Differential Effects of Captopril and Enalapril on Tissue Renin–Angiotensin Systems in Experimental Heart Failure

Alan T. Hirsch, MD; Chris E. Talsness, Angela D. Smith, Heribert Schunkert, MD; Julie R. Ingelfinger, MD; and Victor J. Dzau, MD

Background. Angiotensin converting enzyme (ACE) inhibitor therapy elicits beneficial responses from patients with heart failure. We hypothesized that a major site of action of these drugs is tissue ACE and that ACE inhibitors might differ in their ability to inhibit tissue ACE. To test this hypothesis, we assessed the effects of captopril and enalapril on blood pressure and renal function and on serum and tissue ACE activities in sham-operated rats and rats with heart failure induced by coronary artery ligation.

Methods and Results. During short-term (1-week) treatment, captopril (200 mg·kg⁻¹·day⁻¹) and enalapril (25 mg·kg⁻¹·day⁻¹) elicited equipotent effects on blood pressure and inhibition of serum ACE activity (85%). The effects of long-term treatment (47 days) were then studied beginning 45±5 days after coronary ligation in four treatment groups: sham-operated, vehicle (n=14); heart failure, vehicle (n=10); heart failure, captopril (n=8); and heart failure, enalapril rats (n=7). During long-term treatment, captopril and enalapril caused comparable falls of 12–18 mm Hg in blood pressure (p<0.01 compared with vehicle treatment). There was no change in urine volume or sodium or potassium excretion in vehicle- or captopril-treated heart failure rats; in contrast, enalapril-treated heart failure rats demonstrated 83% and 10% increases in urine volume and daily sodium excretion, respectively, compared with vehicle-treated rats (both p<0.01). No significant changes in blood urea nitrogen or creatinine were observed with either treatment. Enalapril but not captopril elicited a significant decrease in serum and lung ACE activities. Captopril but not enalapril inhibited aortic ACE activity. Both agents caused a comparable inhibition of renal ACE activity. The magnitude of inhibition of renal ACE activity but not serum and vascular (aortic) ACE activities correlated with the long-term blood pressure response. Enalapril but not captopril normalized renal angiotensinogen expression; the magnitude of this effect correlated with the increase in daily urinary sodium excretion (r=−0.43; p≤0.005).

Conclusions. These data suggest that chronic treatment with these two agents elicits differential effects on tissue ACE activities and renal angiotensinogen regulation. The differential renal effects of these agents may be important in the treatment of heart failure. (Circulation 1992;86:1566–1574)

KEY WORDS  • angiotensin converting enzymes  • angiotensinogen  • myocardial infarction  • angiotensin II

Angiotensin II is produced in vivo in the systemic circulation or at local tissue sites. In response to decreases in cardiac function, as in acute or decompensated heart failure, the endocrine renin–angiotensin system is activated. Increased circulating levels of angiotensin II may serve a vasoconstrictor role or promote renal sodium retention. Additionally, acute inhibition of circulating angiotensin converting enzyme (ACE) mediates immediate hypotensive and natriuretic responses. In contrast, circulating levels of angiotensin II are not usually increased in the stable, compensated phase of heart failure.1–4 Despite normal circulating renin and angiotensin II levels in this state, ACE inhibitors have been demonstrated to be efficacious, improving ventricular function and prolonging survival.5–8 We have recently demonstrated that tissue renin–angiotensin systems are activated selectively in the heart and kidney in chronic stable experimental heart failure and cardiac hypertrophy.4,5,10 It has been hypothesized that ACE inhibitors might exert their beneficial effects primarily by inhibiting tissue ACE and thereby altering tissue angiotensin II levels. No studies to date, however, have investigated the effects of ACE inhibitors on tissue renin–angiotensin systems during chronic treatment in experimental heart failure or whether the effects vary between different ACE inhibitors.

Accordingly, in the present study, we hypothesized that long-term treatment with captopril and enalapril, the most widely used ACE inhibitors, would cause
differential effects on the circulating and tissue renin–angiotensin systems. We further hypothesized that the hemodynamic and renal effects of ACE inhibitor treatment during chronic drug administration in rats with stable, compensated heart failure might correlate better with tissue ACE than with circulating ACE. The results of this study demonstrate that 1) chronic treatment with these agents elicits differential tissue-specific inhibition of ACE; 2) the magnitude of inhibition of serum and aortic ACE activities is not related to the long-term hypertensive response; and 3) differential effects of these agents on renal sodium and water excretion may be mediated by effects on components of the intrarenal renin–angiotensin system and thereby the local production of angiotensin II.

