Aldosterone receptor antagonism exacerbates intrarenal angiotensin II augmentation in ANG II-dependent hypertension

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Ortiz RM, Graciano ML, Seth D, Awayda MS, Navar LG. Aldosterone receptor antagonist exacerbates intrarenal angiotensin II augmentation in ANG II-dependent hypertension. Am J Physiol Renal Physiol 293: F139–F147, 2007. First published March 20, 2007; doi:10.1152/ajprenal.00504.2006.—Effects of aldosterone receptor (AR) blockade with eplerenone (epl) on renal Na+ excretion, arterial blood pressure, intra-adrenal and renal ANG II, and plasma aldosterone levels during ANG II-dependent hypertension were evaluated. Rats from one cohort (n = 10/group) 1 control, 2) control + epl (25 mg/day), 3) ANG II (60 ng/min), and 4) ANG II + epl were maintained in metabolic cages for 28 days for daily urine collections. Systolic blood pressure (SBP) was measured weekly by tail-cuff. In a second cohort (n = 12/group), daily SBP was measured by telemetry that epl delayed the onset of the increase in SBP by 2 days and slightly reduced SBP (186 ± 6 vs. 177 ± 8 mmHg). Epl transiently increased Na+ excretion within 24 h of treatment in both normo- and hypertensive rats; however, balance was reestablished within 5 days suggesting that alternative mechanisms for conserving Na+ are activated. Cortical α-epithelial Na+ channel content (α-ENaC) was not altered after 21 days of epl treatment. Epl exacerbated the ANG II-mediated increases in intrarenal ANG II (226 ± 16 vs. 365 ± 38 pmol/g) and further increased intra-adrenal ANG II (3.9 ± 0.3 vs. 8.2 ± 0.9 pmol/mg) and aldosterone (255 ± 55 vs. 710 ± 87 pmol/mg) content. Exacerbation of intrarenal ANG II levels likely contributes to the maintenance of α-ENaC protein content and thus Na+ reabsorption, which helps explain the ineffectiveness of AR blockade in reducing SBP in ANG II-infused models of hypertension.

epleronen, mineralocorticoids; sodium; spironolactone

ALDOSTERONE IS A MAJOR HORMONAL regulator of long-term renal Na+ handling and Na+ balance especially under conditions where the renin-angiotensin aldosterone system is activated. Inappropriately elevated circulating aldosterone concentrations may induce a number of pathophysiological consequences including sodium retention, hypertension, and fibrosis. Mineralocorticoid receptor (MR) antagonism has been shown to ameliorate cardiovascular and renal injury in several hypertensive models (6, 7, 19, 26). Chronic MR antagonism should in theory increase urinary Na+ excretion and thus reduce blood pressure; however, in previous studies chronic treatment with MR antagonists did not acutely (23) nor chronically (10, 21, 23) alter urinary Na+ excretion suggesting that alternative renal mechanisms are invoked to compensate for the chronic blockade of the MR.

In adrenalectomized rats, the specific aldosterone receptor (AR) antagonist, eplerenone (epl), 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% (6). However, in rats on a normal Na+-K+ diet, chronic (5 wk) MR antagonism with spironolactone (20 mg/day) did not alter serum concentrations nor urinary excretion of either Na+ or K+ despite an increase in plasma aldosterone by 2.5-fold (10) indicating that alternative mechanisms may be elicited to regulate electrolyte balance during conditions of chronic MR blockade. Furthermore, in ANG II-infused rats supplemented with 1% NaCl, chronic epl treatment (3 wk) had no effect on urinary electrolyte excretion despite increasing mean serum K+ by 19% (23). In this latter case, however, the use of Na+ supplementation (1% NaCl) confounds the ability to properly evaluate the effects of chronic AR antagonism during eunatremic conditions. The effects of chronic aldosterone receptor blockade during ANG II-dependent hypertension in the absence of sodium loading have not been clearly established.

