Aldosterone receptor antagonism alleviates proteinuria, but not malignant hypertension, in Cyp1a1-Ren2 transgenic rats

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Ortiz RM, Graciano ML, Mullins JJ, Mitchell KD. Aldosterone receptor antagonism alleviates proteinuria, but not malignant hypertension, in Cyp1a1-Ren2 transgenic rats. Am J Physiol Renal Physiol 293: F1584–F1591, 2007. First published August 22, 2007; doi:10.1152/ajprenal.00124.2007.—The contribution of elevated aldosterone to the pathogenesis of malignant, ANG II-dependent hypertension remains uncertain. Therefore, we examined whether chronic mineralocorticoid receptor blockade attenuates the development of malignant hypertension in transgenic (TGRs) with inductive expression of the Ren2 gene [TGR(Cyp1a1Ren2)]. Systolic blood pressure (SBP) was measured by radiotelemetry in male TGRs in three groups: 1) control (n = 9), 2) hypertensives (HT; n = 8), and 3) hypertensives + spironolactone (11 mg·kg−1·day−1 s.c.; HTS; n = 8). Malignant hypertension was induced with dietary indole-3-carbionil (0.3%) for 10 days. Metabolic measurements were taken at the beginning of the study and at days 2 and 9. HT exhibited elevated SBP (125 ± 3 vs. 187 ± 5 mmHg), plasma renin activity (5 ± 1 vs. 29 ± 10 ng ANG I·ml−1·h−1), plasma ANG II (175 ± 39 vs. 611 ± 74 fmol/ml), and plasma aldosterone (0.31 ± 0.04 vs. 5.42 ± 1.02 nmol/l). Urinary aldosterone excretion increased 5.5-fold by day 2 and an additional 90% by day 9. HT was associated with a 1.8-fold increase in proteinuria by day 9 that was alleviated by treatment with spironolactone (25 ± 5 vs. 13 ± 3 mg/day), suggesting that aldosterone contributes to the renal damage observed in malignant hypertension. Urinary Na+ excretion was decreased 76% on day 2, despite a sixfold increase in urinary aldosterone excretion. Decrease in urinary Na+ excretion on day 2 in HT suggests that Na+ reabsorption was increased in response to the increase in aldosterone; however, the lack of a change in SBP between HT and HTS suggests that mechanisms independent of aldosterone stimulation make a greater contribution to the maintenance of elevated arterial pressure in malignant hypertension in Cyp1a1-Ren2 transgenic rats.

angiotensin II; eplerenone; pressure natriuresis; spironolactone

ALDOSTERONE IS PRIMARILY RESPONSIBLE for the long-term regulation of distal nephron Na+ reabsorption and thus Na+ balance. Theoretically, chronic blockade of mineralocorticoid receptors (MR) should increase urinary Na+ excretion (UNaV) and, in turn, reduce blood pressure. In adrenalectomized rats, the specific aldosterone receptor antagonist, eplerenone, acutely (<24 h) reversed the renal actions of aldosterone in a dose-dependent manner by increasing mean urinary Na+/K+ ratio by as much as 57% (5), suggesting that blockade of MR increases UNaV. In rats on a normal Na+/K+ diet, chronic (5 wk) spironolactone treatment (20 mg/day) did not alter serum concentrations or urinary excretion of either Na+ or K+, despite an increase in plasma aldosterone by 2.5-fold (14), suggesting that alternative renal mechanisms were elicited.

In rodent models of hypertension, such as aldosterone infusion (1), Dahl salt-sensitive rats (16), and licorice induced (26), MR blockade is effective in reducing blood pressure. However, in ANG II-dependent forms of hypertension, the data are not consistent. In ANG II-infused hypertensive rats on a normal salt diet, spironolactone reduced blood pressure by 12% (35); however, eplerenone was not effective in reducing blood pressure (24). In ANG II-infused rats supplemented with 1% dietary Na+, with or without G-nitro-L-arginine methyl ester, MR antagonism had virtually no effect on reducing blood pressure (19, 27–29), suggesting that Na+ and/or G-nitro-L-arginine methyl ester impede the ability of MR blockade from ameliorating the hypertension. Thus uncertainty exists regarding the contribution of aldosterone-mediated enhancement of distal nephron salt and water reabsorption to the elevation of arterial blood pressure in ANG II-dependent forms of hypertension, in particular, ANG II-dependent malignant hypertension.

