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AT₁ Receptor-Mediated Augmentation of Urinary Excretion of Endogenous Ang II in Val⁵-Ang II Infused Rats

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Abstract

Rats infused chronically with Val⁵-Ang II exhibit increased urinary excretion of endogenous Ile⁵-Ang II by the 12th day of infusion suggesting the stimulation of endogenous Ang II formation by Val⁵-Ang II infusion. The present study determined the time course of increased urinary Ang II excretion and the effects of AT₁ receptor blockade (candesartan, 2 mg/kg/day) on the urinary excretion rates of Ile⁵-Ang II in Val⁵-Ang II-infused (80ng/min) rats. Ile⁵-Ang II was separated from Val⁵-Ang II by HPLC and measured by radioimmunoassay. Systolic blood pressure increased progressively (215 ± 2 mmHg) in Val⁵-Ang II-infused rats (5) while the candesartan treated group (6) remained normotensive (124 ± 3 mmHg). Candesartan treatment significantly increased the level of plasma Ile⁵-Ang II (24.0±7.6 vs. 156.9±24.6 fmol/ml; p<0.01); in contrast, there was a markedly lower intrarenal Ile⁵-Ang II content (357.9±76.6 vs. 21.1±2.8 fmol/g; p<0.01). Urinary Ile⁵-Ang II excretion rates were elevated by day 9 (2185.7 ± 283.2 fmol/24hr) in Val⁵-Ang II-infused rats but not in candesartan treated rats (740.6 ± 110.3 fmol/24hr). Thus, AT₁ receptor blockade prevents the increase in urinary excretion of endogenous Ang II in rats subjected to chronic Ang II infusion. These data indicate that the increased urinary excretion of endogenous Ang II in Val⁵-Ang II-infused rats is primarily due to AT₁ receptor dependent secretion into and/or de novo formation of Ang II within the tubular lumen.

Keywords

angiotensin II induced hypertension; renin-angiotensin system; high-performance liquid chromatography; intrarenal Ang II; urinary Ang II

Introduction

Previous studies have demonstrated that intrarenal angiotensin II (Ang II) content is significantly elevated in chronic Ang II infused rats.^{1,2,3} The increases in circulating Ang II lead to progressive augmentation of intrarenal Ang II. The plasma renin activity (PRA) is markedly reduced in the Ang II infused rats,^{1,2,4,5,6} while the kidney renin content (KRC) and renin mRNA are not reduced to the same extent which indicates that the sustained

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Disclosures
None.

intrarenal renin activity may contribute to the augmented or sustained intrarenal Ang II levels.^{1,7,8,9} Because AT₁ receptor blockers markedly diminish the increase in intrarenal Ang II content in Ang II infused rats, the augmentation of intrarenal Ang II is partially due to AT₁ receptor mediated binding and internalization of circulating Ang II.^{2,3,7,10,11} In addition, renal angiotensinogen (AGT) activity, AGT mRNA, protein and renal Ang I contents as well as intrarenal angiotensin-converting enzyme (ACE) activity are either increased or not reduced from the control levels suggesting that there is also enhanced de novo formation of Ang II in the kidney.^{2, 7, 12–15}

Our recent study demonstrated that rats infused chronically with Val⁵-Ang II exhibit increased urinary excretion of endogenous Ile⁵-Ang II by the 12th day of infusion, indicating the stimulation of endogenous Ang II formation.¹⁶ Several other studies have reported that the augmentation of intrarenal AGT in chronic Ang II infusion is dependent on activation of AT₁ receptors. Chronic Ang II infusion leads to increases in urinary excretion of AGT and enhanced urinary excretion rates of AGT are the consequence of the stimulation of AGT production by the kidney.^{7,12,17,18} These results provide further evidence that there is an enhanced intrarenal endogenous production of Ang II with subsequent augmentation of endogenous Ang II excretion in the urine in Val⁵-Ang II infused rats. However, the relative quantitative contribution of enhanced endogenous Ang II production versus accumulation of circulating Ang II has not been established. Also, it has not been determined if the increased endogenous Ang II in the urine is due to increased filtered Ang II or increased intratubular formation and/or secretion. Finally, the specific role of AT₁ receptors in mediating the increased intrarenal and urinary endogenous Ang II has not been established. The objective of this study was to determine the time course of increased endogenous urinary Ang II excretion and the effects of AT₁ receptor blockade (candesartan) on the intrarenal endogenous Ang II and urinary excretion of endogenous Ang II in Val⁵-Ang II infused rats. Rats were infused with Val⁵-Ang II, which is not formed by rats, but has the same biological and immunoreactive properties as endogenous Ile⁵-Ang II.^{3,16} The effect of Val⁵-Ang II is equivalent to that of Ile⁵-Ang II in raising arterial pressure and intrarenal Ang II levels.^{3,16} Since these two isoforms can be separated by high-performance liquid chromatography (HPLC), this substitution approach enabled determination of the relative contributions of uptake of exogenously infused Val⁵-Ang II vs. endogenously formed Ile⁵-Ang II to the elevated intrarenal Ang II content and increased urinary Ang II excretion in rats subjected to chronic Ang II infusion.

