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Endothelin B receptors contribute to sex differences in blood pressure elevation in angiotensin II hypertensive rats on a high salt diet

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Summary

1. Female rats are more resistant to blood pressure increases induced by high salt (HS) intake or angiotensin (Ang) II infusion. Since endothelin B (ET_B) receptors on endothelial and epithelial cells mediate tonic vasodilation and sodium excretion, we hypothesized that ET_B receptors limit the hypertensive response and renal injury induced by HS diet alone or with chronic Ang II infusion (Ang II/HS) in female rats compared to males.
2. A 4-week HS diet (4% NaCl) did not significantly change blood pressure (measured by telemetry) in either male or female Sprague-Dawley rats. Administration of the ET_B receptor antagonist, A-192621, during HS feeding caused a dose-dependent elevation of blood pressure in both sexes.
3. In Ang II/HS rats, males had a larger increase in blood pressure compared to females. The increase in blood pressure produced by ET_B receptor blockade in male Ang II/HS rats was not statistically significant. However, A-192621 treatment resulted in a significant further increase in blood pressure in female Ang II/HS rats. Male rats had consistently higher protein excretion rates before and during Ang II/HS, but this was not significantly affected by ET_B receptor blockade in either sex.
4. In conclusion, ET_B receptors play a significantly greater beneficial role in protecting female versus male rats against Ang II-induced hypertension and may contribute to the sex differences of Ang II-induced hypertension.

Keywords

ET_B receptor; angiotensin II; high salt diet; sex; blood pressure; renal injury

Introduction

Premenopausal women have a lower incidence of hypertension and renal injury compared to men at the same age.¹ Women are also less sensitive to salt-induced blood pressure elevation prior to menopause.^{2,3} Similarly, many animal models exhibit a salt-sensitive phenotype that is sex-dependent, including angiotensin (Ang) II hypertension,^{4–6} and deoxycorticosterone

acetate (DOCA)-salt;^{7,8} however, the underlying mechanism of why females display less severe hypertension than males is still unknown.

The endothelin (ET) system plays an important role in cardiovascular and renal function, ultimately playing a large part in blood pressure control. The physiological effects of ET-1 are dependent on the activation and counterbalance of two receptor subtypes, ET_A and ET_B. The stimulation of ET_A receptors on vascular smooth muscle cells and glomeruli leads to vasoconstriction and cell injury. In contrast, activation of ET_B receptors located on endothelial and epithelial cells cause vasodilation, diuresis, and natriuresis as well as reducing inflammation.⁹ In animal studies, loss of ET_B receptor function, especially in the collecting duct, causes salt-sensitive hypertension; however, a majority of these studies only demonstrate the protective role of ET_B receptors in regulating blood pressure during a high salt (HS) diet in male animals.^{10,11} Previously, our group showed that female ET_B receptor deficient rats have a larger increase in blood pressure during HS feeding compared to male counterparts,¹² suggesting that the ET_B receptor function plays a larger protective role against salt-induced blood pressure elevation in females.

Ang II has been extensively used in hypertension research. A subpressor dose of Ang II causes a gradually developing hypertension, which mimics human hypertension.¹³ Moreover, the degree of blood pressure elevation by the subpressor dose of Ang II depends on the level of salt intake.¹⁴ Our group demonstrated that the ET system is involved in hypertensive action of Ang II in combination with a HS diet. The pharmacological blockade of ET_B receptors did not further elevate blood pressure in male rats that received chronic Ang II infusion with a high salt diet (Ang II/HS),¹⁵ indicating that systemic ET_B receptor function is impaired in male Ang II hypertensive rats. More recently, we reported the existence of sex differences in Ang II control of ET receptor function in the renal medulla.⁶ The ability of ET_B receptors in the renal medulla to increase in water and sodium excretion was lost in male Ang II hypertensive rats.⁶ In contrast, ET_B-induced water and sodium excretion was preserved in female Ang II hypertensive rats.⁶ Chronic ET_B receptor blockade is normally accompanied by a marked elevation in blood pressure that is magnified by HS intake.¹⁰ Thus we asked the question of whether the preserved ET_B receptor function could protect female rats from blood pressure elevation and renal injury in response to chronic Ang II infusion. Therefore, the aim of this study was to examine sex differences in ET_B receptor function by examining the blood pressure response and renal damage in normotensive or Ang II hypertensive rats in response to a HS diet. We hypothesized that ET_B receptors play a more protective role in female rats by limiting the hypertensive response and renal injury induced by HS diet alone or with chronic Ang II infusion compared to males.

