Angiotensin converting enzyme inhibition, AT₁ receptor inhibition, and combination therapy with pacing induced heart failure: effects on left ventricular performance and regional blood flow patterns

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Abstract

Background: AT₁ receptor activation has been demonstrated to cause increased vascular resistance properties which may be of particular importance in the setting of congestive heart failure (CHF). The overall goal of this study was to examine the effects of ACE inhibition (ACEI) alone, AT₁ receptor blockade alone and combined ACEI and AT₁ receptor blockade on LV pump function, systemic hemodynamics and regional blood flow patterns in the normal state and with the development of pacing induced CHF, both at rest and with treadmill induced exercise. Methods and results: Pigs (25 kg) were instrumented in order to measure cardiac output (CO), systemic (SVR) and pulmonary vascular (PVR) resistance, neurohormonal system activity, and myocardial blood flow distribution in the conscious state and assigned to one of 4 groups: (1) rapid atrial pacing (240 bpm) for 3 weeks (n = 7); (2) ACEI (benazeprilat, 3.75 mg/day and pacing (n = 7); (3) AT₁ receptor blockade (valsartan, 60 mg/day) and rapid pacing (n = 7); and (4) ACEI and AT₁ receptor blockade (benazeprilat/valsartan, 1/60 mg/day, respectively) and pacing (n = 7). Measurements were obtained at rest and with treadmill exercise (15°, 3 miles/h; 10 min) in the normal control state and after the completion of the treatment protocols. With rapid pacing, CO was reduced at rest and with exercise compared to controls. ACEI or AT₁ blockade normalized CO at rest, but remained lower than control values with exercise. Combination therapy normalized CO both at rest and with exercise. Resting SVR in the CHF group was higher than controls and SVR fell to a similar degree with exercise; all treatment groups reduced resting SVR. With exercise, SVR was reduced from rapid pacing values in the ACEI and combination therapy groups. PVR increased by over 4-fold in the rapid pacing group both at rest and with exercise, and was reduced in all treatment groups. In the combination therapy group, PVR was similar to control values with exercise. Plasma catecholamines and endothelin levels were increased by over 3-fold with chronic rapid pacing, and were reduced in all treatment groups. In the combination therapy group, the relative increase in catecholamines and endothelin with exercise were significantly blunted when compared to rapid pacing only values. LV myocardial blood flow at rest was reduced in the rapid pacing only and monotherapy groups, but was normalized with combination therapy. Conclusion: These findings suggest that with developing CHF, combined ACE inhibition and AT₁ receptor blockade improved vascular resistive properties and regional blood flow distribution to a greater degree than that of either treatment alone. Thus, combined ACEI and AT₁ receptor blockade may provide unique benefits in the setting of CHF. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Cardiac output; Myocardial blood flow; Pig; Neurohormone

1. Introduction

Functional characteristics of severe congestive heart failure (CHF) include left ventricular (LV) pump dysfunction, alterations in systemic hemodynamics, increased neurohormonal system activation, and exercise intolerance [1–7]. With the development of CHF, increased production of angiotensin II (Ang-II) occurs with resultant activation of the AT₁ receptor system [2,6,8,9]. Abundant AT₁ receptors have been located within a number of vascular beds including the pulmonary and coronary vasculature and...
activation of this receptor pathway causes vascular smooth muscle vasoconstriction [9–15]. Thus, heightened AT1 receptor activation with CHF will cause increased vascular resistive properties which in turn may exacerbate the LV pump dysfunction and hemodynamic instability which occur with the development of this disease process. Past clinical studies have clearly demonstrated that angiotensin converting enzyme (ACE) inhibition improves LV function and survival in the setting of CHF [16–18]. ACE inhibition has been demonstrated to produce multiple effects within the vascular system, which include prevention of Ang-II formation, potentiation of bradykinin levels, and modulation of nitric oxide production [14,19–24]. Past studies have demonstrated that combined ACE inhibition and AT1 receptor blockade may influence vascular resistive properties to a greater extent than either treatment alone [25,26]. Whether and to what degree specific interruption of AT1 receptor activity, as well as combined ACE inhibition and AT1 receptor blockade influence systemic hemodynamics and regional blood flow patterns in the setting of CHF remains unclear. Accordingly, the overall goal of this project was to determine the effects of ACE inhibition, AT1 receptor blockade, or combination therapy on LV function, hemodynamics and regional blood flow patterns in the normal state and following the development of CHF; both at rest and with treadmill induced exercise.

Past reports from this laboratory and others have demonstrated that chronic pacing tachycardia in animals causes progressive and time dependent changes in LV geometry and pump function, and neurohormonal system activation [26–34]. While the stimulus which induces CHF in this model is dissimilar to clinical etiologies of CHF, the functional and neurohormonal changes which occur with chronic rapid pacing are similar to the clinical spectrum of this disease process [2–6,26,33,34]. In this model of CHF, the increased neurohormonal system activity is paralleled by increased systemic, pulmonary and coronary vascular resistance [11,26–28,30]. For example within the coronary vasculature, the development of pacing induced CHF is associated with diminished myocardial blood flow reserve [30]. Moreover, this model has been successfully used in order to determine the effects of exercise with the development of CHF [31]. Accordingly, in the present study, this model of pacing induced CHF was employed in order to examine the potentially differential effects of ACE inhibition, AT1 receptor blockade, and combined treatment on systemic hemodynamics and regional blood flow patterns both at rest and with treadmill induced exercise.

