Role of Endothelin in the Maintenance of Blood Pressure in Conscious Rats With Chronic Heart Failure

Acute Effects of the Endothelin Receptor Antagonist Ro 47-0203 (Bosentan)

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Background  Endothelin (ET) is a potent vasoconstrictor, and its concentration is increased in patients with heart failure. The purpose of this study was to investigate the role of endothelin in heart failure by use of a rat model.

Methods and Results  Experiments were performed on rats at 1 through 16 weeks after sham operation or coronary artery ligation. Rats with left ventricular end-diastolic pressures >15 mm Hg were considered to have chronic heart failure (CHF), while the others were considered to have uncomplicated myocardial infarction (MI). There were increased ET-1 concentrations in CHF rats at weeks 1 to 16 (Sham, 20 ± 0.5 pg/mL, n = 45; CHF, 31 ± 2 pg/mL, n = 50; P < .001) and transient increases in ET-3 concentrations at week 1 in both the MI and CHF groups. There were no significant increases in big ET-1 concentrations, suggesting an increased conversion of ET-1 from big ET-1 in the CHF group. At weeks 2 through 8, oral administration of the mixed (ET\textsubscript{A} and ET\textsubscript{B}) endothelin receptor antagonist bosentan significantly decreased mean arterial pressure in conscious CHF rats, an effect that increased over time. Furthermore, bosentan had an additive effect to the angiotensin-converting enzyme inhibitor cilazapril.

Conclusions  Endothelin plays a role in the maintenance of blood pressure in CHF rats, as evidenced by the significant reduction in mean arterial pressure after oral administration of bosentan. Therefore, endothelin antagonists may be useful therapeutic agents in the treatment of CHF. (Circulation. 1994;90:2510-2518.)

Key Words • vessels • endothelium • myocardial infarction • heart failure • endothelin

Endothelin (ET)-1 is a 21-amino-acid vasoactive peptide\textsuperscript{1,2} that can cause potent and prolonged constriction of both venous and arterial smooth muscle and is detectable in the plasma of healthy human subjects.\textsuperscript{3} A role for endothelin has been proposed in a variety of pathophysiological cardiovascular states, including shock (septic and cardiogenic),\textsuperscript{4} angina (stable, unstable,\textsuperscript{5} and variant),\textsuperscript{6} myocardial infarction,\textsuperscript{7-13} and heart failure.\textsuperscript{16-21} Experimental and clinical studies have demonstrated that vasoconstriction and increased concentrations of many circulating neurohumoral factors can contribute to the progression of heart failure. Plasma concentrations of endothelin are increased in patients with heart failure,\textsuperscript{16-21} as well as in some animal models of heart failure\textsuperscript{22-27}; however, the time course and role of these increases have not been evaluated. The availability of new orally active mixed (ET\textsubscript{A} and ET\textsubscript{B}) endothelin receptor antagonists\textsuperscript{28} allows for further investigation of this role.

This study investigates the role of endothelin in heart failure by studying plasma endothelin levels and hemodynamic profiles at different time periods in rats with hemodynamically compensated myocardial infarctions and with heart failure. In addition, bosentan, an orally active mixed (ET\textsubscript{A} and ET\textsubscript{B}) endothelin receptor antagonist, was administered to conscious rats monitored by telemetry to investigate the functional role of endothelin. Therefore, the purpose of our study in this rat model of heart failure was fourfold: (1) to investigate the time course of ET-1, ET-3, and big ET-1 and to possibly gain insight into the effects of heart failure on conversion of big ET-1 into ET-1; (2) to evaluate the relation between the endothelin plasma concentrations and hemodynamic alterations and the presence of significant left ventricular dysfunction; (3) to investigate the physiological role of endothelin in the maintenance of blood pressure in heart failure by evaluating the in vivo effect of bosentan; and (4) to analyze the effects of the endothelin antagonist in combination with angiotensin-converting enzyme (ACE) inhibition.