Methods

Animal preparation and experimental design

Male and female Sprague-Dawley rats were obtained from Harlan Laboratories (Indianapolis, IN). Animals 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 NIH guidelines. After 7 weeks of age, rats were implanted with telemetry transmitters as previously described¹⁰ and allowed to recover for at least one week.

After 4 days of baseline mean arterial pressure (MAP) collection, separate groups of male and female rats were switched from a normal salt diet (0.4% NaCl; Harlan, Teklad, Madison, WI) to a HS diet (4% NaCl; Harlan, Teklad, Madison, WI). Rats were also infused

with either vehicle (saline) or Ang II (150 ng/kg/min; Phoenix, CA) subcutaneously via osmotic minipump (Alzet model 2004, DURECT, Cupertino, CA) for 4 weeks. Following one week of vehicle or Ang II, a subset group of rats received the ET_B receptor antagonist, A-192621 (Abbott Laboratories, Abbott Park, IL), at increasing doses of 1, 3 and 10 mg/kg/day with food (each dose given for a period of 7 days). A-192621 has 636-fold selectivity to ET_B receptor ($K_i = 5600$ nM for ET_A and $K_i = 8.8$ for ET_B).^{16,17} The dose of A-192621 was checked and adjusted, if necessary, three times a week by monitoring food intake. Body weight was measured weekly. On the last two days of each week, rats were placed in metabolic cages for 24-h acclimation followed by a 24-h urine collection to monitor water and food intake, as well as excretory parameters.

Assays

Urinary sodium concentration was measured by atomic absorption spectrometry (model AA200, Perkin Elmer, Boston, MA). Urinary protein concentration was determined using the Bradford colorimetric method (Bio-Rad Laboratories, Hercules, CA). Urinary and plasma creatinine were measured to determine creatinine clearance by picric acid method.^{18,19} Nephlin concentration was determined in urine via ELISA kit (Exocell Inc., Philadelphia, PA). For tubular injury parameters, urinary concentrations of kidney injury molecule-1 (KIM-1) and netrin-1 were analyzed using ELISA kits from R&D Systems (Minneapolis, MN) and Biotang (Waltham, MA), respectively.

Statistical analysis

24-hr MAP, metabolic parameters, urine flow, urinary sodium excretion, and urinary protein excretion were analyzed by repeated-measures ANOVA with two between factors (sex and treatment with ET_B receptor antagonist) and one within factor (week of treatment). The Bayesian Information Criterion (BIC) was used to select the covariance structure that best described the inter-correlations among the weekly observations. Analysis of covariance was used to adjust for baseline values of the dependent variables. Bonferroni-adjusted post hoc tests were used to compare male vs. female rats, as well as to compare rats on a HS diet with or without an ET_B receptor antagonist. Separate analyses were performed for rats on vehicle and for Ang II-treated rats. Urinary concentrations of nephlin, netrin-1 and KIM-1, as well as creatinine clearance, were analyzed by two-way ANOVA followed by Bonferroni post hoc tests. In the presence of significant interactions in the repeated measures ANOVA or two-way ANOVA, comparisons between groups were made using appropriately defined contrasts based on simple effects with a Bonferroni adjustment. Results were expressed as mean $\pm$ SE and results with $p < 0.05$ were considered statistically significant.

