Loss of renal medullary endothelin B receptor function during salt deprivation is regulated by angiotensin II

Wararat Kittikulsuth, Jennifer S. Pollock, and David M. Pollock

Section of Experimental Medicine, Department of Medicine, Georgia Health Sciences University, Augusta, Georgia

Submitted 12 April 2012; accepted in final form 5 June 2012

Kittikulsuth W, Pollock JS, Pollock DM. Loss of renal medullary endothelin B receptor function during salt deprivation is regulated by angiotensin II. Am J Physiol Renal Physiol 303: F659–F666, 2012. First published June 6, 2012; doi:10.1152/ajprenal.00213.2012.—We have recently demonstrated that chronic infusion of exogenous ANG II, which induces blood pressure elevation, attenuates renal medullary endothelin B (ETB) receptor function in rats. Moreover, this was associated with a reduction of ETB receptor expression in the inner medulla. The aim of this present work was to investigate the association of ETB receptor binding with changes in ET system function and receptor expression in rats on a low-salt diet. Rats were placed on a low-salt diet (0.01–0.02% NaCl) for 2 wk to allow an increase in endogenous ANG II. In rats on normal-salt chow, the stimulation of renal medullary ETB receptor by ETB receptor agonist sarafotoxin 6c (S6c) causes an increase in water (<3 ± 0.4 from baseline vs. 10.5 ± 1.3 µl/min following S6c infusion; P < 0.05) and sodium excretion (0.38 ± 0.06 vs. 1.23 ± 0.17 µmol/min; P < 0.05). The low-salt diet reduced the ETB-dependent diuresis (4.5 ± 0.5 vs. 6.1 ± 0.9 µl/min) and natriuresis (0.40 ± 0.11 vs. 0.46 ± 0.12 µmol/min) in response to acute intramedullary infusion of S6c. Chronic treatment with candesartan restored renal medullary ETB receptor function; urine flow was 7.1 ± 0.9 vs. 15.9 ± 1.7 µl/min (P < 0.05), and sodium excretion was 0.4 ± 0.1 vs. 1.1 ± 0.1 µmol/min (P < 0.05) before and after intramedullary S6c infusion, respectively. Receptor binding assays determined that the sodium-depleted diet resulted in a similar level of ETB receptor binding in renal inner medulla compared with rats on a normal-salt diet. Candesartan reduced renal inner medullary ETB receptor binding (1.41 ± 0.5 vs. 0.86 ± 0.5 fmol/mg; P < 0.05). We conclude that endogenous ANG II attenuates renal medullary ETB receptor function to conserve sodium during salt deprivation independently of receptor expression.

low-salt diet; ETB receptor; renal medulla; renin-angiotensin system; natriuresis

THE ENDOTHELIN (ET) and renin-angiotensin system (RAS) play an important role in sodium balance and blood pressure control. ET-1 is highly produced in the kidney, especially by the inner medullary collecting duct (23). ET-1 via ETB receptor activation in the renal medulla stimulates nitric oxide production to facilitate water and sodium excretion (27) via increased renal medullary blood flow (43) and inhibition of epithelial sodium channel activity (ENaC) (8, 9) as well as reabsorption in the medullary thick ascending limb (30). In contrast, ANG II prominently acts as an antinatriuretic hormone at least in part by increasing abundance of sodium transporters and channels along the nephron, mainly mediated by the AT1 receptor-dependent pathway (22).

Differences in salt intake affect both the ET system and RAS. A high-salt diet, which is a low-renin state, activates the renal ET system by increasing renal medullary ET-1 production as well as renal ETB receptor expression (31, 32, 34). A low-salt diet physiologically activates the RAS in the kidney and elsewhere (19). The increase in ANG II is mainly to conserve sodium. ANG II regulates other natriuretic factors, including prostaglandins and bradykinin, under low-salt diet conditions (17, 36). From in vitro studies, we know that ANG II increases ET-1 gene (edn1) activation in both endothelial and epithelial cells (38, 39). However, the interplay between the ET system and RAS during a low-salt feeding is still unknown.

