Angiotensin II, interstitial inflammation, and the pathogenesis of salt-sensitive hypertension

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Angiotensin II, interstitial inflammation, and the pathogenesis of salt-sensitive hypertension. Am J Physiol Renal Physiol 291: F1281–F1287, 2006. First published July 25, 2006; doi:10.1152/ajprenal.00221.2006.—Transient administration of angiotensin II (ANG II) causes persistent salt-sensitive hypertension associated with arteriolopathy, interstitial inflammation, and cortical vasoconstriction; blocking the vascular and inflammatory changes with mycophenolate mofetil (MMF) prevents vasoconstriction. While infiltrating leukocytes during the salt-sensitive hypertension phase express ANG II, the functional role of ANG II during this phase is not known. We examined the acute effect of candesartan on renal hemodynamics during the established salt-sensitive hypertensive phase and related these findings to direct measurement of intrarenal ANG II and inflammatory cells in rats previously exposed to ANG II with or without MMF treatment. Sham controls were also examined. The administration of ANG II, followed by exposure to high-salt diet, resulted in hypertension, cortical vasoconstriction, an increase in interstitial inflammation of ANG II, followed by exposure to high-salt diet, resulted in MMF treatment. Sham controls were also examined. The administration of ANG II causes persistent salt-sensitive hypertension associated with arteriolopathy, interstitial inflammation, and cortical vasoconstriction; blocking the vascular and inflammatory changes with mycophenolate mofetil (MMF) prevents vasoconstriction. While infiltrating leukocytes during the salt-sensitive hypertension phase express ANG II, the functional role of ANG II during this phase is not known. We examined the acute effect of candesartan on renal hemodynamics during the established salt-sensitive hypertensive phase and related these findings to direct measurement of intrarenal ANG II and inflammatory cells in rats previously exposed to ANG II with or without MMF treatment. Sham controls were also examined. The administration of ANG II, followed by exposure to high-salt diet, resulted in hypertension, cortical vasoconstriction, an increase in interstitial inflammation cells (44.8 ± 1.3 lymphocytes/mm², and 30.8 ± 1.2 macrophages/mm²² ANG II vs. 19.6 ± 2 lymphocytes/mm²², and 22 ± 0.7 macrophages/mm²² Sham), and increase in renal ANG II levels (1.358 ± 0.746 pg/ml ANG II vs. 194 ± 9.28 pg/ml Sham). Treatment with MMF during the administration of exogenous ANG II resulted in reduction in renal interstitial inflammation (19.7 ± 0.9 lymphocytes/mm²² and 15.9 ± 0.8 macrophages/mm²²), ANG II levels (436.9 ± 52.9 pg/ml), cortical vasoconstriction, and stable blood pressure levels during the subsequent challenge with a high-salt diet. Acute administration of candesartan similarly reduced renal vasoconstriction and blood pressure. We conclude that the cortical vasoconstriction occurring with salt-sensitive hypertension following exposure to ANG II is mediated by intrarenal ANG II, related, at least in part, to the interstitial inflammation.

renal hemodynamics; candesartan; AT₁ angiotensin II receptor blockade

RECENT STUDIES HAVE SUGGESTED that salt-sensitive hypertension may result from a subtle acquired renal injury that alters the ability to excrete salt (13). One experimental model in which this has been demonstrated is the postangiotensin II model (15, 24). In this model the transient infusion of ANG II results in hypertension with renal vasoconstriction and the development of microvascular and tubulointerstitial injury (15). Interest-

ingly, after the exposure to ANG II, the rats become susceptible to the development of salt-sensitive hypertension despite maintaining a normal glomerular filtration rate (GFR) (9, 24). Specifically, when rats are placed on a high-salt diet (HSD) they rapidly become hypertensive in association with the development of cortical vasoconstriction (9). The hemodynamic changes are associated with the presence of tubulointerstitial inflammation consisting of T cells and macrophages and local oxidative stress (22, 24, 25, 34). Importantly, many of the infiltrating cells express ANG II (9, 21). The administration of the immunosuppressant mycophenolate mofetil (MMF) during the initial ANG II infusion can block these histological changes (21, 24) and prevent the cortical vasoconstriction and hypertensive response to the HSD (21, 26).

