Endogenous Endothelin-1 Depresses Left Ventricular Systolic and Diastolic Performance in Congestive Heart Failure

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ABSTRACT

Endothelin-1 (ET-1) is a positive inotrope in normal hearts; however, the direct cardiac effects of endogenous ET-1 in congestive heart failure (CHF) are unknown. We evaluated the cardioc responses to endogenous ET-1 using an ET<sub>A</sub> and ET<sub>B</sub> receptor blocker (L-754,142) in seven conscious dogs before and after pacing-induced CHF. Before CHF, when the plasma ET-1 was 7.3 ± 1.7 fmol/ml, L-754,142 caused no significant alterations in heart rate, left ventricular (LV) end-systolic pressure, total systemic resistance, and the time constant of LV relaxation (τ). LV contractile performance, measured by the slopes of LV pressure (P)-volume (V) relation (E<sub>ES</sub>), dP/dt<sub>max</sub>-end-diastolic V relation (dE/dt<sub>max</sub>), and stroke work-end-diastolic V relation, was also unaffected. After CHF, when the plasma ET-1 was significantly increased to 14.1 ± 3.0 fmol/ml (p < .05), L-754,142 produced a significant decreases in LV end-systolic pressure (101 ± 11 versus 93 ± 8 mm Hg) and total systemic resistance (0.084 ± 0.022 versus 0.065 ± 0.15 mm Hg/ml/min). The τ (42 ± 12 versus 38 ± 10 ms), mean left atrial P (22 ± 5 versus 18 ± 4 mm Hg) (p < .05), and minimum LVP were also significantly decreased. After CHF, the slopes of P-V relations, E<sub>ES</sub> (3.4 ± 0.4 versus 4.8 ± 0.8 mm Hg/ml), dE/dt<sub>max</sub> (42.4 ± 7.8 versus 50.0 ± 7.8 mm Hg/s/ml), and stroke work-end-diastolic V relation (58.1 ± 3.3 versus 72.4 ± 5.2 mm Hg) (p < .05) all increased after L-754,142, indicating enhanced contractility. Before CHF, low levels of endogenous ET-1 have little cardiac effect. However, after CHF, elevated endogenous ET-1 produces arterial vasoconstriction, slows LV relaxation, and depresses LV contractile performance. Thus, elevated endogenous ET-1 may contribute to the functional impairment in CHF in this canine model.

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Endothelin-1 (ET-1) is a 21-amino-acid peptide, originally isolated from endothelial cells (Yanagisawa et al., 1988), that is also produced by the kidney and heart (Miller et al., 1989; Luscher et al., 1991). It is a potent, arterial, and venous constrictor (Kiowski et al., 1995), and it interacts with the renin-angiotensin system (Miller et al., 1989). Plasma ET-1 levels are increased in both experimental (Cavero et al., 1990; Margulies et al., 1990; Teerlink et al., 1994; Shimoyama et al., 1996) and clinical (McMurray et al., 1992; Rodeheffer et al., 1992; Wei et al., 1994) congestive heart failure (CHF), and endothelin receptors are increased in a rat model of CHF (Sakai et al., 1996). Thus, ET-1 may play an important role in the cardiac response to neurohumoral activation in CHF.

ET-1 exerts a positive inotropic action on normal myocardium (Ishikawa et al., 1988; Watanabe et al., 1989; Neubauer et al., 1990). Thus, “upregulation of endothelin pathways may be beneficial in providing short-term support for the failing myocardium” (Goto et al., 1996). However, several lines of evidence indicate that the effect of ET-1 on normal myocardial contraction may be altered in a pathologic state (Kohmoto et al., 1993; Thomas et al., 1996; Spinale et al., 1997; Ito et al., 1997; Suzuki et al., 1998). We found that angiotensin II has a positive inotropic effect in normal myocytes but depresses contraction in myocytes from dogs with pacing-induced CHF (Cheng et al., 1996). The intracellular signaling responsible for the inotropic effect of angiotensin II and ET-1 in normal myocardium is similar (Fareh et al., 1996; Touyz et al., 1996; Ito et al., 1997) and is altered by myocardial hypertrophy in the rat (Ito et al., 1997). Thus, the inotropic effect of ET-1 on normal myocardium may be altered in a similar fashion in CHF (Thomas et al., 1996; Suzuki et al., 1998).

Currently, endogenous ET-1 has been reported to
produce positive (Sakai et al., 1996) or negative (Toerlink et al., 1994; Shimoyama et al., 1996; Spinale et al., 1997) effects on left ventricular (LV) contraction in animal models of CHF. These inconsistent results may have resulted from the influence of ET-1-produced changes to loading conditions on conventional measures of LV contractile performance and the variable effects of anesthesia. Thus, the inotropic effect of the elevated endogenous ET-1 in CHF remains unclear.

The pacing-induced CHF model has been studied by many investigators (Armstrong et al., 1986; Burchell et al., 1992; Spinale et al., 1994), including those at our laboratory (Cheng et al., 1993, 1996). The biochemical alterations are similar to those reported for volume or pressure overload (O’Brien et al., 1989). Chronic rapid pacing produces time-dependent changes in LV and cardiomyocyte function, structure, hemodynamic compromise, and neurohormonal activation (including ET-1) that are very similar to the clinic spectrum of CHF (Cohn, 1995). The National Institutes of Health has identified this rapid pacing model as one of the most promising for the elucidation of mechanisms that contribute to the initiation of the progression of CHF (Lenfant, 1994).