Malignant hypertension is a severe form of hypertension characterized by rapidly increasing blood pressure, pressure diuresis and natriuresis, severe renal vasoconstriction and ischemia, activation of the renin-angiotensin system (RAS), microangiopathy, and hemolytic anemia (8, 12, 15, 20–22, 25). Given the importance of the activation of the RAS to the development of malignant hypertension and in light of the evidence that ANG II potently simulates aldosterone synthesis and secretion, one could predict that ANG II-mediated enhancement of aldosterone levels contributes to the elevated arterial blood pressure and renal dysfunction in malignant hypertension. However, the contribution of elevated aldosterone levels to the increased arterial blood pressure in rats with ANG II-dependent malignant hypertension remains unclear.

Recently, a transgenic rat (TGR) line [strain name: TGR(Cyp1a1Ren2)] was created that allowed the induction of ANG II-dependent malignant hypertension (15). This TGR line was generated by inserting the mouse Ren2 renin gene, fused to an 11.5-kb fragment of the cytochrome P-450 1a1 (Cyp1al) promoter, into the genome of the Fischer 344 rat. Cyp1al, which catalyzes the oxidation of a wide range of endogenous lipophilic compounds and xenobiotics (3, 32), is not constitutively expressed, but is highly inducible on exposure to various aryl hydrocarbons, such as indole-3-carbionil (13C) (3, 13, 18, 32). Induction of Cyp1al is mediated by the aryl hydrocarbon receptor, which is a basic helix-loop-helix transcription factor
that binds to specific DNA elements in the Cyp1a1 promoter (3, 32). Rats transgenic for the Cyp1a1-Ren2 construct do not constitutively express the Ren2 renin gene. Rather, the Ren2 gene is expressed, primarily in the liver, only on induction of the Cyp1a1 promoter by aryl hydrocarbons such as I3C (15). In essence, induction of the Cyp1a1 promoter by I3C is used to drive hepatic expression of the Ren2 renin gene. In this TGR model, induction of the Cyp1a1 promoter by dietary administration of I3C results in a fixed level of expression of the Ren2 renin gene and in the development of ANG II-dependent hypertension (15, 21). Thus, this inducible TGR model allows genetic clamping of renin gene expression and thus of plasma renin levels that are not subject to the normal physiological feedback mechanisms, regulating the activity of the RAS. At a dose of 0.3% (wt/wt), dietary I3C induces malignant hypertension characterized by loss of body weight, polyuria, polydypsia, lethargy, and piloerection. This model, therefore, allows the induction of ANG II-dependent malignant hypertension using a benign and naturally occurring dietary supplement without the need for surgical intervention, dietary salt manipulation, or the administration of exogenous steroids.

The present study was performed to evaluate the contribution of elevated aldosterone levels to the development of ANG II-dependent malignant hypertension by chronically antagonizing the MR. In previous studies, it has been demonstrated that Cyp1a1-Ren2 TGRs with malignant hypertension exhibit increased kidney ANG II content and elevated plasma levels of ANG II and aldosterone (15, 21). However, little information is available regarding the prevailing organ tissue aldosterone levels or the effects of chronic MR antagonism on these levels in Cyp1a1-Ren2 rats with ANG II-dependent malignant hypertension. Accordingly, an additional objective was to determine the effects of chronic MR blockade with spironolactone on plasma and tissue levels of aldosterone in Cyp1a1-Ren2 rats following induction of malignant hypertension.

METHODS

Experimental procedures performed in this study conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Tulane University Health Sciences Center before initiation of the study. All animals used in this study were bred at Tulane University School of Medicine from stock animals supplied from the University of Edinburgh (Edinburgh, UK). The TGRs used in this study [strain name TGR(Cyp1a1Ren2)] were recently created and generated to allow for the induction of various degrees of ANG II-dependent hypertension (15, 20, 21). Details of this strain and induction of the Cyp1a1 promoter have been published (8, 12, 15, 20–22, 25).

