Gender Differences in Pressure-Natriuresis and Renal Autoregulation
Role of the Angiotensin Type 2 Receptor

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Abstract—Sexual dimorphism in arterial pressure regulation has been observed in humans and animal models. The mechanisms underlying this gender difference are not fully known. Previous studies in rats have shown that females excrete more salt than males at a similar arterial pressure. The renin-angiotensin system is a powerful regulator of arterial pressure and body fluid volume. This study examined the role of the angiotensin type 2 receptor (AT2R) in pressure-natriuresis in male and female rats because AT2R expression has been reported to be enhanced in females. Renal function was examined at renal perfusion pressures of 120, 100, and 80 mm Hg in vehicle-treated and AT2R antagonist-treated (PD123319; 1 mg/kg/h) groups. The pressure-natriuresis relationship was gender-dependent such that it was shifted upward in female vs male rats (P<0.001). AT2R blockade modulated the pressure-natriuresis relationship, shifting the curve downward in male (P<0.01) and female (P<0.01) rats to a similar extent. In females, AT2R blockade also reduced the lower end of the autoregulatory range of renal blood flow (P<0.05) and glomerular filtration rate (P<0.01). Subsequently, the renal blood flow response to graded angiotensin II infusion was also measured with and without AT2R blockade. We found that AT2R blockade enhanced the renal vasoconstrictor response to angiotensin II in females but not in males (P<0.05). In conclusion, the AT2R modulates pressure-natriuresis, allowing the same level of sodium to be excreted at a lower arterial pressure in both genders. However, a gender-specific role for the AT2R in renal autoregulation was evident in females, which may be a direct vascular AT2R effect. (Hypertension. 2011;57:00-00.)

Key Words: angiotensin type 2 receptor ■ gender differences ■ hypertension ■ natriuresis ■ renal blood flow ■ sodium

It is well-established that women are protected from cardiovascular and renal disease relative to men before menopause.1 However, the mechanisms responsible for this gender difference are poorly understood, partly because females remain underrepresented in human clinical trials and animal studies.2–3 Evidence suggests that estrogen plays a protective role against cardiovascular disease in women,4 and previous studies have identified gender differences in the activity of the renin-angiotensin system (RAS),1 a major regulator of arterial pressure.

Studies in rodents have also revealed major gender differences in the expression of RAS components and differences in the way males and females respond to stimulation and inhibition of the RAS under physiological and pathophysiological circumstances.5–8 Recently, with the discovery of angiotensin-converting enzyme 2, a depressor axis to the RAS has been identified9 that incorporates the angiotensin type 2 receptor (AT2R), which is upregulated by estrogen.7,10 Previously, we have demonstrated that the vasodepressor RAS pathways are enhanced in females and that the AT2R has a depressor influence on the response to chronic angiotensin II (Ang II) infusion in female but not in male rats.5 Estrogen also has been shown to protect against Ang II-induced hypertension in female mice, suggesting the importance of the interaction between estrogen and the RAS in this response in females.6,11 Thus, the AT2R appears to play a role in countering the pressor actions of Ang II at the angiotensin type 1 receptor (AT1R) in females via an estrogen-dependent mechanism and may contribute to gender differences in arterial pressure control. However, we know little about how these effects on arterial pressure are mediated.

The kidney plays an integral role in the regulation of arterial pressure through the mechanism of pressure-natriuresis,12 which has been shown to be modulated by the RAS.13 Furthermore, compared to their male counterparts, female rats demonstrate a leftward shift in the pressure-natriuresis relationship such that they excrete the same amount of sodium as males at a lower arterial pressure.14 The
mechanisms underlying this gender difference in sodium excretion are not fully known. However, the AT\textsubscript{2}R has been implicated as a modulator of natriuresis.\textsuperscript{15,16} Thus, it is plausible that the AT\textsubscript{2}R, which is upregulated in females,\textsuperscript{5,7} may modulate the pressure-natriuresis relationship, contributing to gender-associated differences in blood pressure regulation.

