Long-Term Treatment With Low-Dose, But Not High-Dose, Guanethidine Improves Ventricular Function and Survival of Rats With Heart Failure After Myocardial Infarction

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Toyama, Japan

OBJECTIVES
We sought to evaluate the effects of various doses of guanethidine, a sympathoinhibitory drug, on ventricular function and survival in chronic heart failure (CHF) after myocardial infarction (MI) in rats.

BACKGROUND
Direct inhibition of sympathetic outflow by a sympathoinhibitory drug might be an effective approach to therapy of CHF. However, recent clinical trials suggest that excessive suppression of sympathetic activity has an adverse effect on outcome. It remains unclear whether the beneficial effects of the sympathoinhibitory drug would be modified by its dosage.

METHODS
Three doses of guanethidine (low-dose [LG], 1 mg/kg/day; medium-dose, 3 mg/kg/day; high-dose, 10 mg/kg/day) were administered via an osmotic mini-pump for 4 weeks. Hemodynamics, left ventricular (LV) diameters, plasma and myocardial norepinephrine (NE) levels, and survival were determined for four weeks after MI.

RESULTS
As compared with MI rats receiving vehicle, LG suppressed LV dilation (9.2 ± 0.9 mm vs. 11.0 ± 0.8 mm, p < 0.05) and improved LV fractional shortening (25.0 ± 4.5% vs. 16.4 ± 4.7%, p < 0.05) in association with a reduction of plasma NE levels (520 ± 250 pg/ml vs. 1,000 ± 570 pg/ml, p < 0.05), but not with a significant reduction of noninfarcted myocardial NE levels (154 ± 71 ng/g vs. 207 ± 71 ng/g). Low-dose guanethidine reduced 24-h (6%) and 28-day mortality (6%), as compared with untreated MI rats (36% and 52%, respectively). High-dose guanethidine also reduced 24-h mortality (12%) but increased 28-day mortality (91%), in association with a depletion of myocardial NE. Medium-dose guanethidine had no beneficial effects on LV hemodynamics or long-term survival.

CONCLUSIONS
These results indicate that the dosage of the sympathoinhibitory drug might be quite important for the treatment of CHF. (J Am Coll Cardiol 2003;42:541–8) © 2003 by the American College of Cardiology Foundation

Activation of the sympathetic nerve system is one of the major pathophysiologic abnormalities leading to the progression of chronic heart failure (CHF). Activation of sympathetic outflow is initially a hemodynamic adaptation to compensate for decreased cardiac function. However, it leads to an increase in sodium and fluid retention and also in peripheral vascular resistance through the renin-angiotensin-aldosterone system or direct renal and vascular effects (1). Other adverse effects of increased sympathetic activity might be directed toward the heart, resulting in the desensitization or downregulation of beta-adrenergic receptors (2,3), myocyte injury (4,5), and a predisposition to ventricular arrhythmias (6). The activation of sympathetic outflow relates to a poor prognosis in patients with CHF (7). Therefore, direct inhibition of sympathetic activity might be an effective therapy for CHF. Actually, the beneficial effects of sympathoinhibitory drugs in the treatment of CHF have been reported (8,9). Moxonidine is a novel agonist of the central imidazoline receptor and reduces sympathetic outflow and circulating levels of norepinephrine (NE) (10). Based on the beneficial effects of moxonidine on hemodynamics in patients with CHF (11,12), clinical trials were designed to evaluate the effects of moxonidine on plasma NE concentration (Moxonidine Safety and Efficacy [MOXSE] study) (13) and on mortality and morbidity (Moxonidine Congestive Heart Failure [MOXCON] trial) (14) in patients with CHF. Although plasma NE concentrations were significantly reduced, there was a significant increase in adverse effects in the MOXSE study, and the MOXCON study was stopped early because of increasing mortality in the moxonidine group. These findings suggest that sympathoinhibition worsens clinical outcomes in CHF. However, pharmacologic therapy with angiotensin-convertase enzyme inhibitors (15) or beta-adrenergic blockers (16) provided evidence that the inhibi-
tions of sympathetic activation improves morbidity and mortality in patients with CHF. It is not fully elucidated whether the sympathoinhibitory drugs are useful in the treatment of CHF.