In the present study, we used pacing-induced CHF in dogs to evaluate the hypothesis that endogenous ET-1 contributes to a functional impairment of LV contraction and relaxation in CHF independent of its effect on arterial load. To avoid the potential confounding effects of ET-1-produced changes in loading conditions on conventional measures of LV performance, we evaluated LV contractile performance in the pressure-volume (P-V) plane in conscious animals (Kass and Maughan, 1988; Little et al., 1989; Cheng et al., 1996).

### Materials and Methods

#### Instrumentation

Seven healthy, adult, heartworm-negative mongrel dogs (weight, 25–36 kg) were instrumented using the technique that we described previously (Cheng et al., 1990, 1996). Anesthesia was induced with xylazine (2 mg/kg i.m.) and sodium thiopental (6 mg/kg i.v.) and maintained with halothane (0.5–2.0%). They were intubated and ventilated with oxygen-enriched room air to maintain arterial oxygen pressure greater than 100 mm Hg and pH between 7.38 and 7.42. The pericardium was opened through a left thoractomy. Micro-manometer pressure transducers (Konisberg Instruments, Inc., Pasadena, CA) and polyvinyl catheters (1.1 mm I.D.) for transducer calibration were inserted into the left ventricle (LV) through a LV apical stab wound and into the left atrium (LA) through the LA appendage. Three pairs of ultrasonic crystals (5 MHz) were implanted in the endocardium of the LV to measure the anterior-to-posterior, septal-to-lateral, and base-to-apex (long-axis) dimensions (Cheng et al., 1990). Hydraulic occluder cuffs were placed around the posterior, septal-to-lateral, and base-to-apex (long-axis) dimensions (Kass and Maughan, 1988; Little et al., 1989; Cheng et al., 1996).

#### Data Collection

Studies were begun after full recovery from instrumentation (from 10 days to 2 weeks after surgery). The LV and LA catheters were connected to pressure transducers (Statham P23Db; Gould, Cleveland, OH) calibrated with a mercury manometer. The signal from the micromanometer was adjusted to match that of the catheter. The LA micromanometer was adjusted to match LA and LV pressures at the end of long periods of diastasis.

The analog signals were recorded on a 16-channel oscillograph (Astro-Med, West Warwick, RI), digitized with an online analog-to-digital converter (Data Translation Devices, Marlboro, MA) at 200 Hz, and stored on a magneto-optical disk memory system by use of a 486 computer system. Each data acquisition period lasted for 12 to 15 s, spanning several respiratory cycles. The derivatives of LV pressure and volume were calculated using the five-point Lagrangian method (Cheng et al., 1990, 1993, 1996).

#### Experimental Protocol

To evaluate the potential functional role of endogenous ET-1 in the progression of CHF, we used L-754,142, which is a potent mixed ET-1 antagonist with more ETA selectivity (Williams et al., 1995a).

### Studies before CHF

#### Effect of Exogenous ET-1 With and Without Pretreatment of L-754,142

To determine the dosage for the ET-1 antagonist L-754,142, we measured cardiovascular responses during ET-1 infusion with and without pretreatment of L-754,142. Data were initially recorded with the animals lying quietly on their sides without medication to obtain baseline values. Three sets of variably loaded P-V loops were generated by sudden transient occlusion of the cavae. This caused a progressive fall in end-systolic pressure (PES)-LV end-diastolic volume (VEd) over a 12- to 15-s recording period. Immediately after the recording period, the caval occlusion was released, and hemodynamic parameters were allowed to restabilize. After all parameters returned to their baseline levels, ET-1 (600 ng/kg i.v.) was administered. When the arterial pressure had reached a stable level, steady-state and caval occlusion data at rest were again collected. To assess the interactions of ET-1 with autonomic reflexes, the same protocol was repeated after the administration of metoprolol (0.5 mg/kg i.v.) and atropine (0.1 mg/kg i.v.).

On the following days, the adequacy of ET-1 blockade produced by L-754,142 (3 mg/kg plus 3 mg/kg/h i.v.) was tested in the animals both with and without autonomic blockade. First, L-754,142 was administered, and the steady-state and caval occlusion data were collected. Then, ET-1 (600 ng/kg i.v.) was infused. Data were acquired again.

#### Effect of Endogenous ET-1

On the next day, after the collection of control steady-states and caval occlusion data, L-754,142 (3 mg/kg i.v. followed by 3 mg/kg/h infusion) was administered. After 5 min, when the arterial pressure had reached a stable level, steady-state and caval occlusion data at rest were again collected.

#### Studies during Development of CHF

After the completion of the baseline studies, the pacemaker rate was adjusted to 200 to 250 beats/min, using the external magnetic control unit. Three times per week, the pacemaker rate was adjusted below the spontaneous rate. The animal was allowed to equilibrate for 30 min, and then data were collected. After each study, pacing rate was returned to 200 to 250 beats/min. After pacing for 4 to 5 weeks, when the LV end-diastolic pressure (PEd) during nonpaced period had increased by more than 15 mm Hg over the prepping control level, CHF data were obtained. This level of CHF was chosen because the animals had begun to show clinical evidence of CHF (anorexia, mild ascites, and pulmonary congestion).

