The Effects of Endothelin-A Receptor Blockade During the Progression of Pacing-Induced Congestive Heart Failure

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**Objectives.** We sought to identify the effects of endothelin (ET) subtype-A (ET\(_A\)) receptor blockade during the development of congestive heart failure (CHF) on left ventricle (LV) function and contractility.

**Background.** Congested heart failure causes increased plasma levels of ET and ET\(_A\) receptor activation.

**Methods.** Yorkshire pigs were assigned to four groups: 1) CHF: 240 beats/min for 3 weeks; n = 7; 2) CHF/ET\(_A\)-High Dose: paced for 2 weeks then ET\(_A\) receptor blockade (BMS 193884, 50 mg/kg, b.i.d.) for the last week of pacing; n = 6; 3) CHF/ET\(_A\)-Low Dose: pacing for 2 weeks then ET\(_A\) receptor blockade (BMS 193884, 12.5 mg/kg, b.i.d.) for the last week, n = 6; and 4) Control: n = 8.

**Results.** Left ventricle fractional shortening decreased with CHF compared with control (12 ± 1 vs. 39 ± 1%, p < 0.05) and increased in the CHF/ET\(_A\) High and Low Dose groups (23 ± 3 and 25 ± 1%, p < 0.05). The LV peak wall stress and wall force increased approximately twofold with CHF and remained increased with ET\(_A\) receptor blockade. With CHF, systemic vascular resistance increased by 120%, was normalized in the CHF/ET\(_A\) High Dose group, and fell by 43% from CHF values in the Low Dose group (p < 0.05). Plasma catecholamines increased fourfold in the CHF group and were reduced by 48% in both CHF/ET\(_A\) blockade groups. The LV myocyte velocity of shortening was reduced with CHF (32 ± 3 vs. 54 ± 3 \(\mu\)m/s, p < 0.05), was higher in the CHF/ET\(_A\) High Dose group (39 ± 1 \(\mu\)m/s, p < 0.05), and was similar to CHF values in the Low Dose group.

**Conclusions.** ET\(_A\) receptor activation may contribute to the progression of LV dysfunction with CHF.

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Activation of the endothelin (ET) receptor, particularly the subtype A receptor (ET\(_A\)), has been demonstrated to modulate a wide variety of biological processes including vascular tone and myocardial contractile function (1–9). Increased plasma endothelin levels have been identified in patients with significant significant left ventricle (LV) dysfunction and hemodynamic compromise (10–14). Accordingly, it has been postulated that chronic activation of the ET\(_A\) receptor may contribute to the severity of the LV dysfunction in the setting of congestive heart failure (CHF). Chronic pacing tachycardia causes time-dependent changes in LV function and geometry with progressive hemodynamic compromise (15–22). The progression of this cardiac disease process with respect to changes in LV function and neurohormonal activation is similar to the functional and neurohormonal spectrum observed in patients with progressive CHF (10,13,23–26). More importantly, increased plasma endothelin levels have been observed to occur during the progression of pacing-induced CHF (15,18,19,22). The overall goal of this project was to examine whether institution of ET\(_A\) receptor blockade during the progression of pacing-induced CHF would significantly influence LV function.

There are several potential mechanisms by which ET\(_A\) receptor blockade may cause changes in LV pump function with developing CHF. First, ET\(_A\) receptor inhibition may influence systemic vascular resistance properties and thereby LV loading conditions. Second, ET\(_A\) receptor blockade may cause effects on LV contractile performance through local modulation of myocardial ET\(_A\) receptor activity. To determine whether the effects of ET\(_A\) receptor blockade were primarily due to alterations in LV loading conditions, ET\(_A\) receptor blockade was initiated using two different dosing strategies that would cause differential effects on systemic blood pressure. In light of the significant changes in LV loading conditions and neurohormonal status that occur with CHF, determination of...
whether and to what degree ETA receptor blockade influences intrinsic properties of LV contractile function in vivo would be problematic. Accordingly, the present project examined the effects of ETA receptor blockade with the development of pacing-induced CHF on isolated myocyte contractile function and inotropic responsiveness.