We hypothesized that the leftward shift in the pressure-natriuresis relationship that previously has been observed in females as compared to males is attributable to an AT\textsubscript{2}R-mediated mechanism that enables females to excrete the same level of sodium as males at a lower arterial pressure. Therefore, in this study we examined the role of the AT\textsubscript{2}R antagonist PD123319 in females as compared to males, we also examined the renal blood flow (RBF) response to increasing doses of Ang II in the presence and absence of AT\textsubscript{2}R blockade in male and female rats. It was hypothesized that the AT\textsubscript{2}R would modulate the renal vascular response to Ang II infusion in female but not male rats.

Subjects and Methods

Animals

Male and female Sprague-Dawley rats from the Animal Resources Centre (Perth, Western Australia, Australia) were fed a sodium-controlled diet (0.25\% sodium chloride; Specialty Feeds) and received water ad libitum. The rats were housed at 21°C with a 12-hour light/dark cycle and allowed 1 week to acclimatize. Experiments were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Monash University School of Biomedical Sciences Animal Ethics Committee.

Surgical Procedure

The 11- to 12-week-old rats were anesthetized with Inactin (150 mg/kg; thiobutabarbital; Sigma Aldrich) and body temperature was servo-controlled at 37.5°C (DigiSense, Cole Parmer). After fructoseostomy, the left jugular vein was catheterized to deliver maintenance fluids (2\% bovine serum albumin at 4 mL/100 g/h for 30 minutes and then 0.7 mL/h/g; Sigma Aldrich) and tritiated inulin (1.2 μCi/mL at 1 mL/h; Sigma Aldrich) for measurement of glomerular filtration rate (GFR). The left kidney was exposed, placed in a holder, and denervated. An adjustable clamp was positioned around the abdominal aorta above the left renal artery and a loose ligature was placed around the celiac and mesenteric arteries. The left ureter was catheterized for urine collection. A transit-time ultrasound flow probe (0.7 VB; Transonic Systems) was placed around the renal artery for measurement of RBF. Arterial pressure above the level of the aortic clamp (mean arterial pressure [MAP]) and arterial pressure below the level of the aortic clamp (RPP) were continuously measured via catheters placed in the left carotid artery and lower aorta via the right femoral artery, respectively.

Protocol 1: The Effect of AT\textsubscript{2}R Blockade on Pressure-Natriuresis

To examine the effect of AT\textsubscript{2}R blockade on pressure-natriuresis, the AT\textsubscript{2}R antagonist PD123319 (1 mg/kg bolus plus 1 mg/kg/h; Sigma Aldrich) or vehicle (0.9\% saline; 1 mL bolus plus 1 mL/h) was infused intravenously for the duration of the experiment. After a 30-minute equilibration period, RPP was increased to 120 mm Hg by tightening the ligatures around the celiac and mesenteric arteries and then decreased to 100 mm Hg and 80 mm Hg by tightening the aortic clamp above the left renal artery. At each RPP, after a 10-minute equilibration period, urine was collected during a 20-minute clearance period and MAP, RPP, and RBF were recorded. Arterial blood was collected at the end of the period. Urinary and plasma sodium concentrations were measured using a RapidChem 744 Electrolyte Analyser (Bayer Australia Limited). Plasma renin activity (PRA) was measured via radioimmunoassay, as previously described.\textsuperscript{17} At the end of the experiment, the left kidney was weighed. The stage of the estrus cycle of each female rat was also identified by a vaginal smear test and uterine weights were measured at the completion of the experiment.

Protocol 2: Contribution of the AT\textsubscript{2}R to the RBF Response to Graded Ang II Infusion

In a second cohort of male and female rats, the RBF response to graded doses of Ang II was measured during vehicle or AT\textsubscript{2}R blockade treatment. After a 60-minute equilibration period, PD123319 or vehicle was administered intravenously, as described. After 30 minutes, a series of intravenous infusions of Ang II (0, 30, 100, 300, and 1000 ng/kg/min followed by a recovery period) commenced. Each dose was infused for 10 minutes, allowing arterial pressure to equilibrate, and RPP was held at basal levels via manipulation of the aortic clamp. Measurements of RBF were then obtained for the next 5 minutes. At the end of the experiment the left kidney was weighed.

