Chronic endothelin-1 blockade reduces sympathetic nerve activity in rabbits with heart failure


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Liu, J.-L., R. U. Pliquett, E. Brewer, K. G. Cornish, Y.-T. Shen, and I. H. Zucker. Chronic endothelin-1 blockade reduces sympathetic nerve activity in rabbits with heart failure. Am J Physiol Regulatory Integrative Comp Physiol 280: R1906–R1913, 2001.—Endothelin-1 (ET-1) is elevated in chronic heart failure (CHF). In this study, we determined the effects of chronic ET-1 blockade on renal sympathetic nerve activity (RSNA) in conscious rabbits with pacing-induced CHF. Rabbits were chronically paced at 320–340 beats/min for 3–4 wk until clinical and hemodynamic signs of CHF were present. Resting RSNA and arterial baroreflex control of RSNA were determined. Responses were determined before and after the ET-1 antagonist L-754,142 (a combined ETA and ETB receptor antagonist, n = 5) was administered by osmotic minipump infusion (0.5 mg·kg−1·h−1 for 48 h). In addition, five rabbits with CHF were treated with the specific ETA receptor antagonist BQ-123. Baseline RSNA (expressed as a percentage of the maximum nerve activity during sodium nitroprusside infusion) was significantly higher (58.3 ± 4.9 vs. 27.0 ± 1.0, P < 0.001), whereas baroreflex sensitivity was significantly lower in rabbits with CHF compared with control (3.09 ± 0.19 vs. 6.04 ± 0.73, P < 0.001). L-754,142 caused a time-dependent reduction in arterial pressure and RSNA in rabbits with CHF. In addition, BQ-123 caused a reduction in resting RSNA. For both compounds, RSNA returned to near control levels 24 h after removal of the minipump. These data suggest that ET-1 contributes to sympathoexcitation in the CHF state. Enhancement of arterial baroreflex sensitivity may further contribute to sympathoinhibition after ET-1 blockade in heart failure.

baroreflex; ET receptors; autonomic nervous system; myocardial dysfunction; neurohormones

ENDOTHELIN-1 (ET-1) is a 26-amino acid peptide with potent vasoactive properties. ET-1 is produced from vascular endothelial cells and other tissues including neural tissue. ET-1 is elevated in patients and animals with chronic heart failure (CHF) and correlates with the severity of the CHF state (2, 3, 19, 25). Furthermore, ET-1 is elevated in plasma and tissues of rabbits with CHF (6, 35, 40). ET-1 stimulates a variety of tissues primarily through two membrane receptors, namely the ETA and ETB receptors. The therapeutic efficacy of ET-1 antagonists has been evaluated in patients and animals with CHF; ET-1 antagonists have been beneficial in the treatment of CHF (37, 38, 42). ET-1 blockade has been shown to reduce plasma norepinephrine in experimental CHF (21, 22). Furthermore, treatment with the ET-1 receptor antagonist bosentan lowered arterial pressure without an increase in heart rate (HR) or plasma norepinephrine (14). Changes in plasma norepinephrine can be mediated by alterations in both release and uptake as well as changes in the metabolic clearance of this catecholamine. Therefore, it is important to assess the direct effects of ET-1 receptor blockade on sympathetic outflow in a model of CHF. Although one study has shown a reduction in sympathetic nerve activity (27) in response to central administration of ET-1 antagonists in the hypertensive state, there have been no studies in which direct measurements of sympathetic nerve activity have been made in the CHF state after administration of ET-1 antagonists in conscious, chronically instrumented animals.

The present study was therefore carried out to determine if systemic ET-1 blockade altered renal sympathetic nerve activity (RSNA) and arterial baroreflex function in conscious, instrumented rabbits with pacing-induced CHF. The use of two different types of receptor antagonists allowed us to evaluate which receptor subtype was primarily involved in any sympathoinhibition observed.

METHODS

Experiments were carried out on 15 male New Zealand White rabbits weighing between 2.5 and 3.5 kg. All experiments were reviewed and approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee. All experiments conformed to the guidelines for care and use of laboratory animals of the American Physiological Society and the National Institutes of Health.