Materials and Methods

Animal Preparation

Male Sprague-Dawley rats weighing 330 to 350 g (Charles River Laboratories) were housed in wire cages under controlled temperature and lighting conditions. Throughout the experiments, the animals had free access to tap water and standard rat chow (Ralston Purina). All experiments were approved by the Tulane University Animal Care and Use Committee. Rats were divided into two experimental groups: rats infused with Val⁵-Ang II (n = 5), and rats infused with Val⁵-Ang II and treated with AT₁ receptor antagonist, candesartan (n = 6). Candesartan (AstraZeneca, Molndal, Sweden) was administered in the

drinking water at a dose of 2 mg/kg/day to allow chronic treatment throughout the period of Ang II infusion. Ang II was delivered continuously at a rate of 80ng/min via osmotic minipump (model 2002, Durect Corp. Cupertino, CA) that was implanted subcutaneously at the dorsum of the neck. Systolic blood pressure (SBP) was measured in conscious rats by tail-cuff plethysmography (Visitech Systems, Inc. Apex, NC) to monitor the progression of hypertension¹⁶. The rats were decapitated on day 13 and trunk blood was collected and the kidneys were immediately removed, quickly weighed, and homogenized in methanol. The time delay between decapitation and homogenization of the kidneys did not exceed 60 seconds.

Collection and Extraction of Blood, Kidney and Urine Samples

Blood was collected in chilled tubes containing a mixed inhibitor solution (final concentration: 5 mmol/L EDTA, 20 μ mol/L pepstatin, 10 μ mol/L PMSF, 20 μ mol/L enalaprilat, and 1.25 mmol/L 1,10-phenanthroline). After centrifugation at 4° C for 10 min at 1000g, plasma was separated and applied to phenyl-bonded solid-phase extraction columns (Bond-Elut, Varian) that had been prewashed with 90% methanol followed by water. After sample application, each solid-phase extraction column was washed sequentially with water, hexane, and chloroform. Angiotensin peptides were eluted from the solid-phase extraction column with 90% methanol.¹⁹ The eluants were collected, evaporated to dryness under vacuum, and then subjected to HPLC to separate Val⁵-Ang II from Ile⁵-Ang II.^{3,19}

For tissue Ang II measurements, each kidney was immersed in cold methanol (100%), minced and homogenized with polytron tissue tearor immediately after harvesting and weighing. The homogenates were centrifuged and the supernatants from the kidney homogenates were dried overnight in a vacuum centrifuge. The dried residue was reconstituted in 1 ml RIA buffer (19mmol/L monobasic sodium phosphate, 81mmol/L dibasic sodium phosphate, 0.05 mol/L sodium chloride, 0.1% BSA, 0.1% triton x-100, 0.01% sodium azide, pH 7.4). These samples were extracted and evaporated as described above for plasma and subjected to HPLC. Urine was collected in tubes containing the same mixed inhibitor solution as for blood. Collection was carried out for 24 hours on the day before Ang II infusion was started and on day 3, 6, 9 and 12 during the chronic Ang II infusions. Urine was centrifuged and representative 1.0 ml aliquots of the supernatants were extracted and evaporated as described above for plasma and subjected to HPLC.

Separation of Val⁵-Ang II from Ile⁵-Ang II by HPLC

The HPLC and radioimmunoassay methodologies for the measurement of Ang peptides were similar to those previously described with some modification.^{3,11,19–21} Briefly, the dried extract residue from each sample was resuspended with 250- μ l equilibration solvent which included 65% mobile phase A (0.13% HFBA) and 35% mobile phase B (80% acetonitrile / 0.13% HFBA). A 200- μ l aliquot of supernate was chromatographed by isocratic elution at 1 ml/min on a 25 \times 0.46-cm, 5- μ m Vydac C18 reverse-phase HPLC column (Separations Group). Val⁵-Ang II eluted at 9 minutes, and Ile⁵-Ang II had an elution peak at 12 minutes. Fractions were collected from 8 to 10 minutes for Val⁵-Ang II and from 11 to 13 minutes for Ile⁵-Ang II, evaporated to dryness, reconstituted in assay diluent, and

measured directly by radioimmunoassay. For the present study, particular care was given to the prevention of any carry over of the peptides from the calibration procedures to the experimental samples.