Experimental animals and procedures. Male TGRs were randomly assigned to three experimental groups: 1) controls (n = 9) fed normal rat chow diet without I3C (TD 90229, Harlan-Teklad, Madison, WI), 2) hypertensives (HT; n = 8) fed normal rat chow diet with I3C (0.3% wt/wt; TD 00554, Harlan-Teklad), and 3) hypertensives + spironolactone (HTS; n = 8; 11 mg·kg⁻¹·d⁻¹·sc; Innovative Research of America, Sarasota, FL) fed the same diet as HT. This dosage of spironolactone is slightly higher than that previously shown to substantially displace aldosterone from its receptor and thus reduce the actions and specific binding of aldosterone in vivo (4). In addition, this dose is consistent with that used previously to successfully evaluate the effects of aldosterone in stroke-prone hypertensive rats (27). Before and during the experiment, all animals had unrestricted access to their specific food and water. Once assigned, animals were surgically implanted with radiotelemeters (PA-C40; DSI, St. Paul, MN) placed in the abdominal cavity with the catheter end inserted into the descending aorta and fixed in place with a nylon suture and surgical adhesive (24). Animals were allowed 5 days to recover from the surgery before initiation of the study. The study was initiated with 5 days of baseline, normotensive recordings before the hypertension was induced in the HT and HTS groups. On the day hypertension was induced, controls and HT were sham operated, and HTS were implanted with a spironolactone time-release pellet subcutaneously at the nape of the neck. When animals were returned to their cages, their specific diets were made available for the remainder of the study (10 days).

Blood pressure and metabolic measurements. For each individual animal, daily systolic blood pressure (SBP) values represent the mean of 24 measurements taken every hour on the hour for 10 s for the 15 days. Animals were individually housed in plastic cages placed on top of the telemetry receivers. Body mass (BM) measurements were taken daily for the 15 days, and, for the data reported as percent change from baseline, day 4 was considered baseline. On the day before initiation of the hypertension (day −1) and on days 2 and 9, animals were placed in metabolic cages designed to facilitate the collection of urine. On these 3 days, food and water consumption and urine output were recorded. After urine volume was recorded, an aliquot was collected for immediate analysis of Na⁺ and for later analysis of urinary aldosterone and total protein. Urinary excretion values were the product of concentration and urine volume. On days when telemetry animals were maintained in metabolic cages, SBP measurements were calculated from 12-h recordings.

Dissections. After BM measurements were obtained on day 10, animals were decapitated, and trunk blood was collected into chilled vials containing the following: 1) 5 mM EDTA and 2) 5 mM EDTA plus protein inhibitor cocktail (pH = 7.4, 12.5 mM 1–10-phenanthroline, 22 μM pepstatin, 10 μM PMSF, 20 μM enalaprilat). Microcapillary tubes were filled in duplicate from trunk blood to determine hematocrit following centrifugation of tubes. Plasma renin activity (PRA) was measured from the EDTA-treated sample, and plasma ANG II and aldosterone were measured from the EDTA + protein inhibitor cocktail-treated sample. The left kidney and adrenal were removed, cleaned, weighed, placed in separate glass vials containing 10 ml of methanol, and homogenized for extraction of ANG II. The right adrenal and the heart were removed, cleaned, weighed, placed in separate glass vials containing 10 ml of cold PBS, and homogenized for extraction of aldosterone. Kidney and adrenal masses are reported as paired or combined masses.

Analyses. PRA was measured using a commercially available RIA kit (DiaSorin, Stillwater, MN). Adrenal, heart, and urinary aldosterone were extracted from their homogenates with ethyl acetate, as previously described for urinary aldosterone analyses (23). Following dilution of plasma (HT and HTS samples) of 1:20 in assay buffer, plasma, adrenal, heart, and urine aldosterone concentrations were measured by a commercially available RIA kit (DPC, Los Angeles, CA). Kidney and adrenal homogenates and plasma (1.0 ml) were extracted for ANG II measurements, with details of the ANG II extraction and assay procedures described previously (2). Urine and plasma Na⁺ were measured by flame photometry (Instrumentation Laboratories, Lexington, MA; model 943). Total protein was measured by photospectrometry (BioRad, Hercules, CA).

Statistics. Means (±SE) of daily and periodic measurements were compared by analysis of variance adjusted for repeated measures over time. If significant (P < 0.05) group × time interactions were detected, one-way analysis of variance was performed to determine differences on specific days. Means for plasma and organ measurements were compared by one-way analysis of variance. For all cases, if significance (P < 0.05) was detected, Fisher’s protected least significant difference test was applied post hoc. The slopes for the decreases in BM expressed as percent change from baseline were compared by analysis of variance. Significance of correlations was
was induced (day 0), with a diet containing 0.3% indole-3-carbinol (I3C). The arrow indicates when the malignant hypertension was induced (day 0), with a diet containing 0.3% indole-3-carbinol (I3C). *Significant (P < 0.001) difference from control.

determined by simple regression. Statistics were performed using Statview software (SAS, Cary, NC).