An important issue is whether there is a functional increase in ANG II in this model. Classically, HSDs are associated with the expansion of blood volume and a reduction in the activation of the renin-angiotensin axis. However, intrarenal angiotensin is not suppressed by plasma expansion and in fact, it may function as a separate compartment (10, 18). Furthermore, Ruan et al. (27) reported that HSD increases the renal vasoconstrictive response to ANG II by increasing intrarenal expression of AT₁ receptors. Hence, we postulated that an increase in intrarenal ANG II during salt loading, likely related to the renal interstitial inflammation, could be responsible for the cortical vasoconstriction in this model. These experiments were addressed to test this hypothesis.

METHODS

Experimental Protocol

Male Sprague-Dawley rats (350 g) were administered ANG II (Sigma, St. Louis, MO; 435 ng·kg⁻¹·min⁻¹ in Ringer lactate) for 2 wk via subcutaneous miniosmotic pumps (Alzet model 2002, Alza, Palo Alto, CA) as previously reported (9). At day 14, the minipump was removed and the rats were allowed to recover for 5 days on a normal-salt diet (0.4% NaCl). The rats were then placed on a HSD (Harlan, 4% NaCl) for 5 additional wk and micropuncture studies were performed (Fig. 1). Care, use, and treatment of all animals in this study were in strict agreement with the guidelines set for by the Instituto Nacional de Cardiología I Ch. Two sets of experiments were done.

Micropuncture experiments. These included the following groups. ANG II group (n = 10) rats received vehicle (0.5 ml of saline

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Micropuncture studies were done as in the ANG II group above. ANG II during the 5-day washout period until the initiation of a HSD. rats received 30 mg/kg body wt of MMF by gastric gavage during the 2 wk and every 2 wk for the rest of the study.

Blood Pressure Measurements

From normal rats in our hands. previously, the procedure was validated to obtain values were given 5 wk of HSD. Sham-operated (n = 9) rats received vehicle as surgical procedure for insertion of subcutaneous pumps but pumps during the micropuncture studies. Sham-operated (n = 9) rats received 30 mg/kg body wt of MMF by gastric gavage during the 2 wk in which exogenous ANG II infusion was administered and during the 5-day washout period until the initiation of a HSD. Micropuncture studies were done as in the ANG II group above. ANG II + MMF (n = 9) rats received 30 mg/kg body wt of MMF by gastric gavage during the 2 wk of exogenous ANG II infusion was administered and during the 5-day washout period until the initiation of a HSD. Rats received a single dose of 0.25 mg/kg of candesartan diluted in 0.5 ml of saline solution 30–40 min before the glomerular hemodynamic measurements, during the micropuncture studies (5). ANG II + MMF (n = 9) rats received 30 mg/kg body wt of MMF by gastric gavage during the 2 wk in which exogenous ANG II infusion was administered and during the 5-day washout period until the initiation of a HSD. Micropuncture studies were done as in the ANG II group above. ANG II + MMF + Cand (n = 9) rats received MMF (30 mg·kg⁻¹·day⁻¹ by gastric gavage) during the period of ANG II infusion and during the 5-day washout period until the initiation of a HSD. Rats received a single dose of 0.25 mg/kg of candesartan diluted in 0.5 ml of saline solution 30–40 min before glomerular hemodynamics were measured during the micropuncture studies. Sham-operated (n = 10) rats had a surgical procedure for insertion of subcutaneous pumps but pumps were not inserted and received normal-salt diet for 2 wk followed by 5 wk of HSD. At the time of micropuncture, they received vehicle as described above. Sham + Cand (n = 11) included 11 sham-operated rats as mentioned above; they received a normal-salt diet for 2 wk followed by 5 wk of HSD. Candesartan was administered before micropuncture experiments.

Determination of interstitial ANG II. These experiments were done to evaluate the interstitial ANG II concentrations during the salt-driven hypertension that follows 2 wk of exogenous ANG II infusion, with and without concomitant administration of MMF. For this purpose, we studied rats that received exogenous ANG II (n = 10) or ANG II and MMF (n = 12) for 2 wk, as described above, and then were given 5 wk of HSD. Sham-operated (n = 12) rats were used as controls. Previously, the procedure was validated to obtain values from normal rats in our hands.

Blood Pressure Measurements

Systolic blood pressure (SBP) measurements were performed in conscious, restrained rats by tail-cuff plethysmography (Narco Biosystems, Austin, TX). Rats were conditioned twice before the blood pressure was measured at a basal period, every week during the first 2 wk and every 2 wk for the rest of the study.