Statistical Analysis

Data are expressed as mean±SEM. For experiments in protocol 1, ANOVA was used to compare differences in physiological parameters. To compare differences in pressure-natriuresis and renal autoregulation between the vehicle-treated males and females and between the treatment groups, data were analyzed using an analysis of covariance. Standard unpaired Student \( t \) tests were used for group comparisons at equivalent RPP. Plasma renin activity data were analyzed using repeated-measures ANOVA. In protocol 2, for comparisons between treatment groups, data were analyzed using repeated-measures ANOVA. Statistical significance was accepted as \( P<0.05 \).

Results

Protocol 1: Physiological Parameters

Body weight and left kidney wet weight were significantly less in the female vehicle-treated (body weight, 270±11 grams; left kidney wet weight, 0.93±0.04 grams) and AT\textsubscript{2}R antagonist-treated (body weight, 266±8 grams; left kidney wet weight, 0.86±0.03 grams) groups vs the male vehicle-treated (body weight, 421±12 grams; left kidney wet weight, 1.43±0.07 grams) and AT\textsubscript{2}R antagonist-treated (body weight, 452±9 grams; left kidney wet weight, 1.50±0.08 grams) groups (\( P_{\text{gender}}<0.0001 \)). There were no significant differences in basal MAP or basal hematocrit between the male vehicle-treated (MAP, 121±2 mm Hg; hematocrit, 43%±2%), male AT\textsubscript{2}R antagonist-treated (MAP, 117±4 mm Hg; hematocrit, 41%±2%), female vehicle-treated (MAP, 116±4 mm Hg; hematocrit, 40%±1%), and female AT\textsubscript{2}R antagonist-treated (MAP, 114±4 mm Hg; hematocrit, 40%±1%) groups. Uterine weight was not significantly different between the female vehicle-treated (0.59±0.10 grams) and AT\textsubscript{2}R antagonist-treated (0.51±0.05 grams) groups. The female vehicle-treated and AT\textsubscript{2}R antagonist-treated groups each consisted of rats in estrus and anestrus, in equal proportion.

Influence of Gender on Pressure-Natriuresis and Renal Autoregulation

There was an increase in urine flow (UF; \( P_{\text{RBF}}<0.0001 \); Figure 1A) and urinary sodium excretion (\( U_{\text{Na}^+} \);
$P_{RPP<0.0001}$; Figure 1B) from low to high RPP in both the male and female vehicle-treated rats. Gender had a significant effect on the pressure-natriuresis-diuresis relationship such that both UF and $U_{Na^+V}$ were significantly greater in the female group at equivalent levels of RPP ($P_{gender<0.001}$).

In both genders, there were slight increases in RBF ($P_{RPP<0.002}$; Figure 1C), GFR ($P_{RPP<0.02}$; Figure 1D), and renal vascular resistance (RVR; $P_{RPP<0.03}$; data not shown) with increasing RPP. However, RBF, GFR, and RVR were not significantly different between the male and female vehicle-treated rats.

**Influence of AT$_2$R Blockade on Pressure-Natriuresis and Renal Autoregulation**

In males, UF ($P_{RPP<0.01}$; Figure 2A), $U_{Na^+V}$ ($P_{RPP<0.006}$; Figure 2B), and fractional sodium excretion (FE$_{Na^+}$; $P_{RPP<0.02}$; Figure 2C) were significantly less after PD123319 than vehicle treatment. Similarly, in females, UF ($P_{RPP<0.005}$; Figure 2F) and $U_{Na^+V}$ ($P_{RPP<0.008}$; Figure 2G) were significantly less after PD123319 than vehicle treatment. However, FE$_{Na^+}$ was not significantly altered by PD123319 treatment in females (Figure 2H). The pressure-natriuresis and pressure-diuresis relationships thus were significantly shifted downward in both male and female AT$_2$R antagonist-treated rats. The magnitude of this effect did not differ between the genders ($P_{gender*RPP>0.05}$).

