Feedback Regulation of Angiotensin Converting Enzyme Activity and mRNA Levels by Angiotensin II

Heribert Schunkert, Julie R. Ingelfinger, Alan T. Hirsch, Yigal Pinto, Willem J. Remme, Howard Jacob, and Victor J. Dzau

Although renin and angiotensinogen are known to be subject to feedback regulation, the effects of angiotensin II (Ang II) on the regulation of angiotensin converting enzyme (ACE) gene expression and enzymatic activity have not yet been studied. Therefore, the effects of exogenous Ang II infusion and ACE inhibition on ACE mRNA expression were examined. Ang II was infused intravenously in male Sprague-Dawley rats for 3 days at 100 (low dose), 300 (medium dose), or 1,000 (high dose) ng/kg per minute (n=8 for each group). Compared with control (vehicle infusion, n=8), Ang II infusion increased plasma Ang II concentration (62, 101, 126 [p<0.05], and 187 [p<0.05] fmol/ml) and mean arterial blood pressure (106, 119 [p<0.05], 134 [p<0.05], and 125 mm Hg for control, low, medium, and high doses, respectively). Ang II infusion decreased ACE mRNA levels in the lung (57%, 52%, and 51%; p<0.05 for each) and testis (49%, 63%, and 53% of control for low, medium, and high doses, respectively; p<0.05 for each), two major sites of ACE synthesis. There was, albeit less pronounced, a parallel decrease in pulmonary ACE activity (4.38, 3.92, 3.07 [p<0.05], and 3.48 [p<0.05] nM/mg per minute for control, medium, and high doses, respectively). In contrast, serum (54, 50, 48, and 38 [p<0.05] nM/ml per minute) and testicular (2.63, 2.08 [p<0.05], 2.24, and 2.18 nM/mg per minute for control, low, medium, and high doses, respectively) ACE activities displayed only minimal change in animals infused with Ang II. The effects of blockade of Ang II production were studied after 3 days of ACE inhibition by quinapril (10 mg/kg per day) in drinking water. Mean arterial blood pressure decreased significantly (111 versus 99 mm Hg, p<0.05). Quinapril treatment suppressed serum (43% of control, p<0.05) and pulmonary (66% of control, p<0.001) ACE activities. However, ACE mRNA level was induced in the lung (140% of control, p<0.05). Testicular ACE activity was not significantly inhibited by quinapril (88% of control), and no significant difference was seen in the testicular mRNA levels between the two groups (98% of control). To study the effects of discontinuation of ACE inhibition on the regulation of serum ACE activity, additional rats were treated with quinapril (10 mg/kg per day for 3 days, followed by a single gavage of 10 mg/kg). Serum ACE activity was measured 6, 24, 48, 96, and 168 hours after withdrawal of treatment. Serum ACE activity was inhibited at 6 hours after gavage. However, serum ACE activity exceeded baseline values significantly at 48 hours (193% of baseline, p<0.05) and 96 hours (146% of baseline, p<0.05) after discontinuation of ACE inhibition. These studies suggest that pulmonary ACE expression is subject to negative feedback by Ang II. Furthermore, our data are consistent with previous reports that ACE inhibitors do not penetrate into the testis in vivo. (Circulation Research 1993;72:312–318)

KEY WORDS • angiotensin II • angiotensin converting enzyme • gene expression • feedback regulation • angiotensin converting enzyme inhibitor

The synthesis of angiotensin II (Ang II) is principally regulated by the activity of two enzymes: renin and angiotensin converting enzyme (ACE). Although renin cleaves angiotensinogen to form the decapeptidyl angiotensin I, the final step of the renin-angiotensin cascade is catalyzed by the dipeptidyl carboxypeptidase ACE (kininase II, EC 3.4.15.1) to yield the biologically active octapeptide Ang II. 1 ACE gene expression and enzymatic activity are found in tissues of the cardiovascular and urogenital systems, with highest concentrations in the lung and testis. 2–5 The regulation of the activity of other components of the renin-angiotensin system has been studied in considerable detail. Such studies have revealed that Ang II is one of the most potent factors in the regulation of renin and angiotensinogen synthesis. 6–8 Ang II exerts a negative feedback on renin gene transcription and renal renin secretion. 9–14 On the other hand, Ang II has been
shown to exert positive feedback regulation of renal and hepatic angiotensinogen gene expression\textsuperscript{8,14,15} and protein synthesis.\textsuperscript{16,17}

