Blood Pressure and NaCl-Sensitive Hypertension Are Influenced by Angiotensin-Converting Enzyme Gene Expression in Transgenic Mice

Scott H. Carlson, Suzanne Oparil, Yiu-Fai Chen, J. Michael Wyss

Abstract—ACE plays an important role in the regulation of arterial pressure; however, a linear relationship between ACE expression and arterial pressure has not been demonstrated. The present study employed telemetric monitoring in female transgenic mice to determine the influence of partial and complete deletion of the ACE gene on basal arterial pressure and arterial pressure responses to a high-NaCl diet. On the basal NaCl diet, 24-hour mean arterial pressure was significantly correlated with the number of functional copies of the ACE gene; ie, arterial pressure was lowest in 0-copy (80±1 mm Hg), intermediate in 1-copy (100±1 mm Hg), and highest in 2-copy (113±1 mm Hg) ACE mice. The high-NaCl diet significantly increased mean arterial pressure in 0-copy (99±1 mm Hg) and 1-copy (108±1 mm Hg) mice but not in 2-copy mice (114±1 mm Hg). These results demonstrate a copy-dependent relationship between ACE gene expression and both basal arterial pressure and arterial pressure responses to a high-NaCl diet, suggesting that either partial or complete reduction in the ACE gene can alter arterial pressure. (Hypertension. 2002;39:214-218.)

Key Words: angiotensin-converting enzyme □ arterial pressure □ renin-angiotensin system □ sodium

The ACE is a dipeptidyl carboxy-peptidase that both converts the inactive decapeptide angiotensin I to the active octapeptide angiotensin II and inactivates bradykinin by degradative metabolism. These metabolic events promote increased arterial pressure, sympathetic nervous system activity, sodium retention, and mitogenesis of vascular cells. Because of these prohypertensive effects, many pharmacological treatments for hypertension have focused on the inhibition of ACE activity.

Homologous recombination techniques have been used to test the cardiovascular effects of altering expression of components of the renin-angiotensin system. Deletion of either the angiotensinogen1,2 or angiotensin type 1 (AT1) receptor3 gene decreases arterial pressure in a copy-dependent manner, indicating a direct relationship between arterial pressure and the number of functional gene copies. In contrast, studies in ACE knockout mice have reported that although deletion of both copies of the ACE gene significantly reduces basal arterial pressure,4,5 deletion of a single copy of the ACE gene has no effect on basal arterial pressure.4,5 These reports suggest that arterial pressure is not influenced by partial decreases in ACE gene expression, a finding consistent with the common assumption that ACE is not rate-limiting in the renin-angiotensin system.

The interpretation of these results is limited by the relatively low sensitivity of the methods that were used to record arterial pressure, ie, tail-cuff4,6 and acute catheterization.5 Both of these methods require restraint, heating, and/or tethering and are typically used to record arterial pressure only during the daytime, the normal sleep period for the mouse, when arterial pressures are lowest in rodents (ie, comparable to nighttime blood pressures in humans). Thus, these methods do not document important data from the nighttime period, when mice are awake, are active, and have higher arterial pressures.

The recent introduction of telemetric monitoring of arterial pressure in mice provides a much more sensitive technique for assessing arterial pressure and its circadian rhythm. Telemetric methods make possible the continuous recording of arterial pressure and heart rate (HR) from mice without the stress of either handling or tethering. We have developed an implantation protocol by which mice as small as 19 grams can be reliably implanted with telemetry probes and monitored for several weeks.7 Using this technique, the present study tested the hypothesis that arterial pressure is directly related to the number of functional ACE gene copies expressed in mice. Further, previous studies have demonstrated that pharmacological ACE inhibition increases arterial pressure responses to a high-NaCl diet.8–10 Therefore, the second phase of this study tested the hypothesis that reduction in ACE gene copies increases arterial pressure responses to excess dietary NaCl. The results demonstrate a copy-dependent relationship between functional ACE gene copies and both basal arterial pressure and arterial pressure responses to dietary NaCl.

Methods

Animals

Mice (2-copy ACE, n=7; 1-copy ACE, n=7; and 0-copy ACE, n=6) were bred at the University of Alabama at Birmingham animal
facility,\textsuperscript{4,5} from heterozygous F1 offspring (1-copy ACE mutation) of C57BL/6J mice in which exon 14 of the ACE gene was disrupted. The founders were originally obtained from Oliver Smithies (University of North Carolina at Chapel Hill). Mating of a $+/_{\text{H11001}}$ male with a $+/_{\text{H11001}}$ female results in a $+/_{\text{H11001}}$: $+/_{\text{H11001}}$:-/- ratio of 1:1.58:0.34, making generation of the -/- genotype a limiting factor.\textsuperscript{4,5} Female mice were used in all experiments. The breeding produced 2-copy, 1-copy, and 0-copy F2 offspring that were genotyped by Northern blot analysis to determine the number of functional ACE gene copies, as described previously (Figure 1).\textsuperscript{4,5}

Mice were housed in individual cages in a sound attenuated room at constant humidity, temperature, and light cycle (06:00 AM to 6:00 PM). All mice were allowed ad libitum access to tap water and either basal NaCl (0.6%; diet No. 8746, Teklad) or high-NaCl (8%; diet No. 5008, Teklad) diet, as specified in the experimental protocols. All experimental procedures were conducted in accordance with National Institutes of Health guidelines and approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee.