Micro puncture Experiments

For micropuncture studies, the rats were anesthetized with pentobarbital sodium (30 mg/kg ip, and supplementary doses were administered as required). The rats were placed on a thermo-regulated table, and the temperature was maintained at 37°C. Polyethylene tubing was used to catheterize the trachea (PE-240), both jugular veins and femoral arteries (PE-50), and the left ureter (PE-10). The left kidney was exposed, placed in a Lucite holder and sealed, covering the kidney surface with Ringer solution. Mean arterial pressure (MAP) was continuously monitored with a pressure transducer (model p23 LX, Gould Hato Rey, Puerto Rico) and recorded on a polygraph (Grass Instruments, Quincy, MA). Blood samples were taken periodically every 45–60 min and replaced with blood from a normal donor rat.

Rats were maintained euvoicmic by infusion of 10 ml/kg body wt of isotonic rat plasma during surgery, followed by an infusion of 10% polyfructosan (Inutest, Laevosan-Gesellschaft, Austria) in 0.9% sodium saline solution, at a 2.5-ml/h rate. After 60 min, seven timed samples of proximal tubular fluid were obtained to determine flow rate and polyfructosan concentration; intratubular hydrostatic pressure under free flow and stop-flow conditions, and peritubular capillary pressures were measured in other proximal tubules with a servo-null device (Servo-Nulling Pressure System, Instrumentation for Physiology and Medicine, San Diego, CA), as previously described (3). Polyfructosan was measured in plasma samples. Glomerular colloid osmotic pressure was estimated in protein from blood taken from the femoral artery and from the surface of the efferent arterioles.

The ability of candesartan to block the effects of ANG II on blood pressure was tested in five rats. Administration of intravenous 50 ng ANG II produced a rise in MAP of 60 ± 8.7 mmHg; this effect was completely blocked with 0.25 mg candesartan. In addition, at the end of the micropuncture studies in groups that received candesartan, a 50-ng bolus of ANG II did not produce any change in blood pressure.

Polyfructosan concentrations were determined by the Davidson and Sackner (7) technique. The volume of fluid collected from an individual proximal tubule was estimated from the length of the column of fluid in a capillary tube of uniform bore and known internal diameter. The concentration of tubular polyfructosan was measured by the method of Vurek and Pegram (37). The protein concentration in the efferent samples was determined by the Viets et al. (36) method.

Proximal single nephron glomerular filtration rate (SNGFR), intratubular pressure during free flow conditions (FF) and under stopped flow conditions after blocking the tubular lumen with a long oil column (SFP, mmHg), glomerular capillary hydrostatic pressure (PGC), peritubular capillary pressure (PTCP), afferent oncotic pressure (πA), efferent oncotic pressure (πE), glomerular capillary hydrostatic pressure gradient (ΔP), single-nephron filtration fraction, single nephron glomerular blood (Qa) and plasma flow (GPF), afferent (AR) and efferent (ER) resistances, and ultrafiltration coefficient (Kf, ml·s⁻¹·mmHg⁻¹) were all calculated according to equations given elsewhere (2, 8). We estimated PGC by the stopped flow method according to the following equation: PGC = SFP + πA (2, 8).

It should be mentioned that, in micropuncture studies, proximal collections were taken to measure SNGFR, since this is the only available method to evaluate whole glomerular hemodynamics. However, this procedure impairs the tubuloglomerular feedback mechanism (TGF) and results in an overestimation of SNGFR; if this is so, calculated values of glomerular plasma flow are higher and AR and RE lower than the real values. However, the method is generally accepted (3, 21a, 25a). Since the increase of afferent resistance with ANG II is mainly mediated through TGF, and the chronic high intake of salt induces low TGF sensitivity (28), the absence of TGF-mediated effects, on one hand, is a limitation in this study, but on the other hand, it allowed the evaluation of glomerular hemodynamics without the interference of TGF (3, 29). The evaluation of TGF by micropuncture is beyond the scope of this study.
Renal ANG II concentration was determined in the renal cortex through microdialysis, according to the method described by Nishiyama et al., Fox et al., and Siragy et al. (10, 18, 30). The efficiency of the microdialysis probe was determined in vitro, testing ANG II concentrations from 2 to 10 nM, the regression line obtained with the data was $Y = 20.17x + 13.20$, $r^2 = 0.94$. At a perfusion rate of 1.5 $\mu$L/min, the relative equilibrium rate was 66 ± 2% which did not deteriorate with time (data not shown). The in vitro recovery may not be equivalent to the in vivo recovery, but it has been generally accepted as a reasonable estimate for the interstitial concentrations of endogenous compounds (18, 30). The high correlation coefficient ($r^2 = 0.94$) of the linear regression obtained with four different concentrations of ANG II indicates the adequacy of the microdialysis probe. The 66 ± 2% recovery attained may introduce some error for the calculations of recovery in vivo but, at any rate, similar for all the groups studied (18, 30).