One possibility for the negative results of the MOXCON study could be the higher dosage of moxonidine, leading to an excessive reduction in plasma NE, because a dose-related increase in serious adverse events was observed in the MOXSE study. This suggests that excessive suppression of sympathetic activity could be harmful, as increased sympathetic activity would compensate for decreased cardiac function. The MOXCON study raises the question of whether there is an optimal dosage of sympathoinhibitory drug for the treatment of CHF. We hypothesized that the beneficial effects of the sympathoinhibitory drug might be modified by its dosage. The present study was therefore designed to elucidate the effects of various doses of guanethidine on ventricular function and survival in heart failure after myocardial infarction (MI) in rats.

### METHODS

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

**Experimental animals.** Male Wistar rats weighing 300 to 350 g were used for induction of MI. Myocardial infarction was produced by ligation of the left coronary artery under ether anesthesia, as reported previously (17). Briefly, the chest was opened by a left lateral thoracotomy, and the left coronary artery was ligated approximately 2 to 3 mm from its origin with a suture of 6-0 silk. Control rats received a sham operation by using a similar procedure without coronary ligation.

Two days before the surgical procedure, the rats were randomized to the guanethidine group, which received subcutaneous implantation of osmotic mini-pumps (Alzet, ALZA Pharmaceuticals, Palo Alto, California) filled with guanethidine, or the control group, which received vehicle. In our preliminary study, four different doses of guanethidine (0.3, 1.0, 3.0, or 10.0 mg/kg/day) were given for two weeks, using the mini-pumps in normal rats to determine the effects on plasma NE concentrations and left ventricular (LV) NE contents. As shown in Table 1, guanethidine induced a dose-dependent decrease in plasma and myocardial NE levels. Accordingly, a low dose of guanethidine (LG) (1 mg/kg/day) was chosen as the dose not to reduce myocardial NE; a medium dose (MG) (3 mg/kg/day) to deplete myocardial NE moderately; and a high dose (HG) (10 mg/kg/day) to deplete myocardial NE severely. Guanethidine was dissolved in saline and adjusted to provide a daily dose of 1 mg/kg (LG), 3 mg/kg (MG), or 10 mg/kg (HG). Before the induction of MI, rats were classified into four groups: LG (n = 17), MG (n = 20), HG (n = 34), and saline vehicle (n = 25). The sham-operated rats were also classified into two groups: HG (n = 7) and vehicle (n = 6). The mini-pump was replaced every two weeks under ether anesthesia. The administration of guanethidine was continued throughout the study period (i.e., 4 weeks).

**Infarct size** was determined using a technique described by Chien et al. (18). Briefly, after sacrifice of the rats with an overdose of pentobarbital (70 mg/kg intraperitoneally), 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 region of infarction were outlined on a clear plastic sheet for both the endocardial and epicardial surfaces. The difference in weight between the two marked areas on the sheet was used to determine the relative size of MI, which was expressed as a percentage of LV surface area.

**Hemodynamic study and measurement of catecholamines.** Four weeks after MI induction or sham operation, transthoracic echocardiography was performed under ether anesthesia with a 7.5-MHz transducer (SSH140A, Toshiba, Japan), as described previously (17). Left ventricular end-diastolic and end-systolic internal dimensions (LVEDD and LVESD, respectively) 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 signals of LV pressure were digitized on-line at a sampling interval of 2 ms and

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**Table 1. Effects of Guanethidine on Plasma NE Concentration and LV NE Contents in Normal Rats**

<table>
<thead>
<tr>
<th>Guanethidine Dose, mg/kg/day</th>
<th>Plasma NE (pg/ml)</th>
<th>LV NE (ng/g)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(n = 4)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>0.3</td>
<td>600 ± 100</td>
<td>540 ± 160</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td>320 ± 70</td>
</tr>
<tr>
<td>3.0</td>
<td></td>
<td>230 ± 50†</td>
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<tr>
<td>10.0</td>
<td></td>
<td>160 ± 320</td>
</tr>
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</table>

*p < 0.05 vs. 0.3 mg/kg/day. †p < 0.05 vs. 1.0 mg/kg/day. Data are presented as the mean value ± SD.