Methods

Myocardial Infarction and Selection Procedure

Thirteen-week-old male Wistar rats were randomly selected to undergo coronary artery ligation or sham operation by a technique previously described.\textsuperscript{29} In brief, the rats were anesthetized with ether, and a left thoracotomy was performed. Through gentle pressure applied to the right hemithorax, the heart was exteriorized, and a ligature was placed around the proximal left coronary artery. This silk suture was tied securely in the rats randomized to coronary artery ligation but was pulled through in the sham-operated animals. The heart was rapidly replaced into...
the chest, and the thorax was closed. More than 500 rats underwent coronary artery ligation, and at 48 hours after this procedure, 65% of the ligated and 98% of the sham-operated animals had survived. The rats were housed in clear plastic cages with free access to normal rat chow and water and handled according to the “Position of the American Heart Association on Research Animal Use” adopted November 11, 1984, by the American Heart Association.

At 1, 4, and 16 weeks after the coronary ligation or sham procedure, the rats were randomly selected for catheterization. Under ether anesthesia, a microtip pressure transducing catheter (2F, model SPC 320, Millar Instruments Inc) was introduced into the thoracic aorta via the right carotid artery where arterial blood pressure and heart rate (HR) tracings were obtained. The catheter was advanced into the left ventricle, and simultaneous tracings of left ventricular pressure, expanded-scale left ventricular end-diastolic pressure (LVEDP), the maximal rate of rise in left ventricular pressure (+LV dp/dt), and HR were also recorded on a linear chart recorder (Linearcorder Mark VII, model WR3101, Graphtec Corp). Sham-operated rats were randomly selected to enter the study, while only rats that underwent ligation with LVEDPs >15 mm Hg were considered to have chronic heart failure (CHF group). Rats that had undergone the coronary artery ligation procedure but had LVEDPs <15 mm Hg were categorized as the myocardial infarction (MI) group.

**Biochemical Assays and Pathological Studies**

After hemodynamic study, blood samples were collected in a syringe containing 50 µL EDTA/mL blood and were immediately divided into 2-mL aliquots, which were centrifuged for 5 minutes at 13 000 rpm. Plasma was immediately pipetted into plastic storage tubes and placed in dry ice. All samples were stored at −20°C until biochemical assay. ET-1, big ET-1, and ET-3 were measured as described previously. The cross-reactivities of the different antisera with ET-1, ET-3, and big ET-1 were <10%. The extraction recovery as determined by radioimmunoassays were performed as described.31 The cross-reactivity of 5313 with big ET-2 and big ET-3 was <0.01%. The extraction recovery as described above. Hemodynamic Monitoring and Evaluation of Drug Effects

The telemetry system used in this study (Data Sciences International Inc) has been described in detail previously.35 The implantable sensor consisted of a fluid-filled catheter connected to a highly stable, low-conductance strain-gauge pressure transducer, which measured the absolute arterial pressure relative to a vacuum, and a radio frequency transmitter. A receiver platform converted the radio signal to digitized input that was sent to a dedicated personal computer, with which gauge arterial pressures were calibrated by input from a C11PR ambient pressure monitor. Telemetric monitoring of the hemodynamic and activity variables was initiated immediately after implantation of the telemetry units to ensure proper operation of the system. Activity level, HR, and systolic, diastolic, and mean arterial (MAP) pressures were monitored prospectively and at regular intervals with a sampling rate of 500 Hz and a 100 Hz filter cutoff. In a published validation study,33 this system had a >86% success rate after 8 weeks of monitoring, with success defined as >5 mm Hg accuracy for measures of systolic and diastolic pressures and MAP.

At 2, 4, and 8 weeks after the coronary artery ligation or sham operation, blood pressure and HR were obtained every 5 minutes for a 24-hour period, and average hourly values were calculated as described previously.4 Then, either the vehicle (placebo), bosentan (100 mg/kg), cilazapril (10 mg/kg) or cilazapril (10 mg/kg) or placebo was administered at 8 AM by gavage in a randomized three-way crossover design. Hemodynamics were recorded by telemetry for 48 additional hours. The blood pressure and HR values were averaged for the first 6 hours of administration of the randomized treatment, and these values were compared with the average values for the 6 hours before treatment. At week 8, a combination treatment of bosentan (100 mg/kg) and cilazapril (10 mg/kg) was also given by gavage. After completion of the telemetry protocol, the rats were killed and the hearts were removed for pathological analysis as described above.

