Effect of Reduced Angiotensin-Converting Enzyme Gene Expression and Angiotensin-Converting Enzyme Inhibition on Angiotensin and Bradykinin Peptide Levels in Mice

Duncan J. Campbell, Theodora Alexiou, Hong D. Xiao, Sebastien Fuchs, Michael J. McKinley, Pierre Corvol, Kenneth E. Bernstein

Abstract—There is uncertainty about the contribution of angiotensin-converting enzyme (ACE) to angiotensin II formation, with recent studies suggesting that non-ACE enzymes may be the predominant pathway of angiotensin II formation in kidney, heart, and lung. To investigate the role of ACE in angiotensin II formation, we measured angiotensin I and II levels in blood, kidney, and heart of 2 mouse genetic models (ACE.1 and ACE.4) of reduced somatic ACE gene expression and in blood, kidney, heart, lung, adrenal, and brain of mice administered the ACE inhibitor lisinopril. We also measured the levels of bradykinin (1-9) and its ACE metabolite bradykinin (1-7). Reduced ACE gene expression and ACE inhibition had similar effects on angiotensin and bradykinin peptide levels. Angiotensin II levels were reduced by 70% to 97% in blood, 92% to 99% in kidney, 93% to 99% in heart, 97% in lung, and 85% in adrenal and brain. The marked reductions in angiotensin II/angiotensin I ratio indicated that ACE was responsible for at least 90% of angiotensin I conversion to angiotensin II in blood, kidney, heart, lung, and brain, and at least 77% in adrenal. Blood bradykinin (1-9) levels were increased 6.4-fold to 8.4-fold. Heart bradykinin (1-9) levels were increased in ACE.4 mice and the bradykinin (1-7)/bradykinin (1-9) ratio was reduced in kidney and heart of ACE.4 mice and heart of lisinopril-treated mice. These studies demonstrate that ACE is the predominant pathway of angiotensin II formation in blood and tissues of mice and plays a major role in bradykinin (1-9) metabolism in blood and, to a lesser extent, in kidney and heart. (Hypertension. 2004;43:854-859.)

Key Words: mice ■ angiotensin-converting enzyme ■ angiotensin I ■ angiotensin II ■ bradykinin

A ngiotensin-converting enzyme (ACE) inhibitors are of established benefit for the treatment of cardiovascular and renal disease. However, there is continuing uncertainty about the mechanism of their therapeutic benefit and the effect of ACE inhibition on angiotensin II levels. Many patients receiving ACE inhibitor therapy fail to show reduction in angiotensin II levels, leading to the proposal that alternate enzymes such as chymase may convert angiotensin I to angiotensin II.1 In support of this proposal, Wei et al2 reported that although plasma angiotensin II levels of ACE gene knockout (KO) mice were reduced to below the limit of detection, angiotensin II levels in kidney, heart, and lung of ACE KO mice were no different from the levels in wild-type (WT) mice. These authors also reported that chymase levels were increased 14-fold in kidney and 1.5-fold in heart of ACE KO mice, in comparison with WT mice, and they suggested that prolonged suppression of ACE activity may lead to the induction of alternate enzymatic pathways of angiotensin II formation in tissue.2

We and others have shown that ACE inhibition produces marked reduction in angiotensin II levels in blood and tissues of rats and humans.3–6 However, ACE inhibition is reported to have variable effects on angiotensin II levels in mice.7 To further investigate the role of ACE in angiotensin II formation, we measured the levels of angiotensin I and II in blood and tissues of two mouse genetic models of reduced somatic ACE gene expression, and in mice administered the ACE inhibitor lisinopril. Additionally, we measured bradykinin (1-9) and its metabolite bradykinin (1-7) in blood and tissues of these mice.

Methods

Genetic Models of Reduced Somatic ACE Gene Expression
Two genetic models of reduced somatic ACE gene expression were studied. ACE.1 mice are null for all ACE gene expression, producing neither somatic nor testis ACE.8 ACE.4 mice present with a phenotype nearly identical to that of ACE.1 mice, except the males have normal fertility because they express testis ACE. Further details
ACE inhibition experiments were performed using female C57Bl/6 mice aged 3 to 6 months with body weight 29±3 g. The mice were randomly assigned to receive vehicle (isotonic saline) or 1 of 3 lisinopril treatments (1, 10, or 100 mg/kg). There were 8 mice per group. Treatment was administered by intraperitoneal injection 1 hour before collection of blood and tissues. The appropriate institutional review boards for animal experimentation approved all experiments.

