Sex Differences in Renal Medullary Endothelin Receptor Function in Angiotensin II Hypertensive Rats

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

Abstract—We hypothesized that angiotensin (Ang) II hypertensive rats have impaired natriuresis after renal medullary endothelin (ET) B receptor stimulation that would be more evident in male versus female rats. Acute intramedullary infusion of the ET\textsubscript{B} agonist sarafotoxin 6c in normotensive male rats increased sodium excretion from 0.51±0.11 μmol/min during baseline to 1.64±0.19 μmol/min (P<0.05) after S6c. After 2 weeks of Ang II infusion (260 ng/kg per minute SC), male rats had an attenuated natriuretic response to S6c of 0.62±0.16 μmol/min during baseline versus 0.95±0.07 μmol/min after S6c. In contrast, ET\textsubscript{B}-dependent natriuresis was similar in female hypertensive rats (0.48±0.07 versus 1.5±0.18 μmol/min; P<0.05) compared with normotensive controls (1.05±0.07 versus 2.14±0.24 μmol/min; P<0.05). Because ET\textsubscript{A} receptors also mediate natriuresis in normotensive female rats, we examined ET\textsubscript{A} receptor function in female Ang II hypertensive rats. Intramedullary infusion of ET-1 increased sodium excretion in both hypertensive and normotensive female rats, which was partially blocked by the ET\textsubscript{A} antagonist BQ-123. Maximum ET\textsubscript{B} receptor binding in inner medullary membrane preparations was comparable between vehicle and Ang II hypertensive females; however, maximum ET\textsubscript{B} binding was significantly lower in male hypertensive rats (1952±251 versus 985±176 fmol/mg; P<0.05). These results indicate that renal ET\textsubscript{B} function is impaired in male Ang II hypertension attributed, at least in part, to a reduced number of ET\textsubscript{B} binding sites. Furthermore, renal ET receptor function is preserved in female rats during chronic Ang II infusion, suggesting that renal ET receptor function could serve to limit hypertension in females compared with males. (Hypertension. 2011;58:00-00.) ● Online Data Supplement

Key Words: sex ■ ET\textsubscript{B} ■ natriuresis ■ Ang II hypertension ■ renal medulla

Endothelin (ET) 1 is a physiologically important regulator of sodium balance and blood pressure (BP) by acting on ET\textsubscript{A} and ET\textsubscript{B} receptors. ET\textsubscript{A} receptors are generally responsible for the regulation of vascular tone through vasoconstriction. In contrast, ET\textsubscript{B} receptors oppose ET\textsubscript{A} receptor effects by vasodilation.\textsuperscript{1} ET\textsubscript{B} receptors also play a role in promoting sodium excretion within the renal medulla,\textsuperscript{2} specifically the collecting ducts.\textsuperscript{3} ET-1 is involved in the development of hypertension by increased ET\textsubscript{A} and/or reduced ET\textsubscript{B} activity in various animal models of salt-sensitive hypertension, such as deoxycorticosterone acetate (DOCA) salt,\textsuperscript{4} Dahl salt-sensitive,\textsuperscript{5} and chronic angiotensin (Ang) II \textsuperscript{6} rat models.\textsuperscript{7} Numerous studies indicate an interaction between the Ang II and ET systems in the development of hypertension. Ang II increases ET synthesis in endothelial,\textsuperscript{8} vascular smooth muscle,\textsuperscript{9} and renal epithelial cells,\textsuperscript{10,11} ET receptor antagonists have favorable effects in Ang II-dependent hypertension.\textsuperscript{12-14} Both ET\textsubscript{A} selective and dual ET\textsubscript{A/B} antagonists can significantly attenuate the BP elevation, as well as renal injury in chronic Ang II–infused rats.\textsuperscript{12-14} In contrast, a selective ET\textsubscript{B} antagonist increases BP in male Ang II–infused rats during high-salt intake,\textsuperscript{13} albeit to a much lower extent compared with nonhypertensive animals.\textsuperscript{14} These recent findings indicate that the ET\textsubscript{A} receptor is important in the development of hypertension, whereas the protective role of ET\textsubscript{B} receptors may be impaired in Ang II hypertension.