Measurement of Val⁵-Ang II and Ile⁵-Ang II by Radioimmunoassay

The reconstituted fractions were incubated with rabbit anti-Ang II antisera (Peninsula Laboratories Inc) and ¹²⁵I-radiolabeled Ang II (Perkin Elmer Life and Analytical Sciences) for 48 hours at 4°C. Bound and free Ang peptides were separated by dextran-coated charcoal, and the supernatants were counted on a computer-linked gamma counter for 3 minutes. Immunoreactivities of the antibody for Val⁵-Ang II and Ile⁵-Ang II were virtually identical. The sensitivity of the Ang II assay was 0.97 fmol. For the Ang II assays, the specific binding was 76.8%, and nonspecific binding was 6.2%.

Plasma Renin Activity and Kidney Prorenin and Renin Content Assays

For plasma renin activity (PRA) determinations, trunk blood was collected in chilled tubes containing EDTA (5 mmol/L). Plasma was separated and stored at -20°C until assayed with a commercially available GammaCoat Plasma Renin Activity ¹²⁵I-Ang I RIA kit (DiaSorin Inc).^{1,16} For kidney prorenin and renin content (KRC) assessment, each kidney was immersed in cold KRC homogenation buffer (2.6 mmol/L EDTA, 3.4 mmol/L hydroxyquinoline, 5 mmol/L ammonium acetate, 200 μmol/L PMSF, 0.3 μmol/L dithiothreitol), minced and homogenized.¹⁶ The homogenates were centrifuged and the supernatants were used to generate 1:1000 dilutions. 10 μl of diluted kidney extract were incubated with trypsin (0.25mg/ml) in the presence of bovine serum albumin (10mg/ml) and CaCl₂ (5mmol/L) for 1 hour at 4°C. The reaction was stopped by adding SBTI (0.5mg/ml) for 10 minutes at room temperature. The diluted kidney extract treated with trypsin and without trypsin were spiked with 1μM synthetic renin tetradecapeptide substrate and the generated Ang I was assayed with the Diasorin PRA RIA kit for the prorenin and renin measurements.^{22, 23}

Statistical Analysis

Results are expressed as mean ± SE. For figures 1 and 6, the data were analyzed by repeated measures ANOVA with post hoc Newman-Keuls Multiple Comparison test within each group and the data were analyzed by one-way analysis of variance with post hoc Newman-Keuls Multiple Comparison Test between two groups. We used student unpaired T test to analyze the data for figures 2, 4, 5. When compared with the third group from the previous publication in figure 2, we applied a one-way ANOVA. A value of P < 0.05 was considered statistically significant.

Results

Effect of Candesartan on Systolic Blood Pressure During Val⁵-Ang II Infusion

SBP increased significantly from 113±1 to 215±2 mmHg in the Val⁵-Ang II infused rats. In the candesartan treated rats, SBP was slightly lower initially and remained in normotensive range throughout the duration of the experiment (107±3 to 124±3 mm Hg; Fig 1).

Effect of Candesartan on Plasma Renin Activity and Kidney Prorenin and Renin Content During Val⁵-Ang II Infusion

PRA was markedly suppressed in Val⁵-Ang II infused rats compared with sham-operated rats in our previous report (0.2 ± 0.1 vs. 5.7 ± 0.8 ng Ang I /ml/ hr ; $p < 0.01$). In Val⁵-Ang II infused rats treated with candesartan, PRA level was significantly elevated compared with that in Val⁵-Ang II infused rats (3.0 ± 1.3 vs. 0.2 ± 0.1 ng Ang I /ml/ hr; $p < 0.05$), but was not statistically different from the sham-operated rats (Fig 2). The kidney prorenin and renin contents were not statistically different between Val⁵-Ang II infused rats and Val⁵-Ang II infused rats treated with candesartan (Fig 3).