**Study Design and Statistical Analysis**

The overall design of this investigation consisted of two experiments. The first experiment analyzed the relation over time between plasma concentrations of the endothelins and the hemodynamic and physical characteristics of rats with myocardial infarctions with and without heart failure. Three groups of rats (Sham, MI, and CHF) were studied at 1, 4, and 16 weeks after the coronary artery ligation or sham operation. All statistical comparisons were performed with a commercially available general linear model statistical package for the Macintosh personal computer (SUPERANOVA, version 1.11, Abacus Concepts, Inc). Comparison of all variables was done with a two-way ANOVA with time (1, 4, and 16 weeks) and group (Sham, MI, CHF) as main effects and prospectively
planned mean contrast comparisons between the groups at each time point. One-way ANOVA with Duncan's multiple comparison tests was used to analyze differences within a group across time.

In the second experiment, which evaluated the effects of the mixed (ET₄ and ET₆) endothelin receptor antagonist bosentan and compared them with the ACE inhibitor captopril on the hemodynamic response of Sham and CHF rats, paired two-tailed t test comparisons (STATVIEW, version 4.0, Abacus Concepts, Inc) were made between the bosentan- and the placebo-treated rats and between the captopril- and the placebo-treated rats within each group. Unpaired t tests assessed differences between the Sham and CHF groups. Differences were considered significant at a level of P<.05, and results are expressed as mean±SEM.

**Results**

**Hemodynamic and Baseline Characteristics**

Rats in the first part of the study were divided into three groups based on the procedure and degree of left ventricular dysfunction: (1) Sham-operated group (Sham), (2) MI group (coronary artery ligation rats with LVEDP <15 mm Hg), and (3) CHF group (coronary artery ligation rats with LVEDP ≥15 mm Hg). Animals from each of these groups were studied at weeks 1, 4, and 16, and blood samples were obtained. The hemodynamic characteristics of the groups are described in Table 1. The MAP (Table 1) increased progressively with time in the Sham and MI rats, although the MI rats had significantly lower MAP than Sham rats only at week 1 (P<.0001). The CHF rats had significantly decreased MAP compared with both Sham and MI groups at all time points. A similar pattern was noted with the left ventricular systolic pressure (LVSP in Table 1). The LVEDP (Table 1) was a selection criterion; consequently, it is not surprising to observe a marked difference between the groups. The Sham rats had a mean LVEDP of 2.1±0.4 mm Hg, which did not change significantly over time. The MI rats had a significantly elevated LVEDP of 6.4±0.8 mm Hg at week 1 (P=.002), which returned to Sham values at weeks 4 and 16. The CHF group had an overall LVEDP of 16.6±1.1 mm Hg, which also did not increase over time. The maximal rate of rise of left ventricular systolic pressure (+LV dP/dt in Table 1) did not change significantly in the Sham group, with an overall mean value of 13 000±300 mm Hg/s. The +LV dP/dt in the MI rats was significantly lower than in the Sham rats at each time point (P<.0001), although within the MI group there was a progressive increase in the +LV dP/dt over time. However, the CHF group not only had a lower +LV dP/dt than both the Sham and MI groups but also had a progressive decrease in the +LV dP/dt over time.

The size of myocardial infarction (MI in Table 1) in the groups was 18±1% in the MI group and 41±1% in the CHF group; no sham-operated rat had a myocardial infarction. There were no significant differences in infarct size within groups with time. The left ventricular mass indexed for body weight (LV Mass in Table 1) was not significantly different between the Sham and MI groups (overall mean: Sham, 1.88±0.03 g/kg; MI, 1.86±0.03 g/kg). However, the LV mass was significantly increased in the CHF group, with an overall mean of 2.15±0.05 g/kg. There was no significant change over time in these animals. A similar pattern was observed in the right ventricular mass indexed for body weight (RV Mass in Table 1).