In addition, the effects of chronic MR blockade on blood pressure are incongruent. In rodent models of hypertension such as aldosterone infusion (3), Dahl salt-sensitive rats (11), and liquorice-induced (20), epl was effective in reducing systolic blood pressure (SBP). However, in models that include ANG II infusion in the presence of 1% Na+ supplementation with or without Nω-nitro-l-arginine methyl ester (l-NAME), epl treatment had minor or no effects on reducing blood pressure (13, 22, 23) suggesting that ANG II in the presence of high Na+ and/or l-NAME impedes the ability of MR blockade to ameliorate the hypertension.

Therefore, the present study was conducted to evaluate further the effects of specific AR blockade on renal handling of electrolytes, blood pressure, and intra-adrenal and renal ANG II levels during chronic ANG II infusion, independent of Na+ supplementation. Because reports on the effects of epl treatment on renal Na+ handling are scarce, and the few reports in rat models are confounded by the supplementation of NaCl, detailed studies on renal Na+ excretion were performed. Our initial hypothesis was that chronic AR antagonism during ANG II-dependent hypertension would increase Na+ excretion and partially ameliorate the ANG II-induced hypertension. However, when it became clear that the effects of AR blockade on blood pressure and urinary Na+ excretion were relatively...
small, we then considered the hypothesis that further augmentation of intrarenal ANG II levels might occur during ANG II-dependent hypertension with treatment with a MR antagonist that might counteract the effects of blocking ARs.

METHODS

All procedures were approved by Tulane University’s Institutional Animal Care and Use Committee.

Animals and study procedures. One cohort of male Sprague-Dawley rats (200–225 g; Charles River, Wilmington, MA) was maintained in metabolic cages to facilitate the daily collection of urine for electrolyte analysis and their blood pressures were measured weekly by tail-cuff plethysmography. A second cohort of rats had biotelemetry devices implanted for continuous monitoring of blood pressure with only weekly measurements of metabolic parameters. The use of biotelemetry in a second cohort of animals allowed us to develop a more refined blood pressure curve, which included evaluation of earlier time points. Rats in the first group were randomly distributed among four groups (n = 10/group): 1) control, 2) control + epl, 3) ANG II, and 4) ANG II + epl. Mean ± SE body mass did not differ among the four groups (269 ± 2 g). At the onset, animals were either sham operated (controls) or implanted with osmotic minipumps (Durect, Cupertino, CA; model 2004) containing ANG II (Phoenix Pharmaceuticals, Belmont, CA) infusing 60 ng/min for 28 days. Following these procedures, animals were placed in metabolic cages designed to facilitate the collection of daily urine voids. Animals were allowed to acclimate to the cages for 3 days before the initiation of data collection. Blood pressure measurements were taken weekly on days 6, 13, 20, and 27 by tail-cuff plethysmography.

The second cohort of rats were distributed among three groups (n = 12/group): 1) control, 2) ANG II, and 3) ANG II + epl. Body mass of animals used for the telemetry implantation procedure was ~50 g greater (320 ± 5 g) at the onset of the study (when ANG II-filled mini-pumps were implanted) to facilitate the surgical implantation of the telemeter catheter. Once assigned, radiotelemetry devices (6 active/group, 6 nonactive/group) were surgically implanted. Thus direct blood pressure data were only obtained from six animals in each group. Following the surgical procedures, rats were allowed 5 days to recover before initiating the study. For each individual animal, daily SBP values represent the mean of 24 measurements taken every hour on the hour for 10 min for 28 days. Osmotic minipumps were implanted 5 days after recovery from the implantation of the telemeters. Following these procedures, animals were individually housed in plastic cages placed on top of the telemetry receivers.