Effect of Candesartan on Plasma and Kidney Ang II Levels During Val⁵-Ang II Infusion

The total plasma Ang II concentration increased to 306.8 ± 65.4 fmol/ml in the Val⁵-Ang II infused rats with most of it (282.8 ± 57.8 fmol/ml) being due to Val⁵-Ang II as the Ile⁵-Ang II concentrations were quite low (24.0 ± 7.6 fmol/ml). In the Val⁵-Ang II infused rats treated with candesartan, the total Ang II concentration was 373.6 ± 58.7 fmol/ml but the Val⁵-Ang II concentration (216.7 ± 34.0 fmol/ml) was not significantly lower compared with that in Val⁵-Ang II infused rats not treated with candesartan; however the Ile⁵-Ang II concentrations (156.9 ± 24.6 fmol/ml) in candesartan treated group were significantly greater compared with the Ile⁵-Ang II levels (24.0 ± 7.6 fmol/ml) in Val⁵-Ang II infused rats; $p < 0.01$ (Fig 4). There were no differences in the plasma Ile⁵-Ang II levels between the sham-operated rats (44.1 ± 1.5 fmol/ml) and Val⁵-Ang II infused rats.

The total intrarenal Ang II content averaged 743.3 ± 45.8 fmol/g with approximately equal amounts of Val⁵-Ang II (385.4 ± 46.2 fmol/g) and Ile⁵-Ang II (357.9 ± 76.6 fmol/g) in Val⁵-Ang II infused rats. With candesartan treatment, however, the total Ang II content (263.2 ± 35.5 fmol/g) was significantly lower and similar to the content of only the Val⁵-Ang II (242.1 ± 32.7 fmol/g) in the group not treated with candesartan. Importantly, candesartan treatment markedly reduced the intrarenal Ile⁵-Ang II content (21.1 ± 2.8 fmol/g); $p < 0.01$ (Fig 5). Furthermore, the Val⁵-Ang II infusions caused a significant increase of intrarenal Ile⁵-Ang II in the kidney (357.9 ± 76.6 fmol/g) compared with that observed in sham-operated rats (206.1 ± 13.0 fmol/g) in our previous report.¹⁶

Effect of Candesartan on Urine Volume, Urinary Osmolality and Urinary Ang II Excretion Rates During Val⁵-Ang II Infusion

Val⁵-Ang II infusion caused marked increases in urine flow and decreases in urinary osmolality presumably due to the well recognized effect of Ang II to stimulate thirst.²⁴ Candesartan treatment prevented the increase in urine volume, becoming statistically different by day 9 (37.6 ± 2.7 vs. 10.9 ± 1.0 ml/24hr); $p < 0.01$, and also prevented the decreases of urinary osmolality, becoming significantly different by day 3 (860.0 ± 119.9 vs. 1571.6 ± 180.1 mmol/kg) relative to the Val⁵-Ang II infused rats; $p < 0.01$.

The urinary Val⁵-Ang II excretion rates in both groups of the Val⁵-Ang II infused rats increased sharply at day 3, then returned to baseline at day 6 and remained stable until day 12. Candesartan treatment reduced the urinary Val⁵-Ang II excretion rates with the difference achieving statistical significance at day 9 (3018.3 ± 391.1 vs. 581.9 ± 86.7 fmol/

24hr; $p < 0.01$). The urinary endogenous Ile⁵-Ang II excretion rates in the Val⁵-Ang II infused rats increased over the course of infusion with perceptible increases observed by day 9 and day 12 when they were significantly greater than measured before the start of Val⁵-Ang II infusion (1330.7 ± 299.0 vs. 4620.5 ± 828.4 fmol/24hr; $p < 0.01$). Candesartan treatment prevented the increases in urinary endogenous Ile⁵-Ang II excretion rates during Val⁵-Ang II infusion and statistical significance was reached by day 9 compared with Val⁵-Ang II infused rats (2185.7 ± 283.2 vs. 740.6 ± 110.3 fmol/24hr; $p < 0.05$, Fig 6).

Discussion

Previous studies have shown that intrarenal Ang II levels are augmented during Ang II-induced hypertension^{1-3,13} due to AT₁ receptor mediated uptake of circulating Ang II as well as increased formation of endogenous Ang II.^{2,3,11} In our recent study,¹⁶ we confirmed that there was an elevated Val⁵-Ang II in the kidneys of Val⁵-Ang II infused rats. We further reported that there was a significant augmentation of endogenous Ile⁵-Ang II levels in the kidneys which was greater than could be explained from equilibration with the circulating Ile⁵-Ang II concentration, thus demonstrating increased de novo formation of endogenous Ile⁵-Ang II.¹⁶ The present study addressed the important question regarding the role of AT₁ receptor in mediating the augmentation of endogenous Ang II.