In vivo calibrations were performed from 0 to 180 min to test the stability of the ANG II concentrations according to the method described by Nishiyama et al. (18). Thirty minutes after implantation of the probe, the concentrations of ANG II fell from 151.6 ± 6.3 pg/ml to reach a steady state (106.5 ± 9.0 pg/ml), and remained stable up to 180 min (nearly 100 pg/ml). Previous studies have determined that ANG II derived from tubular fluid does not contaminate the dialysate (18).

For this procedure, additional groups of 10–12 rats each were studied at the end of the follow-up (7 wk); normal rats under standard chow were included to determine the values of ANG II in our laboratory. The surgical preparation of the animals was identical to that described previously for the micropuncture studies. A 10-mm polysulfone microdialysis probe (Fresenius Medical Care) with a 33,000-D transmembrane diffusion cut-off was implanted into the renal superficial cortex (17, 25); steel needles were inserted in both ends of the fiber. The inflow of the probe was connected with a polyethylene tubing (PE-10) to a microinfusion pump (Harvard apparatus). The probe was perfused with Ringer solution containing 1% bovine serum albumin (pH 7.4), at a rate of 1.5 $\mu$L/min. The dialysate was collected directly from the outflow steel tubing into a small tube containing a solution of inhibitors (30 $\mu$L of 500 mM EDTA, 15 $\mu$L of 1 mM enalaprilat and 30 $\mu$L of 125 M o-phenanthrolene and 0.2 mM pepstatin A in 95% ethanol), to allow the dialysate effluent to be immediately mixed with the inhibitors. After 90 min of the microdialysis probe implantation, three 60-min sample collections were taken. Immediately thereafter, each sample was vortex-mixed, and 100 $\mu$L of the solution were transferred to a tube containing 1 ml chilled 100% methanol. The samples were stored at −70°C until analyzed.

 Determination of ANG II was performed by enzyme-linked competitive immunoassay using a commercial Kit (SPI Bio, Bertin Group, France), following the procedure recommended by the manufacturer, and according to the method described by Nishiyama et al. and Fox et al. (10, 18). The dialysate samples in methanol were evaporated to dryness with nitrogen, reconstituted in assay buffer, and assayed after extraction in phenyl cartridges; an 8-points standard curve (0 to 125 pg/ml) was used. The intra- and interassay variations were 3.93 ± 0.56 and 5.10 ± 0.58%, respectively. Results are expressed in picogram per milliliter. No cross-reactivity was found with ANG I, ANG I–7, ANG 3–8, or ANG III.

Fig. 2. ANG II interstitial concentrations in the cortex of rats that developed salt sensitivity (ANG II), as well as rats which received ANG II but salt-sensitive hypertension was prevented with MMF treatment (ANG II+MMF), and normotensive rats (Sham rats on high-salt diet and normal rats). Values are means ± SE.
Histological Studies

In six rats from each group, coronal sections of the left kidney were obtained at the time of death. The fragments were fixed in 15% formalin and embedded in paraffin. Lymphocyte and macrophage infiltration was studied with the immunoperoxidase technique described before (23, 25). Briefly, tissues were successively washed and incubated with 20 ml of ExtraAvidin (2.5 mg/ml; Sigma) and 30 ml of 0.001% biotin in PBS. Afterwards, tissues were incubated for 2 h at 37°C with 50 ml of the corresponding primary monoclonal antibody, diluted 1:30 in Tris saline buffer (TSB), pH 7.8. After being washed in TSB for 15 min, tissues were incubated 1 h with 30 ml of rat antinmouse IgG (ab1)’ biotin-conjugated fragments with minimal cross-reactivity with human, horse and rat serum proteins (Accurate Chemical Westbury), and finally for 30 min with 60 ml of peroxidase-conjugated Extravidin. After the final wash, tissues were incubated for 15 min in diaminobenzidine and H2O2 in TBS.