Angiotensin Peptides
The effects of the highest dose of lisinopril are shown for blood and all tissues except brain (Figures 1 to 4), and similar results were obtained for lower doses of lisinopril (data not shown). The effects of 1 mg/kg lisinopril are shown for brain (Figure 4), because samples from mice administered higher doses were lost during assay.

Blood angiotensin peptide levels were 5-fold to 10-fold higher in control mice from the ACE inhibition experiment than in ACE.1 and ACE.4 WT littermates. The higher levels in the ACE inhibition experiment may have been caused in part by the different anesthetics used in these experiments. However, the effects of ACE inhibition on angiotensin peptide levels in blood and tissues were similar to the effects of reduced ACE gene expression (Figures 1 to 4). In comparison with their respective WT littermates or controls, blood levels of angiotensin II, angiotensin I, and angiotensin II/angiotensin I ratio were reduced by 70% in ACE.1 mice, 83% in ACE.4 mice, and 97% in lisinopril-treated mice. Blood angiotensin II levels were reduced by 95% in ACE.1 and ACE.4 mice, respectively, and 76% in lisinopril-treated mice.

Heart angiotensin II levels were reduced by 95% in ACE.1 mice, 93% in ACE.4 mice, and 99% in lisinopril-treated mice (Figure 2). Kidney angiotensin II levels were below the detection limit in all ACE.1 mice and in 6 ACE.4 mice, but were detectable in all lisinopril-treated mice. Kidney angiotensin I levels were unchanged in ACE.1 mice and were reduced in ACE.4 and lisinopril-treated mice. The kidney angiotensin II/angiotensin I ratio was reduced by 98% and 95% in ACE.1 and ACE.4 mice, respectively, and 76% in lisinopril-treated mice.

Heart angiotensin II levels were reduced by 95% in ACE.1 mice, 93% in ACE.4 mice, and 99% in lisinopril-treated mice (Figure 3). Heart angiotensin II levels were below the detection limit in 4 ACE.1 mice, 7 ACE.4 mice, and 7 lisinopril-treated mice. Heart angiotensin I levels were unchanged in ACE.1 and lisinopril-treated mice and were increased in ACE.4 mice. Heart angiotensin II/angiotensin I ratio was reduced by 97% to 99%.

Lung angiotensin II levels were reduced by 97%, being below the detection limit in all lisinopril-treated mice (Figure 4). There was a 16-fold increase in lung angiotensin I levels and the angiotensin II/angiotensin I ratio was reduced by 99.9%. Adrenal angiotensin II levels were reduced by 85% in lisinopril-treated mice but remained detectable in 7 mice, were associated with no change in angiotensin I levels, and produced a 77% decrease in angiotensin II/angiotensin I ratio (Figure 4). Brain angiotensin II levels were reduced by 85% (although not statistically significant) and were below the
detection limit in 6 lisinopril-treated mice, were associated with a 3.9-fold increase in angiotensin I levels, and produced a 98% decrease in angiotensin II/angiotensin I ratio (Figure 4).

Bradykinin Peptides

There were marked changes in blood bradykinin peptide levels in ACE.1, ACE.4, and lisinopril-treated mice (Table). In comparison with their respective WT littermates or controls, blood bradykinin (1-7) levels were increased 3.2-fold in ACE.4 mice and bradykinin (1-9) levels were increased 6.4-fold in ACE.1 mice, 8.2-fold in ACE.4 mice, and 8.4-fold in lisinopril-treated mice. The bradykinin (1-7)/bradykinin (1-9) ratio was reduced by 78% and 58% in ACE.1 and ACE.4 mice, respectively, and by 94% in lisinopril-treated mice.

There were modest changes in tissue levels of bradykinin peptides. Kidney of ACE.4 mice showed a 48% decrease in bradykinin (1-7) levels and 39% decrease in bradykinin (1-7)/bradykinin (1-9) ratio (Table). Additionally, heart of ACE.4 mice showed a 57% increase in bradykinin (1-9) levels and 39% decrease in bradykinin (1-7)/bradykinin (1-9) ratio. There was also a 50% decrease in bradykinin (1-7)/bradykinin (1-9) ratio in heart of lisinopril-treated mice.