For all animals, the diet (Purina rodent chow no. 5002) of the epl-treated animals was switched to a feed (Purina rodent chow no. 5002) containing 0.1% (100 mg kg⁻¹ day⁻¹) epl (Pfizer, St. Louis, MO) on day 8. While specific studies were not performed to confirm the completeness of the MR blockade by epl, this dosage has been determined to result in optimal pharmacokinetic characteristics for effective in vivo inhibition of MR in the rat (3). In addition, this is a commonly used dosage of epl in studies with rats (3, 9, 11, 14, 20–24). All other animals were maintained on the same rodent chow (no. 5002) but without the added epl throughout the study. Body mass and food consumption were taken daily for 28 days in the first cohort to obtain comprehensive metabolic data, while these measurements were only taken weekly in the second cohort to confirm equivalent dosing of drug. Urine output was recorded daily for the first 16 days and then intermittently on days 19, 22, 25, and 28 in the first cohort and weekly in the other. After urine volume was recorded, an aliquot was collected for immediate analysis of Na⁺ and K⁺, and for later analysis of urinary aldosterone. On days when telemetry animals were maintained in metabolic cages, SBP measurements were calculated from 12-h recordings.
RESULTS

Blood pressure. ANG II increased SBP in both ANG II-infused groups by day 6 when measured by tail-cuff plethysmography (Fig. 1A). A further increase was observed by day 13, and blood pressures remained elevated above control (136 ± 5 vs. 207 ± 8 mmHg) levels for the remainder of the study. No sustained significant effect of epl on pressure was observed in either normotensive (126 ± 5 mmHg) or hypertensive (215 ± 5 mmHg) rats after 27 days (Fig. 1A). In the telemetry cohort, no differences were detected during the initial preepiranolone treatment phase. Following treatment, ANG II induced a significant elevation in SBP on day 8, reaching a plateau on day 15 (Fig. 1B). Epl treatment delayed the onset of the increase in SBP by 2 days, achieving a final value of 177 ± 8 mmHg, which was slightly but significantly lower than that measured in the ANG II-infused group (186 ± 6 mmHg, P < 0.05). During the course of the epl treatment, SBP in the ANG II + Epl group was 7.4 ± 0.8% lower than the ANG II group (P < 0.05).

Body mass and food and water consumption. Infusion of ANG II induced a decrease in body mass of 10% (406 ± 10 vs. 365 ± 13 g; nonimplanted cohort; Table 1) and of 14% (448 ± 14 vs. 385 ± 11 g; telemetry implanted cohort). Treatment with epl did not affect body mass in either cohort (405 ± 10 vs. 363 ± 7 g; nonimplanted cohort and 407 ± 21 g; telemetry implanted cohort). No differences in mass-specific food consumption were detected among the different groups of animals (Table 1). Treatment with epl did not affect food consumption in either normotensive or hypertensive animals (Table 1). Water consumption increased 83% (41 ± 2 vs. 75 ± 3 ml/day) with ANG II infusion but was not further altered with epl treatment (75 ± 5 ml/day; Table 1).

Intraorgan ANG II and aldosterone. As previously reported (32–34), ANG II infusion was associated with a twofold increase (104 ± 8 vs. 226 ± 16 fmol/g) in intrarenal ANG II content (Fig. 2A). With epl treatment, mean intrarenal ANG II content increased nearly 3.5-fold or an additional 62% (365 ± 38 fmol/g) vs. the ANG II-treated group (Fig. 2A). Similar to intrarenal content, ANG II infusion induced a 70% increase (2.3 ± 0.2 vs. 3.9 ± 0.3 fmol/mg) in mean intra-adrenal ANG II content that was exacerbated an additional twofold (8.2 ± 0.9 fmol/mg) with epl treatment (Fig. 2B). Mean intrarenal (120 ± 19 fmol/g) and intra-adrenal (2.3 ± 0.2 fmol/mg) ANG II were not affected by epl treatment in normotensive conditions (Fig. 2, A and B). Under normotensive conditions, epl treatment increased mean intra-adrenal aldosterone 72% (83 ± 13 vs. 143 ± 11 pmol/mg; Fig. 3). Mean intra-adrenal aldosterone increased 3-fold (255 ± 55 pmol/mg) above control following infusion of ANG II and increased an additional 2.8-fold (710 ± 87 pmol/mg) with epl treatment in hypertensive rats (Fig. 3).

