Renin–Angiotensin System

Angiotensin Type 2 Receptor Stimulation Increases Renal Function in Female, but Not Male, Spontaneously Hypertensive Rats

Lucinda M. Hilliard, Charis L.E. Chow, Katrina M. Mirabito, U. Muscha Steckelings, Thomas Unger, Robert E. Widdop, Kate M. Denton

See Editorial Commentary, pp 227–228

Abstract—Accumulating evidence suggests that the protective pathways of the renin–angiotensin system are enhanced in women, including the angiotensin type 2 receptor (AT_2R), which mediates vasodilatory and natriuretic effects. To provide insight into the sex-specific ability of pharmacological AT_2R stimulation to modulate renal function in hypertension, we examined the influence of the AT_2R agonist, compound 21 (100–300 ng/kg per minute), on renal function in 18- to 19-week-old anesthetized male and female spontaneously hypertensive rats. AT_2R stimulation significantly increased renal blood flow in female hypertensive rats ($P_{Treatment}<0.001$), without influencing arterial pressure. For example, at 300 ng/kg per minute of compound 21, renal blood flow increased by 14.3±1.8% from baseline. Furthermore, at 300 ng/kg per minute of compound 21, a significant increase in urinary sodium excretion was observed in female hypertensive rats (+180±59% from baseline; $P<0.05$ versus vehicle-treated rats). This was seen in the absence of any major change in glomerular filtration rate, indicating that the natriuretic effects of AT_2R stimulation were likely the result of altered renal tubular function. Conversely, we did not observe any significant effect of AT_2R stimulation on renal hemodynamic or excretory function in male hypertensive rats. Finally, gene expression studies confirmed greater renal AT_2R expression in female than in male hypertensive rats. Taken together, acute AT_2R stimulation enhanced renal vasodilatation and sodium excretion without concomitant alterations in glomerular filtration rate in female hypertensive rats. Chronic studies of AT_2R agonist therapy on renal function and arterial pressure in hypertensive states are now required to establish the suitability of AT_2R as a therapeutic target for cardiovascular disease, particularly in women. (*Hypertension*. 2014;64:378-383.) ● **Online Data Supplement**

Key Words: hypertension ■ natriuresis ■ receptor, angiotensin, type 2 ■ renal circulation ■ renin–angiotensin system ■ sex characteristics

The development and progression of hypertension differs between men and women. Before menopause, arterial pressure is lower in women than in men of similar age.[1] However, after menopause, this cardioprotection in women is lost, and a higher proportion of women than men have hypertension after the age of 65 years.[1] Similar differences in arterial pressure control have been observed in other mammalian species, including spontaneously hypertensive rats (SHRs).[2,3] These sex-related differences in the development of hypertension have been strongly linked to sexual dimorphism in the renin–angiotensin system (RAS), which plays a pivotal role in the long-term regulation of arterial pressure.[3,4]

 Classically, angiotensin II (AngII) acts via the angiotensin type 1 receptor (AT_1R) to cause vasoconstriction and sodium retention. Activation of the pressor RAS is a key mediator in

the development of hypertension, and drugs that target this system are mainstays of current antihypertensive therapy. More recently, a depressor arm of the RAS has been recognized, which counterbalances the actions of AngII at AT_1R. AngII together with other biologically active angiotensin peptides, including angiotensin (1–7) and angiotensin III, interact with angiotensin type 2 receptor (AT_2R) to evoke vasodilatation and natriuresis. Overwhelming evidence suggests that the depressor RAS is upregulated in women by estrogen, and this contributes to sexual dimorphism in arterial pressure control.[3,4] It is, therefore, plausible that enhancement of the depressor RAS, and in particular the AT_2R, may represent a novel therapeutic target in the treatment of hypertension, particularly in women.

 Our recent data from normotensive rats support the notion that AT_2R plays an integral, yet sexually dimorphic, functional

role in the kidney. Acute systemic blockade of AT$_2$R shifted the pressure–natriuresis relationship rightwards in both female and male rats.[5] Moreover, AT$_2$R blockade blunted the autoregulation of renal blood flow (RBF) and glomerular filtration rate (GFR) at low renal perfusion pressures and enhanced the renal vasoconstrictor response to AngII in female rats only.[5] This led to our conclusion that AT$_2$R modulates sodium excretion in both sexes and may contribute to the mechanisms by which female sex is protected against AngII-induced vascular alterations and hypertension. In addition, we recently demonstrated that acute administration of the selective AT$_2$R agonist, compound 21 (C21), produced significant vasodilatory and natriuretic effects in the kidney of both female and male normotensive rats.[6] The ability of C21 to induce natriuresis was similar between the sexes; however, C21 induced a greater increase in RBF in female rats. Remarkably, these effects were observed in the absence of any AT$_1$R blockade, demonstrating the ability of C21 to directly modulate renal function in male and female normotensive rats.[6]

Despite this evidence for a significant functional role of AT$_2$R in the kidney in male and female normotensives, the sex-specific ability of pharmacological AT$_2$R stimulation to modulate renal function in hypertension remains unknown. Therefore, we aimed to provide further insight into the ability of pharmacological AT$_2$R stimulation using C21 to modulate renal hemodynamic and excretory function in hypertension using the SHR model.