The primary antibodies used were anti-CD5 (mouse monoclonal anti-rat thymocytes and lymphocytes) and anti-ED-1 (mouse monoclonal anti-rat monocytes and macrophages), purchased from Biosource International (Camarillo, CA). Cellular infiltration was evaluated in tubulointerstitial areas and expressed as positive cells per glomerular cross-section or positive cells per square millimeter (mm²).

Statistical Analysis

Groups were evaluated by one-way ANOVA followed by a Tukey-Kramer posttest. Statistical significance was defined at $P < 0.05$. Results in tables and figures are expressed as means ± SE.

RESULTS

Salt-Sensitive Hypertension Is Associated with Increased Intrarenal ANG II and Tubulointerstitial Inflammation

As previously reported (9, 15), rats transiently infused with ANG II developed hypertension when placed subsequently on a HSD. Control (sham-operated) rats and rats that received MMF during the ANG II infusion did not develop salt-sensitive hypertension (Fig. 1). We previously reported that the administration of MMF blocks ANG II-induced tubulointerstitial inflammation and tubular injury and that this correlates with the prevention of the subsequent salt-sensitive hypertension (9,

Fig. 5. Effects of MMF and candesartan on glomerular hemodynamics of rats with salt-sensitive hypertension.
Table 1. Renal hemodynamics in rats with salt-sensitive hypertension, rats with simultaneous MMF treatment, and normotensive sham rats on a high-salt diet after 7 wk of follow up, and effect of a bolus of candesartan on glomerular dynamics of similar groups at the end of the study.

| Group       | SBP, mmHg | MAP, mmHg | GFR, mU/min | AR, 10^{-5} | ER, Dyns^{-1} | Qa, mmHg | PGC, mmHg | SNFR, mmHg | K_e, mmHg
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<tbody>
<tr>
<td>ANG II + V</td>
<td>165±4*</td>
<td>158±1*</td>
<td>156±2*</td>
<td>0.93±0.06</td>
<td>4.75±0.2*</td>
<td>2.09±0.1*</td>
<td>82.7±2.5*</td>
<td>51.6±1</td>
<td>26.0±0.9*</td>
</tr>
<tr>
<td>ANG II + Cand</td>
<td>164±3*</td>
<td>157±2*</td>
<td>127±1</td>
<td>1.18±0.05</td>
<td>2.63±0.21*</td>
<td>1.50±0.1*</td>
<td>115.9±4.5*</td>
<td>52.2±0.7</td>
<td>33.6±0.9*</td>
</tr>
<tr>
<td>ANG II + MMF</td>
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<td></td>
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<tr>
<td>+ V</td>
<td>152±4†</td>
<td>144±2†</td>
<td>142±3†</td>
<td>0.93±0.07</td>
<td>3.82±0.2*</td>
<td>2.07±0.1*</td>
<td>104.1±6.3*</td>
<td>55.2±1.0‡</td>
<td>33.5±1.1‡</td>
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<tr>
<td>ANG II + MMF</td>
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<td></td>
</tr>
<tr>
<td>+ Cand</td>
<td>148±7†‡</td>
<td>137±2†‡</td>
<td>115±3‡</td>
<td>0.84±0.04*</td>
<td>3.72±0.4†</td>
<td>2.42±0.2†</td>
<td>72.9±5.3*</td>
<td>48.0±1*</td>
<td>26.0±0.9*</td>
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<tr>
<td>Sham + V</td>
<td>125±3</td>
<td>125±1</td>
<td>123±2†</td>
<td>0.94±0.05</td>
<td>2.51±0.1</td>
<td>1.66±0.1</td>
<td>118.0±4.9</td>
<td>53.0±0.8</td>
<td>34.4±1.4‡</td>
</tr>
<tr>
<td>Sham + Cand</td>
<td>120±5</td>
<td>126±4</td>
<td>108±3</td>
<td>0.86±0.06</td>
<td>2.30±0.7</td>
<td>1.65±0.1</td>
<td>101.0±4.4</td>
<td>49.7±1.3</td>
<td>27.4±1.3</td>
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</table>