Many studies have shown that female rats are more resistant to BP elevation than males in animal models such as spontaneously hypertensive rats,\textsuperscript{16,17} DOCA salt,\textsuperscript{18,19} and chronic Ang II infusion.\textsuperscript{20,21} In the DOCA-salt model, there is further evidence that ET\textsubscript{B} receptors may account for the attenuated increase in BP observed in female rats.\textsuperscript{19} Recently, we reported that renal medullary ET\textsubscript{A} receptors also facilitate sodium excretion in female rats, which does not exist in males, most likely because males exhibit a reduction of medullary blood flow (MBF) in response to intramedullary infusion of ET-1.\textsuperscript{22} It is not clear whether the antihypertensive capability of the renal medullary ET system is intact in the chronic Ang II model of hypertension or whether sex differences exist under such conditions.

It has been shown that in vitro incubation of vascular tissue\textsuperscript{23} and renal collecting ducts\textsuperscript{24} with Ang II decreases ET receptor expression. Inhibition of the Ang-converting enzyme can restore ET\textsubscript{B} receptor density in the kidney of...
and urinary sodium excretion (Figure 1A and 1C; \( P < 0.05 \)) compared with intramedullary infusion of saline (vehicle). Male Ang II–infused rats had higher MAP than the vehicle-treated group, yet intramedullary infusion of S6c failed to increase urine flow or sodium excretion (Figure 1A and 1C). The intramedullary infusion of S6c did not affect MAP (Figure 1E) or MBF in any group (Figure S1, available in the online Data Supplement).

GFR was measured in a subset of animals to determine whether reduced sodium delivery to the collecting duct could account for impairment of sodium excretion during intramedullary infusion of S6c in male Ang II hypertension. GFR was similar between vehicle and Ang II hypertensive rats during intramedullary infusion of saline (Figure S2). S6c infusion into the renal medulla had no effect on GFR in vehicle or Ang II hypertensive rats (Figure S2).

In conscious rats, there was no difference in systolic BP between male and female rats that received 14 days of vehicle infusion. However, female rats had lower systolic BP than males in response to Ang II infusion (Table S1; \( P < 0.05 \)).

### Renal Medullary ET\(_B\) Receptor Function in Female Ang II Hypertensive Rats

Similar to male normotensive rats, intramedullary infusion of S6c significantly increased urine flow and sodium excretion in female vehicle-treated rats (Figure 1B and 1D; \( P < 0.05 \)). In contrast to males, S6c infusion into the renal medulla significantly increased urine flow and sodium excretion in female Ang II hypertensive rats (Figure 1B and 1D; \( P < 0.05 \)). The increase in urine flow and sodium excretion during S6c infusion in females was similar between vehicle and Ang II hypertensive rats. S6c infusion did not affect MAP (Figure 1F) or MBF (Figure S1) in female Ang II hypertensive rats.

### Renal Medullary ET\(_A\) Receptor Function in Female Ang II Hypertensive Rats

Because we reported previously that ET\(_A\)-mediated diuresis and natriuresis are found only in female rats and without hemodynamic changes,\(^{27}\) we further examined whether renal medullary ET\(_A\) receptor function was preserved in female Ang II hypertension. ET-1 infusion into the renal medulla of female vehicle-treated rats significantly increased urine flow and sodium excretion (Figure 2A and 2C; \( P < 0.05 \)). The coinfusion of ET-1 with BQ-123, a selective ET\(_A\) antagonist, totally abolished ET-1–induced water excretion (Figure 2A; \( P < 0.05 \)) and partially reduced sodium excretion compared with intramedullary infusion of ET-1 alone in female vehicle-treated rats (Figure 2C). Similarly, intramedullary infusion of ET-1 in female Ang II hypertensive rats significantly increased urine flow and sodium excretion (Figure 2B and 2D; \( P < 0.05 \)). The increase of urine flow was significantly attenuated when ET-1 was coinfused with BQ-123 (Figure 2B; \( P < 0.05 \)), whereas the attenuated ET-1–induced natriuresis was not significantly different from ET-1 alone (Figure 2D). Intramedullary infusion of ET-1 with or without BQ-123 did not affect MAP (Figure 2E and 2F) or MBF in any group (Figure S3).
ET Receptor mRNA Expression After Chronic Ang II Infusion