Inhibition of ACE has been shown to be a valuable therapeutic tool in treatment of both high and low renin hypertension,\textsuperscript{18-20} and ACE inhibitors have been demonstrated to improve survival in both experimental and clinical heart failure and to attenuate left ventricular dilatation after myocardial infarction.\textsuperscript{21-23} Yet, despite its central role in Ang II synthesis, little is known about the regulation of tissue or circulating ACE activity or gene expression. The focus of the present investigation is to study whether Ang II influences ACE gene expression and activity as well.

Materials and Methods

Eight-week-old male Sprague-Dawley rats weighing 250–300 g were studied. Animals were allowed free access to a 0.4% sodium diet (regular Purina chow, Purina Mills, St. Louis, Mo.) and water ad libitum. The studies were approved by the institutional standing committee on animal research and performed according to US Public Health Service guidelines.

Experimental Protocols

In the first protocol, Ang II (Sigma Chemical Co., St. Louis, Mo.) was infused intravenously for 3 days at a low dose (100 ng/kg per minute), medium dose (300 ng/kg per minute), or high dose (1,000 ng/kg per minute) \((n=8\) for each group). Control animals received saline vehicle \((n=8)\). Under pentobarbital anesthesia, PE-10 tubing was inserted into both femoral vein and artery. The venous line was filled with the respective infusate and attached to an Alzet osmotic minipump (Alza Corp., Palo Alto, Calif.) at a flow rate of 1.02 ml/hr. The arterial line was tunneled subcutaneously to the back of the neck for subsequent direct blood pressure measurement. Direct mean arterial blood pressure was measured in conscious animals on the third study day with a Statham Gould pressure transducer (Statham Instruments, Puerto Rico) connected to a polygraph (Grass Instrument Co., Quincy, Mass.). Rats were then decapitated. Blood and tissues were rapidly collected, frozen in liquid nitrogen, and stored at \(-75^\circ\text{C}\) for subsequent analyses.

In the second protocol, animals were treated with quinapril (10 mg/kg per day in drinking water, \(n=8\)) or placebo \((n=7)\) for 3 days. Animals were instrumented, and blood pressure was recorded on the third day in identical fashion.

In the third protocol, animals were treated with quinapril (10 mg/kg per day in drinking water, \(n=20\)) for 3 days. To further ensure acute ACE inhibition, each rat also received an additional dose of quinapril by gavage (10 mg/kg under light ether anesthesia) on the next morning (time 0). Animals were then studied 6, 24, 48, 96, and 168 hours after gavage \((n=4\) at each time point). A control group \((n=8)\) was allowed free access to drinking water and received saline gavage at time 0. Control rats were studied at 6 and 168 hours after gavage \((n=4\) at each time point).

Biochemical Studies

Serum and tissue ACE activity was measured by the rate of generation of His-Leu from Hip-His-Leu sub-
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>0.90 were accepted. The blots were washed and rehybridized with β-actin cDNA. The signal for ACE mRNA was divided by the signal for the β-actin mRNA for each sample, and results were expressed as ACE/β-actin mRNA ratios. Our intrablot and interblot coefficient of variations were 8% and 9%, respectively.

**Statistical Analysis**

All data are presented as mean±SEM. Average slopes of slot-blot autoradiographs were directly compared by Student’s unpaired t tests. A two-way analysis of variance and Fisher’s exact test for posthoc analyses were used for multiple comparisons in case of three or more comparisons between groups. The relations between Ang II plasma levels or blood pressures and pulmonary ACE mRNA levels were determined by rank correlation analysis (Spearman’s ρ). Significance was accepted at p<0.05.