Surgical Instrumentation

Rabbits were instrumented as described earlier (16, 18). In brief, using sterile technique we performed a left thoracotomy through the fourth intercostal space. A pair of 2-mm piezoelectric crystals (Sonometrics, London, Ontario, Canada) was sutured across the left ventricle from anterior to posterior.
posterior near the base of the ventricle. The crystals were used to monitor changes in left ventricular diameter during the development of heart failure. A platinum-wire pacing electrode was secured to the apical surface of the left ventricle. The chest was closed in layers, and the air was evacuated. All wires were tunnelled beneath the skin and exited in the mesocardiac area. The animals were treated with the antibiotic Baytril (Bayer, Shawnee Mission, KS) 2.3 mg/kg im twice per day for 3 postoperative days and allowed to recover for ~2 wk before being used in any experiment.

**Induction of Heart Failure**

After recovery from surgery, each rabbit in the heart failure groups was paced with a small, lightweight pacing unit of our own design. The pacing rate was adjusted and monitored according to the cardiac dimension tracings. In general, each rabbit was paced at 320 beats/min for the first week to determine if it would tolerate this rate. If so, after the first week, the pacing rate was increased to 340 beats/min and left at this rate for the remainder of the protocol. The rabbits were continually paced for ~4 wk, at which time a second surgery was performed to implant the renal nerve recording electrodes and vascular catheters. To monitor the degree of cardiac dilation during the pacing protocol, we took recordings of cardiac dimensions and HR twice per week. During experiments, the rabbits were placed into Plexiglas boxes that limited movement. The pacemaker was turned off for 30 min before any data were recorded.

**Renal Sympathetic Nerve and Arterial Pressure Recording**

The renal sympathetic nerve recording electrodes were implanted as described previously (16, 18). In brief, pacing was temporarily halted during the surgery and was reinstated after recovery from anesthesia. Teflon-coated (except at the distal 1–2 mm) wire electrodes were wrapped around one or two renal sympathetic nerves that ran along the renal artery. A ground electrode was secured to the nearby muscle or perirenal fat. The entire electrode assembly was then covered in a silicone gel (Wacker Sil-Gel 401 A and B; Wacker-Chemie, Munich, Germany). The electrode wires were tunneled beneath the skin and exited in the mesocardiac area.

A Tygon (Fischer Scientific, Houston, TX) catheter was implanted into the left carotid artery and jugular vein so that arterial and central venous pressures could be recorded and drugs could be administered during the experiment. Catheters were filled with heparin (1,000 U/ml) and sealed until the day of the experiment. Cannulation of one carotid artery did not alter baroreflex function in conscious rabbits. In more recent studies (J.-L. Liu et al., unpublished observations), we saw similar baroreflex sensitivity (BRS) when arterial pressure was measured from an implanted thoracic aortic catheter.

**Protocol**

Experiments were carried out 2–3 days after electrode implantation. The rabbits were divided into three groups of five animals each. Two CHF groups received either L-754,142 or BQ-123 as described below. The third sham group received L-754,142. On the day of the experiment, the rabbit was placed in the Plexiglas box as described in Induction of Heart Failure. Baseline recordings of RSNA, arterial pressure, central venous pressure, and HR were taken for several minutes. We determined maximal RSNA in each rabbit by observing its response to a bolus injection of sodium nitroprusside (SNP; 100 μg/kg iv) that lowered arterial pressure to between 45 and 50 mmHg. Arterial baroreflex control of RSNA was determined by the response to a bolus injection of SNP (100 μg/kg iv) and phenylephrine (PE; 30 μg/kg iv) given in random order.

This protocol was repeated 24 and 48 h after implantation of an osmotic minipump (Alzet model 1003D; Alza Pharmaceuticals, Palo Alto, CA) containing the combined ETA and ETB receptor antagonist L-754,142 (0.5 mg·kg⁻¹·h⁻¹) or the selective ETA receptor antagonist BQ-123 (0.5 mg·kg⁻¹·h⁻¹). Finally, the experiment was repeated 24 h after removal of the osmotic minipump. In a separate group of rabbits (n = 4), these doses of L-754,142 and BQ-123 produced an 86.8 ± 10.8% and 28.0 ± 9.5% reduction in the maximum pressor response to an intravenous injection of 1.84 mg/kg of ET-1 (Sigma), respectively.