We next assessed mRNA expression of both ETA and ETB receptors in renal inner medulla of both male and female rats receiving chronic infusion of vehicle or Ang II. In vehicle-treated rats, ETA mRNA expression was higher in female rats compared with males (Figure 3A; $P<0.05$). Ang II infusion did not affect ETA mRNA expression in either male or female rats (Figure 3A). ETB mRNA expression was also comparable in both male and female rats receiving vehicle or Ang II infusion (Figure 3B).

ET Receptor Binding in Renal Inner Medulla After Chronic Ang II Infusion

**Saturation of $[^{125}]$ET-1 or $[^{125}]$ET-3 Binding**

Preliminary experiments were performed using membrane preparations from renal inner medulla to determine the optimal membrane protein concentration for the receptor binding assay with 1 nmol/L of $[^{125}]$ET-1 or $[^{125}]$ET-3. The B$_{max}$ for inner medullary tissue of both male and female rats that received vehicle and Ang II infusion was achieved at 0.3 µg for $[^{125}]$ET-1 binding and 1 µg for $[^{125}]$ET-3 binding (data not shown). Specific binding in membrane preparations from inner medulla of vehicle and Ang II hypertensive rats in both males and females rose with increasing $[^{125}]$ET-1 or $[^{125}]$ET-3 concentrations and reached saturation at 0.3 nmol/L. Specific binding of both $[^{125}]$ET-1 and $[^{125}]$ET-3 in the inner medullary preparation was higher in vehicle than in Ang II hypertensive male rats (Figure S4). However, $[^{125}]$ET-1 and $[^{125}]$ET-3 bindings were comparable in inner medulla of vehicle and Ang II–treated female rats. Scatchard transformation of the specific binding of $[^{125}]$ET-1 or $[^{125}]$ET-3 of inner medullary preparations from both male and female vehicle and Ang II–treated rats revealed a monophasic curve (Figure S4), thus suggesting a single binding site or 2 binding sites with similar affinity, as has been reported previously.$^{29,30}$

**ET Receptor Binding Sites in Renal Inner Medulla**

ET-1 has equal affinity to bind both ETA and ETB receptors, whereas ET-3 has higher affinity to ETB receptors and binds only the ETB receptor at low concentrations.$^{29}$ For these reasons, B$_{max}$ values for ET-1 binding represents total ETA and ETB receptor binding sites, and B$_{max}$ values for ET-3 binding represents ETB receptor binding. For each membrane preparation, B$_{max}$ values for ET-3 binding were subtracted from B$_{max}$ values for ET-1 binding to determine the B$_{max}$ for ETA receptors. ETA receptor binding was comparable between vehicle and Ang II hypertensive rats, whether male or female. Female rats had significantly fewer ETA receptor binding sites compared with males in vehicle-treated groups (Figure 4A; $P<0.05$).

Compared with the vehicle-treated group, male rats that received chronic Ang II infusion had a significant reduction...
in the number of ETB receptor binding sites in renal inner medulla (Figure 4B; $P<0.05$). In contrast, female rats had a similar number of ETB receptor binding sites between vehicle and Ang II treatment. Female Ang II hypertensive rats had a greater number of ETB receptor binding sites than male Ang II hypertensive rats.

**Dissociation Constant**

The binding affinity of $[^{125}]$ET-1 in renal inner medulla was significantly higher in male Ang II–treated rats compared with male vehicle-treated animals (Figure 4C; $P<0.05$). However, there was no difference in binding affinity of $[^{125}]$ET-1 between groups of female rats (Figure 4C). In vehicle groups, female rats had higher binding affinity of $[^{125}]$ET-1 than males (Figure 4C; $P<0.05$). There was no difference between sexes in the binding affinity of $[^{125}]$ET-3 between vehicle and Ang II–treated rats (Figure 4D).