#### Studies After Onset of CHF

#### Effect of Endogenous ET-1

Studies in dogs with CHF were performed after the animal stabilized for at least 30 min after discontinuation of pacing. After recording of the baseline, steady-state, and caval occlusion data, the same amount of L-754,142 as used in the studies before CHF was administered. At 5 min after drug...
administration, when the arterial pressure had reached a stable level, resting steady-state and cava occlusion data were again collected.

Effect of Nitroprusside. To assess the direct cardiac effect of ET-1 blocker, independent of its effect on systolic load, we compared the equal hypotension caused by nitroprusside and L-754,142. Nitroprusside (0.5–2.0 μg/kg/min) was administered to obtain a similar decrease in LV PES. Data were collected in a similar way for the L-754,142 study.

Plasma ET-1 Measurements. The plasma ET-1 concentrations were measured before and after heart failure in five dogs. Before and 5 min after drug administration, 5 ml of blood was obtained from the LA catheter, immediately placed into an EDTA tube on ice, and centrifuged at 2500 rpm at 4°C. Plasma was separated and stored at −20°C until assay. Then, 1 ml of 20% acetic acid was added to the 1-ml plasma samples. The acidified samples were vortexed and centrifuged for 15 min at 2600g. Samples were applied to Si-C18 Sep-Pak cartridges (500 mg C18 in a 3-ml syringe) that had been pretreated with 3 ml of metenolone, 3 ml of water, and 3 ml of 10% acetic acid. Columns were washed with 3 ml of 10% acetic acid and 6 ml of ethyl acetate. Columns were eluted with 3 ml of 80% methanol/20% 0.05 M ammonium bicarbonate. Eluted samples were dried overnight in a Savant Speed-Vac centrifugal evaporator. Dried samples were assayed for immunoreactive ET-1 using an Amersham RIA kit (RPA 545) (Wei et al., 1994).

Data Processing and Analysis

LV volume (V_{LV}) was calculated as a modified general ellipsoidoid using the following equation:

\[ V_{LV} = \left( \frac{\pi}{6} \right) D_{AP} D_{SL} D_{LA} \]

where D_{AP} is the anterior-to-posterior LV diameter, D_{SL} is the septo-to-lateral LV diameter, and D_{LA} is the long-axis LV diameter. We previously demonstrated that this method gives a consistent measure of V_{LV} despite changes in LV loading conditions, configurations, and heart rate (Little et al., 1989; Cheng et al., 1993). To account for respiratory changes in intrathoracic pressure, steady-state measurements were averaged over the 12- to 15-s recording period that spanned multiple respiratory cycles. End-systole was defined as the relative minimum of LV pressure occurring after the A wave. End-systole was defined as the top left corner of the LV P-V loop, identified using the iterative technique described by Kono et al. (1984). The time of mitral valve opening was defined to be when LV pressure fell below LA pressure. LV end-diastolic, end-systolic, and minimum pressures and volumes were measured. LA pressure was measured at the time of mitral valve opening (peak V wave) and at the peak of the A wave. The mean LA pressure was determined.

The derivatives of LV pressure (dP/dt) were calculated using the five-point Lagrangian method (Little et al., 1989; Cheng et al., 1993). Stroke volume was calculated as V_{ES} minus V_{ED}. Cardiac output was determined as stroke volume multiplied by heart rate. LV stroke work (SW) was also calculated by point-by-point integration of the LV P-V loop for each beat. The rate of LV relaxation was analyzed by determining the time constant of the isovolumic fall of LV pressure.

Statistical Analysis

Statistical comparisons were made with Student’s t test for paired observations and ANOVA with the Bonferroni method of multiple-paired comparisons as appropriate. Significance was accepted when p < .05. Data for steady-state and plasma ET-1 are expressed as mean ± S.D., and values for LV P-V relations are expressed as mean ± S.E.M.

Results

Effects of Exogenous ET-1 With and Without Pretreatment of ET-1 Receptor Blocker Before CHF

As shown in Table 1, before CHF, with reflexes intact, the infusion of ET-1 (600 ng/kg i.v.) produced significant increases in LV P_{ES} (103 ± 2 versus 114 ± 8 mm Hg, p < .05), LV P_{ED} (11.6 ± 2.7 versus 16.1 ± 2.4 mm Hg, p < .05), minimum LVP (1.2 ± 1.1 versus 3.3 ± 0.4 mm Hg, p < .05), and TSR (0.063 ± 0.023 versus 0.070 ± 0.022 mm Hg/ml/min, p < .05), indicating a vasoconstriction, and in E_{ES} (5.5 ± 0.6 versus 6.8 ± 0.7 mm Hg/ml, p < .05), dE/dt_{max} (80.6 ± 8.4 versus 107.7 ± 11.0 mm Hg/s/ml, p < .05), and M_{SW} (71.5 ±
Intravenous administration of L-754,142 caused no significant changes in the P-V loops. Before CHF (left), administration of L-754,142 did not significantly affect P-V loops. In contrast, after CHF (right), administration of L-754,142 produced a decrease in P_{ES}, V_{ED} and minimum pressure.

Furthermore, there were no significant differences in \( \tau \).