In the Val⁵-Ang II infused rats, the systolic blood pressure begin to increase by day 3 and progressively increased over the course of the two weeks; candesartan treatment to rats infused with Val⁵-Ang II for 13 days prevented the progressive increase in SBP. The plasma renin activity levels were markedly suppressed in the Val⁵-Ang II infused rats, but not in the candesartan treated rats; thereby indicating the importance of AT₁ receptor activation in the Ang II induced negative feedback effect on renin release.^{1,6,25-28} Interestingly, the kidney prorenin and renin contents were not suppressed in Val⁵-Ang II infused rats. These results are consistent with previous reports that the kidney renin mRNA, renin protein, and KRC are not suppressed to the same extent as PRA.^{1,8,26,27} Indeed, renin expression in collecting duct cells of the distal nephron actually increases in Ang II infused rats.^{8,9} Thus, the KRC was not suppressed in the Val⁵-Ang II infused rats, which would allow the possibility of increased endogenous intrarenal Ang II formation. In the Val⁵-Ang II infused rats treated with candesartan, the plasma endogenous Ile⁵-Ang II levels were elevated which can be explained by the increased circulating renin activity.^{2,11} In contrast, the candesartan treated Val⁵-Ang II infused rats exhibited reductions in kidney endogenous Ile⁵-Ang II content by 94% as well as in Val⁵-Ang II content by 37%. The 37% reduction of Val⁵-Ang II in the kidney reflects the blockade of AT₁ receptor mediated uptake of circulating Val⁵-Ang II.^{2,11} The marked reduction in endogenous Ile⁵-Ang II was due to both reduction of endogenous Ile⁵-Ang II uptake and also a substantial reduction in intrarenal de novo formation of Ile⁵-Ang II in the Val⁵-Ang II infused rats treated with candesartan. Zhou et al¹³ showed that Ang II levels in renal cortical endosomes and intermicrovillar clefts are markedly increased in Ang II infused hypertensive rats and concurrent administration of candesartan prevented the increase in endosomes and intermicrovillar cleft Ang II levels. This study demonstrated that there is increased AT₁ receptor-mediated intracellular trafficking/accumulation of circulating and/or intrarenal formed Ang II into cortical tubular endosomes during Ang II-dependant hypertension which is blocked by an ARB. The

internalized Ang II or Ang II-AT₁ receptor complex may migrate to the nucleus to exert genomic effects^{29–31} because there are nuclear binding sites for Ang II in renal cells.³² In addition, chronic Ang II infusions increased intrarenal AGT mRNA levels causing enhanced intrarenal production of AGT, suggesting additional de novo Ang II generation which contributes further to the increased intrarenal Ang II levels.^{7,12,14,28,33} In addition, AGT and Ang II are present in very high concentrations in proximal tubular fluid.^{17,18,34,35} In our current study, candesartan had a more profound effect on Ile⁵-Ang II production than uptake of Val⁵-Ang II into renal tissue, which provide quantitative evidence supporting that there is a positive amplification mechanism resulting in the increases in de novo intrarenal endogenous Ang II formation in Ang II induced hypertension.

The present study demonstrates that the urinary Ile⁵-Ang II excretion rates by day 12 were significantly greater than those measured in the same rats on the day before the start of Val⁵-Ang II infusion. Furthermore, the urinary Ile⁵-Ang II excretion rates increased progressively and were significantly elevated by day 9 in the Val⁵-Ang II infused rats and significantly greater than in the candesartan treated rats. In candesartan treated rats, the Ile⁵-Ang II excretion rate was reduced by 82% and Val⁵-Ang II excretion rate was reduced by 30%. These percentage reductions are similar to the reductions of intrarenal Ang II content; being 94% for Ile⁵-Ang II and 37% for Val⁵-Ang II. These results indicate that the increased urinary Ang II excretion rate is positively related to the elevation of intrarenal Ang II during Ang II induced hypertension and that changes in the urinary Ang II excretion rates are a reflection of changes in intrarenal Ang II levels. Zou et al² reported that in Ang II infused rats, plasma Ang II levels were elevated by day 3 of Ang II infusion, however, the intrarenal Ang II, measured at day 3, 7, 10 and 13, increased slightly by day 7 and were significantly elevated by day 10. The differences in the time-related changes in circulating and intrarenal Ang II levels suggested a slowly developing effect of circulating Ang II to enhance intrarenal Ang II. The time pattern of the augmentation of intrarenal Ang II observed by Zou et al is closely matched by the enhanced urinary Ile⁵-Ang II excretion rates which we observed. Also, urinary excretion rates of AGT in Ang II infused rats become significantly increased at day 8 compared with sham rats³³ and basal renal plasma flow (RPF) and glomerular filtration rate (GFR) levels in chronic Ang II infused rats and those treated with a ARB are similar.³⁶ These results support the hypothesis that the augmented urinary endogenous Ang II originates from the kidney and not from the filtration of circulating endogenous Ang II which was actually decreased in the Val⁵-Ang II infused rats.