2. Methods

2.1. Model of CHF and exercise

The present study employed a rapid pacing model of CHF in pigs which has been well described by this laboratory previously [26,30,32]. Twenty-eight Yorkshire pigs (25 kg, male) were chronically instrumented in order to measure hemodynamics and LV pump function in the conscious state. The pigs were anesthetized with isoflurane (3%/1.5 l/min) and a mixture of nitrous oxide and oxygen (50:50), intubated with a cuffed endotracheal tube and ventilated at a flow rate of 22 ml/kg/min and a respiratory rate of 15/min. While maintaining a sterile field, the thoracic aorta was exposed through a left thoracotomy and a catheter connected to a vascular access port (Model GPV, 9F, Access Technologies, Skokie, IL) advanced into the aorta and sutured in place. Catheters were placed in the pulmonary artery and the left atrium in a similar fashion. The access ports were then placed in a subcutaneous pocket. A 20-mm flow probe (Transonic, Ithaca, NY) was placed around the pulmonary artery immediately distal to the pulmonary artery catheter and the electrical connection exteriorized through the thoracolumbar fascia. A shielded stimulating electrode was sutured onto the left atrium, connected to a modified programmable pacemaker (8329, Medtronic, Minneapolis, MN) and buried in a subcutaneous pocket. The thoracotomy was closed in layers and the pleural space evacuated of air. 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, 1996).

Following a 14–21 day recovery from the surgical procedure, the animals were then returned to the laboratory for baseline studies as described in the following section. Following measurements under normal resting conditions and with exercise, the pigs were then randomly assigned to the following 4 treatment groups: (1) rapid atrial pacing (240 bpm) for 3 weeks (n = 7); (2) concomitant ACE inhibition (benazeprilat, Novartis, Basle, 3.75 mg/day) and rapid pacing (n = 7); (3) concomitant AT1 receptor blockade [35] (valsartan, Novartis, Basel 60 mg/day) and rapid pacing (n = 7); and (4) concomitant ACE inhibition and AT1 receptor blockade (benazeprilat/valsartan, 1/60 mg/day, respectively) and rapid pacing (n = 7). The drug treatment protocols were begun at the initiation of pacing and continued for the entire 21-day pacing protocol. To maintain a constant steady-state blood level of all compounds used in this study, osmotic minipumps (2ML1, Alza Corp) were implanted in the peritoneal cavity [26]. This study employed a dosing strategy for ACE inhibition, AT1 receptor blockade, and combined ACE inhibition and AT1 receptor blockade which has been demonstrated previously to obtain approximately a 50% inhibition of the Ang-I and Ang-II pressor response, but would not produce significant differential effects on resting blood pressure [26]. Cardiac auscultation and an electrocardiogram were performed frequently during the pacing protocol in order to ensure proper operation of the pacemaker and the presence of 1:1 conduction.

2.1.1. Measurements at rest and with exercise

On the day of the study, the animals were sedated with diazepam (20 mg, p.o., Valium, Hoffmann–La Roche,
Nutley, NJ) and placed in a custom designed sling which allowed the animal to rest comfortably. This laboratory has demonstrated previously that sedation with benzodiazepines produced no significant effect on resting hemodynamics [36]. All studies were performed in the conscious state without additional use of sedation. An ECG was established, and the pacemaker deactivated (pacing groups only).

After a 30-min stabilization period, LV function was measured by two-dimensional and M-mode echocardiographic studies (ATL Ultramark VI, 2.25-MHz transducer, Bothell, WA) [30]. The vascular access ports were entered using a 12-gauge Huber needle (Access Technologies, Skokie, IL), the flow probe was connected, and basal hemodynamics were recorded. The flow probe was connected to a digital flow-meter (T106, Transonics, Ithica, NY) as well as digitized to a computer for processing. From the digitized flow signal, stroke volume was computed on a beat-to-beat basis and averaged from a minimum of 25 ejections.

During the last minute of exercise, hemodynamics and measurements of neurohormonal profiles, or valsartan levels. All of the tissue samples were fixed in 10% formaldehyde in order to facilitate slicing. The mid-region of the LV free wall was separated into endocardial and epicardial layers weighing approximately 3 g each. Samples of approximately 3–5 g were also collected and prepared from the basal regions of the lung, kidney, diaphragmatic muscle, latissimus dorsi, and gluteus maximus. The tissue samples were carefully weighed and then digested using a potassium hydroxide solution as previously described [37,38]. The aortic blood samples were extracted using an identical digestion solution. The fluorescence of the extracted samples were then determined by spectrofluorimetry (Gilford Fluoro IV, Oberlin, OH). The fluorescent microspheres used in this study with respect to excitation/emission characteristics were: blue–green, 428/457 nm; orange, 534/550 nm; red, 580/594 nm; and scarlet, 650/674 nm, respectively. These fluorescent microspheres were chosen since a spectral scan which conformed to a Gaussian distribution could be uniformly obtained, minimal spectral cross-over occurred, and they provided equivalent sensitivity [38]. Regional blood flow computations were determined using the standard formula: \( \frac{Q_{\text{flow}}}{Q_{\text{in}}} = \frac{A_{\text{flow}}}{A_{\text{in}}}. \) Where \( Q_{\text{in}} \) is the blood flow in ml/min, \( A_{\text{flow}} \) is the fluorescence of the aortic reference sample, \( A_{\text{in}} \) is the fluorescence of the tissue sample, and \( Q_{\text{in}} \) is the withdrawal rate of the reference sample. Final blood flow values were normalized to sample weights and expressed as ml/min/g. Coronary vascular resistance was determined as the mean aortic pressure divided by LV myocardial blood flow and expressed as (mmHg min/ml g) [30].

2.2. Neurohormonal profiles and drug levels

The plasma samples were assayed for renin activity, endothelin concentration, and catecholamine levels. Plasma renin activity was determined by computing angiotensin I production using a radioimmunoassay procedure (ARUP Laboratories, Salt Lake City, UT). For the endothelin assays, the plasma was first eluted over a cation exchange column (C-18 Sep-Pak, Waters Associates, Milford, MA) and then dried by vacuum-centrifugation. The samples were reconstituted in 0.02 M borate buffer. A high sensitivity radioimmunoassay was performed to determine endothelin concentrations (Amersham, Arlington Heights, IL). Plasma norepinephrine and epinephrine were measured using high performance liquid chromatography (HPLC) and normalized to pg/ml of plasma. Plasma concentrations of valsartan were determined by an AT\(_1\) receptor binding assay using smooth muscle cell membrane preparations as described previously [40].