**P-V Analysis.** As shown in Table 3 and Fig. 2, in normal dogs, L-754,142 produced no significant increases in the slopes of the P_{ES}-V_{ED} relation (5.3 \pm 0.7 versus 5.3 \pm 0.8 mm Hg/ml), the dP/dt_{max}-V_{ED} relation (82.0 \pm 12.8 versus 82.3 \pm 11.4 mm Hg/ml), and the SW-V_{ED} relation (78.1 \pm 4.4 versus 81.2 \pm 7.5 mm Hg/ml). There also were no significant alterations in the positions of all three relations with rela-

**Table 2**

Effects of ET-1 blocker on steady-state hemodynamics and ET-1 plasma concentration before and after CHF

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>ET-1 blocker</th>
<th>After CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>114 \pm 3</td>
<td>114  \pm 3</td>
<td>114  \pm 7</td>
</tr>
<tr>
<td>Peak +dP/dt (mm Hg/s)</td>
<td>2468 \pm 110</td>
<td>2597 \pm 63</td>
<td>2560 \pm 93</td>
</tr>
<tr>
<td>Peak -dP/dt (mm Hg/s)</td>
<td>2162 \pm 49</td>
<td>2252 \pm 175</td>
<td>2293 \pm 58</td>
</tr>
<tr>
<td>LVP_{ES} (mm Hg)</td>
<td>11.6 \pm 2.7</td>
<td>16.1 \pm 2.4*</td>
<td>10.6 \pm 2.8</td>
</tr>
<tr>
<td>LVP_{ES} (mm Hg)</td>
<td>109 \pm 2</td>
<td>114 \pm 8*</td>
<td>106 \pm 4</td>
</tr>
<tr>
<td>Minimum LVP (mm Hg)</td>
<td>1.2 \pm 1.1</td>
<td>3.3 \pm 0.4*</td>
<td>0.7 \pm 0.6</td>
</tr>
<tr>
<td>TSR (mm Hg/ml/min)</td>
<td>0.063 \pm 0.023</td>
<td>0.070 \pm 0.022a</td>
<td>0.063 \pm 0.017</td>
</tr>
<tr>
<td>E_{a} (mm Hg/ml)</td>
<td>5.5 \pm 0.6</td>
<td>6.8 \pm 0.7*</td>
<td>5.4 \pm 0.3</td>
</tr>
<tr>
<td>V_{ED} (ml)</td>
<td>7.4 \pm 2.0</td>
<td>9.2 \pm 1.5*</td>
<td>10.4 \pm 1.4</td>
</tr>
<tr>
<td>dE/dt_{max} (mm Hg/s/ml)</td>
<td>80.6 \pm 8.4</td>
<td>107.7 \pm 11.0b</td>
<td>72.6 \pm 14.9</td>
</tr>
<tr>
<td>V_{0,SW} (ml)</td>
<td>5.3 \pm 1.0</td>
<td>6.6 \pm 0.9*</td>
<td>4.1 \pm 1.3</td>
</tr>
<tr>
<td>M_{SW} (mm Hg)</td>
<td>71.5 \pm 4.7</td>
<td>81.1 \pm 5.6*</td>
<td>80.4 \pm 6.1</td>
</tr>
<tr>
<td>V_{0,ES} (ml)</td>
<td>22.2 \pm 3.2</td>
<td>22.4 \pm 3.1*</td>
<td>26.2 \pm 4.7</td>
</tr>
</tbody>
</table>

LVP, LV pressure; V_{0,ES}, intercept with volume axis; dE/dt_{max}-V_{ED} relation; V_{0, dP/dt}, intercept with volume axis; V_{0,SW}, intercept with volume axis.

Values are mean \pm S.E. (n = 4). 

* P < .05, ET-1 versus corresponding control value.

** P < .05, ET-1 versus corresponding control value.

**P** values indicate a significant difference from baseline values before CHF (L-754,142).

**Control ET-1 blocker Control ET-1 blocker**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>ET-1 blocker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>111 \pm 17</td>
<td>114 \pm 17</td>
</tr>
<tr>
<td>Peak +dP/dt (mm Hg/s)</td>
<td>2804 \pm 295</td>
<td>2549 \pm 282</td>
</tr>
<tr>
<td>Peak -dP/dt (mm Hg/s)</td>
<td>-2295 \pm 300</td>
<td>-2261 \pm 280</td>
</tr>
<tr>
<td>LVP_{ES} (mm Hg)</td>
<td>10.9 \pm 2.9</td>
<td>10.7 \pm 3.3</td>
</tr>
<tr>
<td>LVP_{ES} (mm Hg)</td>
<td>109 \pm 4</td>
<td>107 \pm 6</td>
</tr>
<tr>
<td>Minimum LVP (mm Hg)</td>
<td>0.6 \pm 0.7</td>
<td>0.7 \pm 1.3</td>
</tr>
<tr>
<td>Mean LAP (mm Hg)</td>
<td>4.2 \pm 2.0</td>
<td>3.4 \pm 2.7</td>
</tr>
<tr>
<td>V_{ED} (ml)</td>
<td>49.4 \pm 11.2</td>
<td>48.6 \pm 11.0</td>
</tr>
<tr>
<td>V_{ES} (ml)</td>
<td>32.5 \pm 7.5</td>
<td>31.8 \pm 7.8</td>
</tr>
<tr>
<td>Stroke volume (ml)</td>
<td>16.9 \pm 4.4</td>
<td>16.7 \pm 3.9</td>
</tr>
<tr>
<td>E_{a} (mm Hg/ml)</td>
<td>6.9 \pm 1.9</td>
<td>6.8 \pm 1.8</td>
</tr>
<tr>
<td>TSB (mm Hg/ml/min)</td>
<td>0.063 \pm 0.016</td>
<td>0.059 \pm 0.013</td>
</tr>
<tr>
<td>( \tau ) (ms)</td>
<td>311 \pm 3.7</td>
<td>309 \pm 3.7</td>
</tr>
<tr>
<td>CBF (ml/min)</td>
<td>33.6 \pm 6.4</td>
<td>34.1 \pm 7.0</td>
</tr>
<tr>
<td>Plasma ET-1 (nmol/l)</td>
<td>7.3 \pm 1.7</td>
<td>18.2 \pm 1.7**</td>
</tr>
</tbody>
</table>

LVP, LV pressure; LAP, LA pressure; E_{a}, arterial elastance; \( \tau \), time constant of LV relaxation; and CBF, coronary blood flow. Values are mean \pm S.D. (n = 7).