**Methods**

**Dose determination studies.** To determine the dosage strategy for ETA receptor blockade, 15 Yorkshire pigs (20 kg, male) were chronically instrumented in order to measure arterial blood pressure in the conscious state as described previously (15). Following a recovery period of 7 to 10 days, the animal was returned to the laboratory for an endothelin-1 pressor response study. For these studies, the animals were sedated with diazepam (20 mg, p.o., Valium, Hoffmann-La Roche, Nutley, NJ) and placed in a custom-designed sling that allowed the animal to rest comfortably. All studies were performed in the conscious state without additional use of sedation. The vascular access port was entered using a 12-gauge Huber needle (Access Technologies, Skokie, IL) and basal, resting arterial pressure and heart rate were recorded. Pressures from the fluid-filled aortic catheter were obtained using an externally calibrated transducer (Statham P23ID, Gould, Oxnard, CA). The electrocardiogram (ECG) and pressure waveforms were recorded using a multichannel recorder (Western Graphtec, FWR3701, Irvine, CA) as well as digitized on computer for subsequent analysis at a sampling frequency of 250 Hz (80386 processor, Zenith Data Systems, St. Joseph, Michigan).

Following these baseline measurements, a bolus of endothelin-1 (10 μg, Sigma, St. Louis, MO) was administered, and heart rate and blood pressure recorded at 0 to 20 min following endothelin-1 (ET-1) administration. The animals were allowed to recover from the ET-1 pressor challenge for 48 h and then entered into the dose determination protocol. For this portion of the study, 10 pigs were assigned to receive either a high dose of the ETA receptor antagonist (50 mg/kg, b.i.d.) or a low dose (12.5 mg/kg, b.i.d.) for 3 days. The animals were returned to the laboratory and baseline blood pressure and ET-1 pressor response studies repeated.

In the group receiving the low dose of the ETA receptor antagonist, resting blood pressure was reduced from control values (91 ± 4 vs. 102 ± 3 mm Hg, p < 0.05) and the ET-1 pressor response was reduced by approximately 50% (Fig. 1). The high dose of the ETA receptor antagonist reduced basal state mean arterial pressure from both control and low dose ETA antagonist values (84 ± 4, p < 0.05) and eliminated the ET-1 pressor response (Fig. 1). In preliminary titration and pharmacokinetic studies, the half-life of this ETA receptor antagonist was computed to be approximately 6 h. Although these studies provided the basis for dose selection in the following pacing CHF studies, it must be recognized that the ET-1 pressor response is an indirect assessment of adequate ETA receptor inhibition.

**Experimental protocol and animal preparation.** Following the selection of the high and low dose of the ETA receptor antagonist to be used, the second part of this study was initiated. Age- and weight-matched pigs (Yorkshire, 20 to 21 kg) were randomly assigned to each of four groups: 1) rapid atrial pacing (240 beats/min) for 3 weeks (n = 7); 2) rapid pacing for 2 weeks at which time the ETA receptor antagonist was administered at 50 mg/kg, b.i.d.-p.o. for the final 7 days of the pacing protocol (n = 6); 3) rapid pacing for 2 weeks at which time the ETA receptor antagonist was administered at 12.5 mg/kg, b.i.d.-p.o. for the final 7 days of the pacing protocol (n = 6); and 4) sham controls (n = 8). The time point for institution of either ETA receptor blockade was chosen based upon past studies that have demonstrated significant LV pump dysfunction and neurohormonal activation by day 14 of rapid pacing (17,19,22).

Pacemakers were implanted or sham procedures performed with animals anesthetized as described in the previous section, and through a left thoracotomy, a shielded stimulating electrode was sutured onto the left atrium, connected to a modified programmable pacemaker (8329, Medtronic, Inc., Minneapolis, MN) and buried in a subcutaneous pocket. Vascular access...
ports were also implanted as described in the previous section. Seven to 10 days following recovery from the surgical procedure, a baseline LV ECG study was performed as described in the following section, and the pacing protocols were initiated. Cardiac auscultation and an electrocardiogram were performed frequently during the pacing protocol to ensure proper operation of the pacemaker and the presence of 1:1 conduction. The sham-operated controls were cared for in identical fashion with the exception of the pacing protocol. All animals were treated and cared for in accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals” (National Research Council, Washington, DC, 1996).