**Discussion**

The current study provides evidence that renal medullary ET receptor function is affected differently in male and female rats in response to Ang II hypertension. In males, Ang II hypertension impaired ETB-dependent sodium excretion, which was accompanied by a reduction of ETB receptor binding in renal inner medullary tissue and independent of any changes in GFR. These results provide a clear explanation that the reduction in receptor availability contributes to reduced ETB function in Ang II hypertension in male rats and, furthermore, allows us to speculate that salt sensitivity in Ang II–dependent hypertension is related to a lack of ETB

![Figure 2.](image-url) Effect of intramedullary infusion of endothelin (ET) 1 alone or ET-1 with BQ-123 on urine flow (A and B), sodium excretion (C and D), and mean arterial pressure (E and F) in anesthetized female vehicle (A, C, and E) and angiotensin (Ang) II hypertensive rats (B, D, and F); n=6 to 7 rats per group. $^*P<0.05$ vs baseline in the same group, $^†P<0.05$ vs saline in the same time, and $^‡P<0.05$ vs ET-1 in the same time.

![Figure 3.](image-url) mRNA expression for endothelin (ET) A (A) and ETB (B) receptors in renal inner medulla of male and female rats that received vehicle or angiotensin (Ang) II infusion. All of the values normalized to GAPDH; n=5 to 6 rats per group. $^*P<0.05$ vs males in the same treatment.
receptor control of sodium excretion. In contrast to males, renal medullary ETB function was preserved in female rats after chronic Ang II infusion. Furthermore, ETA-facilitated sodium excretion was maintained in female rats with Ang II hypertension. These findings suggest that renal medullary ET receptor function could serve to limit hypertension in females compared with males. The degree of BP elevation in Ang II–induced hypertension depends on the level of sodium intake. In a variety of animal models, females often have lower BP compared with males, including in response to Ang II infusion.20,21 Although the cause of this sex difference is still unknown, our findings support the hypothesis that maintenance of ET receptor function in females could be a contributing factor.

Interstitial infusion into the renal medulla is a well-known technique for evaluating receptor or transporter function in the renal medulla in vivo.22,23 The ability of the ETB receptor to facilitate sodium excretion in the renal medulla can be demonstrated by interstitial administration of a pharmacological ETB receptor activator or inhibitor.33 In our previous study, infusion of S6c significantly increased urine flow and sodium excretion without affecting BP or MBF.2 At a dose of S6c that produces a clear diuresis and natriuresis in male Ang II hypertensive rats, we were unable to observe any changes in MBF in either male or female rats, we did not expect to see any change in GFR. Thus, the impairment of ETB-dependent natriuresis in male Ang II hypertensive rats is not attributed to a decrease in GFR. We have observed previously that intramедullary infusion of ET-1 and S6c in both males and females; n = 3 to 4 rats per group. *P < 0.05 vs vehicle in the same sex, †P < 0.05 vs males in the same treatment.

GFR can influence urinary sodium and water excretion by adjustments of fluid delivery to later parts of the nephron. In anesthetized rats, GFR was not different between vehicle and Ang II–infused rats, which is consistent with previous findings.33,34 We also observed that intramedullary infusion of S6c did not affect GFR in any groups, although because S6c was delivered specifically to the renal medulla, we did not
In contrast to males, we observed that Ang II hypertension had no effect on ET\textsubscript{B} receptor expression in female rats. Estrogen has been reported to increase ET\textsubscript{A} receptor expression in rabbit coronary arteries\textsuperscript{26} but to decrease ET\textsubscript{B} receptor expression in smooth muscle cells of hypertensive rats.\textsuperscript{27,28} Our previous study demonstrated that ovariectomy attenuated the natriuretic response to ET-1, suggesting that estrogen may be important for maintaining ET\textsubscript{B} and/or ET\textsubscript{A} receptor expression in the renal medulla. It does not appear as though Ang II hypertension influences estrogen-dependent maintenance of ET\textsubscript{B} receptor function, because the natriuretic response to S6c was maintained in female rats. Whether estrogen regulation protects female rats from ET receptor dysfunction in Ang II hypertension is not easily addressed, because ovariectomy attenuates the response even in normotensive rats.