Serum Na⁺, plasma hormones, and hematocrit. Significant changes in mean serum Na⁺ concentrations were not observed among the different groups (Table 2). Infusion of ANG II in both untreated and epl-treated groups suppressed plasma renin

Table 1. Mean metabolic data and urinary electrolyte excretion values during 28 days of ANG II-induced hypertension and 21 days of Epl treatment

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control + Epl</th>
<th>ANG II</th>
<th>ANG II + Epl</th>
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<td>Body mass, g</td>
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<td>Day 4</td>
<td>273 ± 3</td>
<td>273 ± 3</td>
<td>265 ± 4</td>
<td>264 ± 5</td>
</tr>
<tr>
<td>Day 8</td>
<td>304 ± 4*</td>
<td>301 ± 4*</td>
<td>298 ± 6*</td>
<td>295 ± 4*</td>
</tr>
<tr>
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<td>26 ± 1</td>
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<td>Water intake, ml/day</td>
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<td></td>
<td></td>
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<td>39 ± 1</td>
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<td>44 ± 4†</td>
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<td>39 ± 2</td>
<td>42 ± 2</td>
<td>57 ± 6*†</td>
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<td>19 ± 2</td>
<td>22 ± 4</td>
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<td>20 ± 2</td>
<td>21 ± 3</td>
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<tr>
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<td>19 ± 2</td>
<td>56 ± 4*†</td>
<td>55 ± 4*†</td>
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<td>UnO₂, mmol/day</td>
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<td>Day 4</td>
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<td>Day 8</td>
<td>2.5 ± 0.1</td>
<td>3.2 ± 0.1†</td>
<td>2.5 ± 0.1</td>
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</tr>
<tr>
<td>Day 28</td>
<td>2.2 ± 0.1</td>
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<td>2.6 ± 0.1</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Ue, mmol/day</td>
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<td></td>
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<td>Day 8</td>
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<td>4.8 ± 0.2</td>
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</tr>
<tr>
<td>Day 28</td>
<td>4.8 ± 0.2</td>
<td>5.0 ± 0.1</td>
<td>4.8 ± 0.3</td>
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Values are means ± SE of metabolic data and urinary electrolyte excretion values during 28 days of ANG II-induced hypertension (ANG II) and 21 days of eplerenone (Epl) treatment. ANG II infusion (60 ng/min) began on day 1 and Epl treatment (25 mg/day) began on day 8. *Significant (P < 0.05) time effect within a group. †Significant (P < 0.05) difference from controls. BM, body mass.
activity (PRA) to almost undetectable levels (Table 2). In normotensive rats, epl treatment did not significantly increase mean PRA (Table 2). Consistent with the virtually complete suppression of PRA, significant differences in plasma ANG II were not detected among the different groups (Table 2). The changes in mean plasma aldosterone paralleled those observed for intra-adrenal aldosterone. Epl treatment increased mean plasma aldosterone 6-fold in normotensive rats and 3.5-fold in hypertensive rats over the already elevated ANG II-induced levels (Table 2). ANG II infusion induced a ninefold increase in mean plasma aldosterone (Table 2). ANG II infusion induced a significant increase in mean hematocrit; however, epl treatment did not elicit any further effects (Table 2).