**Methods**

Male Sprague-Dawley rats (weight, 200–250 g; Charles River Laboratories, Wilmington, Mass.) underwent thoracotomy and left coronary artery ligation at 2 months of age. All animals were housed individually in a 12-hour dark/light cycle controlled room. They were allowed free access to a 0.4% sodium diet (Purina rat chow 5001, Purina Mills, St. Louis, Mo.), and water was provided ad libidum. These studies were approved by the institutional Standing Committee on Animals and were performed according to US Public Health Service guidelines and recommendations of the American Association for the Accreditation of Laboratory Animal Care and the American Physiological Society.

**Experimental Heart Failure Model**

The chronic compensated heart failure model was prepared by standard methods. Under ether anesthesia, a thoracotomy was performed, the heart exteriorized, and the left anterior coronary ligated with 6-0 prolene suture. Surgical attrition from this procedure is approximately 40%. One week after surgery, the surviving animals were subgrouped by a modified 10-lead ECG into sham-operated (SO) or heart failure (HF) study cohorts. Group stratification was in all cases confirmed postmortem by both gross pathology and quantitative left ventricular histopathology.

**Experimental Protocol**

The first protocol was designed to evaluate equipotent doses of captopril and enalapril with regard to the magnitude of blood pressure reduction and inhibition of serum ACE activity in this normotensive strain. The short-term hemodynamic and serum ACE activity dose–response relations for captopril and enalapril were thus evaluated in 52 heart failure animals studied approximately 1.5 months after coronary ligation.

The baseline indirect tail cuff blood pressure was studied daily for 7 days. The individual daily and/or diurnal drinking behavior was assessed to determine the time of peak drug ingestion to serve as a guide for when to kill the animals (Figure 1). Tail-artery blood was collected at the end of the baseline period for assessment of the pretreatment serum ACE activity. In these studies, rats then received either captopril for 1 week at 10, 25, 100, or 200 mg · kg⁻¹ · day⁻¹ or enalapril at 15 or 25 mg · kg⁻¹ · day⁻¹ doses based on individual drinking behavior (n=8–10 in each cohort). At the end of each weekly dosing period, indirect blood pressure was determined and blood was collected by tail bleed for measurement of serum ACE activity. Equi potent effects on blood pressure and serum ACE activity were achieved at the captopril 200 mg · kg⁻¹ · day⁻¹ and enalapril 25 mg · kg⁻¹ · day⁻¹ doses.

The effect of chronic ACE inhibitor treatment was then evaluated in a second protocol. The goal of this protocol was to evaluate the hemodynamic and renal effects of captopril and enalapril in animals with chronic heart failure at doses that had been demonstrated to have equipotent short-term effects on blood pressure and circulating ACE. The effects of these treatments on tissue ACE and components of the intrarenal renin–angiotensin system were also assessed. Animals were stratified into four study groups: 1) sham-operated rats treated with tap water vehicle (SO-V, n=14); 2) heart failure rats treated with vehicle (HF-V, n=10); 3) heart failure rats treated with captopril, 200 mg · kg⁻¹ · day⁻¹ (HF-C, n=8); and 4) heart failure rats treated with enalapril, 25 mg · kg⁻¹ · day⁻¹ (HF-E, n=7). All animals were allowed to recover without treatment for 45±5 days after surgery to establish chronic heart failure. Assessment of baseline blood pressure and heart rate was performed, and animals were maintained in individual metabolic cages for assessment of drinking behavior and for urine collection. The concentrations of captopril (kindly provided by E.R. Squibb and Sons, Princeton, N.J.) and enalapril maleate (kindly provided by Merck, Sharp and Dohme, West Point, Pa.) were then individually adjusted every other day to ensure adequate administration of each agent despite differing drinking behaviors. Approximate drug concentrations for enalapril and captopril were 0.2 mg/ml and 2 mg/ml of drinking water, respectively.

At the end of the baseline period, blood (400 μl) was obtained by tail bleed, snap frozen, and stored at −70°C for later ACE activity assay. Chronic treatment was maintained for 47±2 days, and hemodynamic and metabolic assessments were repeated during the final 10 days of study. Animals in each study cohort were treated for an identical duration and were killed at a comparable age.
At the end of treatment, all rats were killed in the early morning by decapitation. Trunk blood was collected for determination of serum ACE activity during treatment, and all tissues were collected within 3 minutes of death. The heart was removed en bloc, and the atria were dissected free from remaining heart at the atrioventricular ring. The right ventricle was dissected from the left ventricle, and ventricular masses were determined. A 2.0-mm (40-mg) specimen of interventricular septal tissue was obtained, and right ventricle and interventricular septal specimens were used for tissue ACE determinations. The entire left ventricle was fixed in 10% buffered formalin. The thoracic aorta was stripped of its adventitia. The lung was dissected to exclude major pulmonary trunks. The kidneys were dissected free of their capsule and bisected; half was used for assay of tissue ACE, and half was flash frozen for later messenger RNA (mRNA) determinations. All tissues used for assessment of tissue ACE activity were immediately placed in ice-cold potassium phosphate buffer and processed without delay.