Values are means ± SE. Exp., experimental; SBP, systolic blood pressure; MAP, mean blood pressure; AR, afferent resistance; ER, efferent resistance; Qa, glomerular filtration rate; PGC, peritubular capillary pressure; K_e, ultrafiltration coefficient; V, vehicle. 1) Candesartan (ANG II + Cand) reduced MAP, AR, ER, and increased Qa, single-nephron glomerular filtration rate (SNFR), and K_e than in the ANG II + V group (P < 0.05). 2) Mycophenolate mofetil (MMF) treatment (ANG II + MMF + V) reduced AR and increased SNFR and K_e than in the ANG II + V group (P < 0.05) to levels comparable to those obtained in the ANG II + Cand group. 3) The addition of Cand after MMF (ANG II + MMF + Cand) did not modify the MMF-induced changes in AR and ER but reduced PGC, SNFR, and K_e (P < 0.05). 4) The addition of Cand to the Sham-operated group (Sham + Cand group) reduced MAP, SNFR, and K_e (P < 0.05). *P < 0.05 vs. Sham. †P < 0.05 vs. ANG II. ‡P < 0.05 vehicle vs. Cand.

Effects of Candesartan on Renal Hemodynamics

To evaluate the functional role of ANG II in salt-sensitive hypertension phase, glomerular hemodynamics were assessed following the acute administration of candesartan. As previously reported, the administration of ANG II followed by exposure to a HSD resulted in profound cortical vasoconstriction with an increase in afferent and efferent arterial resistances, in association with a reduction in SNFR and blood flow. These abnormal findings were significantly ameliorated in the rats treated with MMF (Fig. 5). The acute administration of candesartan also completely blocked the arteriolar vasoconstriction (afferent and efferent) in the postangiotensin II salt-sensitive hypertension model (Fig. 5). Similarly, the ultrafiltration coefficient (K_e) was reduced in the ANG II group (ANG II group = 0.027 ± 0.001, vs. sham = 0.034 ± 0.001, P < 0.05) but was significantly improved in MMF-treated rats (0.033 ± 0.002) and the ANG II group that received an acute injection of candesartan (0.033 ± 0.001). Indeed, the increase in K_e in these latter two groups was similar to that observed in sham-operated rats (0.034 ± 0.001).

In contrast with rats treated with ANG II alone, in ANG II + MMF rats, candesartan administration was associated with no effects on vascular tone; in fact, afferent and efferent resistances were not different when compared with ANG II + MMF rats receiving vehicle. However, since MAP was decreased, glomerular plasma flow, PGC, SNFR, and K_e were 26, 13, 22, and 20% lower, respectively, than in ANG II + MMF group that received vehicle. Similar effects were observed in sham rats, i.e., no effects on vascular resistance, associated with a slight fall in glomerular plasma flow, PGC, SNFR and K_e (Table 1 and Fig. 5). Candesartan treatment 1 h before death did not alter the cellular infiltration in any group (Table 2).

DISCUSSION

We previously proposed that many forms of salt-sensitive hypertension are due to subtle renal microvascular and interstitial inflammatory injury (13, 15). We have been able to demonstrate the engagement of this pathway in both genetic and experimentally induced models of hypertension (15, 23, 24–26). A key finding in these models is the presence of renal ischemia and the infiltration of immune cells that express oxidants and ANG II (5, 6, 17, 19, 21a, 22). Indeed, the blockade of the inflammatory cell infiltration with agents such as MMF (9, 21), or the inhibition of local oxidants (35), can ameliorate the salt-sensitive hypertension. While we have also proposed a role for intrarenal ANG II in this process based on

Table 2. Glomerular and tubulointerstitial inflammatory infiltration observed after 5 wk of a high-salt diet in rats with salt-sensitive hypertension, rats with concomitant MMF treatment, and normotensive sham rats, and inflammatory infiltration during acute treatment with candesartan.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glomeruli</th>
<th>Intersitum</th>
<th>Glomeruli</th>
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<tbody>
<tr>
<td>ANG II</td>
<td>0.65±0.05*</td>
<td>44.86±1.3*</td>
<td>0.30±0.11</td>
<td>30.87±1.2*</td>
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<td>ANG II + Cand</td>
<td>0.56±0.08*</td>
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<td>ANG II + MMF</td>
<td>0.37±0.6</td>
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</tr>
<tr>
<td>ANG II + MMF + Cand</td>
<td>0.33±0.09</td>
<td>22.15±1.6</td>
<td>0.19±0.7</td>
<td>19.59±2.8</td>
</tr>
<tr>
<td>Sham</td>
<td>0.28±0.07</td>
<td>19.92±1.9</td>
<td>0.25±0.95</td>
<td>22.00±0.7</td>
</tr>
<tr>
<td>Sham + Cand</td>
<td>0.27±0.12</td>
<td>25.25±1.5</td>
<td>0.28±0.12</td>
<td>22.56±0.8</td>
</tr>
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Values are means ± SE. *P < 0.05 vs. Sham.
the presence of ANG II-positive cells, a formal testing of this hypothesis has not been performed.