Between the male vehicle-treated and AT$_2$R antagonist-treated rats, no significant difference in either RBF (Figure 2D) or GFR (Figure 2E) was observed at any given RPP. RVR was also similar between the male groups at each RPP (data not shown). In contrast to males, however, AT$_2$R blockade had a significant effect on GFR in the female rats ($P_{RPP<0.04}$; Figure 2J). This effect was particularly evident at 80 mm Hg ($P<0.01$), resulting in a lesser GFR in females receiving PD123319. Similarly, RBF was also significantly less in the female AT$_2$R antagonist-treated rats at 80 mm Hg compared to the vehicle-treated rats ($P<0.05$; Figure 2I). This loss of autoregulation at lower RPP in the female AT$_2$R-antagonist-treated rats was also evidenced by increased RVR at 80 mm Hg (data not shown).

From low to high RPP, PRA decreased in all 4 groups (Figure 3). Notably, treatment with PD123319 had a significant effect on PRA such that in the male and female AT$_2$R antagonist-treated rats, PRA was considerably greater than in the vehicle-treated rats ($P_{treat<0.02}$).

**Protocol 2: Contribution of the AT$_2$R to the Response to Graded Ang II Infusion**

Graded intravenous infusion of Ang II was accompanied by a dose-dependent increase in MAP in all groups ($P_{Ang II<0.0001}$; Figure 4A, 4D). No statistically significant differences were observed in the MAP response to the graded Ang II infusion between the treatment groups in either the males or the females. RPP was maintained near baseline levels by adjustment of the suprarenal aortic clamp and did not differ significantly between the groups (Figure 4B, 4E).

Baseline RBF was similar between the male vehicle-treated and AT$_2$R antagonist-treated groups (3.5±0.2 and 3.9±0.4 mL/min/g kidney weight, respectively). In male vehicle-treated and AT$_2$R antagonist-treated rats, Ang II infusion was accompanied by a dose-dependent decrease in RBF (by 67%±6% and 69%±2% at 1000 ng/kg/min Ang II, respectively; $P_{Ang II<0.0001}$; Figure 4C), which was not significantly influenced by PD123319 treatment. Similar to that in males, baseline RBF did not differ significantly between the female vehicle-treated and AT$_2$R antagonist-treated groups (3.8±0.5 and 3.5±0.6 mL/min/g kidney weight, respectively). As in males, Ang II dose-dependently reduced RBF in females ($P_{Ang II<0.0001}$; Figure 4F). However, in the presence of AT$_2$R blockade, RBF was reduced to a greater extent in response to Ang II (by 82%±5% at 1000 ng/kg/min Ang II compared to 61%±2% in female vehicle-treated rats; $P_{treat<0.02}$).

**Discussion**

There were 2 major findings in the present study. First, AT$_2$R blockade was found to blunt pressure-natriuresis in both
Figure 2 (Continued).
A major novel finding in this study was that AT$_2$R blockade blunted the pressure-natriuresis relationship in both males and females to a similar extent. In male and female rats, AT$_2$R blockade using the specific antagonist, PD123319, resulted in a significant rightward shift in the pressure-natriuresis relationship. To the best of our knowledge, this is the first study that has examined the effect of AT$_2$R blockade on pressure-natriuresis in female rats. Our findings in male rats corroborate earlier findings from experiments performed in male AT$_2$R knockout mice and their wild-type littermates. Gross et al$^{13}$ found that wild-type mice excreted 3-fold more sodium and water than AT$_2$R knockout mice at similar perfusion pressure, providing evidence for a significant rightward shift in the pressure-natriuresis relationship in the AT$_2$R knockout group. In another study, Siragy et al$^{16}$ demonstrated that the absence of the AT$_2$R in AT$_2$R knockout mice led to sustained sodium retention and hypertension in response to exogenous Ang II infusion compared to wild-type control mice, highlighting the protective role of the AT$_2$R against the antinatriuretic and pressor actions of Ang II.