Hemodynamic Studies

Indirect systolic blood pressure (in millimeters of mercury) was determined by the tail-cuff method and a piezoelectric palpatator. Reported values represent the mean of individual recordings performed at the same time of day by the same investigator on five consecutive days. The correlation of indirect blood pressures with direct femoral arterial pressure measurements in our laboratory achieves a correlation coefficient of \( r = 0.98 \) and is comparable to data reported previously.\(^1\) Heart rate (beats per minute) was calculated from the mean RR interval derived from 10 consecutive cardiac cycles of the indirect blood pressure tracing.

Metabolic Studies

Animals were maintained in individual cages with minimal manipulation. Reported values represent the mean of data obtained over 5 days and represent steady-state values. As noted, drug doses were calculated individually to provide the same daily dose of the drug based on the drinking behavior of each rat. Urine was collected for determination of daily urine volume (UV, ml \( \cdot \) kg\(^{-1} \cdot \) day\(^{-1} \)), sodium excretion (UNa\(^{\text{+}} \cdot \) V, meq \( \cdot \) kg\(^{-1} \cdot \) day\(^{-1} \)), and potassium excretion (UK\(^{\text{+}} \cdot \) V, meq \( \cdot \) kg\(^{-1} \cdot \) day\(^{-1} \)), which were indexed to body weight. Urinary sodium and potassium concentrations were determined by flame photometry (Model 443, Instrumentation Laboratories, Inc., Lexington, Mass.). Blood urea nitrogen (BUN) and serum creatinine concentrations (in milligrams per deciliter) were determined by spectrophotometric methods via autoanalyzer (Ektachem 700 Clinical Analyzer, Eastman Kodak, Rochester, N.Y.).\(^{14}\)

Biochemical Studies

Plasma renin concentration (PRC) was determined as previously described.\(^{15,16}\) Serum and tissue ACE measurements were performed by a modified fluorimetric method.\(^{17,18}\) Baseline and treatment blood samples were collected into chilled Eppendorf tubes and cold centrifuged for 5 minutes, and serum was then aliquoted for either storage at \(-70^\circ\)C (baseline sample) or immediate assay (sample at death). Baseline and treatment serum ACE determinations from individual animals were performed within a single assay to minimize interassay variability. Tissues were washed with cold potassium phosphate buffer to remove any contaminating blood. All tissues were assayed immediately the morning the animal was killed to minimize possible dissociation of each ACE inhibitor from ACE. Because we and others have observed that captopril can dissociate ex vivo from ACE during storage or during assay procedures,\(^9\) all serum samples and tissue homogenates were kept ice cold until incubation. Additional control rats were also treated with either captopril (50 mg/kg) or vehicle by gavage and were killed 1 hour later (synchronously with chronically treated rats), and tissues were processed concurrently to ensure sustained ex vivo inhibition of ACE during assay. Each tissue was immediately homogenized and incubated for 10 minutes at 37°C, pH 7.50, in the presence of Hip-His-Leu; the reaction was stopped by the addition of NaOH, pH 8.5. His-Leu product was then tagged with 0.1% phthalaldehyde and quantified fluorometrically (SLM 8000C, SLM Instruments, Inc., Urbana, Ill.) at an excitation wavelength of 386 nm and emission wavelength of 436 nm. This assay is linear between 0.02 and 15 mM of His-Leu product.

RNA Studies

Renal tissue was snap-frozen in liquid nitrogen within 3 minutes and stored at \(-70^\circ\)C until it was studied. Tissue homogenization and RNA extraction were performed as described.\(^{20}\) Comparisons of relative mRNA levels were made in reference to the same amount of total RNA applied per sample and quantified by absorbance at 260 nm in duplicate. For Northern blot analysis, aliquots of total RNA were run by the formaldehyde–agarose method.\(^{21}\) Gels were transblotted onto nylon filters by capillary action with 10 SSC (1 SSC equals 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0) for 16 hours. Efficiency of transfer was confirmed by ethidium bromide stain and examined before and after transfer. RNA on nylon filters was crosslinked by UV light. For additional quantification, slot blot hybridization analyses were performed. Samples were formaldehyde denatured and then serially diluted in 15 SSC. Three concentrations from each sample (8, 4, and 2 \( \mu \)g) were blotted in duplicate to nylon filters with a slot blot apparatus (Schleicher and Schuell, Peterborough, N.H.). After prehybridization for 4 hours, the blots were hybridized overnight in a buffer to which \( ^{32} \)P complementmentary DNA (cDNA) probes were added.\(^{21}\) After hybridization, blots were washed in 0.2 SSC with 0.1% sodium dodecyl sulfate at room temperature for 10 minutes, then three times at 65°C for 30 minutes. Blots were exposed for 5 days to x-ray film (Kodak XAR, Eastman Kodak). All studies of angiotensinogen mRNA were performed with a full-length rat liver angiotensinogen cDNA (pRang 1650). For renin mRNA determinations, we used renin cDNA pDD 1D-2, a full-length mouse submaxillary gland renin cDNA that readily crosshybridizes with rat renal renin mRNA.\(^{16}\) To control for possible sample variability, identical slot blots were performed and hybridized with a control (\( \beta \)-actin) cDNA probe.
**Quantification of mRNA**