**Data Analysis**

RSNA. All parameters were recorded on a MacLab data acquisition and analysis system (model 8S; ADInstruments, Mountain View, CA). Hemodynamic parameters were digitized at 100 samples/s. RSNA was digitized at 200 samples/s and preamplified with a Grass P15 preamplifier with the bandwidth set between 30 Hz and 1 KHHz. The raw nerve activity was rectified and integrated. In addition, the frequency of nerve activity was determined by setting a cursor ~10% above the noise level (determined by a bolus of PE). The MacLab system saves the position of the cursor so that it appears in the same place each time an experiment is conducted on a given rabbit. Over the 4 days of this experiment, the noise level did not change. Both frequency and integrated nerve activity were recorded continuously along with the raw nerve activity. The baseline and baroreflex data were expressed as a percentage of maximum activity.

Cardiac dimensions. Left ventricular external dimensions were recorded with a Triton Electronics sonomicrometer (model system 6 200–1000; San Diego, CA). End diastolic, end systolic, and mean diameters were recorded on the MacLab system. The maximum velocity of diameter change (dD/dt max; mm/s) was computed. For all parameters, the average of five consecutive beats was determined when the rabbit was standing quietly in the box.

Arterial baroreflex. Arterial baroreflex curves were constructed in our laboratory as described previously (17, 26). In brief, several points for HR and RSNA were taken during the fall or increase in arterial pressure after the administration of SNP and PE, respectively. A logistic regression curve as described by Kent et al. (13) was fit to the data using the following equation:

\[
\text{RSNA or } HR = \frac{A}{[1 + \exp(B(MAP-C))] + D}
\]  

(1)

where A is the RSNA or HR range, B is the slope coefficient, MAP is mean arterial pressure, C is the pressure at the midpoint of the range (BP50), and D is minimum RSNA or HR (13). The peak slope (or maximum gain) was determined by taking the first derivative of the baroreflex curve described by the equation above. The first derivative is described by the equation:

\[
\text{Slope} = \frac{[A \times B \times \exp(B(MAP-C))] / ([1 + \exp(B(MAP-C))] - D)}
\]  

(2)

The mean value of each curve parameter was used to derive a composite curve for each group of rabbits before and after drug administration.
Statistical Analysis

Data are expressed as the means ± SE. The differences between time periods within a group were determined by a two-way ANOVA for repeated measures where the two factors were group and time. The Bonferroni procedure was used for post hoc analysis. The differences in baseline parameters between normal and CHF rabbits were determined by a two-tailed Student’s t-test. A P value of <0.05 was considered statistically significant.

RESULTS

Table 1 shows the resting hemodynamic, cardiac dimension, and heart weight data, and Figure 1 shows representative dimension tracings from the same rabbit before and after chronic ventricular pacing. Although MAP and HR were not different between these groups, there was a clear cardiac dilation as evidenced by an increase in end diastolic and end systolic cardiac dimension as well as a significant reduction in dD/dt max and fractional shortening.

In an effort to determine an index of resting sympathetic nerve activity, we evaluated RSNA in two ways, first in absolute terms in spikes/s and second as a percentage of the maximum activity in response to hypotension. There was a significant increase in resting RSNA in the CHF group compared with normals when these data were analyzed in absolute terms (76.0 ± 8 vs. 25.4 ± 3.9 spikes/s; Student’s t = 5.69, P < 0.01). Because this measurement depends on the number of active units in a given nerve and on the recording conditions, the RSNA was also expressed as a percentage of maximum activity. The data shown in Fig. 2 demonstrate a significantly greater RSNA in rabbits with CHF compared with normal rabbits before L-754,142 was administered (F = 3.11; P < 0.05).

The hemodynamic and RSNA responses before and after administration of L-754,142 are also shown in Fig. 2. Although arterial pressure fell slightly 24 h after minipump implantation in both groups, it reached significance only after 48 h of infusion in the rabbits with CHF (F = 4.39; P < 0.01). Removal of the osmotic minipump caused MAP to rise toward the baseline level in the rabbits with CHF. Despite the fall in MAP in the rabbits with CHF, there was no significant change in HR. Figure 2 demonstrates the marked effect of ETAB blockade on RSNA in CHF rabbits. A significant decrease in RSNA was observed at 24 h (F = 31.44; P < 0.01), and a further decrease was noticed at 48 h in the CHF group (P < 0.01). Removal of the osmotic minipump returned RSNA to near the baseline level within 24 h. L-754,142 had no effect on RSNA in normal rabbits (Fig. 2).