Discussion

This study demonstrates, both in mice with reduced ACE gene expression and in lisinopril-treated mice, that ACE is the predominant pathway of angiotensin II formation. The marked reductions in angiotensin II/angiotensin I ratio indicate that ACE is responsible for at least 90% of angiotensin I conversion to angiotensin II in blood, kidney, heart, lung, and brain, and at least 77% in adrenal. Our lisinopril experiments were performed to produce acute ACE inhibition with minimal opportunity for induction of alternative enzymatic pathways of angiotensin II formation. We studied a broad range of lisinopril doses because of the need to obtain effective tissue levels of lisinopril during the 1-hour period of exposure to the drug. The similarity in angiotensin peptide levels measured after acute ACE inhibition and in mice with persistently suppressed ACE gene expression provides no support for the proposal by Wei et al.2 that chronic suppression of ACE activity leads to induction of alternative enzymatic pathways of angiotensin II formation. Our measured angiotensin peptide levels are in agreement with those reported by Mazzolai et al.7 These authors collected blood from conscious mice with arterial catheters and measured plasma angiotensin II levels similar to the levels we measured in blood of WT mice anesthetized with ketamine/xylazine. Moreover, Mazzolai et al.7 found angiotensin peptide levels in kidney and heart similar to those measured in the present study. However, Mazzolai et al.7 reported variable effects after 4 weeks of ACE inhibition (0.05 mg/mL ramipril in drinking water) on angiotensin II levels. Although ramipril
reduced plasma, kidney, and heart angiotensin II levels by 77%, 82%, and 75%, respectively, in mice administered ramipril from age 4 to 8 weeks, the reductions in angiotensin II levels were only 50%, 50%, and 18%, respectively, in mice administered ramipril from age 8 to 12 weeks. The lesser reductions in angiotensin II levels in mice administered ramipril, as opposed to those with lisinopril treatment in the present study, may have been caused by the use of a lower dose of ACE inhibitor by Mazzolai et al. 7

Dell’Italia and Husain offer 3 lines of physiological evidence for a chymase-angiotensin II system.1 First, they refer to the failure of ACE inhibitor therapy to completely suppress angiotensin II levels. Second, they refer to the report by Wei et al2 of similar angiotensin I and II levels and angiotensin II/angiotensin I ratio in kidney, heart, and lung of ACE KO and WT mice. Third, they refer to the failure of captopril to modify the high angiotensin II levels in microdialysate from dog cardiac ventricle.10 Our findings of a marked suppression of angiotensin II levels and angiotensin II/angiotensin I ratio in kidney and heart of ACE.1 and ACE.4 mice are in contrast to the findings of Wei et al.2 The differences between our results and those of Wei et al may have a methodological basis in that the boiling of frozen tissue in 1 mol/L acetic acid11 by Wei et al may permit angiotensin I generation and conversion to angiotensin II by lysosomal enzymes released by the thawed tissue. The much higher tissue levels of angiotensin I measured by Wei et al (∼250 femtomoles [fmol] per gram in heart and 400 fmol/g in kidney and lung) in comparison with levels measured by Mazzolai et al7 (<20 fmol/g in heart, ∼130 fmol/g in kidney) and ourselves (<30 fmol/g in heart, <80 fmol/g in kidney, and <5 fmol/g in lung) support the possibility of angiotensin I generation during sample preparation in the experiments of Wei et al.2 We and others have shown that much lower levels of angiotensin peptides are measured in tissues when precaution is taken to prevent thawing of frozen tissue during sample processing.12,13 In our experiments, we took particular care to prevent artifactual generation of angiotensin peptides by avoiding the freezing of tissue and immediately homogenizing the tissue in GTC, a potent chaotropic agent that effectively prevents peptide generation and degradation.

