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

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Submitted 18 December 2006; accepted in final form 28 February 2007

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http://www.ajprenal.org

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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 eunatrietic 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% NaCl-supplementation with or without N⁵-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 mini-pumps (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 s for the 28 days. Osmotic mini-pumps were implanted 5 days after recovery from the implantation of the telemetry. 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.

Dissections. Tissue harvesting procedures were identical for both cohorts. Following final measurements on day 28, animals were decapitated and trunk blood was collected into chilled vials containing 5 mM EDTA plus protease inhibitor cocktail (pH 7.4, 12.5 mM 1–10-phenanthroline, 22 μM pepstatin, 10 μM PMSF, 20 μM enalaprilat). An additional aliquot of trunk blood was collected in an untreated tube for serum electrolyte analyses. Microcapillary tubes were filled in duplicate from trunk blood to determine hematocrit following centrifugation of tubes. Aldosterone and ANG II were measured from the collected plasma. The right kidney was removed, cleaned, and weighed. A 3- to 5-mm section was dissected sagittally through the center of the kidney and the cortex was removed from the medulla under a light microscope. The cortex was prepared for determination of cortical α-ENaC protein content by Western blot analysis. 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 was removed, cleaned, weighed, placed in a glass vial containing 10 ml of PBS, and homogenized for extraction of aldosterone.

Western blot analysis. All chemicals and reagents used in Western blot analyses were obtained from Bio-Rad (Hercules, CA), Pierce (Rockford, IL), and Sigma (St. Louis, MO), unless otherwise noted. Cortical kidney segments were harvested as described above followed by homogenization in 1 ml hypotonic saline in the presence of a protease inhibitor cocktail. The homogenate was spun down and the pellet was resuspended in 1 ml lysis buffer containing the protease inhibitor cocktail and 1% Triton X-100. The soluble fraction was collected and protein concentration was determined using the BCA assay. Tissue homogenates containing 50 to 100 μg of total protein were mixed with loading buffer containing 3% SDS, and 20 mM dithiothreitol, heated at 85°C for 10 min and used for electrophoresis. Two samples from each group were run on a single gel for a total of eight animals per group. Electrophoresed membrane proteins were transferred to nitrocellulose and probed with ENaC-specific antibodies as previously described (1). The α-ENaC antibody (Affinity Bioreagents, Golden, CO) was diluted 1/2,000 (0.5 μg/ml final concentration) in blocking buffer and used to probe membranes for 1 h at room temperature. ENaC-expressing Madin Darby canine kidney (MDCK) cells were used as a positive control. Membranes were then probed with a secondary horseradish peroxidase-conjugated antibody (Kirkegaard and Perry Laboratories, Gaithersburg, MD) at a concentration of 20 ng/ml. Blots were developed with enhanced chemiluminescent (Pierce, SuperSignal West Dura) and Kodak BioMax Light-1 film. Digital images of blots were generated with a HP ScanJet 4200C (Houston, TX). Band intensity was quantified with densitometric scanning using SigmaGel (Jandel Scientific, San Rafael, CA).

Aldosterone, ANG II, and Na⁺ analyses. Adrenal and urinary aldosterone were extracted from the homogenate with ethyl acetate as previously described for urinary aldosterone analyses (18). Aldosterone concentrations of plasma, adrenal, and urine 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 (4). Urine and serum Na⁺ were measured by flame photometry (Instrumentation Labs, Lexington, MA; model 943).

Calculations. Urinary excretion values were the product of concentration and urine volume. Renal Na⁺ balance for the purpose of this study was calculated as the difference between dietary Na⁺ consumption and urinary Na⁺ excretion. Sodium consumption was the product of food consumption and percent Na⁺ (0.3%) in the diet. Data for urinary Na⁺ excretion and renal Na⁺ balance are presented as change from baseline where day 4 is considered baseline since animals were allowed to acclimate to handling procedures for the first 3 days of the study.

Statistics. Means (±SE) of daily measurements were compared by ANOVA adjusted for repeated measures over time. If significant (P <
0.05) group × time interactions were detected, two-way ANOVA was performed to determine differences on specific days. Although only means of metabolic data for specific, representative days are presented in Table 1, statistical analyses accounted for all measured days. Means for plasma/serum, organ, and densitometry measurements were compared by one-way ANOVA. For all cases, if significance (P < 0.05) was detected, a Fisher’s protected least significant difference test was applied post hoc. Statistics were performed using Statview software (SAS, Cary, NC).

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 preepi 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).