Surgical Procedures
Female mice were chronically instrumented with telemetry probes (TA11-PA20, Data Sciences International) that were placed into the right common carotid artery, as described previously.\textsuperscript{7} After recovery, the mice were returned to their cages for a 1-week period.

Experimental Protocol
After surgery, mice were maintained on a basal (0.6%) NaCl diet for a 1-week period, after which the telemetry probes were turned on, and mean arterial pressure (MAP) and HR were monitored for 3 days. The probes were then turned off, and the mice were fed the high-NaCl (8%) diet for 1 week, after which the probes were turned on and MAP and HR were monitored for an additional 3 days.

Data Acquisition and Analysis
MAP and HR data were collected and analyzed as described previously.\textsuperscript{7,11,12} Ten-second waveforms of MAP and HR were sampled every 5 minutes during the 3-day monitoring periods, and hourly averages and SD were then calculated. Circadian rhythm analysis of the individual hourly MAP and HR data was performed using the nonlinear, least-squares fitting program PHARMFIT,\textsuperscript{13} and the best fit model was defined as the one with the lowest number of harmonics that had a confidence value of at least 0.05, as determined by the subprogram SYNOPS.\textsuperscript{14} All PHARMFIT analyses were based on data for 3 consecutive days, thus facilitating comparisons of the circadian rhythms in each group, including the estimation of the midline estimating statistic of rhythm, amplitude, and acrophase (clock time of peak amplitude) of the 24-hour adjusted rhythm.

Statistical Analysis
Data were evaluated by ANOVA for repeated measures (significance criteria of $P<0.05$), with appropriate post hoc tests (Newman-Keuls) to determine the source of main effects and interactions. An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results
Body weights were not significantly different between the groups at any time during the study (final body weights: 2-copy, 25.8±1.1 g; 1-copy, 23.8±0.6 g; and 0-copy,

![Figure 2](http://hyper.ahajournals.org/) Hourly MAP (top tracings) and the PHARMFIT-generated MAP circadian rhythm (lower tracings) in female 2-copy, 1-copy, and 0-copy ACE mice. MAP was significantly lower in 0-copy ACE mice and moderately lower in 1-copy ACE mice compared with 2-copy ACE mice fed a basal (0.6%) NaCl diet. Exposure to the high-NaCl (8%) diet increased MAP the most in 0-copy ACE mice, increased MAP less in 1-copy ACE mice, and did not affect MAP in 2-copy mice. The black bars on the horizontal axis indicate the nighttime (lights off) period.
TABLE 1. The Circadian Rhythm of MAP in 2-Copy, 1-Copy, and 0-Copy ACE Knockout Mice Maintained on a Basal NaCl (0.6%) or High-NaCl (8%) Diet

<table>
<thead>
<tr>
<th>Diet/Rhythm</th>
<th>2-Copy (n=7)</th>
<th>1-Copy (n=7)</th>
<th>0-Copy (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal NaCl Diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MESOR, mm Hg</td>
<td>112.6±0.9*</td>
<td>99.5±0.8*</td>
<td>79.8±1.0*</td>
</tr>
<tr>
<td>Amplitude, mm Hg</td>
<td>27.0±1.2</td>
<td>20.9±1.2*</td>
<td>25.6±1.5</td>
</tr>
<tr>
<td>Acrophase, hr</td>
<td>01:16±0.5</td>
<td>01:01±0.7</td>
<td>02:11±0.6</td>
</tr>
<tr>
<td>Peak, mm Hg</td>
<td>124.3±3.6*</td>
<td>111.1±1.0*</td>
<td>90.3±1.8*</td>
</tr>
<tr>
<td>Nadir, mm Hg</td>
<td>97.3±4.1</td>
<td>90.2±2.9</td>
<td>64.7±0.7*</td>
</tr>
</tbody>
</table>

High-NaCl Diet

| MESOR, mm Hg  | 114.3±1.3    | 108.0±0.9†   | 98.8±1.1‡  |
| Amplitude, mm Hg | 28.3±1.8*   | 42.9±1.2†    | 12.9±1.5†  |
| Acrophase, hr  | 01:59±0.8    | 03:55±0.3†   | 01:45±7.6  |
| Peak, mm Hg    | 126.2±1.8    | 125.9±3.0†   | 104.5±1.3† |
| Nadir, mm Hg   | 97.9±1.5*    | 83.0±2.6†    | 91.6±3.1‡  |

Values are mean±SEM. MESOR indicates midline estimating statistic of rhythm.