 Autoradiograms generated by slot blots were scanned with an LKB microdensitometer (Paramus, N.J.) with background set to zero for each autoradiograph. Regression lines were calculated from the integral values obtained by scanning the serial concentrations of each sample. The relative signals of the specific mRNA were estimated from the slope of the regression line, and only values of \( r^2 \geq 0.90 \) were accepted. Slopes of specific mRNA for each condition were compared as relative ratios. Sufficient material was available to study specimens with multiple sample applications. Our intrablot and interblot coefficients of variation were 8% and 9%, respectively.

**Morphometric Analysis of Infarct Size**

 The left ventricle was sectioned transversely in at least five planes, stained with Masson’s trichrome and hematoxylin/eosin, and projected for quantitative planimetry of infarct size. The mean of the endocardial and epicardial scar circumferences was compared with total left ventricular circumference to calculate total infarct size (in percent). All infarctions were transmural and involved only the free wall of the left ventricle. Only rats with infarcts greater than 10% of the left ventricular circumference were included in the heart failure groups.

**Statistical Analysis**

 Data are presented as mean±SEM. Mean slopes of slot blot autoradiographs were compared by an unpaired Student’s t test. Hemodynamic and plasma ACE responses were compared by a Student’s t test for paired data. Intergroup variables (SO-V, HF-V, HF-C, and HF-E) were compared by a randomized ANOVA with a Newman-Keuls post hoc test for significant differences between individual groups. Linear regression analysis was performed to evaluate the relations between selected variables. Significance was accepted at the 95% confidence limit (\( p<0.05 \)).

**Results**

 In our initial investigation, we determined equipotent doses of captopril and enalapril during short-term (1-week) treatment of HF rats based on the magnitude of the hypotensive effect and degree of inhibition of serum ACE. During the second protocol, the effects of chronic (7-week) ACE inhibitor treatment with these drugs were assessed in SO and HF rats to determine the relevance of the tissue renin–angiotensin system to the chronic hemodynamic and renal responses.

**Protocol 1**

 In HF-V rats, there were no changes in blood pressure (129±2 versus 125±5 mm Hg, baseline versus treatment, respectively; \( p=NS \)) or serum ACE activity (89±10 versus 91±15 nM · ml⁻¹ · min⁻¹; \( p=NS \)) before or during treatment (Table 1). At lower doses (10 and 25 mg · kg⁻¹ · day⁻¹), captopril treatment caused a fall of approximately 20 mm Hg in blood pressure in all rats (\( p=NS \) between these two doses); the serum ACE response was not assessed in these treatment subsets. Captopril treatment at 100 and 200 mg · kg⁻¹ · day⁻¹ caused blood pressure to fall by an amount comparable to that observed at the lower two doses. Serum ACE was inhibited by 45% and 81% at the 100- and 200-mg · kg⁻¹ · day⁻¹ doses, respectively (\( p<0.005 \) compared with baseline).

 Enalapril treatment for 7 days also caused a hypotensive response of 12–27 mm Hg, and a stepwise increase in serum ACE inhibition was demonstrated. The magnitudes of the hypotensive and serum ACE inhibition responses were comparable for captopril at 200 mg · kg⁻¹ · day⁻¹ and for enalapril at 25 mg · kg⁻¹ · day⁻¹ (\( p=NS \) for both responses between drugs).

**Protocol 2**

 At baseline, animals in each of the four study groups were comparable with regard to age at operation (61 days), age at the initiation of treatment (108 days), and age at the time of death (154 days). Baseline weights and urinary volume and sodium and potassium excretion were also not different between groups (Table 2). The mean infarct size in the total study group was 24±4%. SO rats had a mean infarct size of 3.0±0.3%. The mean infarct sizes in the HF-V, HF-C, and HF-E groups were 17±3%, 33±4%, and 25±3%, respectively. The mean infarct sizes of the captopril and enalapril HF

<table>
<thead>
<tr>
<th>Table 1. Dose-Titrations Studies With Oral Captopril and Enalapril in Heart Failure Rats</th>
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<tr>
<td><strong>Group</strong></td>
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</tr>
<tr>
<td>SO-V</td>
</tr>
<tr>
<td>C10</td>
</tr>
<tr>
<td>C25</td>
</tr>
<tr>
<td>C100</td>
</tr>
<tr>
<td>C200</td>
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<td>E15</td>
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<td>E25</td>
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BP, tail cuff blood pressure; ACE, angiotensin converting enzyme activity. Animals were allowed free access to drinking water (V, vehicle); captopril (C) at 10, 25, 100, or 200 mg · kg⁻¹ · day⁻¹; or enalapril (E) at 15 or 25 mg · kg⁻¹ · day⁻¹ for 1 week. Percent ACE inhibition during treatment was calculated as (baseline serum ACE activity–treatment serum ACE activity)/(baseline serum ACE activity). NA, not available. Data are presented as mean±SEM. \( p<0.05 \) compared with baseline value. \( *\ p<0.005 \) compared with baseline value.

