Effects of Angiotensin-Converting Enzyme Inhibitor and Angiotensin Type 1 Receptor Antagonist in Deoxycorticosterone Acetate–Salt Hypertensive Mice Lacking Ren-2 Gene

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Abstract—We previously reported that inhibition of angiotensin-converting enzyme (ACE) prevented the hypertension and left ventricular hypertrophy induced by deoxycorticosterone acetate–salt (DOCA-salt) in 129/SvEvTac mice, which have 2 renin genes (Ren-1 and Ren-2). In the present study, we induced hypertension by uninephrectomy and DOCA-salt in mice having only the Ren-1 gene (C57BL/6J) and investigated the effect of an ACE inhibitor (ramipril, 4 mg · kg⁻¹ · d⁻¹) and an angiotensin type 1 (AT₁) receptor antagonist (L-158809, 4 mg · kg⁻¹ · d⁻¹) on the development of hypertension, cardiac hypertrophy, and renal injury. After 4 weeks of treatment, systolic blood pressure in DOCA-salt mice was significantly increased (128±2 mm Hg) compared with controls (109±2 mm Hg) (P<0.001), while plasma renin concentration was decreased by 97% (P<0.001). DOCA-salt also induced left ventricular and renal hypertrophy and renal damage as manifested by proteinuria. Collagen content in the left ventricle and kidney was significantly higher in DOCA-salt mice (P<0.001). Urinary albumin (P<0.05) and proliferating cell nucleic antigen–positive cells in the tubules and interstitium of the renal cortex (P<0.001) were significantly increased in the DOCA-salt group. Neither the ACE inhibitor nor the AT₁ antagonist had any antihypertensive effect; however, they partially prevented cardiac hypertrophy and completely inhibited left ventricular collagen deposition. In the kidney, both the ACE inhibitor and AT₁ antagonist partially reduced the increase in collagen but had no effect on hypertrophy. They also significantly prevented the effect of DOCA-salt on urinary albumin and proliferating cell nucleic antigen expression in the kidney. Despite the lack of an antihypertensive effect, both ACE inhibitor and AT₁ antagonist prevented cardiac remodeling and renal damage. Our results indicate that ACE inhibitors and AT₁ antagonists exert beneficial effects on the heart and kidney in DOCA-salt hypertensive mice independently of their effects on blood pressure. (Hypertension. 2001;37:974-980.)

Key Words: mice ■ deoxycorticosterone ■ hypertension ■ angiotensin-converting enzyme inhibitors ■ receptors, angiotensin ■ renal injury ■ cardiac remodeling

Deoxycorticosterone acetate–salt (DOCA-salt) treatment induces hypertension and decreases plasma renin concentration (PRC) to a very low level in rats,1 dogs,2 and humans.3 Moreover, Barrett et al4 found that PRC was also decreased to a very low level in DOCA-salt–treated Balb/c mice (one copy of the renin gene). On the other hand, we previously reported that in DOCA-salt hypertensive 129/SvEvTac mice with 2 renin genes (Ren-1 and Ren-2), PRC was only partially decreased and the development of hypertension was partially prevented by an angiotensin-converting enzyme (ACE) inhibitor.5 In contrast to 129/SvEvTac mice, it has been reported that in DOCA-salt hypertensive rats, ACE inhibitors and angiotensin type 1 (AT₁) receptor antagonists did not alter increased blood pressure (BP) but did prevent deterioration of renal function and cardiac fibrosis.1,6,7 Therefore, in DOCA-salt hypertensive animals the effects of ACE inhibitors or AT₁ antagonists on BP might be different, depending on whether they have 1 or 2 renin genes; furthermore, it appears that the protective effect of ACE inhibitors or AT₁ antagonists on the heart and kidney is independent of their effect on BP.