The report of Dell’Italia et al10 that microdialysate from dog cardiac ventricle has high angiotensin II levels that are not altered by captopril supports a role for non-ACE enzymatic pathways in angiotensin II formation in the heart. However, there is some doubt about the reliability of measurement of angiotensin peptide levels in microdialysate in these experiments. Dell’Italia et al reported that angiotensin II levels in cardiac microdialysate (6333 fmol/mL)10 were 200-times the levels in whole ventricular tissue (∼28 fmol/g).14,15 This would require that the angiotensin II content of 1 g of cardiac ventricle be contained in a compartment with a total volume no greater than 5 μL, and that the investigators be able to place their microdialysis probe within this 5 μL compartment and collect microdialysate without dilution from other compartments within the heart. It is of note that Schuijt et al16 measured angiotensin II levels in pig cardiac tissue (∼22 fmol/g) similar to the levels reported for dog heart,14,15 but angiotensin II levels in microdialysate from pig heart were below the limit of detection (<30 fmol/mL).16 Further evidence against an important role for chymase in angiotensin II formation in the heart is the report by Kokkonen et al17 that interstitial fluid contains protease inhibitors that potently inhibit chymase activity.

Although we have demonstrated that ACE has a predominant role in angiotensin II formation, the persistence of measurable levels of angiotensin II in mice with reduced ACE gene expression or ACE inhibition indicates that non-ACE enzymes also make a small contribution to angiotensin II formation. It is well recognized that ACE inhibition can fail to reduce angiotensin II levels.18 McDonald et al19 demonstrated the operation of a non-ACE enzymatic pathway of conversion of angiotensin I to angiotensin II in humans. However, Jorde et al20 showed that the persistent pressor effect of angiotensin I in heart failure subjects receiving ACE inhibitor therapy was largely caused by a failure to produce complete ACE inhibition. Moreover, we found that ACE inhibitor therapy effectively reduces angiotensin II levels and the angiotensin II/angiotensin I ratio in cardiac tissue from subjects undergoing coronary artery graft surgery.4 The contribution of non-ACE enzymes or residual uninhibited ACE to angiotensin II formation during ACE inhibition depends on the prevailing angiotensin I levels, which are dependent on the levels of renin and angiotensinogen. A non-ACE enzymatic pathway that normally contributes to only 1% of angiotensin II formation, or 1% residual uninhibited ACE, may maintain angiotensin II levels at 30% of
control if angiotensin I levels are increased 30-fold, as we have demonstrated in our studies of the effects of ACE inhibition on plasma angiotensin peptides in rats. With the exception of lung, the increases in tissue angiotensin I levels during ACE inhibition are much less than the increase in plasma angiotensin I levels and are therefore less likely to drive angiotensin II formation in tissues during ACE inhibition. In the present study, the increases in blood angiotensin I levels with reduced ACE gene expression or ACE inhibition were less than the increases we observed in rats treated with ACE inhibitor. The lesser increase in angiotensin I levels may have been caused by the lower plasma angiotensinogen levels in mice. These lower angiotensinogen levels are likely to reflect increased consumption by the much higher renin levels in mice and may have attenuated any increase in angiotensin I levels caused by increased renin levels that result from reduced ACE gene expression or ACE inhibition.

To our knowledge, this is the first report of bradykinin peptide levels in mice. In agreement with our previous studies of ACE inhibition in rats, ACE.1 and ACE.4 mice and lisinopril-treated mice all showed marked increases in blood bradykinin (1-9) levels and reduction in bradykinin (1-7)/bradykinin (1-9) ratio, indicating a major role for ACE in metabolism of bradykinin (1-9) in blood. There were much smaller changes in tissue bradykinin peptide levels, with increased bradykinin (1-9) levels in heart of ACE.4 mice and reductions in bradykinin (1-7)/bradykinin (1-9) ratio in kidney and heart of ACE.4 mice and heart of lisinopril-treated mice, indicating a role for ACE in bradykinin (1-9) metabolism in these 2 tissues. It should be noted that the change in bradykinin (1-7)/bradykinin (1-9) ratio provides an underestimate of the contribution of ACE to bradykinin (1-9) metabolism, because ACE also metabolizes bradykinin (1-7), thereby accounting for the increase in blood bradykinin (1-7) levels seen in ACE.4 mice.

**Perspectives**

Angiotensin II plays a major role in cardiovascular physiology and pathology. An improved understanding of the mechanisms of its formation is important for the optimal use of therapeutic strategies that impact on the renin angiotensin system. Our demonstration of the predominant role of ACE in angiotensin II formation in blood and tissues, and its role in...
bradykinin metabolism, will assist understanding of the mechanism of the therapeutic benefits of ACE inhibition.

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