* P < .05, control values after CHF versus corresponding control values before CHF.

** P < .05, ET-1 versus corresponding control value.
Effects of Pacing-Induced CHF

After the development of CHF at rest, the mean \( P_{ES} \) increased from 10.9 ± 2.9 to 27.3 ± 7.7 mm Hg \((p < .05)\) (Table 2). The minimum LVP (0.6 ± 0.7 versus 10.5 ± 2.0 mm Hg, \( p < .05 \)) and mean LAP (4.2 ± 2.0 versus 22.0 ± 5.3 mm Hg, \( p < .05 \)) also increased. The LV \( V_{ES} \) and \( V_{ED} \) increased, whereas cardiac output was decreased due to the marked reduction in stroke volume (16.9 ± 4.4 versus 11.4 ± 4.1 ml). \( \tau \) increased (31.1 ± 3.7 versus 41.9 ± 12.1 ms, \( p < .05 \)). LV contractility was also significantly impaired as indicated by the decreased slopes and rightward shifts of the P-V relations (Table 3).

Effects of ET-1 Blocker in Dogs with CHF

**Steady-State Measurements.** Steady-state hemodynamic response produced by L-754,142 at rest after CHF is summarized in Table 2 and displayed in Fig. 1. L-754,142 had no significant effect on HR. After CHF, L-754,142 produced a decrease in PES (101 ± 11 versus 93 ± 10 mm Hg, \( p < .05 \)) and \( E_A \) (9.6 ± 2.9 versus 7.9 ± 2.3 mm Hg/ml, \( p < .05 \)) and an increase in SV (11.4 ± 4.1 versus 13.0 ± 4.6 ml, \( p < .05 \)). TS-R (0.084 ± 0.022 versus 0.065 ± 0.015 mm Hg/ml/min, \( p < .05 \)) decreased and CBF increased with L-754,142. This indicated that L-754,142 caused marked arterial dilation of both the systemic and coronary arteries. In addition, L-754,142 caused a marked improvement in LV diastolic performance as indicated by a significant decrease in \( \tau \) (41.9 ± 12.1 versus 37.7 ± 10.4 ms, \( p < .05 \)), a decrease in minimum LVP, and an increase in \( \Delta P/dt_{max} \). In addition, with L-754,142, \( P_{ES} \) and mean LAP (22.0 ± 5.3 versus 17.7 ± 4.2 mm Hg, \( p < .05 \)) also were significantly reduced.

### Table 3

**Effects of ET-1 blocker on the \( P_{ES} \)-\( V_{ES} \), \( dP/dt_{max} \)-\( V_{ED} \), and SW-\( V_{ED} \) relations before and after CHF**

<table>
<thead>
<tr>
<th></th>
<th>( P_{ES} )-( V_{ES} ) Relation</th>
<th>( dP/dt_{max} )-( V_{ED} ) Relation</th>
<th>SW-( V_{ED} ) Relation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( E_{ES} )</td>
<td>( V_{0,ES} )</td>
<td>( V_{100,ES} )</td>
</tr>
<tr>
<td><strong>Before CHF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.3 ± 0.7</td>
<td>9.0 ± 2.6</td>
<td>29.4 ± 3.1</td>
</tr>
<tr>
<td>ET-1 blocker</td>
<td>5.3 ± 0.8</td>
<td>8.8 ± 3.0</td>
<td>29.6 ± 3.2</td>
</tr>
<tr>
<td><strong>After CHF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.4 ± 0.4*</td>
<td>15.7 ± 0.6*</td>
<td>47.3 ± 3.5*</td>
</tr>
<tr>
<td>ET-1 blocker</td>
<td>4.8 ± 0.8**</td>
<td>17.4 ± 0.9**</td>
<td>41.2 ± 3.9**</td>
</tr>
</tbody>
</table>

\( V_{0,ES} \), intercept with volume axis; \( V_{100,ES} \), volume associated with a \( P_{ES} \) of 100 mm Hg; \( dE/dt_{max} \), slope of \( dP/dt_{max} \)-\( V_{ED} \) relation; \( V_{0,dP/dt} \), intercept with volume axis, and \( V_{1000,SW} \), volume associated with SW of 1000 mm Hg ml. Values are mean ± S.E. \((n = 7)\).

* \( p < 0.05 \), control value after CHF versus corresponding normal control value before CHF.