**Plasma Endothelin Concentrations and Hemodynamic Correlates**

Plasma ET-1 concentrations (Fig 1A) were not significantly different between the Sham and MI groups at any time point. However, in the CHF group, plasma

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**Table 1. Physical Characteristics and Hemodynamic Variables**

<table>
<thead>
<tr>
<th>Time</th>
<th>MI, %</th>
<th>Body Weight, g</th>
<th>LV Mass, g/kg</th>
<th>RV Mass, g/kg</th>
<th>HR, bpm</th>
<th>MAP, mm Hg</th>
<th>LVSP, mm Hg</th>
<th>LVEDP, mm Hg</th>
<th>+LV dP/dt, ( \times 10^2 ) mm Hg/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham (n=19)</td>
<td>0±0</td>
<td>355±5</td>
<td>1.81±0.02</td>
<td>0.45±0.01</td>
<td>365±6</td>
<td>117±2</td>
<td>136±2</td>
<td>2.1±0.4</td>
<td>12.5±0.2</td>
</tr>
<tr>
<td>MI (n=32)</td>
<td>17±2</td>
<td>307±5</td>
<td>1.90±0.03</td>
<td>0.56±0.02</td>
<td>388±5</td>
<td>102±3</td>
<td>117±3</td>
<td>6.4±0.8</td>
<td>9.5±0.5</td>
</tr>
<tr>
<td>CHF (n=23)</td>
<td>40±1</td>
<td>307±7</td>
<td>2.10±0.09</td>
<td>0.88±0.06</td>
<td>380±5</td>
<td>94±3</td>
<td>109±3</td>
<td>16.6±1.1</td>
<td>7.5±0.7</td>
</tr>
<tr>
<td>Week 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham (n=13)</td>
<td>0±0</td>
<td>383±8</td>
<td>1.83±0.07</td>
<td>0.47±0.03</td>
<td>379±7</td>
<td>119±3</td>
<td>136±3</td>
<td>3.1±0.8</td>
<td>13.9±0.7</td>
</tr>
<tr>
<td>MI (n=13)</td>
<td>21±2</td>
<td>389±6</td>
<td>1.87±0.05</td>
<td>0.45±0.01</td>
<td>369±7</td>
<td>118±3</td>
<td>133±3</td>
<td>3.7±0.7</td>
<td>10.2±0.5</td>
</tr>
<tr>
<td>CHF (n=15)</td>
<td>42±1</td>
<td>366±7</td>
<td>2.11±0.07</td>
<td>0.87±0.08</td>
<td>357±7</td>
<td>98±4</td>
<td>111±4</td>
<td>18.0±1.9</td>
<td>6.5±0.7</td>
</tr>
<tr>
<td>Week 16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham (n=13)</td>
<td>0±0</td>
<td>427±9</td>
<td>1.83±0.04</td>
<td>0.46±0.03</td>
<td>377±7</td>
<td>124±1</td>
<td>143±2</td>
<td>1.6±0.3</td>
<td>13.8±0.3</td>
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<tr>
<td>MI (n=20)</td>
<td>19±2</td>
<td>433±8</td>
<td>1.82±0.06</td>
<td>0.42±0.01</td>
<td>349±7</td>
<td>119±2</td>
<td>138±2</td>
<td>4.3±0.6</td>
<td>10.4±0.5</td>
</tr>
<tr>
<td>CHF (n=12)</td>
<td>41±2</td>
<td>396±14</td>
<td>2.30±0.11</td>
<td>0.95±0.12</td>
<td>355±7</td>
<td>91±4</td>
<td>105±4</td>
<td>17.7±1.9</td>
<td>5.9±0.5</td>
</tr>
</tbody>
</table>