Several previous studies have adopted pharmacological blockade or stimulation of the AT$_2$R to investigate its contribution to the regulation of pressure-natriuresis in male rats but have produced conflicting results. For example, Lo et al$^{20}$ previously reported that in the presence of AT$_2$R blockade with PD123319, the pressure-natriuresis relationship was shifted leftward, thereby promoting sodium and water excretion. Conversely, pressure-natriuresis was blunted in rats treated with the AT$_2$R agonist, CGP 42112B.$^{20}$ Similar results were found in a later study by these investigators using an alternative AT$_2$R agonist, T2-(Ang II 4-8).$^{12}$ The effects of this agent were reversed by PD123319 treatment.$^{21}$ One possible explanation for the disparity in these findings relates to experimental conditions. In particular, these latter studies were each performed in the presence of set levels of sodium and water-retaining hormones, ie, vasopressin, norepinephrine, aldosterone, and cortisol, to characterize the pressure-natriuresis relationship under controlled neural and hormonal conditions. Gross et al$^{15}$ have shown that application of this “neurohumoral clamp” has a major impact on the pressure-natriuresis relationship. We did not include this “hormone cocktail” in our current study because it would have prevented examination of the gender differences in pressure-natriuresis by suppressing the release of hormones possibly responsible for these differences.

In terms of the mechanisms that mediate pressure-natriuresis, our understanding is currently incomplete, as reviewed extensively elsewhere.$^{12}$ Briefly, however, it has been proposed that pressure-natriuresis may be associated with increased renal interstitial hydrostatic pressure, which has been linked to endothelial nitric oxide (NO)-induced increases in medullary blood flow.$^{12}$ Increased renal interstitial hydrostatic pressure, along with direct effects of NO on tubular transport,$^{23}$ inhibits sodium reabsorption. There is strong evidence that the effects of NO are mediated by extracellular renal interstitial cyclic GMP.$^{24,25}$ Given that the AT$_2$R functions via an NO-mediated pathway to produce cyclic GMP and increase sodium excretion and vasodila-
Figure 4. Effects of graded angiotensin (Ang) II infusion on mean arterial pressure (MAP), renal perfusion pressure (RPP), and renal blood flow (RBF) in male (A–C) and female (D–F) vehicle-treated (closed symbols) and AT2R antagonist-treated rats (open symbols) rats. Data are presented as mean ± SEM and were analyzed using a repeated-measures ANOVA (for MAP and RPP from Ang II 0–1000 ng/kg/min and for RBF from Ang II 100–1000 ng/kg/min) using the factors treatment (P_treat), Ang II (P_Ang II), and the interaction between treatment and Ang II (P_treat*Ang II). n = 6 to 7 per group. RBF values are expressed per gram of left wet kidney weight.

...and GFR at low RPP in females but not in males. We believe that this finding may be related to RAS activation and reflects a role for the AT2R to blunt AT1R-mediated vasoconstriction by endogenous Ang II in response to acute hypotension in females, but not in males. This suggestion is supported by our observation that RAS activity was highest at 80 mm Hg, as indicated by PRA. Interestingly, PRA was significantly greater in the AT2R antagonist-treated rats during the pressure-natriuresis experiments relative to the levels obtained from the vehicle-treated group. This may be explained by earlier observations that have provided evidence that the AT1R inhibits renal biosynthesis in the kidney, thereby reducing Ang II formation. Second, in our latter set of experiments, AT1R blockade enhanced the renal vasoconstrictor effects of exogenous Ang II in females but not in males. Specifically, intravenous infusion of Ang II in male and female normotensive rats in the presence and absence of AT2R blockade was accompanied by dose-dependent decreases in RBF in all 4 groups when RPP was not allowed to increase, with the largest decrease by far observed in the female AT2R antagonist-treated rats. Thus, AT1R appear to have gender-dependent effects on renal vascular responsiveness to Ang II. Human studies that have been conducted to determine whether gender differences exist in the renal vascular response to Ang II also have suggested that the AT1R plays a greater role in females than in males. In a study performed by Miller et al.13 that examined the renal response of young healthy subjects to graded Ang II infusion, GFR was maintained in men, whereas in women GFR declined. More recently, Miller et al.14 demonstrated that in the presence of AT1R blockade, women exhibited a significantly blunted RBF response to Ang II compared to men. Sexual dimorphism in the nonmodulation phenotype of hypertension,15 which is characterized by a lowered RBF response to Ang II...
with a high-salt diet, also has been observed. Nonmodulation is less frequent in women than in men, whereas after menopause there is no gender difference.\textsuperscript{35} It has been speculated that activation of the AT\textsubscript{2}R or NO or both could be mediating these effects.\textsuperscript{34,35} Our results are consistent with such a conclusion.