** ** \( p < 0.05 \), ET-1 blocker versus corresponding control value.
**P-V Analysis.** The effect of L-754,142 on LV P-V relations after CHF is summarized in Table 3. A typical example of the effect of L-754,142 on variably loaded P-V relations from one animal with CHF is shown in Fig. 3. After CHF, L-754,142 produced a markedly leftward shift of the $P_{ES}-V_{ES}$ relation with an increased slope (3.4 ± 0.4 versus 4.8 ± 0.8 mm Hg/ml, $p < .05$). In addition, L-754,142 also increased the slopes of the $dP/dt_{max}-V_{ED}$ relation (42.4 ± 7.8 versus 50.0 ± 7.8 mm Hg/s/ml, $p < .05$) and the $SW-V_{ED}$ relation (58.1 ± 3.3 versus 72.4 ± 5.2 mm Hg, $p < .05$). This result shows that blocking endogenous ET-1 with L754,142 produces marked augmentation of LV contractility in CHF.

**Effect of Nitroprusside after CHF.** As shown in Table 4, nitroprusside produced similar decrease in LV PES (99 ± 4.4 versus 90 ± 4.3 mm Hg, $p < .05$) and a similar increase in coronary blood flow (43.3 ± 4.7 versus 49.2 ± 4.9 ml/min, $p < .05$) as produced by L-754,142. However, nitroprusside produced no significant increases in the slopes of $P_{ES}-V_{ES}$ (3.9 ± 0.7 versus 3.7 ± 0.7 mm Hg/ml), $dP/dt_{max}-V_{ED}$, and $SW-V_{ED}$ (59.5 ± 2.6 versus 58.5 ± 3.4 mm Hg) relations.

**LV-Arterial Coupling and Work Efficiency of LV.** We evaluated LV-arterial coupling and the $SW/PVA$ ratio in normal dogs and dogs with CHF at rest. Data are summarized in Table 5. In normal dogs, L-754,142 had no significant alternations in EES/EA, and $SW/PVA$. However, in dogs with CHF, L-754,142 significantly increased the EES/EA ratio (0.59 ± 0.07 versus 0.36 ± 0.03, $p < .05$). The SW/PVA ratio was also significantly augmented (0.45 ± 0.03 versus 0.37 ± 0.03, $p < .05$).

**Plasma ET-1 Activation.** In five of the seven studied dogs, the plasma ET-1 level was measured. The resting levels of ET-1 were 7.3 ± 1.7 fmol/ml before CHF and increased 2-fold to 14.1 ± 3.0 fmol/ml ($p < .05$) after CHF.

**Discussion**

We found in conscious dogs that the plasma ET-1 levels approximately double after the induction of CHF. Before CHF, ET-1 blockade has no direct effect on LV contractility and relaxation. However, after pacing-induced CHF, ET-1 blockade with L-754,142 improves LV contraction and relaxation independent of its vasodilatory effect. These results suggest that endogenous ET-1 may contribute to the functional impairment of both systolic and diastolic performance in CHF.

**Increased plasma ET-1 levels have been found in experimental (Cavero et al., 1990; Margulies et al., 1990; Teerlink et al., 1994; Shimoyama et al., 1996) and clinical (McMurray et al., 1992; Rodeheffer et al., 1992; Wei et al., 1994) CHF.**

**TABLE 4**

Effects of nitroprusside on the steady-state hemodynamics and P-V relations after CHF

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Nitroprusside</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>117 ± 8</td>
<td>118 ± 9</td>
</tr>
<tr>
<td>Peak $+dP/dt$ (mm Hg/s)</td>
<td>1629 ± 116</td>
<td>1741 ± 84*</td>
</tr>
<tr>
<td>Peak $-dP/dt$ (mm Hg/s)</td>
<td>-1666 ± 109</td>
<td>-1712 ± 114*</td>
</tr>
<tr>
<td>LVPE (mm Hg)</td>
<td>26.6 ± 4.0</td>
<td>19.4 ± 3.0*</td>
</tr>
<tr>
<td>LVPes (mm Hg)</td>
<td>97.0 ± 4.1</td>
<td>85.1 ± 4.9*</td>
</tr>
<tr>
<td>Minimum LVP (mm Hg)</td>
<td>9.9 ± 0.5</td>
<td>6.3 ± 1.4</td>
</tr>
<tr>
<td>TSS (mm Hg/ml/min)</td>
<td>0.088 ± 0.018</td>
<td>0.082 ± 0.019*</td>
</tr>
<tr>
<td>CBF (ml/min)</td>
<td>43.3 ± 4.9</td>
<td>49.2 ± 4.9*</td>
</tr>
<tr>
<td>$E_{ES}$ (mm Hg/ml)</td>
<td>3.9 ± 0.7</td>
<td>3.7 ± 0.7</td>
</tr>
<tr>
<td>$V_{0,ES}$ (ml)</td>
<td>13.1 ± 4.8</td>
<td>12.0 ± 4.3</td>
</tr>
<tr>
<td>$dE/dt_{max}$ (mm Hg/s/ml)</td>
<td>43.8 ± 2.2</td>
<td>43.3 ± 1.9</td>
</tr>
<tr>
<td>$V_{0, dP/dt}$ (ml)</td>
<td>9.4 ± 5.2</td>
<td>11.1 ± 5.2</td>
</tr>
<tr>
<td>$M_{SW}$ (mm Hg)</td>
<td>59.5 ± 2.6</td>
<td>58.5 ± 3.4</td>
</tr>
<tr>
<td>$V_{0,SW}$ (ml)</td>
<td>29.7 ± 4.9</td>
<td>29.5 ± 5.3</td>
</tr>
</tbody>
</table>

LVP, indicates LV pressure; CBF, coronary blood flow; $V_{0,ES}$, intercept with volume axis; $dE/dt_{max}$, slope of $dP/dt_{max}-V_{ED}$ relation; $V_{0, dP/dt}$, intercept with volume axis; $V_{0,SW}$, intercept with volume axis. Values are mean ± S.E. ($n = 4$).