Our findings support the notion that renal ET\textsubscript{A}/ET\textsubscript{B} receptor imbalance may contribute to renal sodium retention and BP elevation and is consistent with findings in other models of hypertension. Stroke-prone spontaneously hypertensive rats on a high-salt diet have an increase in the ET\textsubscript{A}/ET\textsubscript{B} receptor ratio.\textsuperscript{39} Likewise, Dahl salt-sensitive rats have reduced renal medullary ET\textsubscript{B} receptor expression.\textsuperscript{40} In the present study, we found that chronic Ang II infusion in male rats reduced the expression of the ET\textsubscript{B} receptor, but, in contrast, female Ang II hypertensive rats have similar levels of both ET\textsubscript{A} and ET\textsubscript{B} receptor expression compared with vehicle groups. This increase in ET\textsubscript{A}/ET\textsubscript{B} receptor ratio in male rats could, at least in part, explain why chronic Ang II produces a greater hypertension in males versus female rats.\textsuperscript{20,21} In the DOCA-salt model of hypertension, female rats have lower BP than males, and this difference is abolished in rats with genetic modification that renders the ET\textsubscript{B} receptor dysfunctional.\textsuperscript{19}

Although males had higher BP than females during chronic Ang II infusion in conscious rats, this BP difference between male and female Ang II hypertensive rats was absent under inactin anesthesia. Anesthesia can reduce BP and renal function in rats.\textsuperscript{41} This highlights a limitation of the current study in that renal medullary ET receptor function was assessed after administration of ET peptides in anesthetized rats. Although the reasons for the difference in BP measures between conscious and anesthetized rats is not clear, the lack of a difference in BP and GFR between male and female rats indicates that these variables cannot account for the sex difference in ET\textsubscript{B} receptor function. It is important to note that our model of acute stimulation of ET receptors is a measure of receptor capacity that could serve to influence the chronic pressure-natriuresis relationship and not moment-to-moment control.

**Perspectives**

Nonfunctional ET\textsubscript{B} receptors produced by genetic mutation and pharmacological blockade of ET\textsubscript{B} receptors results in elevated BP that is highly salt dependent.\textsuperscript{15,42,43} Moreover, the genetic deletion of collecting duct ET\textsubscript{B} receptors, which are highly expressed in the renal medulla, causes BP elevation that is exacerbated by high-salt intake.\textsuperscript{3} However, endothelial cell ET\textsubscript{B} receptor knockout mice, which display endothelial dysfunction, have a similar level of BP compared with genetic controls.\textsuperscript{44} These results suggest that renal epithelial but not endothelial ET\textsubscript{B} receptors play a role in sodium excretion and BP control. Our findings are consistent with the hypothesis that a decrease in renal ET\textsubscript{B} receptor function may be responsible for the salt sensitivity associated with Ang II hypertension. However, ET\textsubscript{B} receptors in the renal medulla are localized on both endothelial cells of vasa recta and renal tubules,\textsuperscript{45} and so the site of ET\textsubscript{B} receptor dysfunction and downregulation in this model has yet to be confirmed. We further suggest that the sex differences in ET receptor function could serve to account for the sex-dependent response to chronic Ang II infusion. It will be important to determine how ET receptor function may be affected in human kidneys, specifically, in patients with salt-sensitive hypertension.

**Acknowledgments**

We acknowledge Drs Erika Boesen, Kelly Hyndman, and Daisuke Nakano for their useful advice.

**Sources of Funding**

This work was supported by the National Institutes of Health (grants HL60653, HL69999, and HL95499), a grant from the Cardiovascular Discovery Institute of the Georgia Health Sciences University, and an American Heart Association predoctoral fellowship (to W.K.).