Urine output, electrolyte and aldosterone excretion, and renal Na+/H+ balance. During ANG II infusion, urine output was increased 2.7-fold (21 ± 2 vs. 56 ± 4 ml/day; Table 1) but was not altered by epl treatment in normotensive (19 ± 2 ml/day) or hypertensive (55 ± 4 ml/day) animals. By day 4 of ANG II infusion, mean urinary aldosterone excretion was elevated [22 ± 4 (control) and 24 ± 3 (control + epl) pmol/day] in both ANG II-infused groups [70 ± 18 (ANG II) and 60 ± 14 (ANG II + epl) pmol/day] in the nonimplanted cohort before epl treatment (Fig. 4A). Within 24 h (day 8) of treatment with epl, increases in mean urinary aldosterone excretion were detected in both normo- (17 ± 4 vs. 79 ± 7 pmol/day) and hypertensive (88 ± 35 vs. 177 ± 16 pmol/day) rats and remained elevated throughout the study (Fig. 4A). Consistent with the increases in intra-adrenal and plasma aldosterone content, urinary excretion of aldosterone was also increased with epl treatment in both normo- (33 ± 8 vs. 139 ± 13 pmol/day) and hypertensive (368 ± 59 vs. 511 ± 68 pmol/day) animals in the telemetry implanted cohort (Fig. 4B). Examination of the time course of the response to epl indicated significant increases in urinary Na+ excretion 24 h after initiating epl treatment in both control (P < 0.001) and

![Fig. 1. A: mean (±SE) systolic blood pressure measurements taken by tail-cuff plethysmography on days 6, 13, 20, and 27 of the 28-day study in control-, control + eplerenone (epl)-, ANG II-, and ANG II + epl-treated animals (n = 10/group). B: means (±SE) systolic blood pressure measurements taken by telemetry in control-, ANG II-, and ANG II + epl-treated animals (n = 12/group). Chronic infusion of ANG II (60 ng/min) began on day 1 and epl treatment (25 mg/day) began on day 8 (indicated by the arrow). *Significant (P < 0.05) difference from control. †Significant (P < 0.05) epl effect.](https://www.ajprenal.org/content/293/1/F142)

![Fig. 2. Mean (±SE) intrarenal (A) and intra-adrenal (B) ANG II content in control-, control + epl-, ANG II-, and ANG II + epl-treated animals (n = 22/group; n = 10 in control + epl) following 28 days. Chronic infusion of ANG II (60 ng/min) began on day 1 and epl treatment (25 mg/day) began on day 8. *Significant (P < 0.05) difference from control. †Significant (P < 0.05) epl effect.](https://www.ajprenal.org/content/293/1/F143)
ANG II (P < 0.01) groups (Fig. 5, A and B). These increases in urinary Na\(^+\) excretion were associated with decreases in renal Na\(^+\) balance in both control (P < 0.001) and ANG II (P < 0.05) groups (Fig. 6, A and B). These changes returned to baseline within the next collection period (another 24 h). Urinary K\(^+\) excretion was neither acutely nor chronically altered by either ANG II infusion (4.8 ± 0.2 vs. 4.8 ± 0.3 mmol/day) or epl treatment (5.0 ± 0.1 vs. 4.8 ± 0.2 mmol/day).

Renal cortical α-ENaC. After 28 days of ANG II infusion and 21 days of epl treatment, significant changes in mean cortical α-ENaC content were not detected (Fig. 7).

DISCUSSION

Aldosterone is considered a major hormonal regulator of renal Na\(^+\) handling, and therefore chronic blockade of the aldosterone receptor would be expected to increase urinary Na\(^+\) excretion, leading to negative Na\(^+\) balance and amelioration of the ANG II-induced hypertension. To compensate for the chronically elevated urinary excretion of Na\(^+\), dietary Na\(^+\) intake would have to increase or alternative renal mechanisms would have to be activated. The findings of the present study demonstrate that chronic blockade of aldosterone receptors is not associated with sustained elevations in Na\(^+\) excretion or increased food intake suggesting that alternative renal mechanisms must have been induced to prevent sustained increases in urinary Na\(^+\) excretion. Most importantly, chronic AR antagonism caused further augmentation of intrarenal ANG II levels during ANG II-dependent hypertension that likely contributed to the maintenance of renal Na\(^+\) reabsorption, resulting in only a transient phase of increased urinary excretion and the maintenance of elevated arterial blood pressure. Furthermore, epl