In the present study, we tested whether chronic blockade of AT₁ receptors and ACE activity in DOCA-salt–treated mice with 1 renin gene might not prevent hypertension but would prevent cardiac remodeling and renal injury and improve renal function. To test this hypothesis, we used C57BL/6J mice (which express only the Ren-1 gene) to establish a DOCA-salt hypertensive mouse model and investigated the effect of an ACE inhibitor (ramipril) and an AT₁ receptor antagonist (L-158809) on BP, cardiac hypertrophy, collagen deposition, and renal damage. Since most recent advances in transgenic and gene knockout techniques have been done in
mice,\textsuperscript{8,9} we believed it was very important to establish a model of renin-independent hypertension in mice, similar to other species with only 1 renin gene.

Methods

Animals and Experimental Design

Twelve-week-old male C57BL/6J mice (weight, 26 to 27 g; Jackson Laboratories) were anesthetized with sodium pentobarbital (50 mg/kg IP), and the left kidney was removed. A silicon rubber sheet (silicone:DOCA ratio, 3:1) was implanted subcutaneously at a dose of 10 mg DOCA per 10 g body wt.\textsuperscript{5} Mice receiving DOCA (Sigma) were given 1% NaCl and 0.2% KCl to drink. Control mice were also uninephrectomized, but DOCA and saline were not given. Animals were divided into 4 groups: (1) controls receiving tap water, (2) mice receiving DOCA-salt, (3) mice receiving DOCA-salt+ramipril (ACE inhibitor; 4 mg · kg\textsuperscript{-1} · d\textsuperscript{-1} in drinking water), and (4) mice receiving L-158809 (AT\textsubscript{1} receptor antagonist; 4 mg · kg\textsuperscript{-1} · d\textsuperscript{-1} in drinking water). Dosage was based on previous data from our laboratory showing that in C57BL/6J mice, L-158809 at 3.0 mg · kg\textsuperscript{-1} · d\textsuperscript{-1} for 4 weeks reduced the pressor effect of exogenous angiotensin II (Ang II) (5 to 50 ng Ang II per mouse) by 90%, while ramipril at 2.4 mg · kg\textsuperscript{-1} · d\textsuperscript{-1} in drinking water). Therefore, 4 mg · kg\textsuperscript{-1} · d\textsuperscript{-1} was chosen as the dose for both ramipril and L-158809. Treatment started immediately after surgery and continued for 4 weeks. This study was approved by the Henry Ford Hospital Care of Experimental Animals Committee.

Measurement of Systolic BP and Mean BP

Systolic BP (SBP) was measured by tail cuff (BP-2000, Visitech Systems).\textsuperscript{5,10} Mice were trained for 1 week, after which SBP was measured in the morning 3 times a week for 4 weeks. Each session included 2 sets of 10 measurements for each mouse; to include each set of measurements, the computer had to identify a BP in at least 6 of the 10 trials within the set. We averaged the SBP data for 3 days per week and expressed them as 1 SBP per week for each mouse. Mean BP (MBP) was measured during the fifth week; for this, mice were anesthetized with 50 mg/kg methohexital sodium, and a modified polyethylene catheter (PE-10 fused to PE-50; Clay-Adams) was passed into the aorta via the femoral artery and subcutaneously brought out the back of the neck. Mice were allowed to recover from the anesthesia for 24 hours, and MBP was then measured in unrestrained conscious mice as described previously.\textsuperscript{5}

Measurement of Urinary Volume, Urinary Sodium Excretion, and Urinary Albumin

After 4 weeks of drug treatment, metabolic cages especially designed for mice were used to collect urine for 24 hours. Urinary volume (UV) was determined gravimetrically and expressed as mL/24 h. Urinary sodium excretion (UNa V) was measured with a NOVA-1 ion electrolyte analyzer (Nova Biochemical), and sodium excretion was calculated and expressed as nmol/min. Urinary albumin was measured with an enzyme-linked immunosorbent assay kit (Exocell) and expressed as micrograms albumin per gram body weight per 24 hours.