RESULTS

Blood pressure and metabolic data. During the initial 5-day baseline period, SBP was the same in the three groups (Fig. 1). Within 24 h of induction with I3C, mean SBP increased 21% in HT and 22% in HTS (Fig. 1). Mean SBP reached a plateau within 24 h of induction with I3C, mean SBP increased 21% in HT and 22% in HTS (Fig. 1). Mean SBP reached a plateau within 24 h of induction with I3C, mean SBP increased 21% in HT and 22% in HTS (Fig. 1). Within 24 h of induction of hypertension, mean BM decreased 3% in HT and 2% in HTS (Fig. 2). By the end of the study, mean BM had only increased (P < 0.005) 9% in controls, but had decreased (P < 0.001) 22% in HT and 24% in HTS (Fig. 2). The reductions in mean BM (% change) were linear and defined by the following equations: $y = -2.18x + 2.66$ ($R = 0.9969; P < 0.0001$), and $y = -2.15x + 3.15$ ($R = 0.9977; P < 0.0001$) (Fig. 2). The slopes of the two equations were the same ($P > 0.10$). Mean food consumption did not differ with time or among the groups (Table 1). Mean water consumption had nearly doubled by day 2 and remained elevated on day 9 in both hypertensive groups (Table 1). Mean urine output paralleled the changes in water consumption (Table 1).

Organ masses. Absolute mean heart mass was not different among the three groups; however, relative heart mass increased (P < 0.01) 21% in both hypertensive groups compared with controls (Table 2). Absolute mean paired-kidney mass was 21% less (P < 0.05) in HT and 24% less (P < 0.05) in HTS compared with controls; however, the 14% increase in relative mean paired kidney mass observed in the hypertensive groups was significant (P < 0.05) (Table 2). Absolute mean paired adrenal mass was not different among the three groups; however, relative mean paired adrenal mass was increased (P < 0.01) 59% in both hypertensive groups (Table 2). No spironolactone treatment effects were observed among any of the organ masses.

Plasma analyses and hematocrit. Induction of the Ren2 gene with I3C resulted in over a fivefold increase in mean PRA in both hypertensive groups (Fig. 3A). Thusly, concomitant increases in mean ANG II (threefold) and aldosterone (18-fold) were observed in the hypertensive groups (Fig. 3B and C). Spironolactone treatment did not alter the concentrations of any of these hormones. Plasma Na* was not altered by hypertension (160 ± 4 vs. 159 ± 9 mM) or treatment of spironolactone (151 ± 4 mM). Hypertension increased (P < 0.05) Hct (43 ± 1 vs. 49 ± 1%), but it was not further altered by treatment with spironolactone (49 ± 1%).

Intraorgan hormone content. Mean intra-adrenal and intrarenal ANG II were increased three- and fourfold, respectively, with hypertension (P < 0.01) (Fig. 4, A and B, respectively). Mean intra-adrenal and intracardiac aldosterone were increased three- and twofold, respectively, with hypertension (P < 0.05) (Fig. 5, A and B, respectively). Treatment with spironolactone

Table 1. Body mass, food and water consumption, and urine output in control, hypertensive, and hypertensive + spironolactone-treated transgenic rats

<table>
<thead>
<tr>
<th></th>
<th>Day − 1</th>
<th>Day 2</th>
<th>Day 9</th>
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<tbody>
<tr>
<td>Body mass, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>286±9</td>
<td>291±8</td>
<td>305±8</td>
</tr>
<tr>
<td>HT</td>
<td>306±15</td>
<td>290±13</td>
<td>244±12*</td>
</tr>
<tr>
<td>HTS</td>
<td>299±12</td>
<td>286±11</td>
<td>240±11*</td>
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<tr>
<td>Food consumption, g/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14±3</td>
<td>20±1</td>
<td>17±1</td>
</tr>
<tr>
<td>HT</td>
<td>15±2</td>
<td>12±1</td>
<td>14±2</td>
</tr>
<tr>
<td>HTS</td>
<td>15±3</td>
<td>13±2</td>
<td>13±1</td>
</tr>
<tr>
<td>Water consumption, ml/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>18±3</td>
<td>25±2</td>
<td>24±2</td>
</tr>
<tr>
<td>HT</td>
<td>16±2</td>
<td>42±8*</td>
<td>56±10*</td>
</tr>
<tr>
<td>HTS</td>
<td>20±2</td>
<td>47±6*</td>
<td>47±11*</td>
</tr>
<tr>
<td>Urine output, ml/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9±1</td>
<td>10±1</td>
<td>10±1</td>
</tr>
<tr>
<td>HT</td>
<td>9±2</td>
<td>31±5*</td>
<td>46±6*</td>
</tr>
<tr>
<td>HTS</td>
<td>9±2</td>
<td>36±5*</td>
<td>35±8*</td>
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</table>