To determine if this decrease in RSNA was specific to blockade of the ETA receptor, we carried out a series of experiments on five CHF rabbits with the specific ET A receptor antagonist BQ-123. Figure 3 shows MAP, HR,

### Table 1. Hemodynamics, heart dimension, and heart weight in normal rabbits and in rabbits with CHF

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 5)</th>
<th>CHF (n = 10)</th>
<th>P</th>
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<tbody>
<tr>
<td>MAP, mmHg</td>
<td>78.0 ± 2.5</td>
<td>77.8 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>258.6 ± 7.0</td>
<td>253.9 ± 9.3</td>
<td>NS</td>
</tr>
<tr>
<td>CVP, mm Hg</td>
<td>−0.9 ± 0.4</td>
<td>5.9 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EDD, mm</td>
<td>21.6 ± 1.0</td>
<td>24.4 ± 0.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ESD, mm</td>
<td>19.5 ± 1.1</td>
<td>23.3 ± 0.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MD, mm</td>
<td>20.6 ± 1.1</td>
<td>23.9 ± 0.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>%Shortening</td>
<td>15.8 ± 3.4</td>
<td>4.6 ± 0.3</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>dD/dt max, mm/s</td>
<td>−15.8 ± 3.5</td>
<td>−9.7 ± 2.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HW/BW, g/kg</td>
<td>2.4 ± 0.06</td>
<td>3.3 ± 0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV/BW, g/kg</td>
<td>0.9 ± 0.01</td>
<td>1.1 ± 0.04</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE. CHF, chronic heart failure; NS, not significant; MAP, mean arterial pressure; HR, heart rate; CVP, central venous pressure; EDD, end diastolic diameter; ESD, end systolic diameter; MD, mean diameter; dD/dt max, first derivative of diameter change; HW/BW, heart wt-body wt ratio; LV/BW, left ventricular wt-to-body wt ratio.

Fig. 1. Original strip chart recordings of external cardiac diameter, the first derivative of diameter change (dD/dt) and heart rate (HR; beats/min) in a rabbit before (prepace) and 4 wk after chronic ventricular pacing at 320 beats/min. Both end diastolic and end systolic diameter are increased, and maximum dD/dt is reduced after 4 wk of pacing.
and RSNA data from these rabbits. A reduction in RSNA in response to BQ-123 was seen at the 24-h period ($P < 0.05$). At 48 h and during the recovery period, RSNA was not significantly decreased from control. Therefore, the response to BQ-123 was more transient than that to L-754,142.

**Arterial BRS**

To determine the effects of ET receptor blockade on BRS, we constructed baroreflex function curves from infusion of SNP or PE in CHF and normal

![Graphs showing changes in MAP, Heart Rate, and RSNA over time](image1)

**Fig. 2.** Changes in renal sympathetic nerve activity [RSNA, expressed as a percentage of maximum, normalized to the sodium nitroprusside (SNP) response], HR, and mean arterial pressure (MAP) in rabbits with chronic heart failure (CHF) and normal rabbits after osmotic minipump infusion of L-754,142 for 48 h and subsequent removal of the pump for 24 h. *Significantly different from the control state (see RESULTS for details).**

![Graphs showing changes in MAP, Heart Rate, and RSNA over time](image2)

**Fig. 3.** Changes in RSNA expressed as a percentage of maximum normalized to the SNP response, HR, and MAP in CHF rabbits after osmotic minipump infusion of BQ-123 for 48 h and subsequent removal of the pump for 24 h. *Significantly different from the control state (see RESULTS for details).**

rabbits before and after 48 h of treatment with L-754,142. Table 2 presents arterial baroreflex data for BP$_{50}$ and peak slope for the control of RSNA in normal rabbits and in rabbits with CHF before and during treatment with L-754,142. Figure 4 shows composite baroreflex curves for the control of RSNA in normal and CHF rabbits. These curves were constructed from the average curve parameters for each group and time period. It is clear from the BP$_{50}$ and slope data presented in Table 2 that L-754,142 caused a shift in the curve to a lower arterial pressure and at the same time increased the maximum gain or BRS in rabbits with CHF but had no significant effect in normal rabbits. After removal of the osmotic minipump, all curve parameters returned toward the pretreatment level.
The baroreflex control of HR was affected less than that for RSNA after treatment with L-754,142. These curves are shown in Fig. 5, and the BP50 and slope are shown in Table 3. L-754,142 caused an increase in maximum gain in the rabbits with CHF \((P, 0.05)\). Although there was a slight downward shift in the curve, the change in BP50 was not significant. There were no significant changes in baroreflex control of HR in normal rabbits in response to administration of L-754,142.