LV function and hemodynamic measurements. On the morning of day 21 of the study protocol and 2 h following the AM drug dose (ET\textsubscript{A} receptor antagonist groups only), the animals were brought to the laboratory, an ECG established, and the pacemaker deactivated (pacing groups only). After a 30-min stabilization period, two-dimensional and M-mode echocardiography studies (ATL Ultramark VI, 2.25 MHz transducer, Bothell, WA) were used to image the LV from a right parasternal approach (20,21). Arterial blood pressure was simultaneously measured from the arterial access port. From the arterial catheter, 30 cc of blood was drawn into chilled tubes containing EDTA (1.5 mg/ml), and centrifuged (2,000 g, 10 min, 4°C). The plasma was placed in separate tubes, frozen in liquid nitrogen, and stored at −80°C until the time of neurohormonal assay. A second arterial sample was drawn for a room air blood gas determination. From the simultaneously measured LV echocardiogram and aortic pressure recordings, LV fractional shortening, LV peak wall stress, and wall force were determined. The LV fractional shortening was calculated as (end-diastolic dimension – end-systolic dimension)/end-diastolic dimension and was expressed as a percent. Peak circumferential wall stress was computed using a spherical model: $\sigma = \frac{P}{2\pi r^2} \times 1.36$; where $P =$ arterial peak systolic blood pressure, $D =$ minor axis dimension at end-diastole, and $h =$ end-diastolic wall thickness. Assuming a spherical geometry for the LV, net LV wall force at peak systole was computed as: $\text{Force} = \frac{P \pi r^2}{2} \times 1.36$; where $P =$ arterial systolic peak blood pressure and $r =$ is the internal LV chamber radius (27,28).

To obtain a full hemodynamic profile, catheterization studies were also performed under identical anesthetic conditions. The animals were anesthetized with isoflurane (0.5%/1.5 L/min) through a nonrecirculating anesthesia circuit. A multilumened thermodilution catheter (7.5F, Baxter Healthcare Corp., Irvine, CA) was positioned in the pulmonary artery via the right external jugular vein. Using this catheter, thermodilution-derived cardiac output, pulmonary artery pressures, and pulmonary capillary wedge pressure were measured. To ensure no damping of the arterial pressure trace occurred, a 20-cm sheath was placed into the left carotid artery and advanced to the ascending aorta for additional pressure measurements. The ECG and pressure waveforms were recorded and digitized as described in the previous section. Following these measurements and maintaining a surgical plane of anesthesia, a sternotomy was performed, and the heart quickly extirpated and placed in a phosphate-buffered ice slush. The great vessels were removed at the aortic and pulmonary valves, and the LV was quickly weighed. The region of the LV free wall perfused by the circumflex artery (5 × 5 cm) was excised and prepared for myocyte isolation.

Neurohormonal assays. Plasma renin activity (PRA) was determined by computing angiotensin I production using a radioimmunoassay (NEA-026, New England Nuclear, Boston, MA). The interassay variation for the PRA measurements was 15%. For the ET assays, the plasma was first eluted over a cation exchange and ET determined by high sensitivity radioimmunoassay as described previously by this laboratory (18). The interassay variation was 10% and the intraassay variation was 9% for the ET radioimmunoassay procedure. In preliminary studies, the ET\textsubscript{A} antagonist (BMS-193884) was added to normal plasma at a final concentration of 5 μM and the ET assay performed. The variation in ET levels between normal plasma and plasma spiked with the ET\textsubscript{A} antagonist was less than 10%. Thus, the presence of the ET\textsubscript{A} receptor antagonist in plasma samples did not interfere with the ET immunoassay procedure. Plasma norepinephrine and epinephrine were measured using high performance liquid chromatography and normalized to pg/ml of plasma.