Previously, we have shown that chronic infusion of a pressor dose of ANG II impairs the ETB-dependent natriuretic response, which is associated with a reduction of ETB receptor binding in the renal inner medulla (20). However, the pathological conditions induced by exogenous ANG II are also associated with a variety of changes, such as increased inflammation and oxidative stress that could produce changes in the ET system independently of a direct action of ANG II (5, 24). Such conditions mimic chronic kidney disease, which also affects ETB receptor expression in the kidney (35). However, the physiological regulation of the renal ET system by the RAS has not been investigated.

The present study was designed to examine the effect of the endogenous RAS on the renal medullary ET system. We hypothesized that renal medullary ETB receptor function, as well as renal ET-1 production, is reduced during salt deprivation through activation of the RAS. We tested this hypothesis by determining the effect of specific AT1 receptor blockade on the natriuretic and diuretic response to renal medullary ETB receptor activation.

METHODS

Animal Preparation

Male Sprague-Dawley rats (8–10 wk old) from Harlan Laboratories (Indianapolis, IN) were housed under conditions of constant temperature and humidity and exposed to a 12:12-h light-dark cycle. All experiments and surgery procedures were approved and monitored by the Georgia Health Sciences University Institutional Animal Care and Use Committee in accordance with the American Physiological Society’s Guide for the Care and Use of Laboratory Animals. Rats were placed on a normal-salt (0.4% NaCl, Harlan Teklad, Madison, WI) or low-salt diet (0.01–0.02% NaCl, Harlan Teklad) for 2 wk. Another set of rats received candesartan (5 mg·kg⁻¹·day⁻¹ in the drinking water) with a normal- or low-salt diet for 2 wk. Water and food intake were measured daily. The dose of candesartan was adjusted twice a week if necessary to ensure proper dosing.

Metabolic and Renal Function Measurement

Rats were placed in metabolic cages for 24-h acclimation followed by a 24-h urine collection during day 0 (baseline) or day 14 of a
normal- or low-salt diet with or without candesartan treatment. After the baseline urine collection, a blood sample was collected from the tail vein (<50 μl). After 2 wk of treatment, rats were anesthetized with pentobarbital sodium (50 mg/kg ip). Plasma was collected from the descending aorta to measure ANG II concentration. The renal inner medulla was collected for receptor-binding analyses. Urinary and plasma creatinine were measured to determine creatinine clearance as previously described (2, 7). Urinary ET-1 excretion was measured by radioimmunoassay via the manufacturer’s protocol (Peninsula Laboratories, San Carlos, CA).

Plasma ANG II Concentration

ANG II was measured in plasma samples from rats that received a normal- and low-salt diet. Plasma ANG II was extracted with methanol and measured by an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) as previously described (40).

Intramedullary Infusion of an ET\(_{B}\) Receptor Agonist

Surgery and intramedullary infusion was performed in anesthetized rats (100 mg/kg ip) as previously described (20). The jugular vein was catheterized for infusion of saline (0.9% NaCl) containing 4% BSA at a rate of 30 μl/min and changed to 15 μl/min after surgery to maintain euvoolemia. A midline incision was made, and a catheter was inserted 5 mm in the left kidney to infuse saline or saline containing the ET\(_{B}\) receptor agonist sarafotoxin 6c (S6c; American Peptide, Sunnyvale, CA) at the rate of 0.5 ml/h into the renal medulla as described below. Urine was collected from the ureter of each kidney. After surgery, saline was infused into the renal medulla of the left kidney. Following a 60-min equilibration period, a baseline urine collection was obtained (40 min) followed by an experimental period where saline or S6c (0.45 μg·kg\(^{-1}\)·h\(^{-1}\)) was infused into the renal medulla for an additional 80 min. Urine from the final 20 min of the baseline and experimental periods was used for analysis. Urinary sodium concentration was measured by atomic absorption. Mean arterial pressure was measured continuously via a femoral artery catheter.