Intrarenal 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 |
|------------------|------------------|------------------|------------------|
|                  | Control          | Control + Epl    | ANG II           | ANG II + Epl     |
| Body mass, g     | 273 ± 3          | 273 ± 3          | 265 ± 4          | 264 ± 5          |
| Day 4            | 273 ± 3          | 273 ± 3          | 265 ± 4          | 264 ± 5          |
| Day 8            | 304 ± 4*         | 301 ± 4*         | 298 ± 6*         | 295 ± 4*         |
| Day 28           | 406 ± 10*        | 405 ± 10*        | 365 ± 13*†       | 363 ± 7*†        |
| Food intake, g/100 g BM |                |                  |                  |                  |
| Day 4            | 27 ± 1           | 28 ± 1           | 24 ± 1           | 25 ± 1           |
| Day 8            | 28 ± 1           | 28 ± 1           | 27 ± 1           | 27 ± 1           |
| Day 28           | 27 ± 1           | 29 ± 1           | 26 ± 1           | 26 ± 1           |
| Water intake, ml/day |                  |                  |                  |                  |
| Day 4            | 35 ± 1           | 39 ± 1           | 39 ± 2           | 44 ± 4†          |
| Day 8            | 38 ± 2           | 39 ± 2           | 42 ± 2           | 57 ± 6*†         |
| Day 28           | 41 ± 2           | 42 ± 2           | 75 ± 3*†         | 75 ± 5*†         |
| Urine output, ml/day |                  |                  |                  |                  |
| Day 4            | 15 ± 1           | 15 ± 2           | 19 ± 2           | 22 ± 4           |
| Day 8            | 16 ± 2           | 20 ± 2           | 21 ± 3           | 38 ± 6*†         |
| Day 28           | 21 ± 2           | 19 ± 2           | 56 ± 4*†         | 55 ± 4*†         |
| UrNa, mmol/day   |                 |                  |                  |                  |
| Day 4            | 2.4 ± 0.2        | 2.3 ± 0.1        | 2.0 ± 0.1        | 2.3 ± 0.2        |
| Day 8            | 2.5 ± 0.1        | 3.2 ± 0.1*       | 2.5 ± 0.1        | 3.1 ± 0.1*       |
| Day 28           | 2.2 ± 0.1        | 2.4 ± 0.1        | 2.6 ± 0.1        | 2.5 ± 0.2        |
| UrK, mmol/day    |                 |                  |                  |                  |
| Day 4            | 4.9 ± 0.4        | 4.9 ± 0.2        | 4.1 ± 0.2        | 4.5 ± 0.2        |
| Day 8            | 5.3 ± 0.2        | 5.0 ± 0.2        | 4.8 ± 0.2        | 5.0 ± 0.1        |
| Day 28           | 4.8 ± 0.2        | 5.0 ± 0.1        | 4.8 ± 0.3        | 4.8 ± 0.2        |

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) 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\(^+\) 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 nonimplanted cohort (Fig. 4A) and in hypertensive (502 ± 58 vs. 726 ± 53 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
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...
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-
tively, the elevated plasma aldosterone levels may have stimulated renal Na$^+$ reabsorption via binding of GR as has been suggested for in vitro models under specific physiological conditions (8); however, the in vivo data would not support this possibility (17).

Although ANG II (2) and aldosterone (14) have been shown to increase α-ENaC content, our study did not reveal any significant changes in cortical α-ENaC content in either the ANG II-infused rats or those treated with epl. However, in the previous study that reported an increase in α-ENaC following ANG II infusion (2), the infusion duration was only 3 days compared with 28 days for the present study providing sufficient time to initiate compensatory mechanisms. Additionally, treatment with spironolactone for 10 days in NaCl-restricted rats elicited a decrease in α-ENaC abundance (16), but again the difference in time may account for the discrepancy in α-ENaC responses. In the present study, epl in normotensive or ANG II-dependent hypertensive rats treated for 21 days did not significantly alter cortical α-ENaC content. Consistent with these data is the lack of a change in urinary Na$^+$ excretion that Nielsen et al. (16) explain is indicative of compensatory mechanisms allowing Na$^+$ balance to be reestablished despite continuous MR blockade. Because aldosterone was recently shown to increase the abundance of the Na-CI cotransporter (NCC) (17), an increase in NCC could be one potential compensatory change in response to the chronic blockade of AR that sustained Na$^+$ reabsorption.