* $P < .05$, ET-1 versus corresponding control value.

**TABLE 4**

Effects of nitroprusside on the steady-state hemodynamics and P-V relations after CHF

**Fig. 3.** LV P-V loops produced by transient caval occlusion after development of pacing-induced heart failure in same dog. L-754,142 produced leftward shifts of $P_{ES}-V_{ES}$ (top), $dP/dt_{max}-V_{ED}$ (bottom left), and $SW-V_{ED}$ (bottom right) relations with increased slopes. This indicates that L-754,142 improved LV contractile performance after CHF.
Consistent with these findings, we observed that plasma ET-1 levels increased about 2-fold in pacing-induced CHF. Both norepinephrine and angiotensin II (which are elevated in CHF) increase the expression of prepro-ET-1 mRNA in cultured endothelial cells (Masaki et al., 1991). Thus, these neurohormones may contribute to the increased ET-1 in CHF. In addition, shear stress and stretch stimulate endothelial cell production in ET-1 (Emori et al., 1991) and may be an increased conversion of big ET to ET-1 in CHF (Teerlink et al., 1994).

Although ET-1 increases systemic vascular resistance (Miller et al., 1989; Luscher et al., 1991; Kiowski et al., 1995) and coronary resistance, we found that blocking the effect of the low endogenous level of ET-1 in normal animals had no detectable effect on systolic pressure, systemic arterial resistance, and coronary flow or resistance. This is consistent with previous observations (Shimoyama et al., 1996). However, after CHF, ET-1 blockade decreased PES and TSR and increased coronary flow. These data indicate that endogenous ET-1 does not normally play a substantial role in the regulation of resting arterial blood pressure. However, after CHF, the elevated ET-1 levels contribute to both systemic and coronary vasoconstriction.

To avoid the potentially confounding effects of the influence of ET-1 on loading conditions on conventional measures of LV performance, we evaluated LV contractile performance in the P-V plane (Kass and Maughan, 1988; Little et al., 1989). We found that before CHF, exogenous ET-1 infusion caused an increase in the slope of LV P-V relations, which is consistent with previous studies of normal myocardium (Watanabe et al., 1989; Neubauer et al., 1990). However, ET-1 blockade had no effect on LV P-V relations, suggesting that the low levels of ET-1 had no direct cardiac effects in normal subjects. In contrast, after CHF, ET-1 blockade produced a significant improvement in LV contractile performance, as indicated by the increased slopes and leftward shift of the LV P-V relations. These effects are independent on the alterations of loading conditions and coronary blood flow because equally hypotensive doses of nitroprusside produced a similar increase in coronary blood flow, but there was no change in the LV P-V relations. Thus, it appears that the beneficial effect of blocking ET-1 receptors is due to a removal of the direct inhibition by ET-1 of LV contraction and relaxation. This view is supported by the study of the effect of chronic ET α receptor blockade in the rabbit with pacing-induced CHF (Spinale et al., 1997). They found that chronic rapid ventricular pacing, plus concomitant ET α receptor blockade (without marked change in blood pressure), significantly improved LV and cardiomyocyte functional performance, normalized myocyte inotropic responses to calcium and β-adrenergic stimulation, and improved survival.

In the present study, after CHF, the plasma ET-1 was doubled. However, the local levels (in which myocardium exposed) of ET-1 might be much higher than the plasma ET-1 concentrations. For example, Loffler et al. (1993) found that the ET concentration in ventricle was roughly 4 orders of magnitude higher than the plasma concentration in normal rabbits (Loffler et al., 1993). Furthermore, in CHF, the myocardial ET system is up-regulated, producing much higher cardiac ET-1 levels than in normal (Wei et al., 1994; Kiowski et al., 1995; Sakai et al., 1996). Thus, it is likely that the much elevated cardiac ET-1 levels are responsible for the endogenous ET-1-induced cardiac depression in CHF. This finding is in agreement with the studies performed in anesthetized dogs, in which Lerman et al. (1991) demonstrated that a 2-fold increase in circulating levels of ET-1 was sufficient to locally reach a threshold that facilitate the appearance of coronary spasm. Similarly, Yang et al. (1990) concluded that subthreshold concentration of ET-1 could facilitate the appearance of human vascular spasm. It has also been shown that low ET-1 levels (0.1 nM) inhibited substance pase-increased dilation in middle cerebral canine arteries.

The present observations of endogenous ET-1-depressed LV contraction and relaxation in CHF are similar to our previous observations with angiotensin II (Cheng et al., 1996), that the inotropic effect is reversed in CHF, but the magnitude of the angiotensin II-induced cardiac depression was higher. A similar reversal of the inotropic effect of ET-1 has been observed in immature myocytes (Kohmoto et al., 1993), in hypertrophy (Ito et al., 1997), and in isoproterenol-induced CHF (Suzuki et al., 1998). It is possible that there would be an additive effect of ET-1 and angiotensin II type 1 receptor blockade.