Receptor-Binding Assay to ET-1 and ET-3

As previously described (20), inner medullary tissue was homogenized and plasma membrane fractions were prepared for binding studies. Plasma membrane and wheat germ agglutinin polyvinyltoluene beads (PerkinElmer, Boston, MA) were added into each well and incubated for 3 h. Then, \(^{125}\text{I}\)-ET-1 (PerkinElmer) at a final concentration of 0.01–1.0 nM or \(^{125}\text{I}\)-ET-3 (PerkinElmer) at a final concentration of 0.06–1.0 nM was added and incubated for 18 h. Total binding of \(^{125}\text{I}\)-ET-1 or \(^{125}\text{I}\)-ET-3 was determined using 0.3 or 1 μg plasma membrane protein, respectively. Nonspecific binding was determined in the presence of 10 μM unlabeled ET-1 or ET-3 ligand (American Peptide). All measurements were performed in duplicate.

Statistical Analysis

Urine flow rate, urinary sodium excretion, and mean arterial pressure in response to intramedullary infusion of saline or S6c were analyzed by two-way repeated-measures ANOVA followed by Bonferroni post hoc tests among rats receiving a normal- or low-salt diet with or without candesartan. Metabolic parameters, urinary ET-1 excretion, and receptor binding data were analyzed by two-way ANOVA followed by Bonferroni post hoc tests to compare the effects of candesartan on either a normal- or low-salt diet. Results are expressed as means ± SE, with \(P < 0.05\) being considered statistically significant.

RESULTS

Plasma ANG II Concentration

To confirm that a sodium-depleted diet activates the RAS, we measured plasma ANG II in rats fed a normal- or low-salt diet for 2 wk. We found that rats on a low-salt diet had significantly elevated plasma ANG II levels compared with rats on a normal-salt diet (611 ± 78 vs. 279 ± 68 pg/ml; \(P < 0.05\)).

Intramedullary ET\(_{B}\) Receptor Function in Rats on a Low-Salt Diet

As previously reported, intramedullary infusion of the ET\(_{B}\) receptor agonist S6c produced a significant increase in urine flow rate (\(P < 0.05\); Figure 1A) and urinary sodium excretion (\(P < 0.05\); Fig. 2A) in rats on a normal-salt diet. Moreover, the increases in water and sodium excretion during S6c infusion were significantly greater than those observed during saline infusion at the same time period (\(P < 0.05\)). In contrast, intramedullary infusion of S6c did not increase the urine flow rate (Fig. 1B) or urinary sodium excretion (Fig. 2B) in rats on a salt-depleted diet. Mean arterial pressure remained unchanged during intramedullary infusion of saline or S6c for rats on either diet (Fig. 3. A and B).

For the time control group, intramedullary infusion of saline produced a modest, but significant increase in the urine flow rate over time (\(P < 0.05\); Fig. 1A); however, urinary sodium excretion remained stable during saline infusion compared with the baseline period in rats on a normal-salt diet (Fig. 2A). In contrast, saline infusion did not increase the urine flow rate (Fig. 1B) or urinary sodium excretion (Fig. 2B) in a low-salt diet group.

AT\(_1\) Receptor Blockade and ET\(_{B}\) Function in Rats on a Low-Salt Diet

To test the hypothesis that AT\(_1\) receptor activity regulates renal medullary ET\(_{B}\) receptor function during low-salt feeding, the AT\(_1\) receptor antagonist candesartan was given to rats on a normal- or low-salt diet for 2 wk. Intramedullary infusion of S6c significantly increased urine flow (Fig. 1C; \(P < 0.05\)) and urinary sodium excretion (Fig. 2C; \(P < 0.05\)) in rats on a normal-salt diet treated with candesartan, indicating that AT\(_1\) receptors do not affect ET\(_{B}\) receptor function with a normal-salt diet. Interestingly, candesartan restored the S6c-induced urine flow rate (Fig. 1D; \(P < 0.05\)) and urinary sodium excretion (Fig. 2D; \(P < 0.05\)) in rats on a low salt diet. Mean arterial pressure was slightly increased over the course of the acute period of both saline and S6c infusion in rats on a low-salt diet (Fig. 3D; \(P < 0.05\)).