Myocyte isolation and contractile function. The LV isolated myocytes were prepared as described previously (17–21). Isolated LV myocyte contractility was then examined using computer-assisted video-microscopy (19–21). Variables computed from the LV myocyte contraction profiles include percent shortening, velocity of shortening, velocity of relengthening, time to peak contraction, and duration of contraction. Through the use of increased extracellular Ca\textsuperscript{2+} or beta-adrenergic receptor (β-receptor) stimulation, the capacity of the myocyte to respond to an inotropic stimulus can be examined (17–21). The development of CHF in patients and animals has been reported to be associated with abnormalities in inotropic responsiveness (16,21,25). Accordingly, myocyte response to a specific inotropic stimulus was determined in all four treatment groups. Following the determination of baseline contractile function, myocyte contractile function was examined in the presence of either 8 mM extracellular Ca\textsuperscript{2+}, 25 nM isoproterenol (-Isoproterenol, Sigma, St. Louis, MO) or 200 pM ET-1 (E7764, Sigma, St. Louis, MO). The concentrations for isoproterenol and ET-1 used in this study had been demonstrated previously to provide near maximal contractile response for this myocyte preparation (18,21).

Data analysis. Indices of LV and myocyte function were compared among the treatment groups using analysis of variance. For the myocyte function studies, an analysis of variance (ANOVA) using a randomized block split-plot design was employed. The treatment effects were pacing and ET\textsubscript{A} receptor antagonist therapy. Each pig was considered a complete block. Thus, the numbers of myocytes studied from each animal were considered repeated observations within each block. If the ANOVA revealed significant differences, pairwise
and ETA low-dose receptor blockade was associated with a
crease by 100% from rapid-pacing-only values. Rapid pacing
animals at the conclusion of the study protocol (Table 1). In
0.6% and LV end-diastolic dimension was 3.4
pigs prior to randomization into the study protocol was 39.5
Baseline LV fractional shortening in the 27
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tests of individual group means were compared using Bonferroni probabilities. For comparisons of neurohormonal profiles, the Student-Newman-Keuls’ test was employed. All statistical procedures were performed using the BMDP statistical software package (BMDP Statistical Software Inc., Los Angeles, CA). Results are presented as mean ± standard error of the mean (SEM). Values of p < 0.05 were considered to be statistically significant.

Results

LV function with pacing CHF: Effects of short-term ET<sub>A</sub> receptor blockade. Baseline LV fractional shortening in the 27 pigs prior to randomization into the study protocol was 39.5 ± 0.6% and LV end-diastolic dimension was 3.4 ± 0.7 cm; these values were unchanged in the time-matched sham control animals at the conclusion of the study protocol (Table 1). In the chronic rapid-pacing groups, which underwent ET<sub>A</sub> receptor blockade with either a high or a low dose of receptor antagonist, resting heart rates were lower when compared to the rapid-pacing-only group. In the rapid-pacing group receiving the low dose of ET<sub>A</sub> receptor antagonist, mean arterial pressure was higher than in the rapid-pacing-only group. In the high dose ET<sub>A</sub> receptor blockade group, mean arterial pressure was similar to the untreated rapid-pacing group. The LV end-diastolic dimension was similar in the rapid-pacing groups receiving either the high or low dose of ET<sub>A</sub> receptor antagonist when compared to the rapid-pacing-only group. In the rapid-pacing groups receiving either the high or low dose of ET<sub>A</sub> receptor antagonist, LV fractional shortening was increased by 100% from rapid-pacing-only values. Rapid pacing and ET<sub>A</sub> low-dose receptor blockade was associated with a significant reduction in LV peak systolic wall stress when compared to rapid-pacing-only values. Cardiac output was significantly increased in the rapid-pacing and ET<sub>A</sub> receptor blockade groups when compared to rapid-pacing-only values, but remained lower than sham control values. Pulmonary vascular resistance was reduced by nearly 50% in the rapid-pacing and ET<sub>A</sub> receptor blockade groups when compared to rapid-pacing-only values. Pulmonary vascular resistance was lower in the rapid-pacing and high dose ET<sub>A</sub> receptor blockade group when compared to the low dose ET receptor blockade group, but this did not reach statistical significance (p = 0.065). Systemic vascular resistance fell by approximately 50% in the rapid-pacing and ET<sub>A</sub> receptor blockade groups when compared to the rapid-pacing-only group and was not different from the sham control group. Systemic vascular resistance was lower in the high dose ET<sub>A</sub> blockade group when compared to the low dose ET<sub>A</sub> receptor blockade group, but this did not reach statistical significance (p = 0.104). However, dose-dependent relationships of ET<sub>A</sub> receptor blockade were not evaluated in the present study owing to the fact that direct assessment of adequate ET<sub>A</sub> receptor inhibition was not directly measured in the pacing CHF animals.