**Results**

In this investigation, we examined the feedback regulation of ACE expression by Ang II, the effector peptide of the renin-angiotensin system. ACE activity was measured in serum, lung, and testis, and ACE mRNA levels were examined in lung and testis, the tissues with the highest ACE levels.

**Protocol 1: Effect of Exogenous Ang II Infusion**

Ang II (100, 300, or 1,000 ng/kg per minute) infused intravenously by osmotic minipumps over 3 days increased plasma Ang II concentrations in a dose-dependent manner (Table 1). Mean arterial blood pressure increased as well but was more variable with the highest dose of Ang II (Table 1). The infusion of Ang II decreased ACE activity in the lung, as compared with the vehicle-infused control value (Figure 2). In parallel, pulmonary ACE mRNA levels decreased significantly in animals receiving the Ang II infusion (Figure 2). In contrast, the effects of Ang II infusion on serum and testicular ACE activities were less pronounced. Testicular ACE activity tended to decline; however, a significant reduction was observed only with the lowest dose (Figure 3). In contrast, testicular ACE mRNA levels were significantly decreased by Ang II infusion (Figure 3). Serum ACE activity displayed no significant difference after 3 days of Ang II infusion with the low or medium dose (control, 54.3±5.8 nM/ml per minute; low dose, 50.2±2.8 nM/ml per minute; medium dose, 48.3±4.7 nM/ml per minute). However, the high dose of Ang II infusion resulted in a 30% decrease in serum ACE activity (38.2±3.3 nM/ml per minute, p<0.05).

**Protocol 2: Effect of ACE Inhibition**

In this protocol, ACE activity and ACE mRNA levels were studied after 3 days of ACE inhibition with quinapril (10 mg/kg per day) in drinking water. Blood pressure decreased significantly in quinapril- versus vehicle-treated animals (Table 1). Plasma Ang II levels tended to decline, but this did not reach significance (Table 1). However, quinapril treatment decreased se-
Table 1. Serun Angiotensin Converting Enzyme Activity, Plasma Angiotensin II Concentration, and Mean Arterial Blood Pressure After 3 Days of Intravenous Infusion of Vehicle or Angiotensin II or After 3 Days of Oral Vehicle or Quinapril Treatment

<table>
<thead>
<tr>
<th>Angiotensin II Infusion</th>
<th>Low dose (100 ng/kg per minute)</th>
<th>Medium dose (300 ng/kg per minute)</th>
<th>High dose (1,000 ng/kg per minute)</th>
<th>ACE inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ACE (nM/ml per minute)</td>
<td>54±5</td>
<td>50±2</td>
<td>48±4</td>
<td>38±3*</td>
</tr>
<tr>
<td>Plasma Ang II (fmol/ml)</td>
<td>62±13</td>
<td>101±10</td>
<td>126±17*</td>
<td>187±21*</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>106±4.1</td>
<td>119±1.2*</td>
<td>134±9.6*</td>
<td>125±11</td>
</tr>
</tbody>
</table>

ACE, angiotensin converting enzyme; Ang II, angiotensin II. Values are mean±SEM. *p<0.05 vs. respective control value.

rum (Table 1, p<0.05) and pulmonary (Figure 2, p<0.001) ACE activities; in contrast, pulmonary ACE mRNA levels increased significantly as compared with levels in vehicle-treated control animals (Figure 2). Interestingly, quinapril treatment did not change either testicular ACE activity or mRNA levels (Figure 3).

Combined data from both protocols showed a significant negative correlation between pulmonary ACE mRNA levels and plasma Ang II levels (r=-0.554, p<0.005) and mean arterial blood pressure (r=-0.442, p<0.05) (Figure 4). Furthermore, animals receiving Ang II infusion displayed a negative correlation between pulmonary ACE activity and plasma Ang II levels (r=-0.428, p=0.01) and mean arterial pressure (r=-0.390, p<0.05).