Effects of AT\(_1\) Receptor Blockade on 24-h Intake and Excretion During Salt Deprivation

As depicted in Table 1, the baseline intake and excretion variables before any diet manipulations were similar among groups. After 14 days of treatment, a low-salt diet significantly reduced food consumption and urinary sodium excretion compared with rats on a normal-salt diet (\(P < 0.05\)). Urine volume was comparable between salt diets. There was no difference in creatinine clearance between normal- and low-salt-fed rats. Candesartan did not change food intake, urine flow, or urinary sodium excretion during either normal- or low-salt
feeding. Although candesartan increased creatinine clearance when rats received a normal-salt diet ($P < 0.05$), candesartan did not influence creatinine clearance in rats on a low-salt diet.

### Urinary ET-1 Excretion in Rats on a Low-Salt Diet

After 14 days of treatment, low-salt feeding had no effect on urinary ET-1 excretion compared with a normal-salt diet. Candesartan did not affect urinary ET-1 excretion in rats on a normal-salt diet. However, candesartan significantly reduced urinary ET-1 excretion in rats on a low-salt diet (Fig. 4; $P < 0.05$).

### ET-1 and ET-3 Binding in the Inner Medulla of Rats on a Low-Salt Diet

ET-1 has a similar affinity to both ETA and ETB receptors, while ET-3 has weak affinity for ETA receptors and binds only ETB receptors at low concentrations. Thus the saturation curve of $[^{125}\text{I}]-\text{ET-1}$ was performed to determine ETA and ETB

---

Fig. 1. Urine flow rate before (baseline) and during an intramedullary infusion of saline or the ETB receptor agonist sarafotoxin 6c (S6c) for 80 min in rats on a normal- or low-salt diet. Separate groups of rats were pretreated with the AT$_1$ receptor antagonist candesartan for 2 wk before the study. ns, Not significant. *$P < 0.05$ vs. baseline period in the same group; $n = 5–6$ rats/group.

Fig. 2. Sodium excretion before (baseline) and during an intramedullary infusion of saline or the ETB receptor agonist S6c for 80 min in rats on a normal- or low-salt diet. Separate groups of rats were pretreated with the AT$_1$ receptor antagonist candesartan for 2 wk before the study. *$P < 0.05$ vs. baseline period in the same group; $n = 5–6$ rats/group.
receptor binding, and the saturation curve of [125I]-ET-3 was used to determine ETB receptor binding. Both [125I]-ET-1 and [125I]-ET-3 binding curves reached saturation at 0.3 nM. [125I]-ET-1-specific binding sites in the renal inner medullary preparations were no different between normal- and low-salt diet groups (Fig. 5, A and B). Administration of candesartan to rats on a normal-salt diet did not affect saturation binding for [125I]-ET-1 (Fig. 5A). In rats on a low-salt diet, candesartan reduced [125I]-ET-1 saturation binding (Fig. 5B). Similar to [125I]-ET-1 binding, renal inner medullary preparations from rats on a normal- and low-salt diet had comparable [125I]-ET-3-specific binding curves (Fig. 5, C and D). Candesartan did not affect saturation binding for [125I]-ET-3 in rats on a normal-salt diet (Fig. 5C). [125I]-ET-3 binding was significantly reduced in candesartan-treated rats on a low-salt diet compared with a low-salt diet alone (Fig. 5D).