Measurement of Hematocrit and PRC

Three days before the MBP catheter was implanted, mice were lightly anesthetized with ethyl ether, and blood was collected in a microhematocrit tube by puncturing the retro-orbital plexus. After the tube was spun in a centrifuge (Clay Adams), the hematocrit was measured with a microhematocrit capillary tube reader (Oxford). Plasma was collected and was stored at −70°C, and PRC was determined by Skinner’s method.\textsuperscript{12} Briefly, plasma (2 µL) was incubated with sheep angiotensinogen in buffer (0.1 mol/L sodium phosphate, 0.02 mol/L Na\textsubscript{2}EDTA, and 0.05% PMSF; pH 6.5) at 37°C for 30 minutes. The incubation was stopped by immediately boiling in water for 15 minutes. The incubated mixture was centrifuged at 1680g for 10 minutes, and the supernatants were stored at −20°C until assayed. Generated Ang I was measured by radioimmunoassay,\textsuperscript{12} and the results were expressed as micrograms Ang I per milliliter plasma per hour.

Hydroxyproline Assay for Collagen Content

After MBP measurement, mice were anesthetized with 50 mg/kg sodium pentobarbital; the heart and right kidney were rapidly excised, and the left ventricle (LV) (including the septum), right ventricle (RV), atria, and kidney were weighed and normalized to 10 g body wt. Half of the LV was frozen for measurement of hydroxyproline content. Half of the kidney was fixed in 4% paraformaldehyde solution for morphometric studies, and ~20 mg (wet weight) from the other half was frozen and used to measure hydroxyproline. Collagen content of myocardial and renal tissue was determined by hydroxyproline assay.\textsuperscript{13} For this, tissue was freeze-dried and weighed, then homogenized in 0.1 mol/L NaCl and 5 mmol/L NaHCO\textsubscript{3}, washed 5 times with the same solution, and hydrolyzed in 0.5 mL 6N HCl for 16 hours at 110°C. Samples were filtered and vacuum-dried, then dissolved in distilled water. Hydroxyproline content was determined with a colorimetric assay and a standard curve of 0 to 5 µg hydroxyproline. Data were expressed as micrograms collagen per milligram dry weight, assuming that collagen contains an average of 13.5% hydroxyproline.\textsuperscript{14}

Morphometric Analysis of Proliferating Cortical Cells

Sections 4 µm thick were deparaffinized, rehydrated, and boiled in 0.2% citric acid (pH 6.0) for 10 minutes for antigen retrieval. Sections were washed 3 times in PBS for 5 minutes, preincubated with blocking serum (1% normal serum) for 30 minutes, and then incubated with a mouse monoclonal antibody against proliferating cell nuclear antigen (PCNA) (1:1000 dilution; Chemicon) at 4°C overnight. Each section was washed 3 times in PBS and PCNA assayed with a Vectastain ABC kit and DAB substrate (Vector Laboratories). For each kidney, 12 randomly selected cortical fields were examined under high magnification (×400). Those tubular and interstitial proliferating cells that had dark brown nuclei were counted and expressed as the number of PCNA-positive cells per field.

Statistical Analysis

ANOVA with contrasts was used to compare all treatments with control and to compare DOCA-salt/vehicle with DOCA-salt/ACE inhibitor and DOCA-salt/AT\textsubscript{1} receptor antagonist. Simes’ procedure\textsuperscript{15} was used to adjust for multiple testing. The overall familywise α level was maintained at 0.05. Results are expressed as mean±SE.

Results

BP, Hematocrit, Atrial Weight, RV Weight, LV Weight, and Kidney Weight

One week after DOCA-salt treatment, SBP in the DOCA-salt/vehicle group (n=12) was significantly increased compared with controls (n=8) and remained at this level for 4 weeks. Neither ACE inhibitor (n=10) nor AT\textsubscript{1} receptor antagonist (n=10) significantly prevented hypertension in C57BL/6J mice treated with DOCA-salt (Figure 1). At the end of the study, MBP was similar in mice given DOCA-salt/vehicle (n=8), DOCA-salt/ACE inhibitor (n=8), or DOCA-salt/AT\textsubscript{1} receptor antagonist (n=6) and was significantly higher in these 3 groups than in controls (n=5) (Table).