Neurohormonal profiles with pacing CHF: Effects of short-term ET<sub>A</sub> blockade. Plasma norepinephrine increased by four-fold and epinephrine also increased four-fold with chronic rapid pacing when compared to control values (Table 1). In the chronic rapid-pacing group, plasma ET levels increased by 280% and plasma renin activity increased by 333% when compared to the control group. In both the high and low dose ET<sub>A</sub> receptor blockade and rapid-pacing groups, plasma norepinephrine and epinephrine values were significantly lower than rapid-pacing-only values. Plasma norepinephrine concen-

<p>| Table 1. Left Ventricular Function, Hemodynamics and Neurohormonal Profiles With Rapid-Pacing Heart Failure: Effects of Endothelin Receptor Blockade During the Progression of Heart Failure |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Rapid Pacing</th>
<th>Rapid Pacing and ET-block–High</th>
<th>Rapid Pacing and ET-block–Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting heart rate (beats/min)</td>
<td>114 ± 4</td>
<td>165 ± 4*</td>
<td>147 ± 7*†</td>
<td>147 ± 3*†</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>90 ± 2</td>
<td>70 ± 5*</td>
<td>74 ± 2*</td>
<td>80 ± 3*†</td>
</tr>
<tr>
<td>LV end-diastolic dimension (cm)</td>
<td>3.5 ± 0.6</td>
<td>5.4 ± 0.1*</td>
<td>5.6 ± 0.3*</td>
<td>5.0 ± 0.2*</td>
</tr>
<tr>
<td>LV fractional shortening (%)</td>
<td>38.6 ± 1.3</td>
<td>12.3 ± 0.8*</td>
<td>22.7 ± 3.1†</td>
<td>28.6 ± 1.2†</td>
</tr>
<tr>
<td>LV peak wall stress (g/cm²)</td>
<td>153 ± 7</td>
<td>323 ± 32*</td>
<td>293 ± 23*</td>
<td>265 ± 14*†</td>
</tr>
<tr>
<td>LV peak wall force (g x 10³)</td>
<td>1.49 ± 0.06</td>
<td>2.83 ± 0.30*</td>
<td>3.18 ± 0.28*†</td>
<td>2.64 ± 0.19*†</td>
</tr>
<tr>
<td>LV end-diastolic wall thickness (cm)</td>
<td>0.74 ± 0.02</td>
<td>0.48 ± 0.02*†</td>
<td>0.57 ± 0.02†</td>
<td>0.56 ± 0.03†</td>
</tr>
<tr>
<td>P. capillary wedge pressure (mm Hg)</td>
<td>8 ± 1</td>
<td>28 ± 3*</td>
<td>22 ± 2†</td>
<td>22 ± 3†</td>
</tr>
<tr>
<td>Pulmonary artery pressure (mm Hg)</td>
<td>14 ± 3</td>
<td>37 ± 4*</td>
<td>28 ± 5†</td>
<td>37 ± 2*</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>2.43 ± 0.18</td>
<td>0.93 ± 0.12*</td>
<td>1.95 ± 0.09†</td>
<td>1.72 ± 0.19†</td>
</tr>
<tr>
<td>Pulmonary vascular Res. (d.s.cm⁻²)</td>
<td>427 ± 51</td>
<td>3275 ± 440*</td>
<td>1253 ± 233†</td>
<td>1758 ± 160†</td>
</tr>
<tr>
<td>Systemic vascular Res. (d.s.cm⁻²)</td>
<td>3149 ± 223</td>
<td>6934 ± 940*</td>
<td>3152 ± 1500†</td>
<td>3999 ± 398†</td>
</tr>
<tr>
<td>Plasma norepinephrine (pg/ml)</td>
<td>815 ± 219</td>
<td>6864 ± 1160*</td>
<td>2363 ± 706†</td>
<td>3514 ± 705†</td>
</tr>
<tr>
<td>Plasma epinephrine (pg/ml)</td>
<td>422 ± 199</td>
<td>1648 ± 446*</td>
<td>576 ± 322†</td>
<td>935 ± 266†</td>
</tr>
<tr>
<td>Plasma endothelin (mol/ml)</td>
<td>2.7 ± 0.4</td>
<td>10.4 ± 1.6*</td>
<td>8.5 ± 0.5*</td>
<td>14.1 ± 3.1*</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml/hr)</td>
<td>2.7 ± 0.5</td>
<td>13.4 ± 0.2*</td>
<td>10.1 ± 0.4*</td>
<td>10.9 ± 1.07*</td>
</tr>
<tr>
<td>Sample size (n)</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