Methods

Eighteen- to 19-week-old SHRs were anesthetized and instrumented for measurement of mean arterial pressure (MAP; carotid catheter) and RBF (transonic flow probe). Renal hemodynamic and excretory function was examined in response to vehicle, graded infusion of C21 (100–300 ng/kg per minute), or C21 combined with the AT$_2$R antagonist PD123319 (1 mg/kg bolus plus 1 mg/kg per hour). GFR was measured via [^{3}H]-inulin clearance. In a separate cohort of rats, renal angiotensin receptor mRNA expression was measured by real-time reverse transcription polymerase chain reaction. Detailed methods are in the online-only Data Supplement.

Results

Basal Measurements

Body weight and left kidney wet weight did not differ significantly between any of the male or female treatment groups (Table). Furthermore, uterine weight was similar for each in the female treatment groups. The female treatment groups also consisted of similar numbers of rats in estrus and anestrus, as identified by vaginal smear.

Basal levels of MAP, RBF, and GFR were not significantly different between the male and female treatment groups. However, although baseline urine flow (UF) and urinary sodium excretion (U$_{Na+}$V) levels did not differ significantly for any in the male treatment group, a difference in baseline UF and U$_{Na+}$V was detected between the female C21-treated and C21 plus PD123319-treated groups (P_{Group}<0.01).

Influence of C21 on Renal Hemodynamic Function

In female and male vehicle-treated, C21-treated, and C21 plus PD123319-treated SHRs, MAP did not change significantly from baseline (Figure 1A and 1D). However, in the male SHR treatment group, we observed a small but significant decrease ($\approx$7–9 mm Hg) in MAP over time (Figure 1D; P_{Time}<0.001).

In female SHRs, no significant change in RBF was observed in response to vehicle treatment. Whereas, in response to C21 infusion, RBF increased significantly from baseline at each dose of C21 administered, as compared with the female vehicle-treated SHRs (Figure 1B; P_{Group}<0.001). There was a trend for dose-dependent increase in RBF in response to C21 in female SHRs (P_{Dose}=0.066). Furthermore, this renal vasodilatory response to C21 in the absence of any change in MAP was reflected by a significant reduction in renal vascular resistance, as compared with female vehicle-treated SHRs (Figure 1C; P_{Group}<0.01). Each of these responses was completely abolished by coinfusion of C21 with the AT$_2$R antagonist, PD123319 (Figure 1C; P_{Group}<0.001 for RBF; P_{Group}<0.01 for renal vascular resistance).

Unlike the female SHRs, no significant change in RBF was observed in response to C21 in male SHRs. Instead, RBF remained close to baseline levels, similar to that observed in the male vehicle-treated group (Figure 1E; P_{Group}>0.05). Accordingly, no significant effect of C21 on renal vascular resistance in male SHRs was observed (Figure 1F).

Influence of C21 on Renal Excretory Function

Renal excretory function was examined during baseline and experimental periods with 300 ng/kg per minute of C21. In

Table.   Baseline Measurements of Body and Organ Weights, Mean Arterial Pressure, and Renal Function in Each Experimental Group

	Female Vehicle	Female C21	Female C21 Plus PD123319	Male Vehicle	Male C21	Male C21 Plus PD123319
BW, g	208±3	211±2	202±5	330±9	343±5	335±6
KW, g	0.66±0.01	0.67±0.01	0.65±0.01	1.1±0.05	1.1±0.03	1.1±0.04
UW, g	0.37±0.01	0.39±0.03	0.37±0.03	…	…	…
MAP, mm Hg	168±3	170±3	176±7	190±6	187±4	188±3
RBF, mL/min per g	4.1±0.4	4.7±0.4	5.4±0.4	4.8±0.4	4.6±0.6	4.6±0.5
GFR, mL/min per g	1.5±0.1	1.4±0.1	1.3±0.07	1.1±0.09	1.2±0.03	1.1±0.07
UF, μL/min per g	26.9±6.6	14.15±5.4*	40.1±5.3	15.3±4.7	23.5±3.4	25.6±5.5
U$_{Na+}$V, μmol/min per g	5.6±1.6	2.5±0.9*	8.2±1.5	3.05±1.1	3.9±0.6	4.7±1.1