After 4 weeks of treatment, the hematocrit was significantly decreased in DOCA-salt hypertensive mice (n=8) compared with controls (n=8). Both ramipril (n=10) and...
L-158809 (n=10) partially prevented the decrease in hematocrit produced by DOCA-salt (P<0.01) (Table).

Body weight was not affected by any of these treatments. When cardiac and renal weight were corrected for body weight, LV weight/body weight was significantly increased in the DOCA-salt/vehicle group (n=20) compared with controls (n=13), and the ACE inhibitor (n=15) and AT1 receptor antagonist (n=15) partially suppressed this increase (P<0.005) (Table). Kidney weight/body weight and atrial weight/body weight in DOCA-salt hypertensive mice were also significantly increased compared with controls but were not affected by either treatment. There were no significant differences in RV weight/body weight among the 4 groups.

**Plasma Renin Concentration**

After 4 weeks of treatment, PRC was significantly decreased in the DOCA-salt/vehicle group (n=11) compared with controls (n=7) (P<0.001) and did not increase after treatment with the ACE inhibitor (n=10) or AT1 receptor antagonist (n=10) (Table).

**UV, UNaV, and Albuminuria**

UV and UNaV were increased in the DOCA-salt/vehicle group (n=9) compared with controls (n=5) (P<0.001). Treatment with the ACE inhibitor (n=5) in DOCA-salt mice further increased UV (P<0.05) but not UNaV. The AT1 receptor antagonist (n=5) also tended to increase UV but had no effect on UNaV compared with DOCA-salt alone. Urinary albumin was significantly higher in DOCA-salt mice treated with vehicle compared with controls (1.7±0.2 vs. 0.94±0.2 µg/g body wt per 24 h; P<0.05), and both ACE inhibitor and AT1 receptor antagonist significantly blocked this increase (P<0.05) (Figure 2).

**Collagen Content of the LV and Kidney**

Collagen content of the LV was significantly increased in the DOCA-salt/vehicle group compared with controls, and this increase was completely prevented by both the ACE inhibitor and AT1 receptor antagonist (P<0.001) (Figure 3). We also observed a significant increase in renal collagen in the vehicle group compared with controls, which was partially reduced in mice treated with the ACE inhibitor or AT1 receptor antagonist P<0.05).

**Histological Analysis**

In the controls, PCNA-positive cells were largely restricted to the renal tubular epithelium; however, in the DOCA-salt/vehicle group we also found a few in the interstitial space (Figure 4). PCNA-positive cells were significantly increased in the DOCA-salt/vehicle group compared with controls.
**Figure 2.** Effect of ACE inhibitor or AT₁ receptor antagonist on urinary albumin in controls and DOCA-salt hypertensive mice after 4 weeks of treatment. Urinary albumin was increased with DOCA-salt/vehicle, and both ACE inhibitor and AT₁ receptor antagonist significantly blocked this increase. *P<0.05 vs controls; †P<0.05 vs DOCA-salt/vehicle. BW indicates body weight; other abbreviations are as defined in Figure 1.

**(P<0.005).** Treatment with the ACE inhibitor or AT₁ receptor antagonist significantly decreased the number of PCNA-positive cells in the renal tubular epithelium and interstitium (P<0.001), although they still occurred more frequently than in controls (P<0.005) (Figure 5).