Table 2. Mean serum Na\(^+\), PRA, ANG II, aldosterone, and hematocrit following 28 days of ANG II-induced hypertension and 21 days of Epl treatment

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control + Epl</th>
<th>ANG II</th>
<th>ANG II + Epl</th>
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<tr>
<td>Na(^+), mM</td>
<td>154±1.4</td>
<td>153±2.3</td>
<td>153±2.3</td>
<td>150±2.6</td>
</tr>
<tr>
<td>PRA, nmol ANG I·1(^{-1})·h(^{-1})</td>
<td>3.3±0.7</td>
<td>4.5±0.7</td>
<td>0.2±0.1*</td>
<td>0.1±0.0*</td>
</tr>
<tr>
<td>ANG II, fmol/ml</td>
<td>55±4</td>
<td>55±6</td>
<td>51±4</td>
<td>53±3</td>
</tr>
<tr>
<td>Aldosterone, nmol/l</td>
<td>0.4±0.5</td>
<td>2.1±0.4†</td>
<td>3.0±0.5*</td>
<td>10.7±1.8‡</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>44±0.6</td>
<td>42±0.4</td>
<td>47±0.8*</td>
<td>48±0.7*</td>
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</tbody>
</table>

Values are means ± SE of serum Na\(^+\), plasma renin activity (PRA), ANG II, aldosterone, and hematocrit following 28 days of ANG II-induced hypertension and 21 days of Epl treatment. ANG II infusion (60 ng/min) began on day 1 and Epl treatment (25 mg/day) began on day 8. *Significant difference (P < 0.05) from control. †Significant (P < 0.05) Epl effect.
treatment elicited further increases in intra-adrenal ANG II and aldosterone, resulting in elevated plasma aldosterone, which could partially overcome the receptor blockade or mediate its effects via the unblocked glucocorticoid receptor (8), contributing to the sustained elevation in SBP during ANG II-dependent hypertension.

An important and novel discovery from the present study is the enhanced augmentation of intrarenal ANG II content with AR antagonism during ANG II infusion. Because increased aldosterone has been shown to increase ANG II receptor binding (27) and number (28), and ANG II infusion has been shown to increase intrarenal ANG II content (15, 32–34), the mechanism exists for MR antagonism to exacerbate the intrarenal ANG II content during ANG II infusion. Recently, Nielsen et al. (17) demonstrated in aldosterone-infused rats cotreated with spironolactone and RU486 (glucocorticoid receptor antagonist) a 15-fold increase in plasma ANG II that they suggested could explain the lack of a spironolactone-mediated decrease in α-ENaC protein expression. ANG II has been shown to directly increase α-ENaC protein expression, which is mediated by AT1a because blockade of AT1a with candesartan decreased α-ENaC (2). In addition, AT1a knockout mice have decreased expression of α-ENaC despite nearly twofold greater plasma aldosterone (5) further suggesting that ANG II may be a critical regulator of α-ENaC and thus urinary Na+ excretion. The present study supports and extends these previous findings and further suggests that the epl-induced increases in plasma aldosterone potentiate AT1 receptor-mediated intrarenal uptake of ANG II, resulting in exacerbated intrarenal ANG II content during ANG II infusion. As previously suggested (16, 17), this increased intrarenal ANG II content may have masked any effects of the AR blockade by contributing to the maintenance of sustained Na+ reabsorption, and thus, sustained elevated arterial blood pressure. Alterna-

Fig. 5. A: mean (±SE) change from baseline (day 4) in urinary Na+ excretion in control and control + epl animals (n = 10/group), representing normotensive conditions. B: means (±SE) change from baseline (day 4) in urinary Na+ excretion in ANG II- and ANG II + epl-treated animals (n = 10/group), representing hypertensive conditions. Chronic infusion of ANG II (60 ng/min) began on day 1 and epl treatment (25 mg/day) began on day 8 (indicated by the arrow). *Significance at P < 0.05.