DISCUSSION

The data from the present investigation are to our knowledge the first to provide direct evidence for a sympathoinhibitory role of ET-1 receptor antagonists in the setting of CHF. Although there has been suggestive evidence supporting the view that ET-1 is a sympathoexcitatory substance (21, 22, 40), the current results provide further support for the notion that one mechanism by which ET-1 blockade may be beneficial in the treatment of CHF is through a sympathoinhibitory effect. Not only did ET-1 blockade reduce resting RSNA but it also enhanced BRS. The increase in BRS would predispose to sympathoinhibition. It is significant that these effects of ET-1 blockade were observed only in rabbits with CHF. Although normal rabbits showed a slight reduction in arterial pressure, no effect on RSNA was observed in rabbits with CHF. We did not measure plasma ET-1 concentration in this study; however, in a previous study this laboratory (21) carried out in a canine model of pacing-induced CHF, plasma ET-1 increased, consistent with many clinical and experimental observations including those in rabbits (3, 6, 35, 40).

The Role of ET-1 on the Sympathetic Nervous System

Sympathoexcitation is an important initial compensatory mechanism in CHF. There is clear evidence that elevations in plasma norepinephrine and sympathetic outflow contribute to the morbidity and mortality associated with CHF (30). The mechanisms responsible for elevation in sympathetic outflow are multiple and complex. Although sympathetic outflow is regulated by a variety of negative feedback reflexes such as the arterial baroreflex and cardiopulmonary reflexes, there are also several important humoral substances that modulate sympathetic outflow by a central effect.

<table>
<thead>
<tr>
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<th>Pretreatment</th>
<th>Posttreatment</th>
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<tr>
<td></td>
<td>BP50, mmHg</td>
<td>Peak Slope, %max/mmHg</td>
</tr>
<tr>
<td>Normal</td>
<td>69.8 ± 3.0</td>
<td>4.98 ± 0.14</td>
</tr>
<tr>
<td>CHF</td>
<td>78.1 ± 1.1‡</td>
<td>3.14 ± 0.39§</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>62.2 ± 2.2</td>
<td>5.74 ± 1.1</td>
</tr>
<tr>
<td>CHF</td>
<td>64.2 ± 2.9*</td>
<td>5.98 ± 0.5*</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>66.0 ± 2.1</td>
<td>5.60 ± 0.5</td>
</tr>
<tr>
<td>CHF</td>
<td>72.3 ± 1.8†</td>
<td>2.88 ± 0.4§</td>
</tr>
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Values are means ± SE. BP50, midpoint of blood pressure range; RSNA, renal sympathetic nerve activity. Significantly different from pre- and postdrug, *\(P < 0.005\); significantly different from the normal group, †\(P < 0.05\), ‡\(P < 0.01\), §\(P < 0.005\).

Fig. 4. Representative arterial baroreflex curves for the control of RSNA before, 48 h after, and after recovery from infusion of L-754,142 in CHF and normal rabbits. At bottom, the gain curves (first derivative) for each curve shown at top.
These substances include ANG II, nitric oxide, vasopressin, atrial natriuretic peptide, and ET-1. Animals with CHF that are treated with ET-1 receptor antagonists show an apparent improvement in cardiovascular function and a reduction in plasma norepinephrine (7, 37), although similar observations in humans have not been confirmed (37). In a canine model of CHF, our laboratory previously showed that chronic treatment with a specific ETA antagonist reduced the normal rise in plasma norepinephrine by 50%, seen during 4 wk of rapid ventricular pacing in the dog (21). Similar data have been presented by Moe et al. (22) using the same animal model but a different ETA antagonist. It is not possible from our data or from that of others to determine definitively if the fall in sympathetic outflow is directly mediated by a central effect of the antagonist or due to improvement in the hemodynamic state in the CHF animals. In a relevant study by Nakamura et al. (27), central administration of the ETA antagonist BQ-123 lowered sympathetic nerve activity in conscious, spontaneously hypertensive and in spontaneously hypertensive, stroke-prone rats. These investigators also demonstrated an increase in sympathetic outflow in response to central administration of ET-1 in normal rats. Many studies have now documented a central site of action of ET-1 on sympathetic outflow (8, 15, 20, 23). It is highly likely that the responses to both antagonists used in this study were mediated by blockade in the central nervous system (CNS). The protocol used in this study did not allow us to determine the exact site of action in the CNS, but many areas are candidates, such as the nucleus of the solitary tract and the rostro-ventrolateral medulla (24, 33, 34). In addition, there is evidence that ET-1 may have effects at baroreceptor nerve endings as well as at sympathetic ganglia (1, 5). In this regard, in the present study, ET-1 blockade with L-754,142 enhanced baroreflex control of RSNA by virtue of the fact that the maximum gain was increased during administration of this agent and was returned to depressed levels after removal of the osmotic minipump. The fact that this occurred only in rabbits with CHF strongly suggests that the depression of BRS in the setting of CHF is mediated, in part, by an ET mechanism.