**Interaction effect (P) | 0.855 | 0.0004 | 0.0359 | 0.0587 | 0.0022 | 0.0070 | 0.0001 | 0.0222 | 0.0468**

**Time effect (P) | 0.3783 | 0.0001 | 0.6456 | 0.2620 | 0.0069 | 0.0077 | 0.0027 | 0.7190 | 0.7393**

**Group effect (P) | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001**

**Note:** MI indicates myocardial infarction; LV, left ventricle; RV, right ventricle; HR, heart rate; bpm, beats per minute; MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; and +LV dP/dt, maximal rate of rise of left ventricular pressure. Values are mean±SEM.
ET-1 concentrations were 52% higher than in the Sham rats from weeks 1 through 16. There was no progressive change over time within any of these groups. An overall stepwise regression demonstrated that both the size of the infarction (MI) and the extent of right ventricular hypertrophy (RV) predicted the plasma ET-1 concentration (ET-1, 10.1±0.16 MI+15.4 RV; n=155; r=.65; \( R^2=.42; P<.0001 \)). When this analysis was split by the time groups, the size of the myocardial infarction was the strongest predictor of the ET-1 concentration in the week 1 group (week 1 ET-1, 12.1±0.41 MI; n=74; r=.62; \( R^2=.38; P<.0001 \)). In the week 4 and week 16 groups, the right ventricular mass was the most predictive of ET-1 (week 4 ET-1, 10.0+24.8 RV; n=41; r=.59; \( R^2=.35; P<.0001 \); week 16 ET-1, 9.3+21.9 RV; n=45; r=.83; \( R^2=.69; P<.0001 \)).

Plasma ET-3 concentrations (Fig 1B) were not significantly different between the Sham, MI, and CHF groups at week 4 and week 16. However, at week 1, there was a significant increase in ET-3 in both the MI and CHF groups compared with the Sham rats. Stepwise regression of the week 1 data demonstrated that only the LVEDP correlated with ET-3 concentrations, which was collinear with the extent of myocardial infarction, although this correlation was weak (week 1 ET-3, 24.1±0.70 LVEDP; n=74; r=.27; \( R^2=.07; P=.02 \)).

Plasma big ET-1 concentrations (Fig 1C) were not significantly different between groups within each time period, although there was a significant decrease in big ET-1 concentrations over time. This decrease suggested an age-related effect, and stepwise regression revealed a negative correlation between big ET-1 and time (big ET-1, 213±4.2 weeks; n=155; r=.49; \( R^2=.24; P<.0001 \)).

To estimate the kinetics of endothelin in rats with myocardial infarction and heart failure, the molar ratio of ET-1 to big ET-1 was calculated for each rat. As shown in Fig 2, the CHF rats had significantly higher ratios at each time period than both the Sham and MI groups (\( P<.001 \)). The Sham and MI groups were not significantly different from each other at any time period. There was also a progressive, significant increase in the ratio of ET-1 to big ET-1 in all groups over time, suggesting an age-related increase in endothelin production in these rats.