Results

Metabolic characteristics

As shown in Tables. 1 and 2, body weight of male rats was higher than those of female rats at a similar age. For a given sex, rats treated with the ET_B receptor antagonist, A-192621, had similar body weights as untreated groups. A HS diet with or without chronic Ang II infusion did not affect food intake in either male or female rats compared to the respective baseline period. As expected, water intake was significantly increased in groups receiving a HS diet. Similarly, urine flow and urinary sodium excretion were increased in both sexes in response to HS feeding compared to a normal salt diet during the baseline period. When normalized by 100 g body weight, food and water intake, urine flow and urinary sodium excretion were comparable between male and female rats that received HS diet or AngII/HS. While neither food intake or urinary sodium excretion was influenced by A-192621 treatment in HS treated rats of both sexes, water intake and urine volume were significantly higher in females as compared to males after A-192621 treatment ($p < 0.01$). The reason for

this sex difference is not readily apparent. Administration of A-192621 did not affect food and water consumption, urine flow or urinary sodium excretion in Ang/HS group.

ET_B blockade in male and female normotensive rats on a high salt diet

The interaction between diet and time period was statistically significant after adjusting for baseline ($p < 0.0001$), so simple effects were analyzed. HS diet treatment for 4 weeks did not change blood pressure in either male (Fig. 1a) or female rats (Fig. 1b). Incremental increases in the dose of ET_B receptor antagonist A-192621 caused a dose-dependent elevation in 24-hr MAP in both male and female rats on a HS diet ($p < 0.0001$ for both sexes). The increase in female rats was statistically significant at the doses of 1 (115 ± 2 vs. 111 ± 1 mmHg; $p = 0.0019$), 3 (122 ± 2 vs. 110 ± 2 mmHg; $p < 0.0001$) and 10 mg/kg/d (127 ± 3 vs. 109 ± 2 mmHg; $p < 0.0001$) (Fig. 1b). The elevation in male rats was not different at the dose of 1 mg/kg/d (121 ± 2 vs. 121 ± 2); however, blood pressure was significantly increased at doses of 3 (128 ± 2 vs. 118 ± 2 mmHg; $p = 0.0003$) and 10 mg/kg/d (138 ± 4 vs. 117 ± 2 mmHg; $p < 0.0001$) in male rats (Fig. 1a).

ET_B blockade in male and female rats given chronic Ang II

The interactions between Ang II and time period ($p = 0.0427$) and sex and time period ($p = 0.0362$) were both statistically significant after adjusting for baseline, so simple effects were analyzed. After 4 weeks of Ang II/HS, both male ($p < 0.0001$) and female ($p < 0.0001$) rats had a significant increase in 24-hr MAP (Fig. 2a and 2b). Male rats had significantly higher 24-hr MAP compared to female rats at the end of week 3 of Ang II/HS (170 ± 7 vs. 127 ± 10 mmHg; $p = 0.0137$). The difference in MAP between male and female rats continued to week 4 of Ang/HS ($p < 0.05$). A-192621 did not further elevate 24-hr MAP in male Ang II/HS rats (Fig. 2a). In contrast, the high dose of A-192621, 10 mg/kg/d, significantly increased 24-hr MAP in female Ang II/HS rats compared to female rats receiving Ang II/HS without ET_B blockade (164 ± 7 vs. 138 ± 10 mmHg; $p = 0.0089$; Fig. 2b).

Renal injury following ET_B receptor blockade in male and female rats on a high salt diet

In male rats, a HS diet was accompanied by a time-dependent increase in proteinuria over the 4-week treatment period ($p = 0.0308$; Fig. 1c) and was significantly higher than proteinuria in female rats at a dose of 10 mg/kg/d (61 ± 11 vs. 12 ± 5 mg/d; $p = 0.0137$). In fact, a HS diet had no effect on proteinuria in female rats ($p = 0.6493$; Fig. 1d). Creatinine clearance (C_{Cr}) was comparable between male and female rats on a HS diet (Fig. 3a). There were no significant effects of A-192621 on proteinuria and C_{Cr} in either male or female rats on a HS diet.

Urinary nephrin excretion, a marker of the glomerular injury, was slightly increased in male rats on a HS diet compared to females. Male rats had a significantly higher level of nephrin excretion after A-192621 treatment compared to similarly treated female rats (Fig. 3b). To assess tubulointerstitial injury, the biomarkers KIM-1 and netrin-1 were measured in urine after 4 weeks of a HS diet. Urinary excretions of KIM-1 and netrin-1 were similar between male and female rats (Fig. 3c and 3d). Administration of A-192621 did not affect urinary excretion of KIM-1 and netrin-1 in either sex when rats were on the HS diet alone.