2.3. Regional blood flow measurements

All of the tissue samples were fixed in 10% formaldehyde in order to facilitate slicing.
of neurohormonal profiles, the Student–Neuman–Keul test was employed. All statistical procedures were performed using the BMDP statistical software package (BMDP Statistical Software, Los Angeles, CA). Results are presented as mean ± s.e.m. Values of \( P < 0.05 \) were considered to be statistically significant.

3. Results

3.1. LV function with pacing CHF: effects of ACE inhibition, AT\(_1\) blockade, and combination therapy

All of the pigs enrolled in the present study successfully completed the experimental protocols. In the concomitant AT\(_1\) receptor blockade and rapid pacing group, valsartan plasma levels were 338 ± 101 nmol/l and in the ACE inhibition and AT\(_1\) blockade group, plasma levels were 262 ± 48 nmol/l, with no significant difference between groups (\( P = 0.56 \)). These plasma levels for the AT\(_1\) antagonist fell within the target therapeutic range determined from previously established dose response studies [26].

3.1.1. Resting state

LV size and pump function for the treatment groups are presented in Fig. 1 and hemodynamic indices measured in the resting awake state for all of the treatment groups are summarized in Table 1. Ambient resting heart rate was increased by 37% in the rapid pacing only group when compared to controls. In the combined treatment group, heart rate was lower than AT\(_1\) monotherapy values, but remained increased from control values. Stroke volume was reduced by approximately 50% in the rapid pacing only group when compared to control values. In the ACE inhibition and combination therapy groups, stroke volume was higher than untreated pacing values and similar to control values. Systemic arterial pressure was lower in the rapid pacing only group and all three treatment groups when compared to controls. Pulmonary artery and left atrial pressures were increased by over 2-fold in the rapid pacing only group when compared to controls. While remaining increased from control values, pulmonary and left atrial pressures were reduced in the ACE inhibition and combination therapy groups when compared to rapid pacing only values. Systemic and pulmonary vascular resistances are presented in Fig. 2.

3.1.2. Treadmill exercise

In all groups, respiratory rate significantly increased with treadmill exercise when compared to resting values (Table 1). In all rapid pacing groups, heart rate increased significantly from resting values with exercise, but remained lower than that achieved in the normal control state. In the rapid pacing only group, treadmill exercise resulted in a significant increase in stroke volume, but remained reduced from normal control values. Pulmonary artery and left atrial pressures remained increased in the

![Fig. 1. Left ventricular (LV) end diastolic dimension and fractional shortening in conscious pigs under ambient resting conditions. With the development of pacing induced congestive heart failure (CHF), end diastolic dimension increased, and fractional shortening decreased. With ACE inhibition (ACEI), LV end diastolic dimension was decreased and fractional shortening was increased from rapid pacing only values. With AT\(_1\) receptor blockade (AT\(_1\)-block), end diastolic dimension and fractional shortening were similar to rapid pacing only values. With combination therapy (ACEI/AT\(_1\)-block), end diastolic dimension was decreased and fractional shortening was increased when compared to rapid pacing only. LV function was similar in the combination therapy group when compared to the ACE inhibition group, and was significantly different from the AT\(_1\) receptor blockade group. (\* \( P < 0.05 \) vs. control; \*\* \( P < 0.05 \) vs. rapid pacing only; \*\*\* \( P < 0.05 \) vs. rapid pacing and ACEI; \*\*\*\* \( P < 0.05 \) vs. rapid pacing and AT\(_1\)-block).]
Table 1: Systemic hemodynamics, LV function and geometry with pacing induced heart failure: effects of ACE inhibition, AT₁–Ang II receptor blockade, or combined ACE inhibition and AT₁–Ang II receptor blockade during the progression of heart failure

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Rapid pacing</th>
<th>Rapid pacing and ACEI</th>
<th>Rapid pacing and AT₁-block</th>
<th>Rapid pacing and ACEI/AT₁-block</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
<td>Rest</td>
<td>Exercise</td>
<td>Rest</td>
</tr>
<tr>
<td><strong>Heart rate (bpm)</strong></td>
<td>117 ± 3</td>
<td>267 ± 5³</td>
<td>160 ± 7²</td>
<td>217 ± 6⁴</td>
<td>129 ± 7²</td>
</tr>
<tr>
<td><strong>Respiratory rate (min⁻¹)</strong></td>
<td>35 ± 2</td>
<td>72 ± 3³</td>
<td>50 ± 4³</td>
<td>80 ± 5¹</td>
<td>50 ± 7²</td>
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<tr>
<td><strong>Pump function</strong></td>
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<tr>
<td><strong>Stroke volume (ml)</strong></td>
<td>31.4 ± 1.3</td>
<td>30.1 ± 1.4</td>
<td>16.7 ± 1.5³</td>
<td>28.5 ± 2.1¹</td>
<td>25.5 ± 2.4³</td>
</tr>
<tr>
<td><strong>Aorta (mmHg)</strong></td>
<td>97 ± 2</td>
<td>104 ± 2²</td>
<td>91 ± 3³</td>
<td>88 ± 3³</td>
<td>73 ± 3³</td>
</tr>
<tr>
<td><strong>Pulmonary artery (mmHg)</strong></td>
<td>18 ± 1</td>
<td>26 ± 1¹</td>
<td>37 ± 4³</td>
<td>41 ± 4³</td>
<td>27 ± 1³</td>
</tr>
<tr>
<td><strong>Left atrium (mmHg)</strong></td>
<td>8.9 ± 0.6</td>
<td>6.5 ± 0.9⁴</td>
<td>29.2 ± 3.6³</td>
<td>28.3 ± 2.0³</td>
<td>16.0 ± 3.3³</td>
</tr>
<tr>
<td><strong>Neurohormones</strong></td>
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<tr>
<td><strong>Norepinephrine (pg/ml)</strong></td>
<td>122 ± 12</td>
<td>876 ± 101²</td>
<td>1054 ± 199³</td>
<td>3386 ± 889⁵</td>
<td>447 ± 143³</td>
</tr>
<tr>
<td><strong>Epinephrine (pg/ml)</strong></td>
<td>154 ± 25</td>
<td>303 ± 31³</td>
<td>405 ± 99⁴</td>
<td>554 ± 101³</td>
<td>392 ± 60⁴</td>
</tr>
<tr>
<td><strong>Endothelin (fmol/ml)</strong></td>
<td>2.8 ± 0.2</td>
<td>3.1 ± 0.2²</td>
<td>10.1 ± 1.3³</td>
<td>10.5 ± 1.9³</td>
<td>5.1 ± 0.6³</td>
</tr>
<tr>
<td><strong>Renin activity (ng/ml/h)</strong></td>
<td>4.2 ± 0.4</td>
<td>10.8 ± 0.9⁴</td>
<td>35.8 ± 11.0³</td>
<td>30.0 ± 8.8³</td>
<td>16.4 ± 1.7³</td>
</tr>
<tr>
<td><strong>Sample size (n)</strong></td>
<td>28</td>
<td>28</td>
<td>7</td>
<td>7</td>
<td>7</td>
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</tbody>
</table>