**Hemodynamic Effects of Bosentan and Cilazapril**

Seven rats with LVEDP >15 mm Hg at 1 week after myocardial infarction (CHF) and six sham-operated (Sham) rats were monitored by telemetry. The physical characteristics and hemodynamic variables, as well as plasma endothelin concentrations, were not different from those of the 1-week rats from the main study (Table 2); thus, this group was considered to be representative of these animals. After implantation of the telemetry units, baseline MAP and HR were obtained during a 6-hour period for all the rats at 2, 4, and 8 weeks after operation (Fig 3). The MAP increased over time in the sham-operated rats, although there was no change over time in the CHF group. The CHF rats had significantly lower MAP at all time points compared with the sham-operated group (\( P<.01 \)), as well as a significantly higher HR at weeks 2 and 4 (\( P<.05 \)).
TABLE 2. Comparison of Rats From Biochemical Analysis and Telemetry Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Biochemistry Study (n=19)</th>
<th>Telemetry Study (n=6)</th>
<th>Biochemistry Study (n=23)</th>
<th>Telemetry Study (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI, %</td>
<td>0±0</td>
<td>0±0</td>
<td>40±2</td>
<td>43±3</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>355±5</td>
<td>347±3</td>
<td>307±7</td>
<td>299±6</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>365±6</td>
<td>370±10</td>
<td>380±5</td>
<td>390±14</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>117±2</td>
<td>116±2</td>
<td>94±4</td>
<td>92±2</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>136±2</td>
<td>136±2</td>
<td>109±4</td>
<td>106±3</td>
</tr>
<tr>
<td>+LV dP/dt</td>
<td>2.1±0.4</td>
<td>1.1±0.7</td>
<td>16.6±1.1</td>
<td>16.8±0.9</td>
</tr>
<tr>
<td>ET-1, pg/mL</td>
<td>19±1</td>
<td>20±1</td>
<td>30±3</td>
<td>32±2</td>
</tr>
<tr>
<td>ET-3, pg/mL</td>
<td>25±1</td>
<td>28±1</td>
<td>35±6</td>
<td>28±1</td>
</tr>
<tr>
<td>Big ET-1, pg/mL</td>
<td>223±9</td>
<td>231±12</td>
<td>205±19</td>
<td>199±17</td>
</tr>
</tbody>
</table>

CHF indicates chronic heart failure; MI, myocardial infarction; bpm, beats per minute; MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; +LV dP/dt, maximal rate of rise of left ventricular pressure (×10^2 mm Hg/s); and ET, endothelin.

The effects of placebo, bosentan, and cilazapril on the MAP in CHF and Sham rats at 2 weeks are shown in Fig 4. Administration of vehicle placebo resulted in no significant change in MAP in either the Sham or CHF group. However, the endothelin receptor antagonist bosentan caused significant and sustained reductions in MAP in both groups. These effects were sustained over time, such that administration of bosentan at 4 and 8 weeks also produced significant reductions in MAP (Fig 5). Furthermore, the effect of bosentan on MAP in the CHF group increased over time (Fig 5; *P<.01). The ACE inhibitor cilazapril also produced significant reductions in MAP in both groups and at all time points (Figs 4 and 5). The HR was not affected by placebo or

Fig 3. Bar graphs showing baseline pretreatment mean arterial pressure (MAP; A) and heart rate (HR; B) in sham-operated (Sham) and chronic heart failure (CHF) rats measured by telemetry. The CHF group had a lower MAP at all time points, as well as a higher HR at weeks 2 and 4. Values are average of 6-hour means obtained before administration of randomized treatment and are expressed as mean±SEM. †P<.01, *P<.05 vs corresponding Sham values within time period.

Fig 4. Graphs showing effects of placebo, bosentan, and cilazapril on mean arterial blood pressure (MAP) in Sham-operated (column A) and chronic heart failure (CHF) (column B) rats at week 2. Arrow represents the administration of the specified treatment. Values are the average hourly means expressed as mean±SEM. †P<.01 vs corresponding pretreatment value.
bosentan in either group (Fig 5), although there was a markedly increased HR in the cilazapril-treated CHF rats.

The combination of bosentan and cilazapril was given to rats at week 8 to assess the hypothesis that the afterload-reducing effects of these agents were additive in CHF rats. Fig 6 demonstrates that bosentan has an additive vasodilating effect when given with cilazapril compared with either cilazapril (P<.05) or bosentan (P<.01) alone in the CHF group. The dose of cilazapril administered has been shown to produce full inhibition of ACE.35 There was no significant change in HR with the combination treatment compared with the cilazapril-treated rats, although the HR was significantly higher than in the bosentan-treated CHF rats.

Discussion

The present study demonstrates that there were early increases in ET-1 concentrations in rats with heart failure due to coronary artery ligation that most closely correlated with right ventricular mass, an index of pulmonary hypertension, as well as transient increases in ET-3 concentrations at week 1 in both the MI and CHF groups. There were no significant increases in big ET-1 concentrations, suggesting that the increased ET-1 levels were due to increased conversion from big ET-1. The oral administration of the mixed (ET\(_A\) and ET\(_B\)) endothelin receptor antagonist bosentan resulted in significant afterload reduction, as assessed by MAP, an effect that increased over time in rats with heart failure. Furthermore, bosentan had an additive effect to the ACE inhibitor cilazapril.