LV = left ventricular; NE = norepinephrine.

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### Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BW</td>
<td>body weight</td>
</tr>
<tr>
<td>CHF</td>
<td>chronic heart failure</td>
</tr>
<tr>
<td>HG</td>
<td>high-dose guanethidine</td>
</tr>
<tr>
<td>LG</td>
<td>low-dose guanethidine</td>
</tr>
<tr>
<td>LV</td>
<td>left ventricle/ventricular</td>
</tr>
<tr>
<td>LVEDD</td>
<td>left ventricular end-diastolic dimension</td>
</tr>
<tr>
<td>LVESD</td>
<td>left ventricular end-systolic dimension</td>
</tr>
<tr>
<td>MG</td>
<td>medium-dose guanethidine</td>
</tr>
<tr>
<td>MI</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>MOXCON</td>
<td>Moxonidine Congestive Heart Failure trial</td>
</tr>
<tr>
<td>MOXSE</td>
<td>Moxonidine Safety and Efficacy study</td>
</tr>
<tr>
<td>NE</td>
<td>norepinephrine</td>
</tr>
<tr>
<td>RV</td>
<td>right ventricle/ventricular</td>
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</table>

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analyzed with a signal processing computer system (7T-18, NEC, San-Ei, Japan).

After blood sampling for analysis of plasma catecholamines, the chest was opened to remove the heart quickly. The RV and LV were dissected, rinsed in ice-cold saline, and weighed, and the infarct size was then determined as mentioned earlier. Plasma and noninfarcted LV 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 (19). Statistical analysis. Results are expressed as the mean value ± SD. The group differences in body weight, ventricular weight, infarct size, hemodynamic and echocardiographic data, and plasma and myocardial NE levels were tested with one-way analysis of variance without repetition, followed by the Bonferroni test for multiple comparisons. Survival was analyzed by using standard Kaplan-Meier analysis with the log-rank test. The mortality at 24 h and 28 days after MI, with or without guanethidine treatment, was tested by the chi-square test. All analytical results were obtained using StatView for Windows, version 4.5 (Abacus Concepts Inc., Berkeley, California). A p value <0.05 was considered statistically significant.

RESULTS

Hemodynamics and LV geometry. In sham-operated rats, HG reduced body weight (BW) but did not affect LV mass and RV mass indexed for BW (LV/BW and RV/BW, respectively), as shown in Table 2. In MI rats, the infarct size did not differ between untreated and guanethidine-treated rats. The MI rats had a greater RV/BW ratio than sham-operated rats. Low-dose guanethidine did not change the LV/BW or RV/BW ratio. Rats treated with MG had a greater LV/BW ratio. As compared with sham-operated rats, MI rats had elevated LV end-diastolic pressure and decreased maximum and minimum values of the rate of change in LV pressure (dP/dt) (Table 2). Low-dose guanethidine tended to lower LV end-diastolic pressure. However, the hemodynamic indexes of heart rate, mean arterial pressure, or dP/dt were not different among MI rats with or without guanethidine treatment (LG and MG). Hemodynamic and echocardiographic data in MI rats treated with HG were obtained from only three animals, because other rats died during the study period. As shown in Table 2, the surviving rats had a similar infarct size and increased ratio of ventricular weight to body weight, as compared with untreated MI rats. The heart rate or arterial pressure was not decreased with HG. The LV hemodynamics could be obtained from only one animal whose end-diastolic pressure was markedly elevated. An autopsy was performed in nine MI rats with HG that died during the study period. The average infarct size of these rats was 34.7 ± 3.4%, which was not different from that of untreated or LG- or MG-treated MI rats. Most of these rats had pleural effusion and increased lung weight, suggesting pulmonary congestion. In sham-operated rats, HG did not affect the aforementioned hemodynamic indexes.