Renal injury following ET_B receptor blockade in male and female rats with chronic Ang II

The interaction between sex and time period was statistically significant after adjusting for baseline ($p = 0.0087$), so simple effects were analyzed. There was no elevation in proteinuria after the first two weeks of Ang II/HS in male rats; however, protein excretion was abruptly increased at week 3 of Ang II/HS and continued to rise until week 4, yielding a significant upward trend ($p = 0.0080$) (Fig. 2c). Ang II/HS treatment in female rats had no effect on

protein excretion and was significantly less than males at week 2 of Ang II/HS (12 ± 3 vs. 58 ± 10 mg/d; $p = 0.0075$). A-192621 did not produce any additional increase in protein excretion in either male or female Ang II/HS rats (Fig. 2c and 2d).

At week 4 of Ang II/HS, C_{Cr} was unchanged in both male and female rats (Fig. 4a). Male Ang II/HS rats excreted more nephrin compared to female Ang II/HS rats. Interestingly, the sex difference in nephrinuria was abolished with ET_B receptor blockade (Fig. 4b). Administration of A-192621 did not affect C_{Cr} and nephrinuria in male rats, but had a tendency to reduce C_{Cr} and elevate nephrin excretion in female rats. In terms of tubular injury, male rats treated with Ang II/HS have a significant increase in urinary KIM-1 excretion compared to females (Fig. 4c). Administration of A-192621 did not significantly change KIM-1 (Fig. 4c) and netrin-1 (Fig. 4d) excretion in either sex. Compared to HS feeding alone, Ang II/HS rats, especially males, had roughly fourfold greater increases in urinary excretion of nephrin and KIM-1.

Discussion

Hypertension and chronic kidney disease are less common in pre-menopausal women than in men of the same age. In most experimental animal models, females are relatively protected from high blood pressure and renal injury compared to males. One possible mechanism for this protection is the intrarenal ET system, which is highly active in rats on a HS diet and/or subjected to chronic Ang II infusion.²⁰ ET_B receptors play an important role in mediating sodium excretion, vasodilation, and reducing inflammation, and loss of ET_B receptor function contributes to the development of hypertension and renal injury in male rats on a HS diet. The current study further elucidates the role of ET_B receptors in blood pressure regulation as well as potential renoprotective effects in female rats in response to salt and chronic Ang II infusion. The major findings of this study are that 1) ET_B receptors are important in blood pressure control in both male and female rats in response to HS feeding, and 2) ET_B receptor antagonism abolishes the sex differences in the blood pressure response to chronic Ang II infusion with a HS diet in the rat. Thus, we provide further evidence that ET_B receptors have a favorable effect on blood pressure control in female rats on a HS diet compared to males during Ang II induced hypertension.

Endothelial ET_B receptors in the renal vasculature, including afferent arterioles and vasa recta of the renal medulla function to promote vasodilation leading to an increase in total renal and medullary blood flow and sodium excretion.^{21,22} Several lines of evidence indicate that the ET_B receptors play a crucial role in regulating vascular and renal function to restore sodium balance during HS intake, and ultimately maintain normal blood pressure.⁹ A HS diet increases the degree of renal vasodilation compared to a normal salt diet.^{21,22} ET_B receptor activation on aortic endothelial cells results in a more pronounced vasodilation in rats on a HS diet compared to a normal salt diet.²³ Moreover, chronic hypertension produced by ET_B receptor blockade is even greater in rats on a HS diet compared to a normal salt diet in male rats.^{10,11,23} In the current study, we have verified previous findings that feeding rats a 4% HS diet does not increase blood pressure in male or female Sprague-Dawley rats. We further demonstrated that the ET_B receptor antagonism causes a dose-dependent increase in blood pressure in both male and female rats on a HS diet, which reaches significance at a dose of 3 and 10 mg/kg of A-192621. Our results support previous evidence that ET_B receptors play a role in sodium regulation and blood pressure control not only male rats, but also females during a HS feeding, suggesting that there is not a sex difference in the ET_B receptor-mediated blood pressure response to a HS diet.