Collectively, our current observations could be explained by greater AT\textsubscript{2}R expression in the female, relative to male, vasculature. Previous studies that have measured the renal expression of angiotensin receptors in rodents have identified AT\textsubscript{2}R expression in the glomeruli, afferent arteriole, arcuate arteries and veins, and medullary descending vasa recta.\textsuperscript{10,36,37} with evidence of greater renal AT\textsubscript{2}R expression in the female renal vasculature.\textsuperscript{37} We and others previously have reported greater expression of the AT\textsubscript{2}R in the kidney of female vs male rats\textsuperscript{5,37} and mice.\textsuperscript{10}

Given this evidence that the AT\textsubscript{2}R is upregulated in females and protects females against Ang II-induced vasoconstriction, the reduction in RBF and GFR at low RPP during AT\textsubscript{2}R blockade that we observed in females could be the consequence of a number of mechanisms. First, we presume that this effect is at least partly attributable to the ability of AT\textsubscript{2}R blockade to enhance AT\textsubscript{1}R-mediated effects of Ang II. This is because in the presence of PD123319, AT\textsubscript{2}R are blocked and at low RPP Ang II production is likely increased, as indicated by increased systemic PRA, possibly leading to increased stimulation of the AT\textsubscript{2}R. Second, it is likely that the effect of AT\textsubscript{2}R blockade in the renal vasculature occurred predominantly in the preglomerular vasculature. This would result in a reduction in RBF and, in turn, a reduction in glomerular capillary pressure, leading to the reduced GFR we observed. Finally, there may have been a reduction in the glomerular capillary ultrafiltration coefficient, also reducing the GFR. Evidence suggests there may be a role for the AT\textsubscript{2}R in the modulation of glomerular capillary ultrafiltration coefficient because it is affected by NO blockade\textsuperscript{48} and enhanced by Ang II.\textsuperscript{39} whereas an AT\textsubscript{2}R-mediated action on podocytes has been shown to contribute to the maintenance of glomerular barrier function.\textsuperscript{40}

Finally, although our studies indicate a role of the AT\textsubscript{2}R in pressure-natriuresis in both males and females, caution must be applied when interpreting these findings because of the acute nature of these studies. Future examination of the involvement of the AT\textsubscript{2}R in the chronic pressure-natriuresis relationship, established by determining the effects of altered dietary salt intake on arterial pressure, are required to further determine the physiological relevance of this work. This would be best-performed using AT\textsubscript{2}R knockout mice via radiotelemetry. It will also be important in future studies to investigate the more subtle contribution of the intrarenal RAS to gender differences in arterial pressure and renal function. Further, a potential confounding factor in our study design that must be considered is estrus cycle, given that the AT\textsubscript{2}R is upregulated by estrogen.\textsuperscript{7,10} However, our female groups consisted of rats that were in each of the different stages of the estrus cycle; when we compared pressure-natriuresis and diuresis relationships between the estrus and anestrous females (n=3–4) in both the vehicle-treated and AT\textsubscript{2}R antagonist-treated groups, no significant differences were observed. Therefore, variations in estrogen levels between our female groups are unlikely to have confounded our results.

In conclusion, our study confirms that the pressure-natriuresis relationship is gender-dependent, such that it was shifted leftward in female rats as compared to male rats. Our results also indicate that the AT\textsubscript{2}R modulates pressure-natriuresis, allowing the same level of sodium to be excreted at a lower pressure. The AT\textsubscript{2}R modulates this relationship in males and females, although a gender-specific role for the AT\textsubscript{2}R in renal autoregulation was evident in females, which may be associated with a vascular AT\textsubscript{2}R effect.

**Perspectives**

Although it is clear that further investigation into the role of the AT\textsubscript{2}R in the sexual dimorphism of pressure-natriuresis is required, evidence is accumulating that the AT\textsubscript{2}R plays an integral role in arterial pressure regulation and in the mechanisms by which females are protected against Ang II-induced vascular alterations and hypertension. The AT\textsubscript{2}R therefore may be a suitable therapeutic target for the treatment of cardiovascular disease and renal disease, particularly in women.

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**Disclosures**

None.

**References**


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