**Table 2. Baseline Characteristics of Rats in Each Chronic Treatment Group**

<table>
<thead>
<tr>
<th>SO-V</th>
<th>HF-V</th>
<th>HF-C</th>
<th>HF-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>404±11</td>
<td>394±13</td>
<td>388±12</td>
</tr>
<tr>
<td>Urine (ml/day)</td>
<td>45±4</td>
<td>42±4</td>
<td>43±3</td>
</tr>
<tr>
<td>Urea (mg · kg BW⁻¹· day⁻¹)</td>
<td>5.9±0.3</td>
<td>6.4±0.3</td>
<td>6.2±0.4</td>
</tr>
<tr>
<td>Urea (meq · kg BW⁻¹· day⁻¹)</td>
<td>10.2±0.5</td>
<td>10.6±0.5</td>
<td>9.9±0.7</td>
</tr>
</tbody>
</table>

SO-V, sham-operated rats treated with vehicle; HF-V, HF-C, HF-E, heart failure rats treated with vehicle, captopril, and enalapril, respectively; UV, daily urinary volume; Urea, daily urinary sodium excretion; UK*, daily urinary potassium excretion; BW, body weight. Data are presented as mean±SEM. No intergroup differences achieved statistical significance.
groups were comparable and did not differ statistically (p=NS).

These observations are confirmed by the measurement of cardiac weights as displayed in Table 3. The left ventricular and right ventricular weights were slightly greater in the HF-V rats compared to the SO group, but this did not reach statistical significance. This is probably because of the smaller infarct size in the HF-V animals. The left and right ventricular weights of rats chronically treated with captopril or enalapril were not significantly different from those of vehicle-treated animals.

Figure 2 displays the blood pressure responses in these groups at baseline and at the end of treatment. As depicted, there was no change in the blood pressure of rats in the SO-V and HF-V groups (from 124±2 to 125±3 mm Hg in HF-V; from 126±3 to 129±2 mm Hg at baseline and during treatment in each group, respectively; p=NS). There was a comparable hypotensive response of 12–18 mm Hg in the HF-C (from 120±3 to 102±3 mm Hg; p<0.01) and HF-E (from 116±4 to 104±2 mm Hg; p<0.01) groups from baseline to treatment. The magnitude of the hypotensive response did not correlate with the size of the previous infarct (r=0.03 for HF-C rats, and r=0.36 for the HF-E rats, both p=NS). Heart rate did not change significantly in any of the treatment groups: SO-V (from 417±7 to 406±8 beats per minute), HF-V (from 394±10 to 405±15 beats per minute), HF-C (from 388±12 to 377±12 beats per minute), and HF-E (from 388±10 to 392±16 beats per minute).

During vehicle treatment, there was no significant change in the daily urine volume (Figure 3), urinary sodium excretion, or urinary potassium excretion in SO or HF rats (Table 4). Similarly, there was no change in these responses in the HF-C rats. In contrast, HF-E animals demonstrated a modest diuretic and natriuretic response to treatment (Table 4 and Figure 3). Although there was a tendency for the BUN to increase in the ACE inhibitor–treated animals, this did not reach statistical significance. The serum creatinine was unchanged during treatment.

The PRC was not different in HF-V versus SO-V rats (Table 5), as we have previously demonstrated; treatment with captopril and enalapril caused PRC to rise by comparable amounts compared with both vehicle-treated control groups (p≤0.01 for each). Treatment with these two ACE inhibitors resulted in differential effects on serum and tissue ACE activities. Serum ACE activity (Figure 4) did not change during vehicle treatment in the SO and HF rats (serving as a time control). During chronic enalapril treatment, serum ACE was inhibited by approximately 55% compared with baseline values. In contrast, there was no significant inhibition of serum ACE in the HF-C animals after 47 days of treatment. Table 6 displays the effects of captopril and enalapril treatment on tissue ACE activities. Captopril elicited a decrease in aortic ACE activity (31% inhibition), but this was not achieved during enalapril treatment. Both drugs elicited comparable decreases in renal ACE activity (56% during captopril and 44% during enalapril treatment, respectively). Captopril treatment was associated with an induction in pulmonary ACE activity, whereas enalapril treatment elicited a 33% fall in ACE activity in the lung. Neither drug caused a