Values are means ± SE for control (n = 9), hypertensive (HT; n = 8), and hypertensive + spironolactone-treated (HTS; n = 8) transgenic rats. Hypertension was induced on day 0 with a diet containing 0.3% indole-3-carbinol. *Significant (P < 0.05) difference from control.
did not further alter any of the intraorgan hormonal contents (Figs. 4 and 5). Plasma aldosterone and intracardiac aldosterone content were significantly and positively correlated (intracardiac aldosterone content / plasma aldosterone; $R = 0.585; P < 0.005$).

**Table 2.** Body and organ masses following 10 days in control, hypertensive, and hypertensive + spironolactone-treated transgenic rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypertensive</th>
<th>Hypertensive + Spironolactone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body mass, g</strong></td>
<td>305±8</td>
<td>237±15*</td>
<td>233±15*</td>
</tr>
<tr>
<td><strong>Absolute organ masses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart, g</td>
<td>0.85±0.03</td>
<td>0.81±0.05</td>
<td>0.80±0.05</td>
</tr>
<tr>
<td>Kidneys, g</td>
<td>2.26±0.06</td>
<td>1.79±0.17*</td>
<td>1.70±0.04*</td>
</tr>
<tr>
<td>Adrenals, mg</td>
<td>49±1</td>
<td>58±2</td>
<td>59±3</td>
</tr>
<tr>
<td><strong>Relative organ masses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart, g/100 g BM</td>
<td>0.28±0.00</td>
<td>0.34±0.00*</td>
<td>0.34±0.01*</td>
</tr>
<tr>
<td>Kidneys, g/100 g BM</td>
<td>0.74±0.01</td>
<td>0.84±0.01*</td>
<td>0.83±0.01*</td>
</tr>
<tr>
<td>Adrenals, mg/100 g BM</td>
<td>16.0±0.4</td>
<td>25.5±1.3*</td>
<td>25.6±1.6*</td>
</tr>
</tbody>
</table>

Values are means ± SE for control ($n = 9$), HT ($n = 8$), and HTS ($n = 8$) transgenic rats. Hypertension was induced on day 0 with a diet containing 0.3% indole-3-carbinol. BM, body mass. *Significant ($P < 0.05$) difference from control.

**Urinary aldosterone, $Na^+$, and total protein excretion.** Induction of malignant hypertension increased ($P < 0.001$) urinary aldosterone excretion ($U_{aldo\, V}$) sixfold by day 2, which in turn was nearly doubled ($P < 0.01$) by day 9. Spironolactone did not induce any further changes (Fig. 6). $U_{aldo\, V}$ on day 9 was significantly and positively correlated with plasma aldosterone.

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**Fig. 3.** Mean (±SE) plasma renin activity (A), plasma ANG II (B), and plasma aldosterone (C) concentrations in control ($n = 9$), HT ($n = 8$), and HTS ($n = 8$) TGRs following 10 days of malignant hypertension. The hypertension was induced on day 0 with a diet containing 0.3% I3C. *Significant ($P < 0.01$) difference from control.

**Fig. 4.** Mean (±SE) intra-adrenal ANG II (A) and intrarenal ANG II (B) content in control ($n = 9$), HT ($n = 8$), and HTS ($n = 8$) TGRs following 10 days of malignant hypertension. The hypertension was induced on day 0 with a diet containing 0.3% I3C. *Significant ($P < 0.05$) difference from control.
Sterone taken on day 10 (U_{aldosterone} = 40.6 + 0.02 * plasma aldosterone; R = 0.808, P < 0.0001), indicating U_{aldosterone} is a reliable index for evaluating changes in circulating aldosterone concentrations. U_{Na}V was not different over time in control.