Fig. 6. A: mean (±SE) change from baseline (day 4) in renal Na+ balance (consumption − urinary excretion) in control and control + epl animals (n = 10/group), representing normotensive conditions. B: means (±SE) change from baseline (day 4) in Na+ balance (consumption − urinary excretion) in ANG II- and ANG II + epl-treated animals (n = 10/group), representing hypertensive conditions. Chronic infusion of ANG II (60 ng/min) began on day 1 and epl treatment (25 mg/day) began on day 8 (indicated by the arrow). *Significance at P < 0.05.
Fig. 7. Representative Western blot of corticosteroid α-ENaC and means (±SE) densitometry measurements of protein content after 28 days in control, control + epl, ANG II-, and ANG II + epl-treated animals (n = 8/group). Inset: representative Western blot of α-ENaC (n = 2 animals/group/gel). Bottom: summary of detected α-ENaC in the groups shown above (n = 8 animals/group). No significant differences were detected.

Aldosterone receptor blockade and intrarenal ANG II

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Aldosterone receptor blockade and intrarenal ANG II

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Aldosterone receptor blockade and intrarenal ANG II

rone and PRA were increased 4- and 5.5-fold, respectively, following 3 wk of epl treatment in ANG II-infused rats supplemented with 1% NaCl (23) and spironolactone treatment increased PRA and plasma aldosterone concentrations in normotensive rats (16) in as quickly as 1 day (10) suggesting that MR antagonism is renin-mediated in both hypertensive and normotensive conditions. However, plasma aldosterone was not altered following 2 wk of epl treatment in L-NAME/ANG II hypertensive rats supplemented with 1% NaCl (13, 22). In the present study, plasma aldosterone was increased 6-fold in normotensive rats and 3.5-fold in hypertensive rats following 21 days of epl treatment, independent of any increase in PRA indicating that the increase in circulating aldosterone induced by AR-specific antagonism is not mediated by systemic (renal) renin; however, the contribution of adrenal or local renin cannot be discredited by the present study. Furthermore, epl treatment induced an immediate (within 24 h) increase in urinary aldosterone excretion in both normotensive and hypertensive rats that was maintained throughout the study suggesting that the aldosterone response to AR blockade is immediate and sustained for the duration of the AR antagonism, similar to the response observed in spironolactone-treated, normotensive rats (10). In addition, the increases in intra-adrenal aldosterone content in both normotensive and hypertensive rats treated with epl provide compelling evidence to indicate that epl stimulated aldosterone synthesis and release directly at the level of the adrenal gland. Also, the exacerbation of intra-adrenal ANG II observed in the ANG II-infused rats treated with epl suggests that not only did this increase contribute to the exacerbated circulating concentrations of aldosterone but also suggests that AR signaling directly affects the regulation of intra-adrenal aldosterone synthesis.

In conclusion, the present study revealed that MR antagonism with the specific aldosterone receptor antagonist, epl, transiently increased urinary Na+ excretion associated with a parallel decrease in renal Na+ balance. These alterations were reconciled in less than 3 days suggesting that compensatory mechanisms were elicited within this period to restore Na+ balance and demonstrate the kidney’s ability to quickly respond to a perturbation in electrolyte status to recover homeostasis. The lack of a change in cortical α-ENaC content is consistent with the lack of a change in urinary Na+ excretion at the end of the study. Epl treatment exacerbated the ANG II-mediated augmentation of intrarenal ANG II content, likely contributing to the lack of a change in cortical α-ENaC content and to the sustained elevation in arterial blood pressure in the ANG II-infused, epl-treated rats. This increase in intrarenal ANG II also helps explain the absence of a sustained increase in Na+ excretion in the presence of MR blockade during ANG II-dependent hypertension.

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DISCLOSURES

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