Figure 1 shows echocardiographic data. In sham-operated rats, HG did not affect LVEDD (7.1 ± 0.5 mm vs. 6.7 ± 0.2 mm), LVEDD (3.5 ± 0.3 mm vs. 3.3 ± 0.5 mm), or fractional shortening (50.2 ± 3.0% vs. 50.5 ± 6.5%). In untreated MI rats, LVEDD and LVEDV were increased and fractional shortening was decreased, as compared with sham-operated rats. In contrast, LG significantly reduced LVEDD (9.2 ± 0.9 mm vs. 11.0 ± 0.8 mm, p < 0.05) and LVEDV (6.9 ± 0.9 mm vs. 9.2 ± 0.8 mm, p < 0.05) and improved fractional shortening (25.0 ± 4.5% vs. 16.4 ± 4.7%, p < 0.05). Medium-dose guanethidine reduced LVEDD (9.8 ± 0.6 mm, p < 0.05 vs. untreated MI rats) but did not improve fractional shortening (14.6 ± 3.8%). The echocardiographic indexes were not improved with HG (LVEDD = 11.0 ± 0.6 mm, LVEDV = 9.5 ± 0.7 mm, fractional shortening = 13.4 ± 5.0%). Plasma and myocardial NE levels are shown in Figure 2. In sham-operated rats, HG markedly decreased myocardial NE (21 ± 7 ng/g vs. 494 ±

Table 2. Body Weight, Ventricular Weight, and Hemodynamic Data 28 Days After Myocardial Infarction

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 6)</th>
<th>Sham+HG (n = 7)</th>
<th>MI (n = 12)</th>
<th>MI+LG (n = 13)</th>
<th>MI+MG (n = 8)</th>
<th>MI+HG (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>410 ± 32</td>
<td>339 ± 7*</td>
<td>366 ± 32</td>
<td>371 ± 23</td>
<td>341 ± 19*</td>
<td>297 ± 45</td>
</tr>
<tr>
<td>LV/BW (mg/g)</td>
<td>1.83 ± 0.13</td>
<td>2.06 ± 0.12</td>
<td>1.96 ± 0.20</td>
<td>2.10 ± 0.16</td>
<td>2.16 ± 0.27*</td>
<td>2.59 ± 0.17</td>
</tr>
<tr>
<td>RV/BW (mg/g)</td>
<td>0.43 ± 0.05</td>
<td>0.50 ± 0.08</td>
<td>0.88 ± 0.24*</td>
<td>0.78 ± 0.23*</td>
<td>0.93 ± 0.23*</td>
<td>1.15 ± 0.15</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>—</td>
<td>—</td>
<td>33.9 ± 6.7</td>
<td>32.4 ± 8.6</td>
<td>31.9 ± 4.0</td>
<td>32.6 ± 0.9</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>406 ± 22</td>
<td>412 ± 20</td>
<td>395 ± 21</td>
<td>373 ± 31</td>
<td>384 ± 14</td>
<td>389 ± 10</td>
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<td>Mean arterial pressure (mm Hg)</td>
<td>90 ± 11</td>
<td>90 ± 8</td>
<td>83 ± 13</td>
<td>76 ± 9</td>
<td>87 ± 11</td>
<td>92 ± 3</td>
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<tr>
<td>LVSP (mm Hg)</td>
<td>108 ± 6</td>
<td>113 ± 8</td>
<td>104 ± 12</td>
<td>101 ± 8</td>
<td>112 ± 10</td>
<td>117</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>2 ± 2</td>
<td>3 ± 2</td>
<td>18 ± 7*</td>
<td>14 ± 7*</td>
<td>18 ± 12*</td>
<td>24</td>
</tr>
<tr>
<td>LV end-diastolic pressure (dP/dt) (×1000 mm Hg/s)</td>
<td>0.14 ± 1.6</td>
<td>0.19 ± 1.0</td>
<td>0.73 ± 1.6*</td>
<td>0.71 ± 1.4*</td>
<td>0.75 ± 1.8*</td>
<td>7.2</td>
</tr>
<tr>
<td>LV end-diastolic pressure (dP/dt) (×1000 mm Hg/s)</td>
<td>−6.9 ± 1.7</td>
<td>−7.1 ± 0.8</td>
<td>−4.5 ± 1.1*</td>
<td>−4.3 ± 0.1*</td>
<td>−4.4 ± 0.7*</td>
<td>−4.3</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. sham-operated rats. Left ventricular hemodynamic data in MI+HG were obtained from one rat. The difference between MI+HG rats and the other groups was not considered because of the small number. Data are expressed as the mean value ± SD.