The rat model of coronary artery ligation is a well-established model that produces pathophysiological alterations similar to those seen in the most common contemporary cause of heart failure in humans, namely, ischemic heart disease.36 The presence of heart failure as demonstrated by the increased LVEDP was established by catheterization during selection of the rats, and this hemodynamic abnormality was associated with increases in left and right ventricular masses, which corresponds to findings of previous studies.37 The absence of any confounding diseases, such as atherosclerosis, diabetes mellitus, hypercholesterolemia, or hypertension, in this model of segmental myocardial damage provides an opportunity to investigate independently the evolution of the endothelin system in heart failure. The recent advances in electronics have allowed the chronic hemodynamic monitoring of conscious rats through implantable telemetry systems and the observation of drug effects without the confounding factors of anesthesia, tethering or other forms of restraint, or surgical techniques. All of these factors could interfere with the normal physiological responses to these agents. However, these telemetry systems do not allow for determination of cardiac output, ventricular volumes, or filling pressures, and this information will also be important to obtain in the future.

This study is the first to our knowledge to investigate the time course of alterations in ET-1 concentrations in the rat model of myocardial infarction and heart
failure. ET-1 concentrations were markedly increased at week 1 after myocardial infarction in rats with LVEDP >15 mm Hg. At this relatively early time point, these increases may have been due to the acute large myocardial infarction, since this group had an average myocardial infarction of 41%. This hypothesis is supported by the stepwise regression of the week 1 group, which demonstrated that the size of the infarction was the strongest predictor of ET-1 concentration at week 1. The time course of ET-1 concentrations has been well elucidated in humans with myocardial infarction,5,7-15 and levels remain elevated for 24 hours11 to 6 days12 after infarction. Increased plasma ET-1 concentrations correlated with the extent of myocardial infarction as assessed by wall motion abnormalities,9 left ventricular ejection fraction,11,13 other signs of left ventricular failure,14,15 or maximal creatine kinase and MB isoenzymes.13 In the anesthetized open-chest dog model, Miyauchi and colleagues38 found no significant change in peripheral ET-1 concentrations after 1 hour of left anterior descending coronary artery occlusion and 1 hour of reperfusion. However, in another study,39 2 hours of coronary ligation followed by reperfusion resulted in significantly increased ET-1 concentrations. An early study in the rat coronary artery ligation model, consisting of a 1-hour total left coronary artery occlusion followed by 24 hours of reperfusion,40,41 demonstrated an increase in plasma ET-1, peaking at 10 minutes after reperfusion and returning to control levels by 8 hours later, as well as an increase in cardiac tissue ET-1 concentrations. Administration of an ET-1/ET-2 antibody before occlusion and during reperfusion resulted in significantly decreased infarct size, suggesting an important role for ET-1 in myocardial infarction. Another study using the rat model42 demonstrated a 35% decrease in the left atrial ET-1 binding sites at 24 hours after myocardial infarction. The present study extends these observations and demonstrates that the ET-1 concentrations are significantly increased 1 week after myocardial infarction in rats with elevated filling pressures and that this increase most closely correlates with the extent of the myocardial infarction.