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**TABLE 3. Cardiac Weights in Each Chronic Treatment Group**

<table>
<thead>
<tr>
<th></th>
<th>SO-V</th>
<th>HF-V</th>
<th>HF-C</th>
<th>HF-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV, mg/kg BW</td>
<td>2.04±0.04</td>
<td>2.30±0.12</td>
<td>2.11±0.08</td>
<td>2.25±0.19</td>
</tr>
<tr>
<td>RV, mg/kg BW</td>
<td>0.47±0.02</td>
<td>0.55±0.06</td>
<td>0.58±0.06</td>
<td>0.64±0.07</td>
</tr>
</tbody>
</table>

SO-V, sham-operated rats treated with vehicle; HF-V, HF-C, HF-E, heart failure rats treated with vehicle, captopril, and enalapril, respectively; LV, left ventricle; RV, right ventricle; BW, body weight. No intergroup differences reached statistical significance.

**TABLE 4. Urine Electrolyte Excretion, Blood Urea Nitrogen, and Serum Creatinine at End of Treatment**

<table>
<thead>
<tr>
<th></th>
<th>SO-V</th>
<th>HF-V</th>
<th>HF-C</th>
<th>HF-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNa+V (meq · kg BW(^{-1}) · day(^{-1}))</td>
<td>4.9±0.1</td>
<td>5.3±0.1</td>
<td>5.1±0.2</td>
<td>5.8±0.4*</td>
</tr>
<tr>
<td>UK+V (meq · kg BW(^{-1}) · day(^{-1}))</td>
<td>9.3±0.3</td>
<td>9.8±0.4</td>
<td>8.5±0.5</td>
<td>9.6±0.5</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>19±0.5</td>
<td>19±0.8</td>
<td>29±8</td>
<td>22±2</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.5±0.01</td>
<td>0.5±0.01</td>
<td>0.5±0.01</td>
<td>0.5±0.01</td>
</tr>
</tbody>
</table>

SO-V, sham-operated rats treated with vehicle; HF-V, HF-C, HF-E, heart failure rats treated with vehicle, captopril, and enalapril, respectively; UNa+V, daily urinary sodium excretion; UK+V, daily urinary potassium excretion; BW, body weight; BUN, blood urea nitrogen. Data presented as mean±SEM. *p<0.01 compared with SO-V.
significant change in either right ventricular or interventricular septal ACE activities. Serum and tissues from acutely gavaged rats, assayed concurrently with those from chronically treated animals, demonstrated greater than 85% inhibition compared with vehicle gavage.

The long-term hypotensive response (47±2 days of oral treatment) to ACE inhibitor therapy did not correlate with the serum or aortic ACE activities during treatment (r=0.08 and p=NS for serum ACE activity and r=0.15 and p=NS for aortic ACE activity); a moderate correlation was present between the treatment renal ACE activity and the hypotensive response (r=0.48, p<0.05).

The intrarenal renin–angiotensin system was differentially affected by induction of heart failure as well as by chronic ACE inhibitor treatment (Table 6). HF rats demonstrated a 33% induction in renal angiotensinogen expression compared with SO control rats (p<0.05). Renin mRNA expression was unchanged in HF rats compared with SO control rats. This is consistent with the PRC results and confirms our previous data on rats with chronic compensated heart failure. Chronic captopril treatment did not change the renal angiotensinogen expression mRNA level but elicited an increase in renin mRNA expression compared with HF-V rats (67% and 110% compared with SO-V and HF-V, respectively; both p<0.05); PRC increased significantly (to 96±24 ng AI · ml⁻¹ · hr⁻¹; p<0.05 compared with HF-V) during captopril treatment. In contrast, enalapril treatment normalized the renal angiotensinogen mRNA level (p<0.01 compared with both HF-V and HF-C) and also stimulated PRC (to 58±20 ng AI · ml⁻¹ · hr⁻¹; p=NS compared with HF-V).

**Discussion**

It has been demonstrated that ACE inhibitors are particularly effective in the treatment of severe heart failure, in which the plasma renin–angiotensin system is activated. Recent data also demonstrate that ACE inhibitors are effective in mild-to-moderate congestive heart failure and in left ventricular dysfunction without overt heart failure.5–8,23 Because these conditions are characterized by normal plasma renin activity,2,24 the mechanisms underlying the efficacy of ACE inhibitors remain poorly understood. We and others have hypothesized that these agents might modulate tissue renin–angiotensin system activity and thereby beneficially alter end-organ physiological responses.