BW = body weight; HG = high-dose guanethidine; HR = heart rate; LG = low-dose guanethidine; LV = left ventricle; LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure; MG = medium-dose guanethidine; MI = rats with myocardial infarction; Peak +dP/dt and −dP/dt = peak positive and negative value of rate of change in left ventricular pressure; RV = right ventricle; Sham = sham-operated rats.
100 ng/g, p < 0.05). In untreated MI rats, plasma NE was elevated and the NE content of noninfarcted LV was decreased, as compared with sham-operated rats. Low-dose guanethidine significantly reduced plasma NE (520 ± 250 pg/ml vs. 1,000 ± 570 pg/ml, p < 0.05) but did not significantly affect myocardial NE (154 ± 71 ng/g vs. 207 ± 71 ng/g, p = NS) in MI rats. Medium-dose guanethidine reduced plasma (570 ± 230 pg/ml, p < 0.05 vs. untreated MI rats) and myocardial NE (47 ± 18 ng/g, p < 0.05 vs. untreated MI rats). The myocardial NE of surviving MI rats treated with HG was markedly reduced (9 ± 6 ng/g, n = 3).

Mortality. The Kaplan-Meier survival curves of sham-operated rats and MI rats are shown in Figure 3. In sham-operated rats, HG did not affect survival. The survival of MI rats treated with LG improved markedly, as compared with untreated MI rats. The survival of MI rats treated with MG was not different from that of untreated MI rats. In contrast, MI rats treated with HG showed the worst survival, and most of the rats died during the study period. Figure 4 shows 24-h and 28-day mortality after MI. The 24-h mortality rate was 36% in untreated MI rats. Low-, medium-, and high-dose guanethidine reduced 24-h mortality to 6%, 15%, and 12%, respectively. The 28-day mortality rate of untreated MI rats was 52%. Low-dose guanethidine reduced 28-day mortality to 6% (p < 0.05 vs. untreated MI rats), but HG increased mortality to 91% (p < 0.05 vs. untreated MI rats).

DISCUSSION

The major findings of the present study are as follows. First, LG attenuated LV dilation and improved ventricular function after MI, in association with a reduction of plasma NE concentration, but with no significant influence on myocardial NE contents. Secondly, sympathoinhibition by LG, MG, and HG reduced mortality in the acute stage after MI, as compared with untreated MI. As for the long-term effect, however, LG reduced mortality, but HG markedly increased it. These results indicate that the beneficial effects of the sympathoinhibitory drug might be affected by its dosage in the treatment of CHF.

Guanethidine acts on the adrenergic nerve terminal and inhibits release and storage of NE, resulting in a decrease in peripheral vascular resistance and systemic blood pressure (20). As shown in our preliminary study, guanethidine suppressed sympathetic activity in a dose-dependent manner. In sham-operated rats, HG markedly decreased plasma and myocardial NE levels but did not significantly affect the
heart rate, mean arterial pressure, or LV function. This suggests that a level of sympathetic activity in normal rats at rest may be too low for guanethidine to change LV performance.

In the present study, LG attenuated LV dilation and improved ventricular function after MI. There may be several possible explanations for the improvement in ventricular function by guanethidine. First, LG tended to lower plasma norepinephrine (NE) concentrations and NE contents of noninfarcted left ventricle. Figure 2 shows the plasma NE concentration of MI+HG rats was not measured. *p < 0.05 vs. sham-operated rats. †p < 0.05 vs. MI rats. ‡p < 0.05 vs. MI+LG rats. The difference between MI+HG rats and the other groups was not compared because of the small number. Data are presented as the mean value ± SD.