All values presented as mean ± SEM. *p < 0.05 vs. control. †p < 0.05 vs. rapid pacing only. * Rapid Pacing: 21 days of pacing; 240 beats/min. 50 mg/kg, b.i.d. starting at day 14 of pacing. †12.5 mg/kg, b.i.d. starting at day 14 of pacing. d.s.cm⁻²: dyne s cm⁻².
trations in the ET$_A$ receptor blockade and rapid-pacing groups remained higher than control values, but epinephrine values were not significantly different from controls (p = 0.35). No dose-dependent differences occurred in plasma catecholamines with respect to either the high or low ET$_A$ receptor antagonist group (p = 0.45). Plasma ET levels were significantly increased in the ET$_A$ receptor blockade and rapid-pacing groups when compared to controls, but were not different from rapid-pacing-only values. In the low dose ET$_A$ blockade group, plasma ET levels appeared higher than rapid-pacing-only values, but this did not reach statistical significance (p = 0.33). Plasma renin activity was increased in both ET$_A$ receptor blockade and rapid-pacing groups when compared to control values, but was similar to rapid-pacing-only values.

**Myocyte function with pacing CHF: Effects of short-term ET$_A$ receptor blockade.** A total of 1,187 isolated myocytes were studied in the control group, 1,007 in the rapid-pacing-only group, 854 in the rapid-pacing and high dose ET$_A$ blockade group, and 835 in the rapid-pacing and low dose ET$_A$ blockade group (Table 2). In the chronic rapid-pacing group, isolated myocyte length was increased and percent and velocity of shortening were significantly reduced. In both rapid-pacing and ET$_A$ receptor blockade groups, myocyte length was similar to rapid-pacing-only values. In the rapid-pacing and high dose ET$_A$ blockade group, a small but significant increase in myocyte function from basal values in all groups (Table 2). In the presence of isoproterenol, myocyte function remained significantly lower in all rapid-pacing groups when compared to rapid-pacing-only values. In the rapid-pacing and low dose ET$_A$ blockade group, myocyte function was not different from rapid-pacing-only values.