Administration of Ang II increases mRNA expression of renal and hepatic AGT production²⁸ and directly increases AGT production and AGT mRNA levels in cultured proximal tubular cells,³⁷ supporting the hypothesis that intrarenal AGT protein is predominately localized to proximal tubular cells.^{14, 37–44} Furthermore, the increased AGT in the urine in Ang II dependent hypertension indicates that AGT traverses the entire nephron segments.⁷ Komlosi et al⁴⁵ reported that Ang II was formed intraluminally in the collecting duct in the presence of ACE. Furthermore, the increases in renal AGT and urinary AGT excretion rate are mediated by AT₁ receptor activation⁷ and lead to increased AGT secretion into the tubular lumen which would lead to increased spillover of AGT into distal nephron segments where it could then be acted upon by renin and ACE in collecting duct cells leading to enhanced urinary excretion rates of Ang II.⁹ Accordingly, the present data

support the hypothesis that the increased urinary endogenous Ile⁵-Ang II excretion in Val⁵-Ang II infused rats is not derived from the circulating Ile⁵-Ang II but is due to enhanced secretion into and /or de novo formation of intrarenal endogenous Ile⁵-Ang II mediated by AT₁ receptors.

Perspectives

This study demonstrates that chronic Ang II infusions to normal rats significantly increases urinary excretion of endogenous Ile⁵-Ang II in a time-dependent manner that was associated with elevated intrarenal endogenous Ile⁵-Ang II levels and they are both mediated by AT₁ receptors. The urinary excretion of endogenous Ang II may be an indicator of increased distal nephron Ang II production in Ang II -dependent hypertension which could augment distal sodium reabsorption. It is possible that in human subjects, the measurement of urinary excretion of Ang II can be used as one of the biomarkers in hypertensive patients to monitor the efficacy of drugs that block the actions of the renin-angiotensin system and thus contribute to an optimized treatment.

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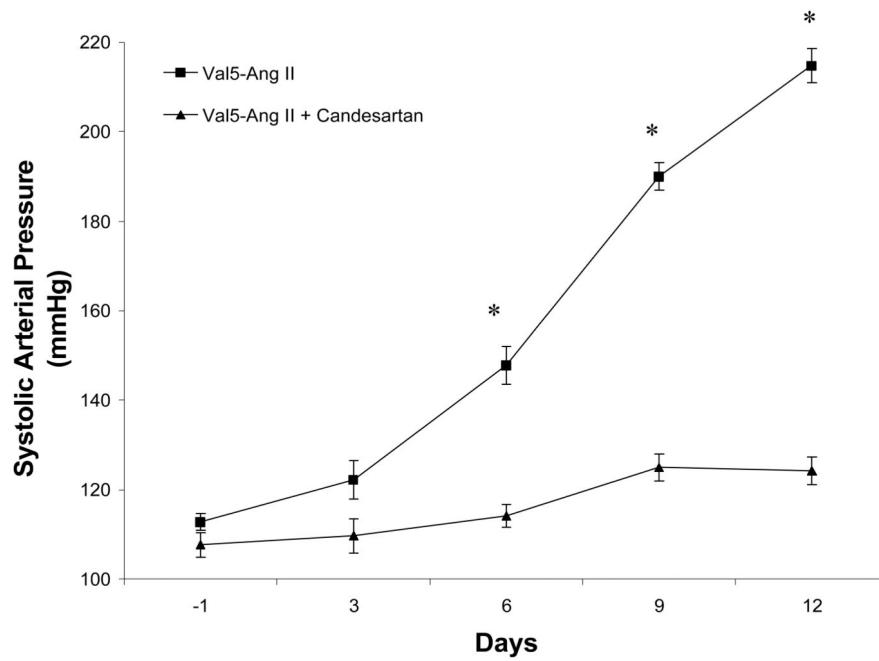


Figure 1. Comparison of systolic blood pressures between Val⁵-Ang II infused rats (n = 5) and Val⁵-Ang II infused rats treated with candesartan (n = 6). Values are mean \pm SE. * p < 0.01 vs. Val⁵-Ang II infused rats + candesartan.