**Disclosures**

D.M.P. has provided advice on endothelin receptor antagonists to Abbott Laboratories, and Speedel Pharmaceuticals, Inc.

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_Hypertension_. published online June 6, 2011;
_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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http://hyper.ahajournals.org/content/early/2011/06/06/HYPERTENSIONAHA.111.172734

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Sex Differences in Renal Medullary Endothelin Receptor Function in Angiotensin II Hypertensive Rats

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Detailed methods

Animals and osmotic minipump implantation

All experiments used male and female Sprague-Dawley rats (6-8 wk old) from Harlan Laboratories (Indianapolis, IN) in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved and monitored by the Georgia Health Science University Committee for Animal Use in Research and Education. Animals were housed under conditions of constant temperature and humidity and exposed to a 12:12-h light-dark cycle. All rats were given free access to regular rat chow and tap water. After 1 wk acclimatization, rats received angiotensin (Ang) II (American Peptide, Inc., Sunnyvale, CA) at a rate 260 ng/kg/min or vehicle (saline) subcutaneously via osmotic mini-pump (Alzet model 2002, DURECT, Cupertino, CA) for 2 wk as previously described.1, 2

Surgical preparation and intramedullary infusion of ET receptor agonist or antagonist in anesthetized rats

After 2 wk of vehicle or Ang II infusion, rats were anesthetized with inactin (100 mg/kg i.p.) and placed on a servo-controlled heating table to maintain rectal temperature constant at 37°C. Surgery was performed as previously described, with minor modifications 3. The jugular vein was catheterized for infusion of PBS (0.9% NaCl) containing 4% BSA at a rate of 30 µl/min and changed to 15 µl/min after surgery to maintain euvoeemia. A midline incision was made and a catheter inserted 5 mm in the left kidney to infused saline (0.9% NaCl) at 0.5 ml/h during baseline period. Then, saline or drugs; ET\textsubscript{B} receptor agonist sarafotoxin 6c (S6c; 0.45 µg/kg/min), ET-1 (0.45 µg/kg/min), or BQ-123 (selective ET\textsubscript{A} antagonist; 4nmol/kg/min); was directly in the renal medulla for 80 min. From preliminary data, the dose of BQ123 at 4 nmol/kg/min inhibited the reduction of medullary blood flow in male rats during intramedullary ET-1 infusion, suggesting the completeness of blocking ET\textsubscript{A} receptor (data not shown). Medullary blood flow was measured by single-fiber, laser Doppler flowmetry 3-5. Urine was collected separately from the ureter of each kidney. Urine collection protocols were started after a 60-min equilibration period. Urine collection for the first two 20-min period was collected in which the last 20-min period was used as baseline excretion. Then, drugs were infused for four-20 min period in which the last 20-min period was reported as experimental period. At the end of each experiment, the kidneys were dissected to ensure the catheter was in the appropriate position within the renal medulla.

Glomerular filtration rate in anesthetized rats

In a subset of animals, glomerular filtration rate (GFR) was determined by plasma clearance of fluorescein isothiocyanate (FITC)-inulin. Surgery was performed as described above. FITC-inulin (20 mg/ml; sigma) dissolved in 4% BSA in PBS (0.9% saline) was infused intravenously at 10 µl/min for 60 min before collected baseline excretion and continued throughout the experiment. Saline or S6c (0.45 µg/kg/h) was infused in to renal medulla of male vehicle or Ang II hypertensive rats. Blood samples were taken at the midpoint of each urine collection period. Collected samples were diluted and the fluorescence signal was measured with a micro-plate reader.

Tail-cuff plethysmography
Systolic blood pressure (SBP) was measured during baseline and day 14 of vehicle and Ang II infusion by tail-cuff plethysmography. The average of four to six independent reading of SBP from each animal was reported.