The β-receptor stimulation with isoproterenol increased myocyte function from basal values in all groups (Table 2). In the presence of isoproterenol, myocyte function remained significantly lower in all rapid-pacing groups when compared to the control group. In the high dose ET$_A$ receptor blockade group, myocyte function was higher following β-receptor stimulation when compared to rapid-pacing-only values. In the low dose ET$_A$ receptor blockade group, myocyte velocity of short-
enning was increased following β-receptor stimulation when compared to rapid-pacing-only values. With increased extracellular Ca\(^{2+}\), myocyte contractile function was significantly lower in all rapid-pacing groups when compared to control values. In contrast to β-receptor stimulation, there was no difference in myocyte function in the presence of increased extracellular Ca\(^{2+}\) between the rapid-pacing-only group and either ETA receptor blockade groups. In the presence of ET-1, myocyte contractile function significantly increased from baseline values in the control group. In marked contrast, in the rapid-pacing-only group, ET-1 resulted in a significant decline in myocyte contractile function from baseline values. In both rapid-pacing and ETA receptor blockade groups, no significant change occurred in myocyte contractile function from baseline values in the presence of ET-1.

**Discussion**

It has been postulated that increased plasma levels of the potent vasoactive peptide endothelin-1, through activation of the ETA subtype receptor, may contribute to the LV dysfunction and exacerbation of symptoms in congestive heart failure (CHF) (6,10–14,29–31). Accordingly, the overall goal of the present study was to determine whether ETA receptor blockade during the progression of pacing-induced CHF would improve indices of LV pump function and to define potential mechanisms for these effects. The significant findings of the present study were threefold. First, ETA receptor blockade initiated during the progression of pacing-induced CHF reduced pulmonary and systemic vascular resistance and improved LV pump function. Second, ETA receptor blockade instituted during the progression of pacing CHF reduced circulating catecholamines from pacing CHF values, but did not reduce plasma ET or renin activity. Third, ETA receptor blockade appeared to confer protective effects on myocyte contractile function and the capacity to respond to an inotropic stimulus, but these effects were most apparent using a high dose of ETA receptor blockade. However, the adequacy of ETA receptor inhibition was not directly assessed in the pacing CHF groups and therefore direct assessment of dose dependency for ETA receptor blockade could not be addressed. Nevertheless, the results from the present study suggest that contributory mechanisms for the improved LV pump function, which was observed with the institution of ETA receptor blockade during the development of pacing-induced CHF, include a reduction in systemic vascular resistance, reduced sympathetic activity and potentially protective effects on myocyte contractile performance.

**Left ventricular function and vascular resistive properties with ETA receptor blockade.** This laboratory has demonstrated previously that chronic rapid pacing in pigs caused progressive LV dilation and pump dysfunction, and severe CHF occurred after a 3-week pacing period (17–21). The ETA receptor blockade during the last 7 days of chronic pacing did not affect the degree of LV dilation, but did significantly improve LV pump function. A likely contributory factor for the improved LV pump function with ETA receptor blockade in this pacing CHF model was reduced systemic vascular resistance. Moreover, results from the present study suggest that the reduction in systemic vascular resistance with ETA receptor blockade was dose dependent. Past experimental and clinical studies have examined the effects of acute ET receptor blockade initiated following the development of CHF (11,29–31). For example, Kiowski et al. (11) reported that acute administration of the nonselective ET receptor antagonist bosentan significantly reduced systemic vascular resistance in patients with CHF. In a canine model of CHF due to coronary microembolization, IV administration of bosentan reduced systemic vascular resistance and increased indices of LV pump function (31). More recently, this laboratory demonstrated that ETA receptor blockade instituted at the onset of chronic rapid pacing in rabbits improved indices of LV and myocyte function (19). Results from the present study build upon these past reports in several important ways. First, results from the current study demonstrated that selective ETA receptor blockade instituted during the evolution of a CHF process improved LV pump function. Second, these beneficial effects of ETA receptor blockade on LV pump performance with developing CHF could be achieved using a dose of ETA receptor that did not significantly compromise mean arterial pressure and cause hemodynamic instability.