**Discussion**

We found that in mice having only the Ren-1 gene (C57BL/6J), DOCA-salt–induced hypertension was not affected by chronic inhibition of ACE or blockade of AT₁ receptors, similar to DOCA-salt hypertensive rats. In addition, DOCA-salt reduced PRC by 97% in C57BL/6J mice, similar to Balb/c mice✿ and rats resigned only 1 copy of the renin gene, and this reduction was not affected by the ACE inhibitor or AT₁ receptor antagonist. In turn, our previous study showed that PRC was only partially reduced in 129/SvEvTac mice (Ren-1/Ren-2) when given DOCA-salt compared with their respective controls, and the development of hypertension was partially prevented by an ACE inhibitor. The remaining PRC in DOCA-salt mice (Ren-1/Ren-2) could be due to the presence of the Ren-2 gene, which expresses renin mainly in the convoluted tubular cells of the submaxillary gland with very low levels in the kidney. While expression of renin from Ren-1 was completely suppressed by DOCA-salt, it appears that expression of renin from Ren-2 is not affected by DOCA-salt in 129/SvEvTac mice. The difference in BP response to the ACE inhibitor might be due to the variable expression of renin genes with DOCA-salt treatment. The absence of decreased BP with ACE inhibitor or AT₁ antagonist treatment and PRC suppression in DOCA-salt hypertensive C57BL/6J mice (Ren-1) suggests that DOCA-salt hypertension in mice with only 1 renin gene is renin-independent, unlike mice with 2 renin genes.

As expected, in C57BL/6J mice DOCA-salt hypertension was associated with (1) cardiac hypertrophy, (2) renal hypertrophy, and (3) increased collagen content in the LV and kidney, similar to DOCA-salt hypertensive rats. Treatment with an ACE inhibitor or AT₁ receptor antagonist in DOCA-salt mice partially prevented LV hypertrophy and renal fibrosis and completely abolished collagen deposition in the LV with no significant effect on BP and renal hypertrophy, in accord with recent reports that inhibition of the renin-angiotensin system (RAS) (1) reversed cardiac fibrosis and (2) significantly decreased renal collagen I and III and fibronectin mRNA in rats treated with DOCA-salt. These results differ from previous studies showing that captopril and losartan did not prevent aldosterone-salt–induced cardiac fibrosis in rats. The dosage of AT₁ receptor antagonist might explain this discrepancy, since high-dose losartan (10 mg · kg⁻¹ · d⁻¹ for 4 weeks), which prevented aldosterone-salt–induced cardiac fibrosis, was 3.3-fold higher than that used by Young and Funder, who showed that losartan did not prevent cardiac fibrosis in the same model. Since AT₁ binding is known to be increased in aldosterone-salt hypertensive rats, it is likely that a high dose of the AT₁ inhibitor is required to prevent Ang II action. In the case of the ACE inhibitor, enalapril (3 mg · kg⁻¹ · d⁻¹ for 5 weeks) had no effect on BP and perivascular fibrosis but decreased subendocardial fibrosis, while Brilla et al reported that captopril (50 mg · kg⁻¹ · d⁻¹ for 8 weeks) did not prevent myocardial fibrosis in aldosterone-salt hypertensive rats. The reasons for this discrepancy are not clear. While we cannot be certain how both ACE inhibitor and AT₁ receptor antagonist partially prevented cardiac hypertrophy and completely normalized collagen content in the LV, we may speculate that the local RAS still played a role in this process. Although PRC was decreased by 97% by DOCA-salt, the remaining PRC (average, 70 to 100 ng Ang I per milliliter per hour) was relatively
high compared with other species such as rats (normal PRC, \( \approx 20 \) ng Ang I per milliliter per hour). Moreover, this low PRC was not involved in the regulation of BP but might affect the heart and kidney in this model of DOCA-salt hypertension, especially since previous studies showed that ACE activity and AT\(_1\) receptor density in the heart and kidney are increased in rats given a high-salt diet combined with mineralocorticoids.\(^{21-23}\)