We recently reported that male Ang II hypertensive rats have impaired renal medullary ET_B receptor function. In contrast, female Ang II hypertensive rats have relatively preserved ET_B

receptor function in renal medulla.⁶ We have also reported that ET_B receptor blockade does not increase blood pressure in male Ang II hypertensive rats on 8% NaCl diet consistent with impaired ET_B receptor function.¹⁵ Even though we used 4% NaCl diet in the current study, we confirmed that male Ang II hypertensive rats did not have a significant blood pressure response to ET_B receptor antagonism. Moreover, in contrast to male rats, we observed that female Ang II hypertensive rats have a significant increase in blood pressure when treated with an ET_B receptor antagonist. Taken together, these data suggest that female rats are relatively protected against Ang II-induced hypertension due to maintained renal medullary ET_B receptor function.

Ballew and Fink²⁴ have previously reported that ET_B receptor blockade increased blood pressure in a similar degree in male rats that received a HS diet or Ang II/HS. They also concluded that the protective of ET_B receptors are not influenced by Ang II infusion in male rats. In contrast, the current study showed that ET_B receptor antagonist caused a significantly increase in blood pressure in male rats on a HS diet, but not in male Ang II/HS. Our results suggest that Ang II impairs ET_B receptor function in male rats. Several possible reasons can be explained the different results between these studies: 1) dosage and route of Ang II administration are different. In our study, the Ang II dose was 150 ng/kg/d delivered subcutaneously via osmotic minipump (increased blood pressure by 70 mmHg at week 4 of Ang II infusion); however, Ballew and Fink administered Ang II intravenously at 5 ng/min (increased blood pressure by 10 mmHg after 5 days of Ang II infusion); 2) amount of sodium intake is higher in our study; and 3) dosage of A-192621 was higher in their study (12 mg/kg twice a day by intra-arterial injection) compared to our use of a dose-response approach reaching a maximum of 10 mg/kg/d delivered in the food. With regard to the dose of Ang II, it is possible that a higher dose of Ang II may be required to diminish ET_B receptor function because a HS diet activates ET_B receptor function.¹⁰ It is unlikely that the dose of ET_B antagonist was not sufficient for adequate receptor antagonism in our study because we have previously shown that our highest dose of A-192621 (10 mg/kg/d) is effective at blocking ET_B-mediated hemodynamic and renal responses.²² Furthermore, we have found that 10 mg/kg/d of A-192621 significantly increased blood pressure in both male and female rats on a HS diet.

After 4 weeks of HS feeding, male and female rats did not have an increase in blood pressure; however, male rats had significantly elevated proteinuria compared to female rats. Since proteinuria is an establish marker for renal injury, these results suggest that male rats are more susceptible to renal injury than females during HS feeding. However, the inhibition of ET_B receptors did not further increase proteinuria in either sex despite the higher renal perfusion pressure. These data is inconsistent with a previous report of increased urinary protein excretion in male rats lacking functional ET_B receptors on a HS diet compare to female counterparts.¹² It is possible that the duration of ET_B receptor antagonist treatment may contribute to the different results between pharmacological inhibition for 7 days versus genetic disruption of ET_B receptors extended over the life of the animal.

The role of ET_B receptors in the sexual dimorphism associated with renal injury in Ang II hypertension has not been established. Similarly to other reports,^{25,26} Ang II-induced proteinuria was more pronounced in male rats compared to females, which may be due to higher blood pressure in male rats and the larger increase in pressure associated with Ang II infusion. In contrast, female rats had lower blood pressures during Ang II infusion along with lower protein excretion. ET_B receptor blockade had no effect on blood pressure or protein excretion in male rats consistent with pressure-dependent effects on proteinuria. However, ET_B receptor blockade produced a significant increase in MAP in female rats but no significant increase in protein excretion. These results suggest that ET_B receptors may play a protective role against hypertension in female rats given Ang II/HS. However, the

mechanisms accounting for protein excretion changes are not as clear. From our results, proteinuria tends to increase in both male and female rats when mean arterial pressure of 140 mm Hg or higher is reached. It is possible that a threshold increase in blood pressure achieved male, but not female Ang II hypertensive rats could be an explanation for the sex difference in protein excretion.