The coronary ligated rat model of experimental heart failure is characterized by a stable compensated low-output state in association with regional blood flow redistribution, renal sodium avidity, and increased mortality.6,25 In this model, as in chronic, stable heart failure, plasma renin activity and serum ACE activity are normal in the compensated state;4,5,25 nevertheless, ACE inhibition normalizes regional blood flow, induces natriuresis, and improves survival.5,7,27,28 Thus, the physiological and neurohormonal status of this model simulates those of human chronic heart failure. This model also demonstrates tissue-specific activation of cardiac ACE in proportion to the size of the myocardial infarction.4

The present investigation contributes to our understanding of the role of tissue ACE in heart failure by demonstrating that 1) the hypotensive response to chronic ACE inhibition is not predicted by effects on circulating ACE activity; 2) the magnitude of chronic tissue ACE inhibition is not predicted by short-term serum ACE inhibition; 3) chronic treatment may elicit the induction of tissue and serum ACE activities; and 4) chronic treatment by these two commonly used ACE inhibitors can result in differential responses of the intrarenal renin–angiotensin system, which may influence sodium and water excretion.

**Blood Pressure Response**

During chronic (7-week) treatment, captopril and enalapril caused blood pressure to fall comparably in HF rats. The magnitude of the hypotensive response at
the target doses (captopril, 200 mg·kg⁻¹·day⁻¹ and enalapril, 25 mg·kg⁻¹·day⁻¹) was approximately equal during 1 week and 7 weeks of treatment. It is notable that this dose of captopril is approximately the same as that used in the chronic survival studies performed by Pfeffer et al.²⁰ (2 g/l). Nevertheless, the magnitude of inhibition of serum ACE during short-term (1-week) treatment was substantially greater than that observed after chronic treatment. Both captopril and enalapril elicited an 85% fall in serum ACE activity during 1 week of treatment. In contrast, despite comparable hypotensive effects, only enalapril elicited a sustained inhibition of serum ACE activity. Thus, serum ACE activity was a poor predictor of the systemic hemodynamic response.

The magnitude of the hypotensive effect was not predicted by the severity of heart failure as assessed by the size of the infarcted myocardium. Additionally, the magnitude of inhibition of serum and vascular (aortic) ACE activity did not correlate with the fall in blood pressure in HF rats. Previous investigations have also observed that the magnitude of serum ACE inhibition poorly predicts the hypotensive response in normotensive and hypertensive animal models.²⁹⁻³⁴ Chronic treatment with both enalapril and captopril has been shown to cause induction of total circulating ACE activity; therefore, this lack of correlation is not unexpected.³⁵⁻³⁷ In our study, the long-term blood pressure response correlated best with renal ACE activity. The nature of this relation is not known. It is interesting to speculate that the reduction in renal ACE activity results in reduced intrarenal angiotensin II concentration and enhanced sodium excretion. Thus, the long-term effect on plasma volume may determine the blood pressure response with chronic therapy.

Chronic ACE Inhibitor Treatment: Effects on the Intrarenal Renin–Angiotensin System

The present study demonstrates differing patterns of renal angiotensinogen expression and sodium and volume excretion during treatment with captopril and enalapril. We have recently reported from a separate investigation that chronic experimental heart failure in this model causes a tissue-specific induction of renal angiotensinogen expression.¹⁰ The present study demonstrates that the increased renal angiotensinogen mRNA level is normalized by chronic enalapril treatment but is not affected by long-term captopril therapy. Renal renin mRNA expression and plasma renin concentration during treatment with these two drugs were comparable. Because the blood pressure was not different between the two treatment groups, differences in intrarenal mechanisms of action of these two drugs are presumed to underlie these different renal angiotensinogen responses. The nature of these differences in intrarenal action is unclear at this time.

The physiological consequences of intrarenal angiotensin production may be extrapolated from the results of the present study. HF-C rats displayed a lower daily excretion of both sodium and water than HF-E rats. Because renal perfusion pressure (arterial pressure) was not different between the two drug treatment groups, an intrarenal effect must differentiate these markedly disparate renal responses; such an intrarenal effect may be the level of angiotensin II. Because the magnitude of renal ACE inhibition was comparable between these two treatment groups, and because renin was present in excess, we speculate that angiotensinogen levels may determine the rate of intrarenal angiotensin synthesis. Intrarenal angiotensin II has been shown to stimulate sodium and water retention via a direct proximal tubular effect. The relation between angiotensinogen mRNA levels and renal sodium excretion is displayed in Figure 5. As shown, there was no correlation between the angiotensinogen mRNA level and sodium excretion during vehicle treatment (r=0.09, p=NS). In contrast, drug-treated HF rats displayed a fairly striking inverse relation, as increased angiotensinogen expression was associated with lower sodium excretion (r=−0.43, p<0.05). Although such a correlation does not delineate either a cause-and-effect mechanism or a common cofactor that directionally alters both angiotensinogen expression and sodium excretion, this finding provides an initial clue to suggest that such links exist. It should be noted that the present 24-hour urinary sodium measurements determined during the final week of treatment may not reflect cumulative changes in chronic sodium balance that likely occur in this model with chronic treatment with either ACE inhibitor.