In the present study, LG attenuated LV dilation and improved ventricular function after MI. There may be several possible explanations for the improvement in ventricular function by guanethidine. First, LG tended to lower plasma norepinephrine (NE) concentrations and NE contents of noninfarcted left ventricle. Figure 2 shows the plasma NE concentration of MI+HG rats was not measured. *p < 0.05 vs. sham-operated rats. †p < 0.05 vs. MI rats. ‡p < 0.05 vs. MI+LG rats. The difference between MI+HG rats and the other groups was not compared because of the small number. Data are presented as the mean value ± SD.

Figure 3. Kaplan-Meier survival curves of sham-operated rats treated with high-dose guanethidine (SH+HG) (n = 7), rats with myocardial infarction receiving vehicle (MI) (n = 25), and MI rats treated with low-dose guanethidine (MI+LG) (n = 17), medium-dose guanethidine (MI+MG) (n = 20), and high-dose guanethidine (MI+HG) (n = 34). Survival of sham-operated rats receiving vehicle (100%) is not shown. *p < 0.05 vs. MI rats. †p < 0.05 vs. MI+MG rats.
mean arterial pressure and LV end-diastolic pressure, suggesting a trend toward a reduction of LV preload and afterload. The changes in the ventricular loading condition by guanethidine might improve LV function. Moreover, decreases in heart rate and LV load condition might have a favorable effect on cardiac energy metabolism. Secondly, inhibition of NE release by LG at the cardiac nerve terminal might decrease the local NE concentration to protect the LV from the adverse effects of NE in MI rats. In heart failure, increased NE release and an impaired re-uptake mechanism at the cardiac nerve terminal result in an increase in the synaptic NE concentration (21). The increased local NE may lead to alteration of beta-adrenergic signaling (2,3,22), myocyte injury (4,5), and cellular hypertrophy (23). Administration of guanethidine early in the development of heart failure partially prevented depression of responsiveness to beta-adrenergic stimulation (24). The suppression of myocyte injury and hypertrophy by the sympathoinhibitory drug moxonidine might reduce interstitial fibrosis (25). These alterations were beneficial in the treatment of CHF. Thirdly, we have previously reported that renal sympathetic denervation suppressed renal sodium reabsorption and thereby improved ventricular function in rats with MI (26). Suppression of renal sympathetic activity by LG may decrease sodium and water retention, leading to attenuation of LV dysfunction after MI.

In contrast to LG, HG unexpectedly worsened the survival of rats with MI. The present data indicate that HG did not attenuate LV dilation and LV dysfunction, despite a marked reduction of myocardial NE. The Valsartan Heart Failure Trial (Val-HeFT) raises a hypothesis that extensive blockade of multiple neurohormonal systems could be deleterious in the treatment of heart failure, because valsartan had an adverse effect on mortality and morbidity in patients receiving both angiotensin-converting enzyme inhibitors and beta-blockers (27). The recent study of nepicastat, a dopamine beta-hydroxylase inhibitor that inhibits synthesis and release of NE, clearly showed the effect was dependent on the dosage (28). That is, low-dose nepicastat prevented progressive LV dysfunction and remodeling, but high-dose nepicastat worsened them, despite greater adrenergic inhibition, as compared with low-dose treatment. Low-dose nepicastat normalized transmyocardial plasma NE, but high-dose nepicastat depleted it below the normal level. These results are consistent with the present study. Low-dose guanethidine that normalized plasma NE but did not deplete myocardial NE was lost when MG or HG that depleted myocardial NE was used. Taken together, these results suggest that excessive inhibition of adrenergic drive in CHF could have an adverse effect on cardiac function and remodeling, and some levels of sympathetic activity might be preserved for the treatment of CHF.