Values presented as Mean ± s.e.m.

a Rapid pacing: 21 days of pacing tachycardia; 240 bpm.
b Rapid pacing and ACEI: benazeprilat 3.75 mg/day.
c Rapid pacing and AT₁-block: valsartan 60 mg/day.
d Rapid pacing and AT₁-block: valsartan 60 mg/day, valsartan 60 mg/day.

P < 0.05 vs. control.
P < 0.05 vs. rapid pacing only.
P < 0.05 vs. rapid pacing and ACEI.
P < 0.05 vs. rapid pacing and AT₁-block.
rapid pacing only group and the AT₁ receptor blockade group when compared to control values. In the ACE inhibition group and combination therapy group, pulmonary and left atrial pressures were reduced from rapid pacing only values. Changes in systemic vascular resistance and pulmonary vascular resistance with treadmill induced exercise are presented in Fig. 2. In the control state, resting systemic oxygen consumption ($V_{\text{O}_2}$) increased by over 4-fold with treadmill induced exercise (7.2 ± 0.4 vs. 30.2 ± 1.5 ml O₂/min/kg, $P < 0.05$). In the rapid pacing only group, $V_{\text{O}_2}$ was similar to control values at rest, but significantly reduced with exercise (19.1 ± 1.1 ml O₂/min/kg). In all treatment groups, resting and exercise $V_{\text{O}_2}$ were similar to rapid pacing only values.

3.2. Neurohormonal activity: effects of ACE inhibition, AT₁ blockade, and combination therapy

3.2.1. Resting state

Plasma norepinephrine and epinephrine values were increased in the rapid pacing only group (Table 1). In all treatment groups, plasma norepinephrine was reduced from rapid pacing only values, but remained increased from control values. In the AT₁ receptor blockade group, plasma norepinephrine was higher when compared to the ACE inhibition or combination therapy groups. Plasma epinephrine was reduced in the combination therapy group when compared to rapid pacing only values, but this did not reach statistical significance ($P = 0.14$). In the rapid pacing only group, plasma endothelin increased by 3-fold from control values. Plasma endothelin was reduced in all treatment groups when compared to rapid pacing only values, but remained increased from control values. Plasma renin activity increased by 8-fold in the rapid pacing only group when compared to normal control values, and remained increased in all treatment groups.

3.2.2. Treadmill exercise

The absolute change in plasma catecholamines in the normal control state and in all of the rapid pacing groups is summarized in Fig. 3. The relative increase in plasma epinephrine was blunted only in the combination therapy group. In the combination therapy group, plasma endothelin levels fell from resting values with treadmill exercise.

3.3. Regional blood flow: effects of ACE inhibition, AT₁ blockade, and combination therapy

3.3.1. Resting state

LV myocardial blood flow was reduced in the rapid pacing only group when compared to normal control values (Table 2). In the monotherapy groups, LV myocardial blood flow remained reduced from control values. How-
ever, in the combination therapy group, LV myocardial blood flow was normalized in both the endocardial and epicardial regions. Coronary vascular resistance is summarized in Fig. 4. Pulmonary parenchymal flow was reduced by 50% from normal control values in the rapid pacing only group. Pulmonary blood flow was normalized in the ACE inhibition and combination therapy groups, but remained reduced in the AT<sub>1</sub> receptor blockade group. Renal blood flow was reduced in the rapid pacing only group and both monotherapy groups, when compared to normal control values. Renal blood flow was normalized in the combination therapy group. In the AT<sub>1</sub> receptor blockade group, skeletal muscle flow was reduced from control and rapid pacing only values.

3.3.2. Treadmill exercise

LV myocardial blood flow increased by approximately 4-fold in the normal control state with treadmill exercise (Table 2). In the rapid pacing only group, LV myocardial blood flow increased with exercise, but was 38% lower than normal control values. LV myocardial blood flow remained reduced from control values in all treatment groups. Coronary vascular resistance with treadmill induced exercise is summarized in Fig. 4. Pulmonary parenchymal flow increased by 4-fold in the normal control state with treadmill exercise, and was significantly blunted in the rapid pacing only group. In the ACE inhibition and combination therapy groups, pulmonary parenchymal flow increased from rapid pacing only values, but remained reduced from control values. Renal blood flow increased by 50% in the normal control state with exercise, but was reduced by 40% in the rapid pacing only group when compared to normal control values. Renal blood flow remained reduced in all treatment groups and was similar to rapid pacing only values. Skeletal muscle blood flow increased by over 5-fold in the control state...
Table 2
Blood flow with pacing induced heart failure: effects of ACE inhibition, AT₁-Ang II receptor blockade, or combined ACE inhibition and AT₁-Ang II receptor blockade during the progression of heart failure