Induction of hypertension decreased U_{Na}V in HT on day 2; however, spironolactone blocked this decrease in U_{Na}V (Fig. 7). By day 9, U_{Na}V was the same between HT and HTS, and 35% greater (P < 0.05) than control (Fig. 7). Induction of hypertension did not increase proteinuria by day 2, but urinary total protein excretion was increased nearly twofold by day 9 in HT (Fig. 8). Chronic treatment with spironolactone completely alleviated the proteinuria (Fig. 8).

**DISCUSSION**

Because aldosterone stimulates the reabsorption of Na⁺ via activation of its receptor, chronic blockade of the MR would be expected to increase U_{Na}V and thus abrogate the elevation of blood pressure in hypertensive states induced by increased activity of the renin-angiotensin-aldosterone system. However, the contribution of aldosterone to the manifestation of increased arterial pressure in ANG II-dependent malignant hypertension remains unexamined. The present study demonstrates that aldosterone does not contribute substantively to the maintenance of the elevated arterial blood pressure in Cyp1a1-Ren2 rats with malignant hypertension, but aldosterone does...
contribute significantly to the proteinuria associated with the malignant hypertension.

In the present study, $U_{Na}V$ on day 2 of I3C induction was reduced in the untreated hypertensive rats by 64 and 76% compared with controls and the spironolactone-treated hypertensives, respectively. This reduction in $U_{Na}V$ was associated with a 5.5-fold increase in $U_{aldoV}$, indicating that increased aldosterone stimulated an increase in renal Na$^+$ reabsorption, resulting in a decrease in $U_{Na}V$. Despite elevated $U_{Na}V$ induced by spironolactone treatment on day 2 in HTS, SBP was not different between the two hypertensive groups, suggesting that this increase in $U_{Na}V$ was not sufficient to alleviate the manifestation of the malignant hypertension in Cyp1a1-Ren2 rats. Thus the present findings are consistent with a number of studies that have demonstrated that increased aldosterone levels do not substantially contribute to the development of ANG II-dependent forms of hypertension (19, 24, 28, 29). By day 9, $U_{Na}V$ rates in the spironolactone-treated hypertensive rats were not different from those in the untreated hypertensive rats, suggesting that the pressure natriuretic effect of the marked increase in arterial blood pressure had overcome the sodium-retaining ability of aldosterone (9, 10). Despite the near doubling in mean $U_{aldoV}$ from day 2 to day 9, $U_{Na}V$ was unaltered, suggesting that the pressure natriuresis was greater than the Na$^+$ retention effects of aldosterone and that renal compensatory mechanisms were quickly invoked to correct for the blockade of MR at the expense of elevated SBP (9, 10, 34). Thus aldosterone does not appear to contribute significantly to the development and maintenance of ANG II-dependent malignant hypertension in Cyp1a1-Ren2 rats.

The parallel increases in PRA, plasma ANG II, and plasma aldosterone are consistent with those reported for induction of the Ren2 gene with I3C in Cyp1a1-Ren2 rats (15, 20), demonstrating marked activation of the RAS. The significant and positive correlation between plasma aldosterone and $U_{aldoV}$ suggests that $U_{aldoV}$ reflects the gross changes in circulating aldosterone concentration. Thus the increase in $U_{aldoV}$ on day 2 in both hypertensive groups suggests that RAS is activated quickly in this model, and the fact that $U_{aldoV}$ is nearly doubled by day 9 further suggests that RAS progressively increases throughout the study. The increase in intra-adrenal aldosterone content is consistent with the observed increases in plasma and urinary aldosterone and is likely attributed to the increase in intra-adrenal ANG II. The increase in intrarenal ANG II is consistent with that observed previously in this model (21). The present study extends these findings to demonstrate that this increase in intraorgan ANG II is not solely limited to the kidney, but includes the adrenals as well, contributing to the increase in circulating aldosterone. While treatment with an angiotensin receptor blocker decreased intrarenal ANG II in Cyp1a1-Ren2 rats with malignant hypertension (21), blockade with an MR antagonist did not alter either intrarenal or intra-adrenal ANG II, suggesting that the augmentation of intraorgan ANG II is mediated by the ANG II receptor (37–39) and not influenced by MR activation in this model. However, it has been shown that MR blockade is associated with increases in plasma aldosterone (11, 14, 24, 29) and with exacerbation of intrarenal and intra-adrenal ANG II (24). These discrepancies could be attributed to differences in experimental models (high vs. low renin), the duration of the hypertension (10 vs. 28 days), and/or the dosage of the antagonists used (i.e., 35).