A Scatchard analysis was used to determine maximum binding (Bmax) values of [125I]-ET-1 binding sites that represent total ETA and ETB receptor binding, and Bmax values of [125I]-ET-3 binding sites represent specific ETB receptor binding as previously described (20). The difference between Bmax values of [125I]-ET-1 and [125I]-ET-3 binding was used to determine ETA receptor binding (Fig. 6A). Bmax values for ETB receptor binding in renal inner medullary tissue were comparable between rats on a normal- and low-salt diet (Fig. 6B). Administration of candesartan did not change Bmax values for ETB receptor binding in rats on a normal-salt diet. In contrast, Bmax values for ETB receptor binding were significantly reduced in inner medullary tissue from rats on a low-salt diet treated with candesartan. Candesartan treatment did not affect Bmax values for ETA receptor binding in tissues from either normal- or low-salt-fed rats (Fig. 6A). Dissociation constants (Kd) for [125I]-ET-1 or [125I]-ET-3 binding were comparable between inner medullary preparations from rats on a normal- or low-salt diet (Fig. 6, C and D, respectively). Candesartan did not change Kd of [125I]-ET-1 or [125I]-ET-3 binding in either group.

Table 1. Metabolic characteristics of rats on a normal-salt diet, a low-salt diet, a normal-salt diet with candesartan, and a low-salt diet with candesartan on day 0 (baseline before any diet manipulations) and day 14 of treatment

<table>
<thead>
<tr>
<th></th>
<th>Normal Salt</th>
<th>Normal Salt + Candesartan</th>
<th>Low Salt</th>
<th>Low Salt + Candesartan</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food intake, g/day</td>
<td>21.6 ± 0.5</td>
<td>21.9 ± 1.5</td>
<td>24.2 ± 0.4</td>
<td>22.0 ± 1.2</td>
</tr>
<tr>
<td>Water intake, g/day</td>
<td>33.0 ± 0.7</td>
<td>31.5 ± 0.7</td>
<td>28.3 ± 2.7</td>
<td>29.4 ± 2.5</td>
</tr>
<tr>
<td>UV, ml/day</td>
<td>15.8 ± 1.0</td>
<td>17.2 ± 1.1</td>
<td>15.2 ± 1.2</td>
<td>16.1 ± 0.6</td>
</tr>
<tr>
<td>UNaV, mmol/day</td>
<td>1.73 ± 0.04</td>
<td>1.88 ± 0.13</td>
<td>1.81 ± 0.12</td>
<td>1.84 ± 1.00</td>
</tr>
<tr>
<td>Creat, ml/min</td>
<td>1.02 ± 0.05</td>
<td>1.01 ± 0.09</td>
<td>1.03 ± 0.10</td>
<td>1.15 ± 0.12</td>
</tr>
<tr>
<td><strong>Day 14</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food intake, g/day</td>
<td>20.8 ± 0.9</td>
<td>21.7 ± 0.6</td>
<td>16.4 ± 1.3*</td>
<td>14.5 ± 0.7*</td>
</tr>
<tr>
<td>Water intake, g/day</td>
<td>30.8 ± 2.2</td>
<td>32.0 ± 2.1</td>
<td>22.9 ± 1.7</td>
<td>31.7 ± 3.8</td>
</tr>
<tr>
<td>UV, ml/day</td>
<td>14.9 ± 1.8</td>
<td>15.3 ± 2.1</td>
<td>11.8 ± 1.7</td>
<td>17.5 ± 2.2</td>
</tr>
<tr>
<td>UNaV, mmol/day</td>
<td>1.67 ± 0.07</td>
<td>1.86 ± 0.25</td>
<td>0.005 ± 0.001*</td>
<td>0.028 ± 0.008*</td>
</tr>
<tr>
<td>Creat, ml/min</td>
<td>0.88 ± 0.06</td>
<td>1.17 ± 0.10†</td>
<td>0.71 ± 0.07</td>
<td>0.74 ± 0.05*</td>
</tr>
</tbody>
</table>

Values are means ± SE. UV, urine flow rate; UNaV, urinary sodium excretion; Creat, creatinine clearance. *P < 0.05 vs. a normal-salt diet. †P < 0.05 vs. the same salt diet alone; n = 5–6 rats/group.
DISCUSSION