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
<td>Rest</td>
<td>Exercise</td>
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<tr>
<td>LV myocardium (ml/min/g)</td>
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<tr>
<td>Endocardial flow</td>
<td>1.89 ± 0.09</td>
<td>8.01 ± 0.49</td>
<td>1.52 ± 0.09</td>
<td>5.03 ± 0.40</td>
<td>1.47 ± 0.13</td>
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<tr>
<td>Epicardial flow</td>
<td>1.51 ± 0.06</td>
<td>6.93 ± 0.41</td>
<td>1.27 ± 0.02</td>
<td>4.11 ± 0.45</td>
<td>1.22 ± 0.14</td>
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<tr>
<td>Average myocardial flow</td>
<td>1.70 ± 0.07</td>
<td>7.47 ± 0.44</td>
<td>1.40 ± 0.05</td>
<td>4.57 ± 0.46</td>
<td>1.34 ± 0.13</td>
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<tr>
<td>Blood flow to regional beds (ml/min/g)</td>
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<tr>
<td>Lung</td>
<td>1.1 ± 0.1</td>
<td>4.1 ± 0.59</td>
<td>0.5 ± 0.1</td>
<td>0.7 ± 0.2</td>
<td>1.0 ± 0.3</td>
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<tr>
<td>Kidney</td>
<td>4.6 ± 0.2</td>
<td>8.4 ± 0.65</td>
<td>3.1 ± 0.5</td>
<td>5.4 ± 1.1</td>
<td>2.3 ± 0.3</td>
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<tr>
<td>Latissimus dorsi</td>
<td>0.41 ± 0.03</td>
<td>1.58 ± 0.14</td>
<td>0.48 ± 0.19</td>
<td>0.53 ± 0.11</td>
<td>0.40 ± 0.06</td>
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<tr>
<td>Gluteus maximus</td>
<td>0.39 ± 0.02</td>
<td>1.72 ± 0.16</td>
<td>0.46 ± 0.16</td>
<td>0.79 ± 0.17</td>
<td>0.39 ± 0.05</td>
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*b* Rapid pacing and ACEI: benazeprilat 3.75 mg/day.

*c* Rapid pacing and AT₁-block: valsartan 60 mg/day.

*d* Rapid pacing and ACEI/AT₁-block: benazeprilat 1 mg/day, valsartan 60 mg/day.

*e* $P < 0.05$ vs. control.

$f$ $P < 0.05$ vs. rapid pacing only.

*g* $P < 0.05$ vs. rapid pacing and ACEI.

$h$ $P < 0.05$ vs. rapid pacing and AT₁-block.

*i* $P < 0.05$ vs. resting state.
and was significantly reduced in all of the rapid pacing groups. This was not affected by any drug treatment.

4. Discussion

The relative contribution of AT₁ receptor activity to the alterations in hemodynamic profiles which occur in the setting of severe congestive heart failure (CHF) is not fully understood. While AT₁ receptor antagonists have been successfully used in patients with CHF [41], confounding influences such as multiple drug therapies, duration and degree of symptoms, duration of treatment, and methodological issues prevent the determination of the direct influences such as multiple drug therapies, duration and successfully used in patients with CHF [41], confounding underestimation of the direct effects of AT₁ receptor blockade on systemic hemodynamic profiles and regional blood flow patterns. Past studies have demonstrated that ACE inhibitors can influence a number of enzymatic pathways in addition to the renin–angiotensin system [19–24,42]. Moreover, clinical and experimental studies have suggested that combined treatment with both ACE inhibition and AT₁ receptor blockade may provide additive effects with respect to vascular resistive properties [25]. Accordingly, the present study determined the effects of ACE inhibition, AT₁ receptor blockade or combination therapy on hemodynamics, neurohormonal profiles, and regional blood flow distribution during the development of CHF. Using a chronically instrumented animal model of pacing induced CHF, several important observations were made. First, monotherapy with ACE inhibition or AT₁ receptor blockade, as well as combination therapy, reduced systemic vascular resistive properties, improved cardiac output, and reduced neurohormonal system activation. Second, with treadmill exercise, combination therapy improved cardiac output and diminished neurohormonal system activity to a greater degree than that obtained with monotherapy treatment. The improvements in pump function and neurohormonal system activity were less pronounced in the AT₁ receptor blockade treatment group when compared to either ACE inhibition or combination therapy. Third, combination therapy instituted during the development of CHF, improved resting myocardial blood flow and reduced coronary vascular resistance. This was not achieved with either ACE inhibition or AT₁ receptor blockade alone. These findings suggest that combined ACE inhibition and AT₁ receptor blockade may provide beneficial effects on regional vascular resistive properties in the setting of developing CHF.

4.1. Systemic vascular resistive properties

In a past study, Symons and Stebbins reported that an acute infusion of losartan in normal pigs reduced systemic vascular resistance and increased myocardial blood flow with treadmill exercise [43]. In dogs with pacing induced CHF, Cheng and colleagues reported a significant increase in stroke volume following acute administration of losartan [44]. In a past report, this laboratory demonstrated that AT₁ receptor blockade reduced systemic and pulmonary vascular resistance in anesthetized pigs with CHF [26]. To our knowledge, the present study is the first to examine both hemodynamic and regional blood flow profiles at rest and with exercise following chronic ACE inhibition or AT₁ receptor blockade in a model of CHF. In the awake chronically instrumented state, chronic ACE inhibition or AT₁ receptor blockade with developing pacing induced CHF reduced systemic and pulmonary vascular resistance both at rest and with treadmill induced exercise. These findings, as well as past reports [8,26,41,44], clearly suggest that Ang-II formation and subsequent AT₁ receptor activation contribute to the increased systemic vascular resistive properties with developing CHF. Furthermore, results from the present study demonstrated that combined ACE inhibition and AT₁ receptor blockade in this model of CHF reduced pulmonary vascular resistance to a greater degree than AT₁ receptor blockade alone and was associated with increased bronchial blood flow. These results suggest that this combination treatment modality may pro-