Our observations are also consistent with several studies showing that acute ET-1 receptor blockade improves LV myocardial function (Kiowski et al., 1995; Cheng et al., 1996; Shimoyama et al., 1996) and survival in CHF (Spinale et al., 1997). The current findings of the beneficial cardiac effect with ET α receptor blockade also are compatible with the recent observations in a study of the chronic actions of ET α receptor blockade (Spinale et al., 1997). Our results differ from the observations of the Li and Rouleau (1996) and Sakai et al. (1996). In an anesthetized left coronary artery ligated rat model of CHF, Sakai and colleagues reported that blocking the ET α receptor with BQ123 induced a negative inotropic action. Similarly, using isolated papillary muscle from normal dogs and from dogs with CHF induced by pacing, Li and Rouleau found that ET-1 caused a positive inotropic response. These inconsistencies may have resulted from the influence of ET-1- or ET-1 receptor blocker-produced changes in loading conditions on conventional measures of LV performance, variable effect of anesthesia, and different levels of CHF.

### Table 5

<table>
<thead>
<tr>
<th></th>
<th>Before CHF</th>
<th>ET-1 blocker</th>
<th>After CHF</th>
<th>ET-1 blocker</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E_P/V</strong></td>
<td>0.78 ± 0.03</td>
<td>0.80 ± 0.07</td>
<td>0.36 ± 0.03</td>
<td>0.59 ± 0.07*</td>
</tr>
<tr>
<td><strong>SW (mm Hg ml)</strong></td>
<td>1566 ± 153</td>
<td>1544 ± 111</td>
<td>985 ± 182</td>
<td>1074 ± 199*</td>
</tr>
<tr>
<td><strong>SW/PVA</strong></td>
<td>0.53 ± 0.02</td>
<td>0.57 ± 0.03</td>
<td>0.37 ± 0.03</td>
<td>0.45 ± 0.03*</td>
</tr>
</tbody>
</table>

*P < .05 versus control after CHF.

E_P, arterial elastance; SW, stroke work; PVA, LV pressure-volume area. Values are mean ± S.E. (n = 7).
Our present study did not address the mechanism of endogenous the action of ET-1 in CHF. Earlier studies suggest that although β-adrenergic receptors are down-regulated and uncoupled in CHF, ET-1 receptors are not reduced in CHF and may even be increased (Wei et al., 1994; Kiowski et al., 1995; Sakai et al., 1996). The effects of ET-1 on myocardial contraction are partially mediated through the inositol triphosphate/protein kinase C pathway that increases the mobilization and reuptake of cytosolic Ca$^{2+}$ and alters Ca$^{2+}$ channel activity and myofibrillar Ca$^{2+}$ sensitivity (Capogrossi et al., 1990; Rogers et al., 1990). These changes may result in the inotropic response seen in the dogs before CHF. In CHF, there is altered Ca$^{2+}$ handling with impaired [Ca$^{2+}$i], hemostasis. Both protein kinase A- and protein kinase C-mediated signal transduction systems are disrupted in CHF (Morgan, 1993; Ishikawa and Homcy, 1997). CHF may alter the protein kinase C activity and expression (Prasad and Jones, 1992). Also, there is a decreased stimulatory G protein but increased inhibitory G protein (Spinale et al., 1994; Ito et al., 1997). Thus, ET-1-induced activation of altered protein kinase C mediates pathway or G protein may exacerbate the dysfunctional Ca$^{2+}$ homeostasis, which might account for the further impairment in myocardial contraction and relaxation that we observed after CHF.

There are several methodological issues that should be considered in interpreting our data. First is the experimental model of CHF. Although rapid pacing produces an animal model of CHF that closely mimics clinical congestive cardiomyopathy (Cheng et al., 1993), we cannot be certain our results apply to CHF that is due to other causes. Incessant tachycardia does lead to clinical CHF in patients.

Second, in the present study, a mixed ETA and ETB receptor antagonist was used. Because ET-1 has two receptors, ETA and ETB, both of which are distributed in various tissues and cells and may be involved in the pressure and cardiac responses (Seo et al., 1994; Beyer et al., 1996), the extent to which endogenous ET-1 affects cardiac performance and hemodynamics through each receptor type (ETA and ETB) in CHF is not addressed in our study.

Third, we studied the effect of acute block of the action of ET-1. Some of the biological actions of ET-1 may take weeks to reverse. These effects, if any, cannot be determined from our study. However, our study does demonstrate that block of the effect of endogenous ET-1 produces an acute hemodynamic response in CHF that is different from the effect before CHF.

Angiotensin-converting enzyme inhibitors are beneficial in patients with CHF (Williams et al., 1995b). These effects have been mainly attributed to a reduction in neurohormonal activation by interfering with the formation of angiotensin II. A recent study indicates an important role of angiotensin II in the activation of ET-1 in cultured cardiomyocyte (Emori et al., 1991). Clavell et al. (1996) have also shown, by using chronic thoracic inferior vena caval constriction in conscious dogs, that chronic angiotensin-converting enzyme inhibition with low-dose enalapril abolishes the increases in circulating and tissue ET-1 as well as angiotensin II concentrations. Our study suggested that the inhibition of the elevated endogenous ET-1 in CHF improved cardiac function. The beneficial effects of angiotensin-converting enzyme inhibition may be achieved partially by the reduction of ET-1 as well as angiotensin II.

In conclusion, the present study demonstrates that the plasma levels of ET-1 are increased in CHF and that ET-1 receptor blockade in a model of CHF has direct beneficial effects on LV contraction and relaxation. Thus, endogenous ET-1, despite its positive inotropic action in normal myocardium, may contribute to the impairment of LV contraction and relaxation in CHF.

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