Physiologically, a low-sodium diet increases activity of the RAS to maintain blood pressure (19). The elevation of ANG II helps to conserve sodium. Our laboratory and others have provided convincing evidence to demonstrate that ETB receptors in the renal medulla, especially in collecting ducts, promote water and sodium excretion as a means of blood pressure regulation (14, 27). The present study provides new evidence that the interaction between the RAS and ET-1 system underlies physiological regulation of sodium retention during salt deprivation. The major findings of the current study are 1) renal medullary ETB receptor function is reduced in rats on a sodium-depleted diet; 2) attenuated ETB-dependent water and sodium excretion during salt deprivation is mediated by ANG II; and 3) renal ET-1 production and renal medullary ETB receptor expression are regulated by ANG II through AT1 receptors during conditions of low-salt intake. Thus this study provides evidence for a contribution of the renal medullary ET system in the ANG II-induced sodium conservation associated with low sodium intake.

We previously reported that chronic infusion of exogenous ANG II at a prohypertensive dose attenuated ETB-dependent natriuresis (20). In the present study, physiological elevation of ANG II activity induced by a shift in dietary sodium intake to a low-salt diet also reduced renal medullary ETB receptor function. Blockade of AT1 receptor activity restored renal medullary ETB receptor function during salt deprivation. These data clearly indicate that the antinatriuretic effects of a low-salt diet on ETB-dependent function are mediated by ANG II and the AT1 receptor. These data are consistent with the hypothesis that renal medullary ETB receptor function is shut down to prevent water and sodium loss.

Several studies have shown that ANG II reduces ETB receptor expression both in vitro and in vivo. Wong et al. (44) demonstrated that ANG II, via the AT1 receptor pathway, downregulated ET receptor binding in cultured inner medullary collecting duct cells. Blockade of ANG II formation by an angiotensin-converting enzyme inhibitor increases ETB recep-

![Figure 4](http://ajprenal.physiology.org/content/doi/10.1152/ajprenal.00213.2012)

**Fig. 4.** Urinary endothelin (ET)-1 excretion (24 h) in rats on a normal- or low-salt diet with (+) or without (−) candesartan treatment for 14 days. *P < 0.05 vs. a low-salt diet alone; n = 5–6 rats/group.

![Figure 5](http://ajprenal.physiology.org/content/doi/10.1152/ajprenal.00213.2012)

**Fig. 5.** Saturation binding curves for [125I]-ET-1 (A and B) or [125I]-ET-3 (C and D) in plasma membrane preparations of renal inner medullary tissue from rats on a normal (A and C) or low (B and D)-salt diet with or without candesartan. Insets: Scatchard analysis of [125I]-ET-1 and [125I]-ET-3; n = 3–4 rats/group.
tor density in the kidney of cardiomyopathic hamsters with moderate heart failure (45). Moreover, our own laboratory demonstrated that chronic infusion of ANG II at a pressor dose decreases ET_B receptor binding in the renal inner medulla (20). In the current study, however, a low-salt diet, which activates the endogenous RAS, had comparable ET_B receptor binding in the renal inner medulla to a normal-salt diet. The reason physiological and pathological increases in ANG II levels differently regulate ETB receptor expression is unknown but could be due to different downstream signaling pathways that are activated under these circumstances. For example, Banescu-Berceli et al. (3) showed that hypertension produced by exogenous infusion of ANG II was dependent upon Janus kinase 2 (JAK2) activation. However, physiological stimulation of endogenous ANG II by a low-salt diet did not activate the JAK2 pathway (3). How such pathways could influence ETB receptor expression and/or signaling has yet to be determined.