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Fig. 4. Coronary vascular resistance fell in all groups with treadmill exercise (P < 0.05 vs. resting state). In the resting state, coronary vascular resistance was increased in the rapid pacing group and the AT₁ receptor blockade (AT₁-block) group when compared to control values. In the ACE inhibition (ACEI) group, coronary vascular resistance was similar to control values. In the combination therapy (ACEI/AT₁-block) group, coronary vascular resistance was reduced from both control and rapid pacing only values. With exercise, coronary vascular resistance remained increased in the rapid pacing group. In all treatment groups, coronary vascular resistance with exercise was similar to normal control values. (* P < 0.05 vs. control; † P < 0.05 vs. rapid pacing only; ‡ P < 0.05 vs. rapid pacing and ACEI; § P < 0.05 vs. rapid pacing and AT₁-block).
vide additional beneficial effects within the pulmonary vasculature in the setting of CHF.

4.2. Myocardial blood flow and coronary vascular resistance

Consistent with past reports [30,45], the present study demonstrated that the development of pacing induced CHF was associated with a significant reduction in myocardial blood flow at rest. This reduction in myocardial blood flow occurred in the absence of a physical obstruction to flow and therefore was likely due to changes in vascular resistive properties of the coronary vasculature. It has been reported previously that in patients with non-ischemic cardiomyopathy, abnormalities in myocardial oxygen delivery/demand exist [46]. Thus, the global reduction in LV myocardial blood flow may be a contributory factor towards the diminished LV performance with pacing induced CHF. In the present study, chronic ACE inhibition improved resting LV epicardial blood flow and coronary vascular resistance, whereas chronic treatment with AT1 receptor blockade failed to provide similar effects. Most importantly, the present study demonstrated that combined treatment normalized resting LV myocardial blood flow and reduced coronary vascular resistance to a greater degree than that of monotherapy with ACE inhibition. This reduction in coronary vascular resistance with combination therapy was achieved without a significant difference in resting blood pressure compared to the monotherapy groups. Thus, combined ACE inhibition and AT1 receptor blockade in the setting of severe CHF may provide a beneficial means by which to improve resting myocardial blood flow without a compromise on systemic hemodynamics.

In a study by Sudhir and colleagues using an anesthetized dog model, the effects of the ACE inhibitor enalaprilat on coronary blood flow was examined [15]. In this past report, intracoronary administration of enalaprilat improved myocardial blood flow by approximately 19% when normalized to maximal flow achieved by adenosine. In patients with dilated cardiomyopathy, Fault and colleagues demonstrated that intracoronary administration of enalaprilat, which had no effects on systemic perfusion pressure, improved coronary sinus blood flow [13]. The present study builds upon these past reports by demonstrating that chronic combined ACE inhibition and AT1 receptor blockade improved resting myocardial blood flow to a greater degree than that of chronic ACE inhibition alone with the development of CHF. In order to more carefully examine LV myocardial blood flow under a physiological stress, measurements were also performed during treadmill induced exercise. Treadmill induced exercise increased relative LV myocardial blood flow in all treatment groups. However, with the development of pacing induced CHF, LV myocardial blood flow was reduced from control values with exercise. Unlike what was observed under resting conditions, LV myocardial blood flow was not significantly increased from pacing CHF values in any of the treatment groups with treadmill exercise. A number of vasoactive substances are locally released with increased myocardial work, which can significantly influence myocardial blood flow characteristics [47]. With the development of pacing induced CHF, a blunted response to endothelial mediated vasodilation as well as to adenosine has been reported [30,48]. In addition, it has been suggested that the diminished coronary flow reserve with pacing induced CHF may be due to increased LV myocardial wall stress during diastole [45]. The findings of the present study would suggest that additional vasoconstrictive as well as mechanical influences, independent of either ACE or AT1 receptor mediated pathways, contribute to the persistent reduction in LV myocardial blood flow during exercise in this model of CHF.

4.3. Regional blood flow distribution

With the development of CHF, pulmonary parenchymal flow was significantly reduced both at rest and with treadmill induced exercise. Parenchymal flow is supplied by the bronchial arteries with venous return through the pulmonary veins. Contributory mechanisms for the reduced bronchial flow with pacing CHF most probably include both hemodynamic and neurohormonal factors. For example, increased hydraulic resistive pressure (left atrial pressure) with pacing CHF likely contributed to the reduction in bronchial flow. In the ACE inhibition group, left atrial pressure was reduced from pacing CHF values, resulted in a normalization of resting bronchial flow. In the ACE inhibition group, left atrial pressure was reduced from pacing CHF values and in turn, was associated with no improvement in bronchial flow at rest or with exercise. Combination treatment, in which resting left atrial pressure was reduced from pacing CHF values and in turn, was associated with no improvement in bronchial flow, both at rest and with exercise. Interestingly, AT1 receptor blockade did not reduce left atrial pressure from pacing CHF values and in turn, was associated with no improvement in bronchial flow at rest or with exercise. Combination treatment, in which resting left atrial pressure was reduced from pacing CHF values, resulted in a normalization of resting bronchial flow. However, despite the significant reduction in left atrial pressure with treadmill exercise in both the ACE inhibition and combination treatment groups, bronchial flow remained lower than control values. In a report by Townsley et al. [49], an enhanced vasoconstrictive response of the pulmonary vasculature to epinephrine was observed following the development of pacing induced CHF. Thus in the present study, inherent defects in the vasodilatory properties of the bronchial smooth muscle may have occurred with pacing CHF, which, in turn, contributed to the persistent defects in bronchial flow. While the contributory mechanisms for changes in bronchial flow with CHF are beyond the scope of the present study, these results do suggest that the defects in bronchial flow which occur with pacing induced CHF were probably not solely due to enhanced AT1 receptor activity.