Twenty-four-hour MAP rhythm (3-day averages): *P<0.05 vs all other groups on same diet; †P<0.05 vs 0.6%-NaCl diet.

The high-NaCl diet caused an increase in arterial pressure that was inversely related to the number of functional gene copies (Figure 2). The high-NaCl diet increased arterial pressure most in the 0-copy mice diet (80.7±3.7 to 98.0±4.6 mm Hg, \( P<0.05 \)), caused a smaller increase in the 1-copy group (100.0±3.5 to 108.0±7.9 mm Hg, \( P<0.05 \)), and had no significant effect on arterial pressure in the 2-copy mice (111.8±4.2 to 113.2±4.8 mm Hg; \( P=NS \)). The high-NaCl diet significantly increased peak nighttime arterial pressures in both 2-copy and 0-copy mice, but it increased the daytime arterial pressure nadir only in the 0-copy group (Table 1). Exposure to the high-NaCl diet also altered the amplitude of the MAP rhythm in 1-copy and 0-copy mice (Table 1). The high-NaCl diet produced comparable decreases in HR in both 2-copy (\( ∼115 \) bpm, \( P<0.05 \)) and 1-copy (\( ∼122 \) bpm, \( P<0.05 \)) mice, and both peak and nadir levels decreased (Table 2). In contrast, the high-NaCl diet resulted in a smaller decrease in HR in 0-copy mice (\( ∼26 \) bpm) than in the other 2 groups, but HR was not significantly different between 2-copy (549±8 bpm) and 1-copy mice (552±14 bpm; Figure 3 and Table 2).
bpm, P<0.05) and the peak HR was unaffected, whereas the reduction in nadir levels was significantly lower compared with those in the other groups (Figure 3 and Table 2). Similarly, the amplitude of the HR rhythm significantly decreased in both 2-copy and 1-copy mice, whereas exposure to the high-NaCl diet significantly elevated the amplitude in the 0-copy mice (Figure 3 and Table 2).

**Discussion**

The present study used mice in which the number of genes coding for ACE were functionally reduced (1-copy) or deleted (0-copy) to test the hypothesis that the level of ACE gene expression plays a role in arterial pressure regulation. The results demonstrate that there is a direct relationship between the number of ACE gene copies and basal blood pressure in mice, and that a reduction in the number of ACE gene copies alters the arterial pressure response to a high-NaCl diet. These data establish for the first time that there is an inverse relationship between the number of ACE gene copies and arterial pressure responses to dietary NaCl.

This study extends the findings of several previous investigations that report a direct relationship between expression of renin-angiotensin system components and basal arterial pressure in transgenic mice. A number of studies have shown that basal arterial pressure is significantly lower in transgenic mice (compared with wildtype) in which either the angiotensinogen,1,2,15–18 ACE,4,6,19 or AT$_1$ receptor1,20,21 genes is functionally deleted (0-copy). Furthermore, insertion of a functional angiotensinogen gene into the angiotensinogen knockout mice rescues the MAP phenotype in these mice; ie, MAP is restored to normotensive values in these animals, the amplitude of the HR rhythm significantly decreased in both 2-copy and 1-copy mice, whereas exposure to the high-NaCl diet significantly elevated the amplitude in the 0-copy mice (Figure 3 and Table 2).

The present study also suggests that deletion of a single ACE gene copy significantly alters arterial pressure in mice. Previous studies in ACE knockout mice have not consistently reported a reduction in blood pressure in 1-copy mice,4,6,19 thereby suggesting that partial reduction of ACE expression does not alter arterial pressure. Although Krege et al initially reported a reduction in arterial pressure in male (but not female) 1-copy ACE knockout mice,4 subsequent studies demonstrated no such relationship,5,6 indicating that only 1-copy of the ACE gene is needed to maintain arterial pressure.6 The failure of these studies to demonstrate that the loss of 1-copy of the ACE gene decreases arterial pressure is likely attributable to the techniques used to record arterial pressure, ie, tail-cuff recording4,6 or acute catheterization.5 Although these techniques can provide an accurate estimate of arterial pressure,25 their sensitivity is limited by the stress they cause to the animal (eg, handling, restraint, surgical, and anesthetic) and because arterial pressure is usually measured at a single point, ie, during the day. In rats, arterial pressure displays a marked 24-hour circadian rhythm that differs among strains and experimental treatments.11,12,14 The present results, along with our previous study,7 indicate that mice have a similar day-night arterial rhythm. Therefore, single daytime measurements only probe arterial pressure when it is at or near its 24-hour nadir. By continuously recording arterial pressure in untethered and unrestrained mice that have fully recovered from surgical and anesthetic effects, the present study clearly demonstrates that arterial pressure is reduced in 1-copy (compared with 2-copy) ACE transgenic mice.