A bolus dosage of epl given by oral gavage in rats was shown to reverse the effects of aldosterone on urinary Na$^+$/K$^+$ ratio suggesting that AR antagonism is effective in acutely antagonizing the renal actions of aldosterone on electrolyte transport (6). Similarly, the present study identified an acute natriuresis associated with an initial period of negative renal Na$^+$ balance at the onset of epl treatment. However, renal Na$^+$ balance was regained within the first week of epl treatment with only intermittent differences. With one exception, differences in urinary Na$^+$ excretion were not detected after day 2 of treatment in both normotensive and hypertensive animals suggesting that other renal compensatory mechanisms favoring Na$^+$ retention were rapidly activated to alleviate Na$^+$ imbalances. The lack of an effect from MR blockade on urinary Na$^+$ excretion after 13 days was also reported in l-NAME/ANG II-dependent hypertensive rats supplemented with 1% NaCl (13). Although Rocha et al. (21) reported a change in urinary Na$^+$ excretion after 10 wk in stroke-prone SHR rats treated with spironolactone, this change was attributed to an increase in excretion by controls and not due to an actual decrease in excretion by treated rats as this group maintained a constant rate of excretion during their hypertensive phase. In ANG II-dependent hypertensive rats supplemented with 1% NaCl, adrenalectomized animals replete with aldosterone exhibited greater urinary Na$^+$ excretion than both normotensive controls (1% NaCl) and hypertensive intact controls (ANG II + 1% NaCl) suggesting that supplementation with 1% NaCl may have confounded the evaluation of MR antagonism on renal Na$^+$ handling in that model. Therefore, the present study design (without NaCl supplementation) was adopted. Results of the present study are also consistent with those observed in aldosterone-infused rats cotreated with spironolactone and RU486 in which a lack of a change in urinary Na$^+$ and K$^+$ excretion was associated with no change in α-ENaC despite elevated plasma aldosterone (17). Collectively, these data indicate that chronic MR antagonism in various rodent models of hypertension does not cause a sustained increase in urinary Na$^+$ excretion, which may partially explain the lack of a blood pressure-lowering effect of MR antagonism in ANG II-infused models.

Epl and spironolactone have been shown to completely reduce SBP in liquorice-induced hypertension (20), whereas epl has been shown to reduce SBP by 18% in aldosterone/1% NaCl-induced hypertension (3), by 14% in nonsalt-loaded SHRSP rats (25), by 14% in Dahl salt-sensitive rats (11), and by 18% in SHR rats (24). In 2K-1C rats that exhibited a mild hypertensive condition (mean SBP 130 mmHg), epl reduced mean SBP by 11.5% after 10 wk (9). However, epl failed to reduce SBP in l-NAME/ANG II/1% NaCl-induced (13, 22) and in ANG II/1% NaCl-induced hypertensive rats (23). An examination of these studies suggests that MR antagonism is not effective in preventing the hypertension induced by ANG II, regardless of the presence or absence of Na$^+$ supplementation. Although the present study detected a small yet significant difference in SBP using telemetry suggesting that aldosterone may contribute a small component to the hypertension, the major hypertensive effect is mediated by ANG II. Thus the inability of epl to effectively reduce SBP during ANG II-dependent hypertension could partially be attributed to the exacerbation of intrarenal ANG II content, which likely counteracts the effects of MR blockade. In addition to the well-documented vasoconstriction effects of ANG II (15, 29, 32–34), the marked impairment of pressure natriuresis induced by ANG II (29) may be one of the mechanisms by which MR antagonism is impeded from alleviating elevated SBP during ANG II-dependent hypertension.

Disruption of the long feedback loop on renin secretion, resulting in increased PRA, is believed to be responsible for increasing plasma aldosterone during MR antagonism, especially in humans (12, 30, 31). However, changes in circulating aldosterone concentrations following epl treatment are not consistent in rodent models of hypertension. Plasma aldoste-
Aldosterone synthesis.

Circulating concentrations of aldosterone but also suggests that not only did this increase contribute to the exacerbated adrenal gland. Also, the exacerbation of intra-adrenal ANG II epl provide compelling evidence to indicate that epl stimulated the response observed in spironolactone-treated, normotensive rats that was maintained throughout the study suggesting 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 and the lack of an effective reduction in SBP. Furthermore, treatment with epl also exacerbated the augmentation of intra-adrenal ANG II, which, at least in the hypertensive conditions, resulted in an increase in intra-adrenal aldosterone content and plasma aldosterone concentrations. In both normo- and hypertensive conditions, urinary aldosterone excretion was elevated within 24 h suggesting that the adrenal response to MR antagonism is immediate. While the benefits of mineralocorticoid antagonism are well documented (3, 6, 7, 9, 11–13, 19, 21–23, 26, 30, 31), the present study indicates that disruption of ANG II signaling may also be necessary in the presence of MR blockade during ANG II-dependent hypertension.

ACKNOWLEDGMENTS

We thank Dr. M. Prieto-Carrascoyro, M. Cabrera, and I. Vukojicic for assistance throughout the study. We also thank Dr. C. Gomez-Sanchez and Dr. A. Nishiyama for critical review of this manuscript draft and extended discussion of results.

GRANTS

R. M. Ortiz was supported by a Ford Foundation Postdoctoral Fellowship followed by National Institutes of Health (NIH) National Research Service Award Grant F32 HL-076985 from National Heart, Lung, and Blood Institute (NHLBI). Research was supported by NHLBI Grant HL-26371 to L. G. Navar, National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-55626 to M. S. Awayda, NIH COBRE Grant P20-RR-017659 from the Institutional Award (IdEA) program of the National Center for Research Resources, and the Millennium Health Excellence Fund from the Louisiana Board of Regents.

DISCLOSURES

The authors thank Drs. M. Heron and E. McMahon of Pfizer (St. Louis, MO) for the generous contribution of epl (SC-66110). None of the authors need to disclose any potential conflicts of interest regarding any aspect of this study.

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