**Quantitative Real-time PCR**

Tissue mRNA was isolated from renal inner medulla of male and female rats received vehicle or Ang II infusion according to the procedure described by the manufacturer (RNase Plus Mini kit, Qiagen, Valencia, CA). RNA (1 µg) was reverse transcribed (QuantiTect RT kit, Qiagen, Valencia, CA). The resulting cDNA was mixed with commercial rat GAPDH, ET<sub>A</sub> and ET<sub>B</sub> receptor primer (Qiagen, Valencia, CA), as well as SYBR green to quantify the relative content of mRNA by real-time PCR (iCycler real-time PCR detection system, Bio-Rad). Fluorescence data were reported in each cycle to determine the cycle threshold (C<sub>T</sub>) values. ET<sub>A</sub> or ET<sub>B</sub> relative to GAPDH mRNA expression was calculated as the change (ΔC<sub>T</sub>) in between C<sub>T</sub> of target gene and C<sub>T</sub> of GAPDH. To calculate –(ΔΔC<sub>T</sub>), the expression in individual animals in all groups was also normalized to the average expression in the male vehicle group. The relative fold expression was calculated as $2^{-\Delta\Delta C_T}$.

**Receptor-binding assay**

Another group of vehicle or Ang II-infused rats in both males and females was used to determine the amount of ET<sub>A</sub> and ET<sub>B</sub> receptors in renal medulla. After 2 wk of infusion, renal inner medulla was harvested and frozen at -80°C. As previously described with minor modification, inner medullary tissues were homogenized and centrifuged in buffer to obtain the membrane fraction. [125I]-ET1 (Perkin Elmer, MA) at the final concentration of 0.02-1 nM or [125I]-ET3 (Perkin Elmer, MA) at the final concentration of 0.06-1.5 nM was added to each well containing membrane preparation, binding buffer, and wheat germ agglutinin polyvinyltoluene beads (Perkin Elmer, MA). Unlabeled ET-1 or ET-3 (American peptide, Sunnyvale, CA) at final concentration of 10 µM was used to determine nonspecific binding. All measurements were performed in duplicate. The maximal binding capacity (B<sub>max</sub>) and the dissociation constant (K<sub>d</sub>) were determined by Scatchard analysis (Prism, GraphPad Software, San Diego, CA). [125I]ET-3 binding was used to determine the B<sub>max</sub> values for ET<sub>B</sub> receptor expression. To determine ET<sub>A</sub> receptor number, the B<sub>max</sub> value for [125I]ET-3 binding was subtracted from the B<sub>max</sub> value for [125I]ET-1 binding. K<sub>d</sub> was determined by receptor binding affinity.

**References**


**Table S1.** Systolic blood pressure of male and female rats before and after 14 day of vehicle and Ang II infusion; n=4-6 rats/group). *p<0.05 vs. day 0 in the same group, †p<0.05 vs. Female in the same treatmet on day 14.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 14</th>
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<td>Ang II</td>
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<td>213±6†</td>
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<td>121±6</td>
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<tr>
<td>Ang II</td>
<td>112±4</td>
<td>157±7*</td>
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**Figure S1.** Medullary blood flow during baseline and medullary infusion of saline or S6c in male (A) and female (B) rats that received vehicle or Ang II infusion; n = 5-7 rats/group.
Figure S2. Effect of renal medullary infusion of saline (A) or S6c (B) on glomerular filtration rate in male vehicle and Ang II-treated rats; $n = 5-6$ rats/group.

Figure S3. Medullary blood flow during baseline and medullary infusion of saline or ET-1 alone or ET-1 with BQ-123 in female vehicle (A) and Ang II (B) infused rats; $n = 6-7$ rats/group.
Figure S4. Specific binding of $[^{125}\text{I}]$ET-1 and $[^{125}\text{I}]$ET-3 to membrane preparations from renal inner medulla of vehicle and Ang II-treated groups in both male and female rats. Insets show Scatchard analysis of $[^{125}\text{I}]$ET-1 and $[^{125}\text{I}]$ET-3 binding curve. Values are means of non-specific binding subtracted from total binding ± SE; $n = 3-4$ rats/group.