These data support the hypothesis that variations in renin-angiotensin system components, including ACE, alter basal arterial pressure in mice. The mechanisms underlying this are unclear, but may include copy-dependent reductions in angiotensin II levels with a subsequent reduction in the direct vasoconstrictor and sympathoexcitatory effects of angiotensin. The significant decrease in HR in the 0-copy mice suggests that a reduction in sympathetic activity contributes to the reduced basal blood pressure, although HR is unchanged in the 1-copy mice. Elevations in circulating bradykinin, caused by decreased ACE levels, may also contribute to the copy-dependent reductions in arterial pressure. However, measurement of these hormones is difficult given the volume of blood needed for determining circulating concentrations and the low ratio of 0-copy offspring produced. Likewise, studies have yet to utilize bradykinin receptor antagonists to address the contribution of bradykinin to basal arterial pressure in these animals. Additionally, Bernstein reported that 0-copy ACE knockout mice have atrophic renal medulla and papilla, leading to the inability of the mouse to effectively concentrate urine and potentially reducing basal arterial pressure. However, renal development is normal in 1-copy (compared with 0-copy) mice,6 which indicates that the basal arterial pressure differences reported in this study are not simply the result of gross renal dysfunction caused by gene deletion.

Over-expression of the renin-angiotensin system results in angiotensin II–dependent hypertension caused by excess volume and sodium retention combined with the sympathoexcitatory effects of angiotensin.23,26 However, suppression of the renin-angiotensin system also can induce/exacerbate NaCl-sensitive hypertension. In angiotensinogen knockout mice, exposure to a high-NaCl diet elevates arterial pressure in 0-copy (but not 2-copy) mice.15–17 Similarly, chronic captopril inhibition of ACE activity produces NaCl-sensitive hypertension in otherwise NaCl-resistant normotensive rats.8 The mechanisms underlying these effects remains ambiguous, but they may include a failure of the angiotensin system to respond to a high-NaCl diet.

Similarly, after chronic sympathetic nervous system blockade with the α$_1$-adrenergic receptor antagonist prazosin, rats become hypertensive without any change in sodium or water balance.27 This indicates that in NaCl-sensitive hypertension, the responsiveness of the sympathetic nervous system to the challenge may be more important than the absolute level of sympathetic nervous system activity. The present results suggest a similar relationship between the renin-angiotensin system and arterial pressure regulation.28 Although in 2-copy mice exposure to the high-NaCl diet did not alter MAP and likely reduced plasma angiotensin II levels, 1-copy mice responded to the high-NaCl diet with a significant increase in blood pressure, and 0-copy mice responded with an even greater increase in MAP. Both the 1- and 0-copy mice are impaired in plasma angiotensin II regulation, suggesting that the responsiveness of the renin-angiotensin system is more
important than the absolute level of circulating angiotensin II. Thus, the inability of the renin-angiotensin system to withdraw circulating angiotensin II levels may promote NaCl-sensitive hypertension in the ACE knockout mice, by eliminating the normal events associated with the NaCl-induced decrease in angiotensin II. Although changes in HR are only an indirect index of sympathetic nerve system activity, the inability of the 0-cm mice to reduce HR in response to the high-NaCl diet indicates that excess sympathetic activity underlies NaCl-sensitivity in ACE knockout mice. However, the observation that similar decreases in midline estimating statistic of rhythm, peak, and nadir HR occurred in 1- and 2-cm mice suggests that NaCl sensitivity is not mediated primarily by the failure of the sympathetic nerve system to respond. Other factors—eg, increased plasma sodium concentration, changes in body fluid volume, increased renovascular tone, and/or increased renin production—may contribute to the observed NaCl-induced rise in arterial pressure. Whether the reduced HR responsiveness in the 0-cm mice contributes to their NaCl-sensitivity remains to be elucidated.

In summary, the present study demonstrates that in mice, either partial or complete deletion of the ACE gene directly affects both basal arterial pressure and arterial pressure responses to a high-NaCl diet. Although it is unclear whether this effect is attributable to lower circulating angiotensin II levels, altered sympathetic outflow, or other factors such as increased plasma bradykinin concentration, the present results demonstrate that the level of ACE expression plays an important role in arterial pressure regulation.

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References

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