*</td>
<td>74±12</td>
</tr>
<tr>
<td>LVDd (mm)</td>
<td>6.9±0.3</td>
<td>10.3±0.4*</td>
<td>9.3±0.8*†</td>
</tr>
<tr>
<td>FS (%)</td>
<td>47±5</td>
<td>21±5*</td>
<td>22±4*</td>
</tr>
</tbody>
</table>

HR=heart rate; MAP=mean arterial pressure; LVSP=left ventricular systolic pressure; LVEDP=left ventricular end-diastolic pressure; +dP/dt and -dP/dt=peak positive and negative value of rate of change in LV pressure; (dP/dt)/P=+dP/dt divided by LV systolic pressure; LVDd=left ventricular end-diastolic dimension; FS=fractional shortening. Values are mean ± SD, *P<0.05 vs Sham-operated rats, †P<0.05 vs MI rats.
decreased LV internal dimension, mean arterial pressure, and LV end-diastolic pressure. Although the maximum value of the rate of change in LV pressure (dP/dt_{max}) did not differ between the enalapril-treated and untreated rats, dP/dt_{max} normalized to the LV systolic pressure, (dP/dt)/P, tended to be higher in the treated rats than in the untreated rats.

**Myocardial NE contents:** Compared with the sham-operated rats, plasma NE tended to be higher in the untreated MI rats, although the difference did not reach statistical significance (0.8±0.3, 1.3±0.5, and 0.9±0.2 ng/mL in sham-operated, untreated MI, and enalapril-treated MI rats, respectively). Myocardial NE contents in non-infarcted LV and RV were markedly decreased in the untreated MI rats. These changes persisted with the enalapril treatment (Figure 1).

**Myocardial MIBG and ICYP accumulation:** MIBG accumulation of non-infarcted LV and RV was lower in the untreated MI rats than in the sham-operated rats. Treatment with enalapril did not affect MIBG accumulation in non-infarcted LV and RV (Figure 2).

ICYP accumulation in non-infarcted LV and RV was not significantly different among the sham-operated, treated, and untreated rats (Figure 2). Also, no significant changes in β-receptor density were observed in the membrane preparation binding study. In sham-operated (n=6), untreated MI (n=7), and enalapril-treated (n=7) rats, B_{max} was 69±11, 69±15, and 73±13 fmol/mg, respectively (P=NS), and K_{d} was 0.26±0.06, 0.19±0.02, and 0.19±0.05 nM, respectively (P=NS).

**Isometric papillary muscle function:** Table III shows the baseline data for the papillary muscle study at a Ca^{2+} concentration of 1mM. In the untreated MI rats, both DT and +dT/dt were significantly reduced to 29% of those in the sham-operated rats.
Figure 2. MIBG and ICYP uptake of non-infarcted left ventricle (LV) and right ventricle (RV). Open bars indicate sham-operated rats \((n=6)\), filled bars untreated rats with myocardial infarction (MI, \(n=7)\), and hatched bars enalapril-treated rats with MI (MI+Enalapril, \(n=7)\). LV infarct size in the untreated and enalapril-treated rats was 36±8% and 39±7%. Results are mean ± SD. * \(P<0.05\) vs sham-operated rats.

Table III. Isometric Function of Papillary Muscles from Sham-Operated Rats, and Untreated (MI) and Enalapril-Treated (MI+Enalapril) Rats with Myocardial Infarction

<table>
<thead>
<tr>
<th></th>
<th>Sham ((n=6))</th>
<th>MI ((n=5))</th>
<th>MI+Enalapril ((n=6))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(DT) (g/mm²)</td>
<td>1.7±0.5</td>
<td>0.5±0.2*</td>
<td>1.5±0.7†</td>
</tr>
<tr>
<td>(+dT/dt) (g/mm²/sec)</td>
<td>15.6±4.4</td>
<td>4.6±1.6*</td>
<td>11.2±4.1†</td>
</tr>
<tr>
<td>(-dT/dt) (g/mm²/sec)</td>
<td>8.5±2.9</td>
<td>3.5±1.5</td>
<td>6.9±2.3</td>
</tr>
<tr>
<td>(TPT) (msec)</td>
<td>205±20</td>
<td>222±31</td>
<td>235±44</td>
</tr>
<tr>
<td>(T_{1/2}R) (msec)</td>
<td>167±18</td>
<td>166±26</td>
<td>194±20</td>
</tr>
<tr>
<td>(L_{max}) (mm)</td>
<td>5.4±1.0</td>
<td>3.5±0.3*</td>
<td>4.3±0.9</td>
</tr>
<tr>
<td>(CSA) (mm²)</td>
<td>1.4±0.3</td>
<td>2.5±0.6*</td>
<td>2.2±0.3*</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>32±2</td>
<td>36±4</td>
<td></td>
</tr>
</tbody>
</table>

\(DT\)=developed tension; \(+dT/dt\) and \(-dT/dt\)=peak positive and negative rates of tension development; \(TPT\)=time to peak tension; \(T_{1/2}R\)=time to half relaxation; \(L_{max}\)=length of papillary muscle at maximum developed tension; \(CSA\)=cross-sectional area of papillary muscle. Values are mean ± SD, * \(P<0.05\) vs Sham-operated rats, † \(P<0.05\) vs MI rats.
ated rats. Enalapril restored the MI-induced deterioration of papillary muscle function.