In this paper, we examined the role of ANG II in a model of salt-sensitive hypertension in which renal injury is induced by a short-term exposure to ANG II. Specifically, the infusion of ANG II for 2 wk results in the acute development of hypertension with renal microvascular and tubulointerstitial injury. While stopping the ANG II results in rapid resolution of the hypertension, the subsequent exposure of the rat to a HSD results in the rapid redevelopment of hypertension. The mechanism was shown to be associated with cortical vasoconstriction (9) and to result in the infiltration of inflammatory cells (9, 24). The administration of MMF was able to prevent the salt-sensitive hypertension, cortical vasoconstriction, and renal interstitial inflammatory changes (9, 22).

We demonstrate that this model is associated with an increase in renal ANG II levels. Since these animals are received a HSD that is known to suppress the systemic renin angiotensin system, the findings represent an example of how intrarenal angiotensin may be dissociated from plasma angiotensin (18). This study also provides evidence that intrarenal ANG II may have a role in maintaining cortical vasoconstriction and sodium retention. Similar findings have been shown in experimental models of volume expansion (10) and in interstitial nephritis (34).

The possibility that the ANG II was mediating the renal vasoconstriction levels was supported by the presence of a strong correlation between the level of ANG II and blood pressure (Fig. 5) and the interstitial inflammatory response. While potential stimulation of ANG II production in tubular cells by the inflammatory reaction has not been studied, one likely source of the intrarenal ANG II is the infiltrating immune cells, as previous studies have shown that many of these cells are ANG II-positive by immunostaining (21, 24). The observation that the renal ANG II levels were reduced by MMF (even though the rats received the same dose of ANG II) strongly argues against the possibility that intrarenal ANG II represents exogenous angiotensin II given nearly 6 wk earlier.

To investigate the functional role of ANG II in the salt-sensitive hypertension model, we examined the effects of acute administration of candesartan in this model. As shown in Table 1, AT₁ receptor blockade decreased blood pressure in all groups, but the magnitude of the fall was larger in the ANG II group; candesartan was able to reverse the cortical vasoconstriction in ANG II-treated rats, suggesting that this pathophysiological state is mediated by the AT₁ receptor. Similar effects were seen with Kₐ in which there was a near complete correction to that observed in controls (Fig. 5). Such a vasodilator response was not observed in the ANG II + MMF + Cand or in Sham + Cand groups, in which the lack of a vasodilatory renal response can be attributed to an increased renal sympathetic nerve activity in the afferent and efferent arterioles, induced by the fall in blood pressure (4). In this regard, since SNGFR was taken from proximal collections, the TGF was prevented (3, 29). Nevertheless, the site(s) at which ANG II interacts with glomerular function is still unclear, since AT₁ receptors are located in macula densa cells, afferent and efferent arterioles and mesangial cells (16, 20). Thus this limitation is partially balanced by the fact that it allows evaluation of glomerular hemodynamics without interference of TGF (3). Thus we can only suggest a defective autoregulatory response to the fall in blood pressure in Sham and ANG II + MMF groups, since TGF is the main component of the autoregulatory response. In addition, it should be recognized that while our results suggest that renal ANG II is responsible for the glomerular hemodynamic findings in the salt-driven hypertension that follows exogenous angiotensin administration, the potential role of incomplete suppression of circulating angiotensin levels (12) was not addressed in this investigation.

In conclusion, we demonstrated that intrarenal ANG II levels are elevated in a model of salt-sensitive hypertension and that this correlates with the presence of intrarenal inflammation and cortical vasoconstriction. Importantly, blocking the AT₁ receptor can ameliorate the renal vasoconstriction. These studies are consistent with the hypothesis that the intrarenal angiotensin system in association with interstitial immune infiltration plays a pivotal role in this model of salt-sensitive hypertension.

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