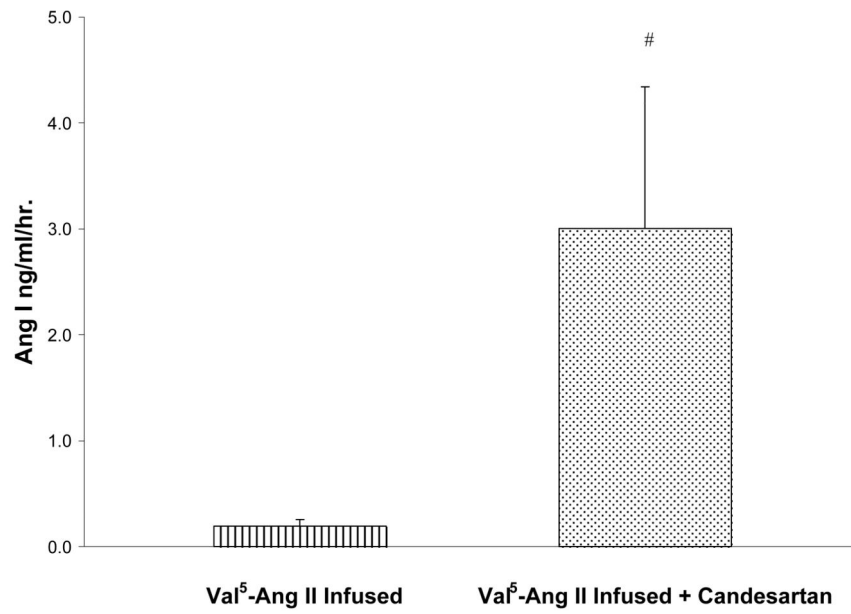


Figure 2. Comparison of plasma renin activity between Val⁵-Ang II infused rats (n = 5) and Val⁵-Ang II infused rats treated with candesartan (n = 6). Values are mean \pm SE. # p < 0.05 vs. Val⁵-Ang II infused rats.

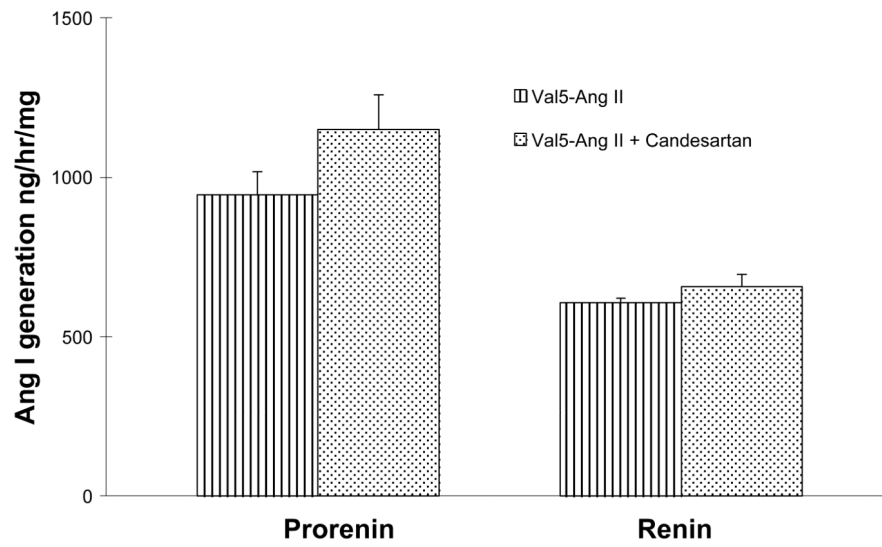


Figure 3. Comparison of kidney prorenin and renin content between Val⁵-Ang II infused rats (n = 5) and Val⁵-Ang II infused rats treated with candesartan (n = 6). Values are mean \pm SE.

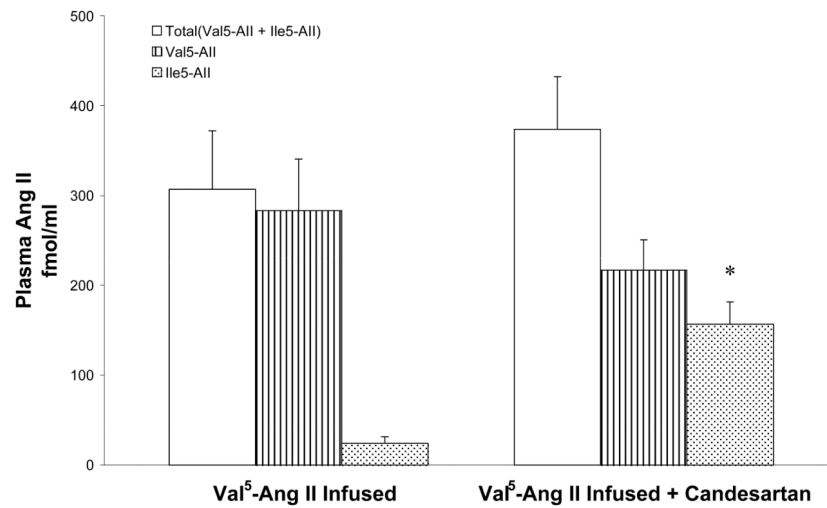


Figure 4. Comparison of plasma Val⁵-Ang II and Ile⁵-Ang II levels between Val⁵-Ang II infused rats (n = 5) and Val⁵-Ang II infused rats treated with candesartan (n = 6). Values are mean ± SE. * p < 0.01 vs. Val⁵-Ang II infused rats.