Figure 3 shows the dose-response curves for isometric parameters to cumulative doses of calcium, isoproterenol, and forskolin. Dose-related increases in DT, +dT/dt, and -dT/dt in response to calcium, isoproterenol, and forskolin were

**Figure 3.** Dose-response curves for isometric papillary muscle function to calcium, isoproterenol, and forskolin 12 weeks after the operation. Data are shown as changes from the value at 0.5 mM Ca\(^{2+}\) in the calcium dose-response curve and as changes from the baseline values before isoproterenol or forskolin at a Ca\(^{2+}\) concentration of 1.0 mM. DT indicates developed tension; +dT/dt, peak positive rate of tension development; -dT/dt, peak negative rate of tension decline. Open circles indicate sham-operated rats (n=6), closed circles untreated rats with myocardial infarction (MI, n=5), open squares enalapril-treated rats with MI (n=6). LV infarct size in the untreated and enalapril-treated rats was 32±2% and 36±4%. Results are mean ± SD. *

\(p<0.05\) vs sham-operated rats.
observed in the sham-operated rats. In the untreated MI rats, however, calcium, isoproterenol, and forskolin failed to produce corresponding increases in DT, +dT/dt, and -dT/dt although there was a slight increase in DT in response to calcium. Treatment with enalapril did not improve the responsiveness of muscle to calcium, isoproterenol, or forskolin.

**DISCUSSION**

Using a rat coronary artery ligation model of heart failure, we have assessed the effects of long-term treatment with enalapril on LV function, sympathetic neuronal function, β-receptor density, and inotropic responsiveness to β-agonists in non-infarcted papillary muscle. We found that enalapril attenuated ventricular dilatation and hypertrophy after MI and improved the baseline isometric muscle contractility of the residual non-infarcted myocardium. These effects of enalapril were associated with a reduction of LV loading conditions without changes in myocardial NE content, cardiac sympathetic neuronal function, or β-receptor density. Moreover, with enalapril treatment the MI-induced blunted isometric muscle responsiveness to isoproterenol, forskolin, or calcium persisted. These data suggest that the beneficial effects of enalapril in the treatment of heart failure after MI are not primarily due to sympathoinhibition and resensitization of β-adrenergic signaling.

Previous studies4,10,22) have reported that ACE inhibitor treatment minimizes ventricular remodeling and improves cardiac hemodynamics in rat models of heart failure after MI. The architectural changes in the ventricle following MI primarily result from sustained elevation in wall stress. ACE inhibitors might reduce both volume and pressure overload, thereby leading to decreased diastolic and systolic wall stress. The combined reduction of preload and afterload by ACE inhibitors might favorably alter the loading conditions of the LV and reduce progressive ventricular enlargement after MI.

Vasodilator therapy may improve hemodynamic function in heart failure. However, administration of the direct-acting vasodilator hydralazine or a calcium antagonist augments cardiac adrenergic drive, and may blunt the potential beneficial effect via pressure reduction.7,23) In contrast, in the present study enalapril did not alter plasma or myocardial catecholamines despite the significant reduction in mean arterial pressure. Thus, vasodilator therapy by ACE inhibitors might not increase adrenergic activity.

MIBG is an analogue of NE and shares neuronal transport and storage mechanisms with NE.24,25) A decrease in myocardial MIBG accumulation and NE has been reported in heart failure21,26-29) and also in experimental models of MI.19,30) In the present study, it is unlikely that reduced MIBG accumulation and
NE content would be due to sympathetic denervation by the surgical procedure because MIBG accumulation and NE content did not decrease in the sham-operated rats. Reduced MIBG accumulation in non-infarcted myocardium might result from increased cardiac sympathetic activity and impaired cardiac neuronal uptake function after MI.