Other possible routes by which ACE inhibitors and AT\(_1\) receptor antagonists prevent cardiac hypertrophy and fibrosis as well as renal fibrosis include mechanisms independent of RAS blockade. Kinins acting through B\(_2\) receptors\(^{24}\) may also participate in the effect of ACE inhibitors in DOCA-salt hypertension, perhaps via the release of prostaglandins and NO.\(^{25,26}\) However, the role of kinins in the antihypertrophic/antifibrotic effect of ACE inhibitors in hypertension is not well established. During blockade of the AT\(_1\) receptors, Ang II may activate the AT\(_2\) receptors, resulting in increased cardioprotection, since the beneficial effects of the AT\(_1\) receptor antagonist were partially prevented by cotreatment with an AT\(_2\) receptor antagonist.\(^{27-29}\) Activation of endothelial AT\(_2\) receptors has been linked to increased kinins and release of NO and prostaglandins,\(^{25,26}\) leading to reduced cardiac hypertrophy and inhibition of collagen synthesis.\(^{27,30}\)

Chronic ACE inhibition is associated with a 5-fold increase in plasma \( N \)-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP).\(^{31,32}\) In addition, we previously reported that Ac-SDKP significantly inhibits cardiac fibroblast proliferation and collagen synthesis in vitro and LV collagen deposition in rats with renovascular hypertension,\(^{33}\) while Yoshioka et al\(^{34}\) reported that Ac-SDKP is a novel antiproliferative peptide in renal fibroblasts. This suggests that increased plasma Ac-SDKP might contribute to the antifibrotic effect of ACE inhibitors in the heart and kidney.

In the present study, both ACE inhibitor and AT\(_1\) receptor antagonist significantly reduced urinary albumin and prevented renal lesions in DOCA-salt mice without lowering BP, just as in DOCA-salt rats.\(^6\) This may be explained by the "hyperfiltration theory."\(^{35}\) ACE inhibitors and other RAS blockers may have a predominantly postglomerular effect, lowering intraglomerular pressure and proteinuria by dilating the efferent arteriole.\(^{36,37}\) In mice, DOCA-salt hypertension reportedly results in significantly increased renal vascular
resistance and lower renal blood flow compared with controls, with the latter causing renal dysfunction. Treatment with an ACE inhibitor may increase the response to vasodilators such as kinins and NO, leading to increased renal blood flow and improved function. Again, AT₁ receptor antagonists may improve renal function by activating AT₂ receptors and thereby mediating vasodilation. Although AT₂ expression in the kidney is dramatically decreased after birth, upregulation may occur under some pathophysiological circumstances.

PCNA is expressed during the S-G₁ stage of the cell cycle and is thought to be a marker of cell proliferation. Both ACE inhibitor and AT₁ receptor antagonists significantly decrease the number of tubular and interstitial cell nuclei that stained positive for PCNA in DOCA-salt hypertensive mice. Although the mechanism(s) by which these 2 drugs reduce PCNA expression is not known, one could postulate that (1) suppression of the mitogenic effect of Ang II and/or (2) increased Ac-SDKP may reduce PCNA.

As expected, in mice given DOCA-salt the hematocrit was significantly lower than in controls; however, the fact that it increased somewhat with ACE inhibitor or AT₁ receptor antagonist treatment might be explained by the diuretic effect of ACE inhibitors or AT₁ receptor antagonists in mice given DOCA-salt. The renal kallikrein-kinin system regulates water excretion, promoting diuresis. Therefore, ACE inhibitors or AT₁ receptor antagonists may increase circulating and/or tissue kinins by inhibiting their degradation (ACE inhibitor) or blocking AT₁ receptors, causing Ang II to bind to and activate the AT₁ receptors. Kinins in turn act on B₂ receptors located on the luminal and basolateral sides of the collecting tubules and cause free water to be excreted.

In conclusion, DOCA-salt hypertension in mice having only the Ren-1 gene (C57BL/6J) is RAS-independent, similar to other species with only 1 renin gene such as rats and humans. Cardiac remodeling, renal hypertrophy, and renal damage were observed in this model. In DOCA-salt hypertensive mice, ACE inhibitors or AT₁ receptor antagonists exert a beneficial effect on the heart and kidney independently of their effects on BP.

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**References**


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