Resting skeletal muscle blood flow was lowest in the AT1 receptor blockade group when compared to either the
ACE inhibition or combination therapy group. This observation was likely due to the fact that ACE inhibition and combination therapy attenuated circulating norepinephrine and endothelin to a greater degree than that of AT₁ receptor blockade, and therefore improved relative muscle perfusion. With treadmill induced exercise, skeletal muscle blood flow was significantly reduced with pacing CHF. This is consistent with a past report in which abnormalities in skeletal muscle perfusion were noted in dogs with pacing CHF during treadmill exercise [28]. Chronic ACE inhibition, AT₁ receptor blockade, or combination therapy did not increase skeletal muscle blood flow from CHF values during exercise. With exercise, significant vascular smooth muscle vasodilation occurs primarily due to the local release of a number of metabolites [50] and the vascular response to these local metabolites has been reported to be abnormal with CHF [7]. These abnormalities in local vasodilatory response at the level of the muscle vasculature likely superseded any potential beneficial effects that inhibition of Ang-II formation or AT₁ receptor activation may have provided with treadmill induced exercise.

4.4. Neurohormonal activity

Consistent with the clinical spectrum of CHF [2–6], the development of pacing induced CHF was accompanied by increased sympathetic nervous system activity as evidenced by elevated plasma catecholamine levels. As reported previously by this laboratory, chronic ACE inhibition with the development of pacing CHF, reduced plasma norepinephrine levels under resting, ambient conditions [26,34]. In addition, and consistent with a recent report [26], the present study demonstrated that concomitant AT₁ receptor blockade with developing pacing CHF did not cause a similar reduction in plasma norepinephrine. In the present study, combination ACE inhibition and AT₁ receptor blockade, significantly reduced plasma norepinephrine from pacing CHF values. With treadmill exercise, plasma norepinephrine increased significantly in the pacing CHF group, and was reduced with either chronic ACE inhibition or combination treatment. Moreover, the relative increase in both norepinephrine and epinephrine with exercise was reduced to a greater degree in the combination therapy group. This overall reduction in sympathetic nervous activity with combined ACE inhibition and AT₁ receptor blockade, particularly with exercise may have contributed to the relative reduction in vascular resistive properties.

With the development of pacing CHF, increased plasma levels of the potent vasoactive peptide, endothelin have been reported [26,51]. One of the important findings of the present study was that combination treatment blunted the relative rise in plasma endothelin levels with treadmill exercise when compared to untreated CHF values or monotherapy treatment values. In patients with CHF, a relationship between circulating levels of endothelin and the degree of pulmonary vascular resistance has been reported [5,51]. For example, Tsutamoto and colleagues demonstrated that in patients with severe CHF, endothelin spillover in the pulmonary circuit occurred and correlated to the degree of pulmonary vascular resistance [51]. Kiowski et al. reported that acute administration of the non-selective endothelin receptor antagonist, bosentan, significantly reduced systemic and pulmonary vascular resistance in patients with CHF [52]. In a model of CHF induced by chronic caval occlusion, Cannan and colleagues demonstrated that the coronary vasoconstrictor effects of endothelin were increased [53]. Thus in the present study, the relative reduction in plasma endothelin levels which occurred in the combination therapy group, particularly during treadmill induced exercise, likely contributed to the reduction in pulmonary and coronary vascular resistance.

With the development of pacing induced CHF, plasma renin activity was increased from control values both at rest and with exercise. With chronic ACE inhibition, AT₁ receptor blockade, or combination treatment, plasma renin activity remained increased from normal control values. This persistent elevation in plasma renin activity with ACE inhibition or AT₁ receptor blockade was not surprising and is consistent with interruption of the renin–angiotensin enzymatic pathway. The development of CHF has been demonstrated to cause changes in plasma and myocardial Ang-II levels [2,6,9,13,33,54–56]. Furthermore, alternative pathways for the local production of Ang-II have been identified [54,55]. Direct assessment of local production of Ang-II levels was not performed in the present study, and therefore the relative production of Ang-II and AT₁ Ang-II receptor activity could not be addressed. Future studies which directly examine Ang-II production within the LV as well as steady-state plasma levels following ACE inhibition, AT₁ Ang-II receptor blockade, or combination therapy in this model of CHF are necessary in order to address this important study limitation.

4.5. LV function and geometry

Consistent with past reports from this laboratory and others [26–34,43,45], chronic rapid pacing caused LV dilation and pump dysfunction. The progressive LV dilation which occurs with chronic rapid pacing results in recruitment of the Frank–Starling mechanism, but this mechanism is exhausted and results in diminished LV stroke volume with prolonged periods of pacing [29]. Concomitant ACE inhibition or combination treatment with chronic rapid pacing reduced the degree of LV dilation and improved LV pump function. The reduction in LV end-diastolic volume which was achieved through chronic ACE inhibition has been demonstrated previously to be paralleled at the cellular level by a reduction in isolated myocyte resting length [26,34]. Because of the persistently elevated basal heart rate in the AT₁ receptor blockade
group, resting cardiac output was higher than pacing CHF values and similar to ACE inhibition and combination treatment. However, consistent with a recent report [26], chronic AT receptor blockade with rapid pacing did not provide similar effects with respect to LV dilation. In a recent study by Weinberg and colleagues, it was demonstrated that AT receptor blockade did not prevent LV myocardial remodeling following chronic aortic stenosis in rats [57], whereas these investigators demonstrated previously that ACE inhibition attenuated the LV myocardial remodeling in this model of hypertrophy [58]. The results from these reports as well as the present study suggest that the mechanisms of action of chronic ACE inhibition with respect to LV dilation and remodeling, are, at least in part, independent of Ang-II formation and subsequent AT receptor activation.