These data should also be interpreted in the context of the study of Packer et al.¹⁰ that demonstrated that enalapril resulted in a higher BUN and serum creatinine than captopril under the conditions of their experiments. In the present study, we did not observe a
difference in renal function between the two drug treatments under the conditions of our experiment. Unlike Packer et al, we did not observe a difference in systemic blood pressures between the two drugs, as expected from the design of our protocol. We did, however, observe a difference in angiotensinogen mRNA expression that may reflect a shorter intrarenal duration of action of captopril. Because we have shown that intrarenal angiotensin II directly stimulates angiotensinogen mRNA expression, the higher renal angiotensinogen mRNA in the captopril rats is consistent with a lesser reduction in angiotensin II in the kidney. Because angiotensin II can also directly stimulate proximal tubule sodium reabsorption, this differential effect on intrarenal angiotensin may also explain the differences in urinary sodium excretion between the two drugs.

Differential Effects of Captopril and Enalapril on Tissue ACE

It has previously been recognized that ACE inhibitors have differential effects on the inhibition of tissue ACE. Previous studies have reported a decrease in cardiac hypertrophy and the prevention of ventricular enlargement with ACE inhibition. Chronic ACE inhibitor treatment in this study did not decrease either total left or right ventricular mass, however, despite prolonged drug therapy. This may not be surprising, in that ACE inhibitors were not initiated until 47 days after myocardial infarction. Anversa et al have shown that cardiac myocyte hypertrophy is an early response to injury, occurring within 3 days of infarction. Additionally, it is interesting that cardiac ACE activity in this study did not appear to be suppressed by either captopril or enalapril. This is probably because of the induction of cardiac ACE during concomitant ACE inhibition. Although both agents may inhibit cardiac ACE when administered as a single dose in spontaneously hypertensive rats, the chronic responses in normetotensive experimental models have not been previously studied.

Aortic ACE activity was inhibited by captopril but not enalapril in HF rats. Thus, one might speculate that captopril would have greater effects on vascular compliance or hypertrophy than enalapril at these administered doses. Although these would be potentially important end-organ effects, these vascular functional responses were not specifically studied in these protocols.

Equipotency: Serum versus Tissue Responses

In clinical trials, the effects of various ACE inhibitors on clinical endpoints have been assessed by titration of drug doses based on the systemic hemodynamic response. This study demonstrates that hemodynamic equipotency cannot always be correlated with equipotent effects at the tissue level. Whereas captopril and enalapril caused differing patterns of tissue ACE inhibition, it is likely that one might achieve comparable tissue ACE inhibition with alternative doses of these agents. Additionally, the duration of treatment by each agent may cause variable induction of serum and tissue ACE activities. Until better methods of assessing the magnitude of this induction are available, true drug equipotency may be difficult to define. Finally, the magnitude of penetration and/or binding of these agents to tissue ACE is difficult to quantify.

Study Limitations

This investigation examined primarily tissue ACE activities; it examined renin and angiotensinogen only in the kidney. It was not realistic to study every component of this system in all tissues. Present techniques do not permit localization of the cell type(s) at which ACE is inhibited. Tissue angiotensin II concentrations were not determined. Concentrations of angiotensin II in tissue homogenates are notoriously difficult to quantify because of the presence of other non-ACE tissue peptides and crossreactivity of most antibodies to angiotensins I, II, and III. Thus, the magnitude of effect of these drugs on angiotensin II biosynthesis must be inferred from surrogate markers (e.g., rate-limiting components of the system). In addition, ACE inhibitors may alter the synthesis and metabolism of kinins and prostaglandins, which may contribute to their beneficial end-organ effects.

Finally, whereas these data describe the effect of these ACE inhibitors at selected doses in a relevant small-animal model of heart failure, these findings should be extrapolated to clinical investigation cautiously. Small-animal pharmacological investigations are often characterized by greater drug doses on a milligram per kilogram basis. The effect of clinically appropriate doses of ACE inhibitors on human tissue ACE is not known.

Conclusions

These findings demonstrate that circulating and tissue ACE levels are probably altered (induced) by chronic drug therapy. Despite comparable acute hemodynamic and serum ACE inhibitory effects, chronic captopril and enalapril treatment elicit quite different patterns of inhibition of tissue ACE in experimental heart failure; this finding has relevance in the design of future clinical or experimental investigations that attempt to compare these or other agents of this class. The beneficial effects of ACE inhibitors are probably a result of the interplay of multiple mechanisms of action of these agents on target end-organs in heart failure and other pathophysiological states. Some of these effects may be attributed to the ability of ACE-inhibitors to alter the expression or activity of tissue renin–angiotensin systems.

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