Because proteinuria may reflect both glomerular and tubular injury, we further examined urinary excretion of nephrin, KIM-1 and netrin-1 to assess whether glomerular and/or tubular injury has occurred as a result of HS feeding as well as chronic Ang II infusion. Nephrin is a unique molecular component of the podocyte slit diaphragm that can be detected in urine following damage to glomerular epithelial cells.²⁷ Several lines of evidence suggest that increased Ang II activity increases urinary excretion of nephrin in male hypertensive and diabetic animal models.^{28–30} KIM-1 and netrin-1 are tubular membrane proteins, both of which are elevated in kidney diseases and serve as biomarkers of proximal tubular damage.^{31–33} In our studies, rats that received Ang II/HS had a 4–5 fold increase in nephrin and KIM-1 excretion compared to a HS alone, suggesting that chronic Ang II infusion leads to both glomerular and tubular damage. Furthermore, in response to Ang II/HS, males tended to have a higher level of glomerular damage as measured by increased nephrin excretion, compared to female hypertensive rats. It is somewhat surprising that ET_B receptor blockade had no significant effect on any of these measures in either sex with or without Ang II hypertension since we did see further elevations in blood pressure observed in female rats.

The physiological actions of ET system are complex because the two different ET receptors, ET_A and ET_B, act in opposite manners to regulate vascular tone and blood pressure. ET_B receptors mediate vasodilation and increased sodium excretion.^{9,34} It is well recognized that the blockade of ET_B receptors in male rodents on a normal or high salt diet causes an increase of plasma ET-1.^{11,35} Moreover, the blockade of ET_B receptors in animals fed with HS diet causes blood pressure elevation, which is attenuated by ET_A receptor antagonism.¹⁰ These findings suggest that an increase in plasma ET-1 enhances ET_A receptor activation during ET_B receptor blockade. These direct and indirect effects of ET_B receptor blockade contribute to an increase in blood pressure. Previous studies from our laboratory and others have shown that the ET_A receptor is important to maintain high blood pressure in male Ang II hypertensive rats.¹⁵ However, the question whether ET_A receptors are involved in blood pressure elevation in female rats that received a HS diet or Ang II/HS with ET_B receptor antagonist has not been sufficiently investigated.

Several studies show that ET_A receptor activation or high blood pressure causes renal injury in rodents.^{36,37} However, these studies have all been conducted in male rats and so little is known about how the ET_A receptors participate in female animals associated with renal injury. Muller *et al.*³⁸ demonstrated that an ET_A receptor antagonist increased survival rate in male rats after 7 day of renal ischemia; however, ET_A receptor blockade increased mortality in females. This result suggested that ET_A receptors have a protective effect against post-ischemic renal failure in female animals. In the current study, we found that ET_B receptor antagonism, which increased blood pressure by 20 mmHg (130 mmHg at 10 mg/kg/d A-192621 vs. 110 mmHg for the control), tended to reduce urinary excretion of nephrin in female rats fed a HS diet, but did not achieve statistical significance. This trend was absent in female Ang II hypertensive rats. It is possible that an increase in ET_A receptor activation may have a beneficial effect against nephrin loss in female rats on a HS diet. Because blockade of ET_B receptors dramatically increased blood pressure (170 mmHg at 10 mg/kg/d A-192621 vs. 135 mmHg for non-treatment group) in the female Ang II hypertensive rat, it is likely that the high blood pressure may have a larger influence on renal

injury and overcome the protective effect of ET_A receptors. However, additional studies will be necessary to explore this idea further.

Our laboratory previously demonstrated that the activation of ET_A receptors increase glomerular permeability.³⁹ It is likely that elevation of plasma ET-1 during ET_B receptor blockade activates ET_A receptors in the glomeruli leading to an increase in glomerular permeability. However, we failed to observe an increase in proteinuria in rats fed with a HS diet alone or with Ang II infusion that received ET_B receptor antagonist. Several possibilities could explain this finding. First, it has been shown that an increase in glomerular permeability does not always produce an increase in proteinuria, especially if the change in permeability is relatively small because proximal tubules can reuptake a finite amount of filtered protein.⁴⁰ It is possible that treatment with the ET_B receptor antagonist may produce only a small change in glomerular permeability via ET_A dependent pathway. It is well established that elevated blood pressure accounts for a large portion of renal injury in Ang II hypertension exacerbating the ability of changes in glomerular permeability to produce a change in protein excretion.^{37,41} It is possible that high blood pressure, especially in Ang II-treated animals, may have a larger influence on glomerular permeability than ET_A-mediated effects in both sexes.