Protocol 3: Effect of Quinapril Withdrawal on Serum ACE Activity

This protocol was designed to study whether the induction of tissue ACE during ACE inhibition will eventually lead to an increase in serum ACE activity after withdrawal of the ACE inhibitor. Animals were treated with 10 mg/kg per day quinapril in drinking water for 3 days, followed by a final dose (10 mg/kg) given by gavage (time 0). As compared with saline-gavaged control animals, quinapril-treated animals displayed a marked decrease of serum ACE activity 6 hours after gavage. However, 48 and 96 hours after discontinuation of quinapril treatment, a significant overshoot of serum ACE activity was observed increasing serum ACE activity up to 193% of control values (Figure 5).

Discussion

Despite the wide clinical use of agents that interfere with the renin-angiotensin system, no previous reports appear to examine the direct effects of Ang II on the regulation of ACE activity. In the present investigation, Ang II infusion was related to decreased tissue ACE activity in the lung, suggesting negative feedback regulation of pulmonary ACE activity. Serum ACE activity decreased to a lesser extent after 3 days of Ang II infusion. However, the estimated half-life of circulating ACE in rats is 72 hours and may mask a more pronounced change.

Indirect evidence for a role of Ang II in the regulation of ACE activity was provided previously by investigations using ACE inhibitors. However, such studies were complicated by interference of the inhibitors with the measurement of ACE activity. Fyhrquist et al removed captopril and enalapril from patient serum or cell media by prolonged storage, chloramine treatment (captopril), or dialysis (enalapril) before measuring ACE activity and observed ACE induction during ACE inhibitor treatment. Jackson and Johnston studied the relation of plasma enalaprilat levels to plasma ACE activity.
inhibition in hypertensive patients. As compared with acute effects of the drug, higher plasma concentrations of enalaprilat were required during long-term treatment to achieve comparable plasma ACE inhibition, suggesting induction of circulating ACE activity. In a more recent study by Hirsch et al., prolonged captopril treatment (7 weeks) in rats with heart failure was associated with increased lung ACE activity; whereas, kidney and vascular ACE remained inhibited. Unfortunately, plasma Ang II levels were not reported in these former studies. The present study extends these observations and provides evidence for a role of Ang II in tissue ACE regulation.

Other factors, in addition to Ang II, such as the hormonal and physiological perturbations can also influence ACE activity. For instance, glucocorticoids, thyroid hormone, and androgen have been shown to stimulate ACE activity in various tissues. Physiological factors that appear to regulate ACE activity include hypoxia and \( \beta \)-adrenergic stimulation. However, ACE enzymatic activity is not merely a consequence of expression of the gene product. Other proteins interact with tissue ACE and may thereby alter ACE activity. Ikemoto et al. have described an endogenous ACE inhibitor in the heart, and Urata et al. have demonstrated an angiotensin-generating enzyme other than ACE. Thus, ACE enzymatic activity determinations in various studies may reflect changes of ACE protein content or measured enzymatic activity.

The recent sequencing of the ACE gene and availability of cDNA probes now provides a highly specific method to study ACE synthesis and regulation. ACE gene expression has been demonstrated in numerous tissues, confirming that tissue ACE activities reflect local ACE synthesis rather than contamination of circulating ACE. In the present investigation, we used these molecular biological methods to study the feedback regulation of ACE by Ang II. Ang II infusion decreased ACE mRNA levels in the lung. The negative feedback regulation of ACE synthesis was confirmed by decreased pulmonary ACE activity. Interestingly, the decrease of tissue ACE mRNA levels was more pronounced than the decrease of tissue ACE activity. Our study does not address the possible mechanisms for this observation; however, the different half-lives of mRNA

**FIGURE 3.** Bar graphs showing testicular angiotensin converting enzyme (ACE) mRNA levels (left panel) and testicular ACE activity (right panel) after 3 days of intravenous infusion of vehicle (veh) or angiotensin II (A II) at 1,000 ng/kg per minute (n=8), 300 ng/kg per minute (n=8), and 100 ng/kg per minute (n=8) (closed bars) or after 3 days of oral vehicle (n=7) or quinapril (Quin) treatment (n=8) (hatched bars). \( *p<0.05 \) vs. respective control value.