The persistent and trending toward progressive increase in plasma ET-1 concentration in the rats with heart failure is an important finding, and the stepwise regression suggests that it may be secondary to pulmonary hypertension, since the extent of right ventricular hypertrophy most closely correlated with the ET-1 concentrations at weeks 4 and 16. Although one study did not observe increases in ET-1,4 it is most studies in patients with heart failure have also demonstrated increased plasma ET-1.16-21 These increases have been noted in patients with idiopathic dilated cardiomyopathy independent of atherosclerotic coronary artery disease15 and are known to persist and progress after heart transplantation,18 although this may be secondary to cyclosporine.19 There is no correlation between plasma endothelin concentrations and serum creatinine19,20 or serum urea.19 A negative correlation between plasma ET-1 concentration and left ventricular ejection fraction has been reported in some studies16,20 but not in others.18 The strongest predictor of plasma ET-1 in patients with heart failure appears to be indexes of pulmonary hypertension,17 as in our study. Elevated ET-1 levels have also been observed in patients with primary pulmonary hypertension4,44 and pulmonary hypertension secondary to congenital heart defects.45 Our findings in this rat model of heart failure are consistent with these studies in humans and suggest that this model may be useful in assessing the effects of heart failure on the endothelin system. Other studies in animals have also shown increases in ET-1 concentrations in the rat aortic valvular high-output heart failure model,24 the rabbit aortic valvular insufficiency and subdiaphragmatic aortic stenosis model,27 the dog rapid ventricular pacing model,22,23 and the dog thoracic inferior vena cava constriction model.25 In the one study in which hemodynamics were analyzed, there was a positive correlation with right atrial and pulmonary capillary wedge pressure in dogs after 8 days of rapid ventricular pacing.23

Plasma ET-3 concentrations were also increased in the present study, but only during week 1. These increased concentrations correlated very weakly with the LVEDP (r=.27; P=.02), which was collinear with the extent of myocardial infarction. Serum levels of ET-3 have not been as completely investigated as those of ET-1, but in one report in patients, there was no change in ET-3 during 14 days after the onset of myocardial infarction.10 Plasma big ET-1 concentrations were not significantly elevated in the myocardial infarction or heart failure rats, although there was a global time-dependent decrease in big ET-1 from week 1 to week 16. Patients with congestive heart failure are known to have increased big ET-1 concentrations that correlated with right atrial pressure, pulmonary capillary wedge pressure, left ventricular ejection fraction, effort capacity, and severity of heart failure.46 Despite the time-dependent decrease in big ET-1, there were increased ET-1 concentrations in rats with heart failure; this finding is represented by a significantly increased ratio of ET-1 to big ET-1 in these rats at each time point. These findings suggest that increased conversion of big ET-1 may play a significant role in heart failure. However, the possibility of decreased degradation (for example, by the lung due to pulmonary congestion or hypertension) and/or elimination (for example, decreased clearance by the kidneys) of ET-1 cannot be ruled out.

The oral administration of a mixed (ET$_{A}$ and ET$_{B}$) endothelin receptor antagonist, bosentan, caused decreased MAP without significant changes in HR in rats with heart failure. There was an increased effect of the bosentan at week 16 compared with week 1, consistent with the trend toward increasing plasma ET-1 levels with time in the heart failure group. These results demonstrate that ET-1 plays an important role in the maintenance of blood pressure and systemic vasoconstriction in heart failure and that this role is not only evident early in the course but also present after 4 months of heart failure.

Therapeutic Implications

This study has shown that plasma ET-1 concentrations are increased in rats with myocardial infarction and heart failure. These increased concentrations appear to be secondary to increased conversion of big ET-1 into ET-1 and to play a role in the maintenance of
blood pressure, as evidenced by the significant reduction in MAP after oral administration of the endothelin receptor antagonist bosentan. Systemic vasoconstriction is a central component in the pathophysiology of heart failure, and the benefits of afterload reduction have been demonstrated in animal models and in patients. This study demonstrates that endothelin receptor antagonists, such as bosentan, may serve a useful role in the treatment of heart failure. Furthermore, since ACE inhibitors are now considered a cornerstone in the management of patients with heart failure, it is important to note that in this study, oral administration of bosentan resulted in further reductions in MAP in rats concurrently treated with the ACE inhibitor cilazapril. This effect of bosentan suggests that endothelin antagonists may be useful therapeutic agents in the treatment of heart failure and may add to the therapeutic value of ACE inhibitors.

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Role of endothelin in the maintenance of blood pressure in conscious rats with chronic heart failure. Acute effects of the endothelin receptor antagonist Ro 47-0203 (bosentan).

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