It is well known that the sexual dimorphism exists in the blood pressure control in both animals and humans. ET_B receptors play a protective role in the control of blood pressure in both male and female rats, especially when salt intake is elevated. This study demonstrated that the sex differences in blood pressure elevation during Ang II/HS may be, at least in part, due to the beneficial effects of ET_B receptors in female rats and the decrease in ET_B receptor function in male rats. We previously found that the sex differences of ET_B receptor function in the renal medulla exist in Ang II hypertension.⁶ For future experiments, it is important to understand the role of ET_B receptor function in the vasculature and central nervous system on blood pressure regulation in both male and female Ang II hypertensive models.

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Figure 1. Effects of A-192621 on 24-hr MAP (a, b) and urinary protein excretion (c, d) in male (a, c) and female (b, d) rats on a high salt diet; n=5–6 rats/group; *p<0.05 vs. high salt feeding alone in the same sex.

Figure 2. Effects of A-192621 on 24-hr MAP (a, b) and urinary protein excretion (c, d) in male (a, c) and female (b, d) rats that received chronic Ang II infusion on a high salt diet; n=5–7 rats/group; *p<0.05 vs. Ang II infusion with high salt feeding alone in the same sex.

Figure 3.

Creatinine clearance (a), urinary excretion of nephrin (b), KIM-1 (c) and netrin-1 (d) in male and female rats after 4 weeks of a high salt diet alone or with a high dose of A-192621; n=5–6 rats/group

Figure 4. Creatinine clearance (a), urinary excretion of nephrin (b), KIM-1 (c) and netrin-1 (d) in male and female rats after 4 weeks of Ang II infusion with a high salt diet alone or with a high dose of A-192621; $n=5-7$ rats/group

Table 1

Effects of ET_B receptor antagonist A-192621 on metabolic characteristics in male and female rats on high salt feeding. Rats were 9 week of age at baseline measurements. After baseline measurement, rats were received a high salt diet for 4 weeks. The subset groups of rats were received A-192621 was administrated at the dose of 1, 3 and 10 mg/kg/d; 7d/dose. All parameters were collected weekly. UV, urine flow; UNaV, urinary sodium excretion; n=5–6 rats/group.

	A-192621 (dose)											
	1	3	10									
Male	High salt					High salt						
	Baseline	wk 1	wk 2	wk3	wk4	Baseline	wk 1	wk 2	wk3	wk4		
	Body weight (g)	255±9	295±10	324±9	341±11	354±6	247±10	292±7	316±6	333±5	345±3	
	Food intake (g/100g BW/d)	9.4±0.5	7.4±0.1	6.5±0.3	6.3±0.3	6.3±0.1	9.7±0.4	7.9±0.3	6.7±0.1	5.7±0.3	6.1±0.2	
	Water intake (ml/100g BW/d)	12.6±0.4	19.7±0.5	17.4±0.8	16.5±1.0	15.3±0.5	13.2±0.6	19.3±0.8	16.8±0.4	15.1±0.7	16.8±0.9	
	UV (ml/100g BW/d)	5.6±9.4	13.4±0.6	12.5±0.6	12.0±0.8	11.2±0.7	5.6±0.2	13.3±0.9	11.9±0.3	11.6±0.4	11.7±0.5	
	UNaV (mmol/100g BW/d)	0.55±0.04	4.20±0.21	3.72±0.12	3.66±0.14	3.32±0.26	0.59±0.06	4.33±0.33	3.77±0.12	3.49±0.17	3.40±0.10	
	Female	Body weight (g)	190±2 *	212±3 *	223±3 *	229±4 *	234±5 *	195±4 *	220±4 *	229±4 *	234±6 *	239±6 *
		Food intake (g/100g BW/d)	9.6±0.7	7.9±05	7.3±0.3	6.6±0.3	6.6±0.4	9.9±0.9	7.8±0.2	7.6±0.6	7.2±0.5	6.8±0.44
		Water intake (ml/100g BW/d)	14.8±1.9	20.4±0.7	18.2±1.3	17.1±0.9	19.4±1.6	16.2±1.1	21.8±1.0	21.2±1.5 *	21.5±1.5 *	21.5±1.5
UV (ml/100g BW/d)		8.2±1.5	14.9±0.8	13.6±1.2	12.9±1.1	14.2±1.7	8.6±0.9	16.6±0.8	17.3±1.5 *	17.4±1.4 *	17.2±1.3 *	
UNaV (mmol/100g BW/d)		0.65±0.04	4.43±0.26	3.86±0.25	3.79±0.18	3.94±0.28	0.58±0.08	4.68±0.11	4.36±0.44	4.39±0.39	4.06±0.24	