Urinary ET-1 excretion reflects ET-1 production within the kidneys (41), and the collecting duct is a major source of ET-1 synthesis (41). In vitro studies suggest that flow or sodium stimulates ET-1 mRNA expression in tubular cells, including in the collecting duct or thick ascending limb (16, 26, 29). Furthermore, animal studies show that increased tubular flow rate (26), dietary salt intake (16, 31), and renal interstitial osmolality (6, 16) elevate renal ET-1 production. These data indicate that renal tubular cells may play a role in renal ET-1 production in response to a high sodium intake. In our experiments, urine flow rate was comparable, while urinary sodium excretion was dramatically reduced with low-salt feeding compared with a normal-salt diet. We expected that urinary ET-1 excretion would be reduced in a low-salt diet. To our surprise, a low-salt diet had comparable urinary ET-1 excretion to a normal-salt diet. We know that a high-salt diet and increases in renal tubular flow rate increase ET-1 production within the collecting duct system, so we presumed that a low-salt diet would decrease urinary ET-1 excretion. However, ANG II activates ET-1 gene expression and production in endothelial (11, 13) and tubular cells (15). Therefore, these effects appear to negate one another such that urinary ET-1 excretion is no different between normal- and low-salt-treated animals. Pharmacological blockade of ANG II activity with candesartan had no effect on ET-1 excretion in rats on a normal-salt diet but reduced urinary ET-1 excretion during low-salt feeding. These data suggest that ANG II contributes to the maintenance of renal ET-1 production during low-salt feeding.

We observed that administration of candesartan decreased blood pressure in both dietary salt groups under inactin anesthesia, an effect that was more dramatic in rats on a low-salt diet. Using telemetry, Klinger et al. (21) also reported that the AT_1 receptor antagonist losartan reduced blood pressure whether on a normal- or low-salt diet (21). It is possible that the reduction of renal ET_B receptor binding and urinary ET-1 excretion in rats on a low-salt diet and treated with candesartan may result from the concomitant decrease in blood pressure. However, the blood pressure effect of candesartan on the ET system is not apparent in rats on a normal-salt diet. We
demonstrated previously that acute reduction of blood pressure did not significantly attenuate urinary ET-1 excretion compared with normal blood pressure (33). Furthermore, Dumont et al. (10) reported that the AT1 receptor antagonist losartan reduced blood pressure and urinary ET-1 excretion in uremic rats; however, treatment with conventional triple therapy, which reduced blood pressure to a comparable level as the losartan-treated group, did not change urinary ET-1 excretion. While these results suggest that urinary ET-1 excretion, which reflects renal ET-1 production, is dependent on the AT1 receptor pathway, we still cannot exclude the blood pressure-lowering effect of AT1 receptor antagonism having some influence in our study.

Activation of ETB receptors leads to an increase in nitric oxide (NO) production, which inhibits sodium channel activity including ENaC (8). In our study, low-salt feeding reduced NO synthase (NOS) activity (42), increase superoxide production (12, 18), and stimulate ENaC expression and activity (4, 25, 28). It is possible that the inhibition of ANG II activity in the kidney by AT1 blockade could oppose or all of these effects to reduce ENaC activity, as well as increase the ETB/NOS1 signaling, thus leading to enhancing ETB-dependent natriuresis under these conditions. Further studies will need to elucidate the postreceptor pathways that are influenced under various dietary salt intakes.

**Perspectives**

The current study highlights the physiological importance of ANG II and ET system interactions in the regulation of sodium balance. In a clinical setting, RAS-modulating therapies are among the most widely prescribed treatments to lower blood pressure in hypertensive patients and are concomitant with the recommendation to reduce salt consumption. Because animal models of salt-sensitive hypertension, including Dahl-salt (37) or ANG II hypertensive rats (20), have an impairment of the prerenal actions of the ET system, it will be important to determine whether any of the beneficial effects of AT1 receptor blockade in hypertension is a function of improved renal medullary ETB receptor function.

**GRANTS**

This work was supported by National Institutes of Health Grants HL06563, HL09999, and HL95499, a grant from the Cardiovascular Discovery Institute of the Georgia Health Sciences University, and an American Heart Association predoctoral fellowship (W. Kitikulthai).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: W.K., J.S.P., and D.M.P. provided conception and design of research; W.K. performed experiments; W.K. and J.S.P. analyzed data; W.K., J.S.P., and D.M.P. interpreted results of experiments; W.K. prepared figures; W.K. drafted manuscript; W.K., J.S.P., and D.M.P. edited and revised manuscript; W.K., J.S.P., and D.M.P. approved final version of manuscript.

**REFERENCES**