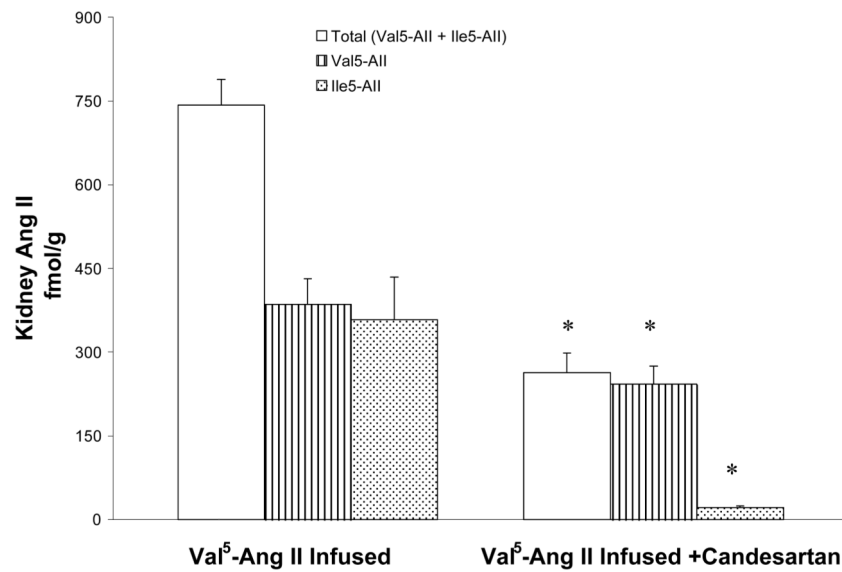


Figure 5. Comparison of kidney Val⁵-Ang II and Ile⁵-Ang II levels between Val⁵-Ang II infused rats (n = 5) and Val⁵-Ang II infused rats treated with candesartan (n = 6). Values are mean ± SE. * p < 0.01 vs. Val⁵-Ang II infused rats.

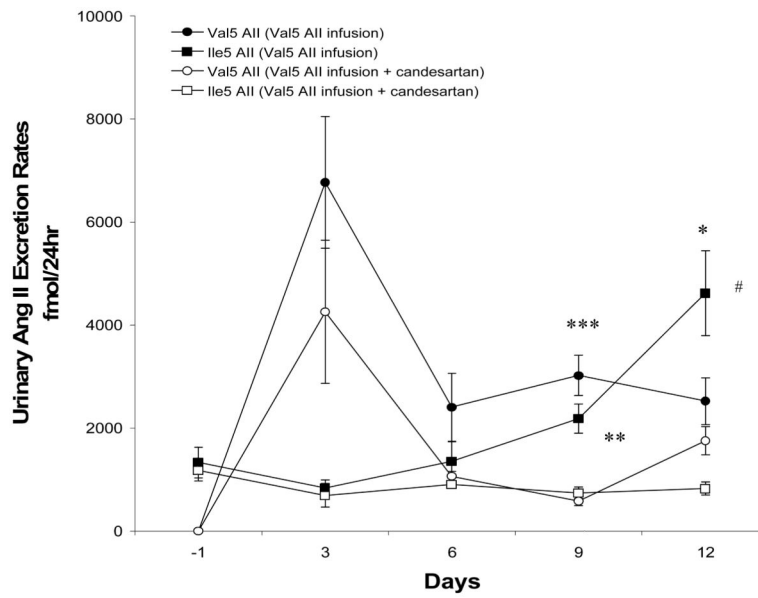


Figure 6.

Comparison of urinary Val⁵-Ang II and Ile⁵-Ang II excretion rates between Val⁵-Ang II infused rats (n = 5) and Val⁵-Ang II infused rats treated with candesartan (n = 6). Values are mean ± SE. * p < 0.01, ** p < 0.05, *** p < 0.01 vs. Val⁵-Ang II infused rats + candesartan. # p < 0.01 vs. Val⁵-Ang II infused rats at day -1.