The dual-tracer method used in the present study provides information on coupling between sympathetic neuronal function and β-receptor density in a living heart.19,21) In the present study, no significant changes in β-receptor density among the sham-operated, untreated MI, and enalapril-treated MI rats were observed using myocardial ICYP accumulation or the membrane preparation. One of the beneficial effects of ACE inhibitors in the treatment of heart failure is considered to be their sympatholytic effect. Böhm, et al31) reported that both ACE inhibitors and angiotensin II antagonists reversed sympathetic neuroeffector defects in renin-induced hypertension. Kawai, et al32) reported that ACE inhibitors improved cardiac sympathetic nerve terminal function and prevented myocardial β-receptor downregulation and desensitization in chronic heart failure dog. In rats with MI, a significant reduction of β-receptor density was reported33,34) and ACE inhibitor treatment restored the reduction.33) However, others35,36) have reported no significant changes in β-receptor density in heart failure after MI, as seen in the present study. These differences may not be due to the period of examination because no β-receptor downregulation was observed in 24 weeks after MI in one of our previous studies.19) Gu, et al37) reported that the decrease in β-receptor density occurred only in border and infarcted regions, not in the non-infarcted region in rats with MI. Inclusion of the border region in the non-infarcted region might affect the results of β-receptor density in this model of heart failure. Another possibility for the lack of β-receptor downregulation or sympathoinhibitory effect of the ACE inhibitor in the present study may be due to the severity of the heart failure or the degree of sympathetic activation. Gilbert, et al38) reported that an ACE inhibitor lowered cardiac adrenergic drive and increased β-receptor density in subjects with increased cardiac adrenergic drive, but had no effects on these parameters in subjects with normal cardiac adrenergic drive. In our previous study in rats with MI,19) the degree of cardiac sympathetic alteration was most potent in the acute stage after MI and decreased in the chronic stage in accordance with hemodynamic stabilization. The lack of potent sympathetic activation in this model of heart failure might cause the absence of the sympatholytic effects of the ACE inhibitor as well as the β-receptor downregulation.

In the present study, the baseline isometric contractility of non-infarcted papillary muscle was depressed, a finding consistent with previous reports.34,35,38,39) The inotropic responsiveness of papillary muscle to isoproterenol, forskolin, or calcium stimulation was severely blunted in MI rats without
alteration in β-receptor densities. This suggests that the depressed contractility in non-infarcted myocardium is due to post-receptor defects. Several mechanisms for the decreased contractility in non-infarcted myocardium have been proposed, including decreased calcium availability or responsiveness, altered excitation-contraction coupling, increased expression of fetal myosin isoforms, and alterations in the extracellular matrix.

Previous studies have demonstrated that chronic captopril treatment in MI rats improves the contractility of non-infarcted myocardium, as was observed in the present results. Enalapril, however, did not improve the muscle responsiveness to either isoproterenol, forskolin, or calcium compared with the untreated MI rats. These results suggest that alterations in the adrenergic signal transduction, calcium handling, or Ca²⁺ responsiveness of myofilaments in MI rats were not restored by long-term treatment with enalapril. The improvement of muscle function with an ACE inhibitor in the present study is related, at least in part, to hemodynamic unloading and the suppression of LV remodeling after MI. The reduction of LV wall stress by the ACE inhibitor might minimize ventricular remodeling and consequently prevent a deterioration of baseline muscle contractility after MI. This was supported by the recent study that hemodynamic unloading with an LV assist device improved myocyte contractile properties and increases β-adrenergic responsiveness in human heart failure. On the other hand, ACE inhibitors might have a direct effect on myocardial tissue, such as the suppression of cardiac ACE activity and angiotensin II, preventing inappropriate growth and hypertrophy. Both the favorable alteration of the LV loading condition and suppression of the renin-angiotensin system by ACE inhibitors might attenuate LV remodeling and play an important role in the treatment of heart failure after MI. In addition, changes in cardiac gene expression, and a reduction of myocardial oxidative stress by ACE inhibitors have been reported.

Some methodological limitations deserve comment when interpreting the present results. First, our data did not determine the precise location of the defects in the β-adrenergic pathway in the non-infarcted myocardium. Various changes in the β-adrenergic signal transduction, including the β-receptor, G proteins, adenylate cyclase complex, and intracellular calcium handling, have been described in heart failure. Our results suggest that an alteration in the β-adrenergic signal pathway distal to cAMP-dependent enzymes may be involved. Further studies will be required to clarify the precise mechanisms. Second, blood sampling for NE measurement was performed under anesthesia. Plasma NE levels in conscious unstrained rats without MI were lower than those in the sham-operated rats in the present study. The influence of anesthesia may account for the lack of significant differences in plasma NE between the sham-operated and MI rats in the present study.
Conclusion: Long-term treatment with the ACE inhibitor enalapril attenuated ventricular remodeling and improved myocardial contractility after MI. However, enalapril did not restore alterations in the cardiac sympathetic neuronal function, β-adrenergic signal transduction, or calcium responsiveness of the muscle after MI. The present results suggest that the major effect of ACE inhibitors in the treatment of heart failure is not due to either sympathoinhibition or the restoration of β-adrenergic signaling in this model of heart failure.

ACKNOWLEDGMENTS

This study was supported by a grant-in-aid for Scientific Research from the Japanese Ministry of Education, Science, and Culture (10670637).

The authors gratefully acknowledge Dr. Che-Ping Cheng and Dr. William C Little of Wake Forest University for their critical review of the manuscript.

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