Increased sympathetic activity could relate to induction of ventricular arrhythmias, especially in cases of acute myocardial ischemia (29). Increased alpha1- and beta-adrenergic receptor activation by NE and heterogeneous sympathetic innervation in the ischemic heart play a role in a predisposition to ventricular arrhythmias (30). Indeed, ventricular arrhythmias such as ventricular tachycardia were frequently observed after coronary artery ligation in the present study. Although we did not determine the frequency of ventricular arrhythmias in detail, rats treated with guanethidine had fewer episodes of ventricular tachycardia for approximately 20 min after coronary ligation. Suppression of NE release by
guanethidine may prevent ventricular arrhythmias and thereby improve mortality in the acute stage after MI.

Guanethidine has several pharmacologic effects unrelated to direct cardiac sympathetic inhibition. First, guanethidine is concentrated in adrenergic nerve terminals and exerts a selective local anesthetic effect at this site (31). This anesthetic effect of guanethidine inhibits NE release at the nerve terminal (31) and might reduce arrhythmic deaths during the first 24 h after MI in guanethidine groups. Secondly, guanethidine initially releases NE and might have a sympathomimetic effect on the cardiovascular system when given intravenously (32). However, this does not occur with oral administration (33). In the present study, we measured blood pressure and heart rate in five rats before and two days after HG administration (before MI induction). After HG administration, there was no increase in systolic blood pressure or heart rate (119 ± 11 to 121 ± 7 mm Hg and 376 ± 21 to 328 ± 13 beats/min, respectively). In the chronic stage, subcutaneous administration of guanethidine did not increase the heart rate in sham-operated rats or MI rats. Therefore, we think the dosage of guanethidine we used in the present study might not have significant sympathomimetic effects. Thirdly, inhibition of neuronal uptake of NE induced by guanethidine might increase the interstitial NE level in the heart. However, in the chronic stage after administration, guanethidine depletes NE storage and reduces NE release in the nerve terminal (34). In the present study, the level of plasma NE was decreased by LG and MG in MI rats. So, we think that a significant increase in myocardial interstitial NE induced by guanethidine did not occur in MI rats, at least in long-term treatment with guanethidine. This might also be supported by the report that long-term guanethidine treatment increases cardiac beta-adrenergic receptors (35).

Some methodologic limitations deserve comment to interpret the present results. First, we could not fully evaluate the effects of HG on LV function and ventricular remodeling in MI rats because of the higher mortality. The mechanism of the adverse effects of HG remains to be elucidated. However, the autopsy of MI rats treated with HG suggests that these rats died of heart failure. Secondly, the mode of action of guanethidine is different from that of other sympathoinhibitory drugs such as moxonidine. Moreover, there was no direct evidence that the effects of guanethidine contribute to sympathoinhibition. A further study will be required to clarify the importance of the degree of sympathoinhibition using a central sympathoinhibitory drug. Thirdly, we did not measure the infarct sizes of some rats that died during the study period. However, almost the same site of the coronary artery was ligated for induction of MI. In our previous study (17), the infarct size was about 36%, using the same methods for induction of MI as in the present study, and did not vary from the experimental groups. This value is similar to that in the present study. Actually, the infarct size of some rats treated with HG that died during the study period was not different from that of the other MI rats. Small differences in infarct size among the four groups of MI might not significantly influence the present results. Fourthly, there was no direct measurement of changes in LV contractility with guanethidine treatment, as the echocardiographic parameters were influenced by the LV loading condition. Finally, in the present study, we started guanethidine treatment two days before the coronary ligation. The sympathetic activation and increase in plasma catecholamines occurred immediately after the coronary occlusion (36). The degree of sympathetic activation might be most potent at the onset of MI because it compensates for acute hemodynamic deterioration. To suppress the sympathetic activation occurring immediately after MI, guanethidine was started before the coronary ligation in this study.

Although limited for these reasons, the present study suggests that the dosage of the sympathoinhibitory drug is quite important in the treatment of CHF.

Acknowledgments
We thank Dr. Makoto Nonomura, Dr. Akira Matsuki, and Dr. Teruo Nakadate for their technical assistance and data acquisition.

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