* p<0.01 vs. male rats at the same treatment and time period.

Table 2

Effects of ET_B receptor antagonist A-192621 on metabolic characteristics in rats received chronic Ang II infusion with high salt feeding. Rats were 9 week of age at baseline measurements. After baseline measurement, rats were received Ang II infusion (150 ng/kg/min, s.c.) with a high salt diet for 4 weeks. The subset groups of rats were received A-192621 was administrated at the dose of 1, 3 and 10 mg/kg/d; 7d/dose. All parameters were collected weekly. UV, urine flow; UNaV, urinary sodium excretion; n=5–7 rats/group.

	A-192621 (dose)										
	Ang II+High salt						Ang II+High salt				
	Baseline	wk 1	wk 2	wk3	wk4	Baseline	wk 1	wk 2	wk3	wk4	
Male											
Body weight (g)	249±10	285±7	310±8	326±10	332±9	247±19	285±18	310±13	331±14	331±17	
Food intake (g/100g BW/d)	9.2±0.6	7.9±0.4	7.1±0.2	6.2±0.5	5.7±0.3	9.1±0.5	8.2±0.7	7.3±0.5	5.3±0.5	5.0±0.6	
Water intake (ml/100g BW/d)	13.1±1.0	22.0±0.6	19.1±1.8	19.7±2.1	21.0±2.8	13.2±1.5	25.5±1.7	21.8±1.1	18.7±1.2	22.9±4.5	
UV (ml/100g BW/d)	6.6±0.4	17.0±0.4	15.5±0.5	16.7±2.1	17.9±3.0	6.8±1.2	19.5±1.1	16.5±1.1	15.5±1.6	19.2±3.5	
UNaV (mmol/100g BW/d)	0.54±0.10	4.73±0.25	4.11±0.19	3.73±0.18	3.36±0.22	0.57±0.05	4.93±0.14	4.16±0.14	3.43±0.23	3.06±0.28	
Female											
Body weight (g)	189±4 *	220±1 *	224±4 *	233±4 *	241±5 *	186±3 *	211±4 *	223±3 *	228±3 *	229±5 *	
Food intake (g/100g BW/d)	8.1±1.4	6.7±1.2	6.2±1.1	5.8±1.1	6.4±1.1	10.2±0.5	8.7±0.5	7.4±0.4	6.7±0.3	6.8±0.4	
Water intake (ml/100g BW/d)	11.0±2.3	22.1±4.3	18.6±3.4	19.9±4.3	19.2±4.0	16.2±1.5	27.8±1.7	23.0±1.3	24.3±1.3	25.9±1.4	
UV (ml/100g BW/d)	6.6±1.4	16.6±3.4	13.8±2.6	15.7±3.6	15.0±3.3	8.7±0.7	21±1.7	16.7±1.3	19.3±1.2	21.1±1.2	
UNaV (mmol/100g BW/d)	0.49±0.08	3.70±0.68	3.22±0.60	3.78±0.66	3.49±0.66	0.52±0.06	4.63±0.26	3.93±0.22	3.89±0.15	3.56±0.20	

* p<0.01 vs. male rats at the same treatment and time period.