4.6. Potential mechanisms for the effects of combination treatment

Chronic ACE inhibition reduced the degree of LV dilation, increased LV pump function and diminished neurohormonal system activity to a greater degree than that obtained with chronic AT receptor blockade with pacing induced CHF. Combination therapy in which ACE inhibition was added to AT receptor blockade yielded beneficial effects similar to that of ACE inhibition alone. The experimental design of the present study was predicated on past studies in which favorable effects on vascular resistance and blood flow have been reported with ACE inhibition [12,13,15]. However, it must be recognized that the effects of ACE inhibition on vascular tone may not be solely due to interruption of Ang-II formation. It is has been well established that ACE inhibitors can influence other enzyme systems and bioactive peptide levels such as bradykinin [14,20–24,42]. For example, in a recent study by Henrion and colleagues, the AT receptor antagonist losartan only caused partial relaxation of rat resistance arteries when compared to ACE inhibition [10]. Furthermore, in this past study, arterial relaxation achieved through ACE inhibition was significantly attenuated in the presence of a bradykinin antagonist. Barra et al. recently demonstrated that AT receptor blockade did not induce similar changes in compliance compared to ACE inhibition in a canine aortic preparation [14]. Hornig et al. reported that the vasodilatory effects of ACE inhibition in the radial artery of normal human subjects was primarily due to an accumulation of bradykinin [42]. In the present study, the differential effects between ACE inhibition and AT receptor blockade with respect to LV function and neurohormonal system activity was probably due to the differences in bradykinin levels. Furthermore, a potential mechanism for the additive effects of combined ACE inhibition and AT receptor blockade on vascular resistive properties is probably due to a potentiated effect of ACE inhibition on alternative enzyme systems, such as the bradykinin pathway. In light of the findings of the present study as well as recent reports [24,42], future studies which employ bradykinin receptor agonists and antagonists in this model of CHF are warranted.

In a recently completed study, receptor binding studies using LV myocardial preparations from normal and pacing CHF pigs revealed that the AT receptor was the predominant Ang-II receptor subtype [26]. Thus, the predominant Ang-II receptor subtype expressed in both normal and failing porcine myocardium was the AT receptor and thereby minimized potential confounding influences of AT receptor activity. However, this past report demonstrated that the absolute LV myocardial AT receptor density was decreased with pacing induced CHF [26]. In a recent study, Asano and colleagues reported a significant reduction in LV myocardial AT receptor density with end-stage human cardiomyopathy [59]. Thus, the downregulation of the AT receptor which occurs with the progression of pacing induced CHF appears to be similar to what occurs in clinical forms of severe CHF. More importantly, these observations may provide an additional explanation by which AT receptor blockade failed to provide similar results to that achieved by ACE inhibition. A relatively high abundance of the AT receptor subtype has been reported in the human myocardium [59,60]. Whether and to what degree changes in relative AT /AT receptor density influence LV myocardial function and remodeling with developing CHF remain unclear. In a past report by this laboratory, chronic combined ACE inhibition and AT receptor blockade returned myocardial Ang-II receptor density to near control levels [26]. Thus, a potential mechanism for the additive effects of combination therapy which were observed in the present study may have been due to favorable effects on AT receptor density and transduction. The controlling mechanisms for AT receptor expression in this model of CHF and the interactive effects of ACE inhibition and AT receptor blockade warrant further study.

4.7. Study limitations and summary

The results of the present study hold clinical significance in light of the use of losartan, an AT receptor antagonist, in the Evaluation of Losartan in the Elderly (ELITE) trial. In the ELITE study, AT receptor blockade was well tolerated in patients with CHF, as evidenced by a low attrition rate. AT receptor antagonist treatment appeared to be associated with a reduction in all-cause mortality [61]. While additional studies using this model are warranted, combined ACE inhibition and AT receptor blockade may be of particular benefit in the setting of CHF.

The present project employed a model of chronic rapid pacing which produced changes in LV functional and
neurohormonal characteristics similar to that of the clinical spectrum of CHF [1–7,49]. Using this animal model of CHF provided an opportunity to determine the acute effects of AT₁ receptor blockade in the absence of confounding influences which may 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 which occur with pacing induced CHF are not similar to clinical forms of CHF due to chronic ischemia or hypertensive disease [62]. Furthermore, this model of chronic rapid pacing for 3 weeks produces a rapidly progressive model of CHF which is in contrast to clinical forms of CHF which occur over a period of months to years. In a past clinical study, AT₁ receptor blockade with losartan reduced systemic vascular resistance in the setting of CHF; which appeared to be dose dependent [41]. Similar to this past clinical study, AT₁ receptor blockade with the development of pacing induced CHF reduced systemic vascular resistance. However, in the present study, the dosage of AT₁ receptor antagonist was selected based upon attenuating the Ang-II pressor response and not producing a significant hypotensive effect when compared to that of ACE inhibition [26]. Thus, whether higher doses of either ACE inhibition, AT₁ receptor blockade, or a combination of both therapies may provide further beneficial effects on hemodynamics and neurohormonal profiles in the setting of CHF could not be addressed by the current experimental design. Furthermore, whether and to what degree ACE inhibition and/or AT₁ receptor blockade may influence ventricular function, neurohormonal activity, and blood flow distribution in the normal myocardium was not addressed in the current experimental design. These limitations not withstanding, the present study demonstrated that in this model of CHF, the effects of ACE inhibition and AT₁ receptor blockade did not provide equivalent effects with respect to hemodynamics and neurohormonal profiles in the resting and exercise state. However, combination treatment improved vascular resistive properties, neurohormonal profiles and regional blood flow to a greater degree than with either treatment alone. The importance of these findings are two-fold. First, these results provide additional evidence that the mechanisms of action of ACE inhibition in the setting of CHF are not solely due to an attenuation of AT₁ receptor activity. Second, combined ACE inhibition and AT₁ receptor blockade may provide unique beneficial effects in the setting of severe CHF.

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References

[18] SOLVD investigators, Konstant MA, Kronenberg MW, Rousseau MF, et al. Effects of the angiotensin converting enzyme inhibitor enalapril on the long-term progression of left ventricular dilation in...


enzyme inhibition prolongs survival and modifies the transition to heart failure in rats with pressure overload hypertrophy due to ascending aortic stenosis. Circulation 1994;90:1410–1422.