**FIGURE 4.** Graphs showing correlation between plasma angiotensin II (ANG II) concentrations and pulmonary angiotensin converting enzyme (ACE) mRNA levels (left panel) and correlation between mean arterial blood pressure and pulmonary ACE mRNA levels (right panel).
and protein or posttranscriptional modifications may account for this finding.

In the present study, pulmonary ACE mRNA levels and Ang II plasma levels were inversely correlated, supporting the role of Ang II in the regulation of pulmonary ACE. ACE mRNA levels were also negatively correlated with mean arterial blood pressure. Since the pulmonary circulation is not exposed to systemic blood pressure, this correlation might be indirect, presumably because of the effect of Ang II on systemic blood pressure. This presumption is supported further by findings of Miyazaki et al., who demonstrated an induction of aortic ACE activity in hypertension, suggesting a positive rather than a negative correlation between arterial blood pressure and ACE induction. However, we cannot rule out that changes in blood pressure achieved by Ang II infusion or ACE inhibition are responsible for the changes in ACE mRNA expression and activity in our experiments; thus, further investigations will be required to study this question.

A limitation of our study is that Ang II infusion may affect other hormonal systems such as adrenocorticotropic hormone, vasopressin, and 11-hydroxycorticosteroid secretion when given short term in the dog as well as 18-hydroxy-11-deoxycorticosterone and aldosterone plasma concentrations in humans. Therefore, alternatively, the mechanism for Ang II effects on pulmonary and testicular ACE activity might be indirect.

To study the effects of blockade of Ang II production, rats were treated with the ACE inhibitor quinapril. Significant ACE inhibition in the lung was accompanied by an induction of pulmonary ACE mRNA. The induction of pulmonary ACE mRNA after 3 days of ACE inhibition was followed by a marked overshoot of serum ACE activity 48 and 96 hours after withdrawal of quinapril, providing more evidence for a negative feedback regulation of ACE by Ang II. According to the plasma half-life of quinapril (0.8 hours) and its active metabolite quinaprilat (1.9 hours), more than 99.9% of the last dose should have been excreted by the 48-hour time point. Therefore, discontinuation of treatment with the ACE inhibitor may unmask the induction of serum ACE during quinapril administration.

Ang II infusion did not result in a dose-dependent decrease in testicular ACE activity, despite a significant decrease in ACE mRNA levels. Furthermore, systemic ACE inhibition did not affect testicular ACE activity. Similar data have been reported by Jackson et al., who studied animals treated with perindopril. This finding is presumably due to a blood–testis barrier, which prevents ACE inhibition in this tissue. Interestingly, the induction of ACE mRNA levels in the quinapril-treated animals was observed only in lung, but not in testis. These findings suggest that factors other than circulating Ang II may be important for regulation of testicular ACE activity during Ang II infusion or ACE inhibition.

It cannot be determined from this study whether the induction of lung and plasma ACE can influence the long-term blood pressure and tissue functional responses to prolonged ACE inhibitor administration. Although no drug tolerance has been reported in hypertension, Packer et al. have described that captopril failed to achieve a sustained hemodynamic response in approximately 25% of patients with heart failure. It is intriguing to speculate that a marked induction of ACE synthesis may overcome the tissue drug effect. Further studies on this possibility are clearly of interest.

In summary, Ang II regulates pulmonary ACE mRNA expression via a negative-feedback mechanism. ACE inhibition causes induction of pulmonary ACE mRNA, as well as an increase of serum ACE content, without an immediate effect on arterial blood pressure. These findings may provide an improved understanding of the feedback regulation of the renin-angiotensin system and novel insights into the action of ACE inhibitors on tissue ACE gene expression and activity.

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