The institution of ETA receptor blockade during the progression of pacing CHF attenuated the degree of LV myocardial wall thinning but did not prevent the LV chamber dilation, which invariably occurs in this CHF model (15–21). Thus, the relative improvement in LV pump function with ETA receptor blockade was not associated with a proportional reduction in LV peak wall stress. Moreover, the LV peak wall force, an index of the load under which the LV must contract against (27,28), was increased with pacing CHF and was not reduced with ETA receptor blockade. Hence, the improved LV pump function with ETA receptor blockade must have been due to additional contributory factors, rather than primarily by a reduction in LV loading conditions. Potential contributory mechanisms for the improved LV pump function with ETA receptor blockade during the progression of pacing CHF include a reduction in basal resting heart rate, reduced sympathetic activation and potential improvements in myocyte contractile processes.

Several past studies have demonstrated that ETA receptor activation may play an important role in modulating pulmonary vascular resistance in patients with CHF (10,14). In the present study, there was a significant reduction in pulmonary vascular resistance with ETA receptor blockade with pacing CHF. In patients with CHF, a relationship between circulating levels of ET and the degree of pulmonary vascular resistance has been reported (10,14). Kiowski et al. (11) reported that acute administration of the nonselective ET receptor antagonist bosentan significantly reduced pulmonary vascular resistance in patients with CHF. Results from the present study suggest that modulation of ETA receptor activity within the
pulmonary circuit may be an important strategy for reducing pulmonary vascular resistance with developing CHF.

**Neurohormonal system activity with ETA receptor blockade.** Increased circulating levels of catecholamines, endothelin and elevated plasma renin activity commonly occur with the development of severe CHF (10–14,23,25). In the present study and consistent with past reports, (15–19,22) pacing-induced CHF was accompanied by a similar profile of neurohormonal activation. Institution of ETA receptor blockade during the progression of pacing CHF reduced plasma catecholamine values but had no effect on plasma renin activity.

The relative reduction in plasma catecholamines was observed with ETA receptor blockade instituted at either the high or low dose. A similar reduction in plasma catecholamines has been reported following the induction of angiotensin converting enzyme inhibition in the setting of CHF (17,23). Whether and to what degree ET-1 production and ETA receptor activation directly contributed to sympathetic activation and enhanced catecholamine production in the setting of CHF remains to be established. The persistent increase in plasma renin activity observed with ETA receptor blockade and chronic rapid pacing may have been secondary to a relative reduction in renal perfusion pressure. In addition, an interaction has been reported to occur in vitro between ET receptor density and perfusion pressure. In addition, an interaction has been reported to occur in vitro between ET receptor density and perfusion pressure. In addition, an interaction has been reported to occur in vitro between ET receptor density and perfusion pressure. In addition, an interaction has been reported to occur in vitro between ET receptor density and perfusion pressure. In addition, an interaction has been reported to occur in vitro between ET receptor density and perfusion pressure.
characteristics similar to that of the clinical spectrum of CHF (15–22). Using this animal model of CHF provided an opportunity to determine the effects of ET\(_A\) receptor blockade in the absence of confounding influences that might be encountered in clinical studies. However, it must be recognized that any animal model will not fully represent the complex clinical spectrum of CHF. Specifically, the changes in LV myocardial structure that occur with pacing-induced CHF are not similar to clinical forms of CHF owing to chronic ischemia or hypertensive disease. Nevertheless, the present study demonstrated that institution of ET\(_A\) receptor blockade in the setting of progressive CHF improves LV pump function and is accompanied by beneficial effects on vascular resistive properties and adrenergic tone. Second, the findings of the present study suggested that the beneficial hemodynamic effects reported in adrenergic tone. Second, the findings of the present study compained by beneficial effects on vascular resistive properties and adrenergic tone. Second, the findings of the present study suggest that beneficial hemodynamic effects reported in both experimental and clinical studies of CHF following administration of a nonspecific ET receptor antagonist (11,29,31) are mediated primarily through the ET\(_A\) receptor subtype. Finally, the results from the present study provide evidence to suggest that ET\(_A\) receptor activation contributes to the continued progression of LV dysfunction in the setting of CHF and that ET\(_A\) receptor blockade may provide favorable effects in this developing disease process.

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References