**ET-1 Receptors Most Likely to Be Involved in Sympathetic Activation in CHF**

Although sympathetic nerve activity decreased with both agents, the time course and magnitude of the response differed slightly between the two agents. The fact that both combined ETA and ETB and a specific ETA receptor antagonist reduced resting sympathetic nerve activity in rabbits with CHF strongly suggests that the phenomenon and the observed effects are most likely

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**Table 3. Arterial baroreflex parameters for the control of HR before and after administration of L-754,142 in normal rabbits and in rabbits with CHF**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Peak Slope, %max/mmHg</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>73.0 ± 2.9</td>
</tr>
<tr>
<td>CHF</td>
<td>73.6 ± 3.0</td>
</tr>
<tr>
<td>48 h</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>71.7 ± 1.9</td>
</tr>
<tr>
<td>CHF</td>
<td>68.2 ± 5.5</td>
</tr>
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</table>

**Posttreatment**

| Normal       | 75.5 ± 1.5            | 4.90 ± 0.26 |
| CHF          | 73.2 ± 3.5            | 2.70 ± 0.54† |

Values are means ± SE. Significantly different from pre- and postdrug, *P* < 0.05; significantly different from the normal group, †P < 0.05.

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**Fig. 5.** Representative arterial baroreflex curves for the control of HR before, 48 h after, and after recovery from infusion of L-754,142 in CHF and normal rabbits. At bottom, the gain curves (first derivative) for each curve shown at top.
mediated by the ET\textsubscript{A} receptor. Interestingly, we achieved a greater blockade with the combined antagonist than with the ET\textsubscript{A} antagonist. It is possible that a higher dose of BQ-123 would be more effective in reducing RSNA and arterial pressure. The stimulation of ET\textsubscript{B} receptors has been associated with transient increases in the synthesis of nitric oxide (11, 12, 43), which is known to be sympathoinhibitory (9, 31, 32). Therefore, it is unlikely that the contribution of ET\textsubscript{B} receptor blockade is significant. ET\textsubscript{B} effects do not outweigh the sympathoexcitatory action due to ET\textsubscript{A} stimulation as seen in the experiments with L-754,142.

In summary, the current data provide evidence for a sympathoexcitatory effect of ET-1 in the setting of CHF. This effect is most likely mediated by the ET\textsubscript{A} receptor. We propose that activation of sympathetic outflow by ET-1 may contribute to the downward spiral of deterioration during the progression of CHF and thus would be a novel target for potential therapeutic intervention.

**Perspectives**

The sympathoexcitation that occurs in the CHF state is complex and multifactorial. There is good agreement that several humoral factors may be responsible for activating the sympathoexcitatory nervous system in CHF. Cardiovascular reflex alterations may also be, in part, responsible for sympathetic stimulation. Over the past several years, it has been repeatedly demonstrated that the peptide ET-1 is elevated in the CHF state. At present, it is unclear why this peptide is elevated in CHF. Although this peptide, like ANG II, is a potent vasoconstrictor, it also possesses other biological effects, including those related to growth and neural activation. The data provided by the current study are the first to document increases, associated with ET-1, in central sympathetic outflow in an animal model of CHF.

Several studies clearly show a beneficial effect of ET-1 blockade on hemodynamics and mortality in the CHF state in both animals and humans (25, 28, 36, 39). Because sympathoexcitation appears to be a deleterious consequence of CHF (4, 30), it is important to understand the mechanisms that are involved in this phenomenon. Blockade of the adrenergic nervous system has proved to be a highly beneficial therapeutic modality in the management of patients with CHF (29). Therefore, it would seem appropriate to reduce sympathetic outflow at its source, namely the CNS. For instance, such an approach has recently been taken for the imidazoline receptors (10, 41). The current study provides an important rationale for a similar approach targeted against the ET receptor.

The authors thank J. H. Hackley and P. Curry for expert technical assistance. We thank Merck for kindly donating a supply of L-754,142 and BQ-123.

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