It is well known that aldosterone contributes to the pathogenesis of cardiovascular disease, but what remains to be clearly identified is whether plasma aldosterone concentrations accurately reflect intracardiac aldosterone content and whether circulating aldosterone contributes to intracardiac content in hypertensive conditions. The existence of an intracardiac steroidogenic mechanism responsible for cardiac actions of both aldosterone and corticosterone has been reported (30, 31, 33, 36). In contrast, other data suggest that intracardiac production of aldosterone is minimal at best and that a majority of aldosterone in the heart is derived from plasma (Ref. 7; see Ref. 6 for editorial). Nonetheless, few studies have examined intracardiac aldosterone content in hypertensive conditions (17), much less the effects of MR blockade on intracardiac aldosterone levels in malignant hypertension. The present study demonstrates that induction of malignant hypertension is associated with over a twofold increase in intracardiac aldosterone levels, similar to that observed in spontaneously hypertensive rats (17). However, where spironolactone reduced intracardiac aldosterone by 30% after 4 mo of treatment in spontaneously hypertensive rats (17), spironolactone did not alter heart aldosterone content in the present study, suggesting that treatment time and hypertensive condition may influence intracardiac aldosterone content. In addition, the positive correlation between plasma aldosterone and intracardiac aldosterone in the present study suggests that the increase in plasma aldosterone associated with this model of hypertension predicts an increase in intracardiac aldosterone levels; however, it does not accurately reflect the magnitude of the change. Moreover, the present data do not allow assessment of the degree to which uptake of circulating aldosterone and/or increased cardiac generation of aldosterone contributed to the elevated cardiac aldosterone levels observed in the hypertensive rats. Further studies are required to address this issue.

As previously observed, induction of malignant hypertension with high-dose I3C in Cyp1a1-Ren2 rats was associated with a marked decrease in BM (15, 20–22, 25). Although the differences in mean food intake between the noninduced controls and the hypertensive groups were not significant, these differences could not account for the nearly 24% decrease in BM observed over the 10 days. The present study reveals that the loss of BM observed in the malignant hypertensive Cyp1a1-Ren2 rats is not attributed to a reduction in food intake, suggesting that increased ANG II contributed, either directly or indirectly, to increased catabolism of fat and/or lean tissue. If the increase in hematocrit reflects a decrease in plasma volume, then a reduction in total body water pool size may partly contribute to the decrease in BM; however, the polydipsia can almost be completely accounted for by the polydipsia, suggesting that the animals are not losing additional mass as urinary water.

In conclusion, the positive correlation between plasma aldosterone and $U_{aldoV}$ suggests that $U_{aldoV}$ reflects the gross changes in circulating plasma aldosterone concentrations in Cyp1a1-Ren2 rats. Therefore, the increase in $U_{aldoV}$ observed on day 2 in untreated hypertensive rats indicates that circulating aldosterone was increased and was responsible for the concomitant decrease in $U_{Na}V$ (increased reabsorption). Conversely, treatment with an MR antagonist inhibited the increase in renal Na$^+$ reabsorption, despite the increase in $U_{aldoV}$. At most, the contribution of aldosterone to the hypertension in this
model is acute and transient and does not significantly contribute to the maintenance of the malignant hypertension. Blockade of MR was sufficient to prevent the proteinuria commonly associated with hypertension, suggesting that aldosterone does contribute to renal damage in Cyp1a1-Ren2 hypertensive rats. While angiotensin receptor blockers have been reported to reduce the intrarenal ANG II content in malignant hypertensive Cyp1a1-Ren2 rats (21), MR antagonism had no effect on intrarenal or intradrenal ANG II content, suggesting that the intragranal augmentation of ANG II is dependent on the ANG II receptor and not the MR in this model. Induction of malignant hypertension in Cyp1a1-Ren2 rats is associated with increased intracardiac aldosterone that likely contributes to the observed relative cardiac hypertrophy. The present findings indicate that MR activation by elevated circulating aldosterone levels does not contribute markedly to the increased arterial pressure following induction of ANG II-dependent malignant hypertension in Cyp1a1-Ren2 TGRs. Rather, the data are consistent with our laboratory’s previous findings (21) that AT1-receptor activation by elevated plasma and intrarenal ANG II levels primarily mediate the development and maintenance of malignant hypertension in Cyp1a1-Ren2 TGRs.

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