Data are presented as mean±SEM and were analyzed using ANOVA with Tukey post hoc test. All RBF, GFR, UF, and U$_{Na+}$V values are expressed per g of KW. BW indicates body weight; C21, compound 21; GFR, glomerular filtration rate; KW, left kidney wet weight; MAP, mean arterial pressure; RBF, renal blood flow; UF, urine flow; U$_{Na+}$V, urinary sodium excretion; and UW, uterine weight.

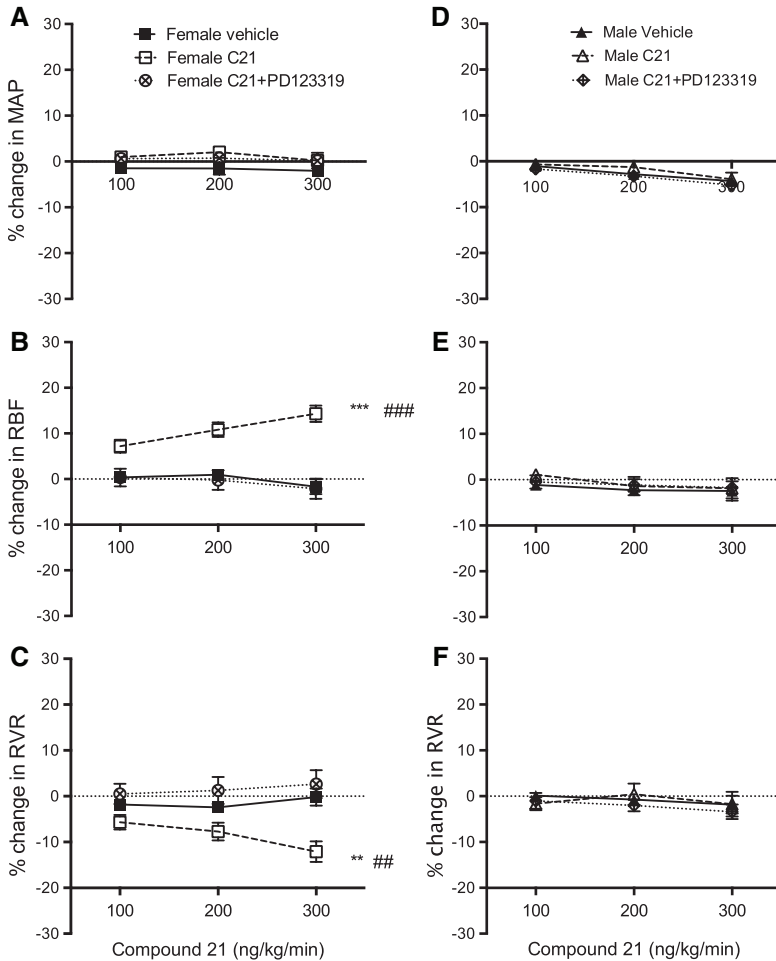*P<0.01 vs female C21 plus PD123319-treated rats; n=6 to 9 per group.

Figure 1. Percent change from baseline for mean arterial pressure (MAP), renal blood flow (RBF), and renal vascular resistance (RVR) in female (**A**–**C**) and male (**D**–**F**) spontaneously hypertensive rats (SHRs) in response to 100 to 300 ng/kg per min of compound 21 (C21). Data are presented as mean±SEM and were analyzed using repeated-measures ANOVA. **$P<0.01$, ***$P<0.001$ vs vehicle-treated SHR; ##$P<0.01$, ###$P<0.001$ vs C21 plus PD123319-treated SHR; n=6 to 9 per group. All RBF and RVR values are expressed per g of left kidney wet weight.

female SHRs, a time-dependent reduction in GFR was observed in response to vehicle treatment. However, a similar reduction in GFR was observed in the female C21-treated group (Figure 2). Overall, this indicates that there was no significant effect of C21 on GFR in female SHRs. Subsequently, there was a greater reduction in filtration fraction in female C21-treated versus vehicle-treated SHRs ($P<0.05$; Figure 2). In contrast, because no significant change in RBF or GFR was observed in response to C21 in male SHRs, no significant difference in filtration fraction was observed between male vehicle-treated and C21-treated SHRs (Figure 2).

Finally, no significant change in UF, $U_{Na+}V$, or fractional sodium excretion (FE_{Na+}) was observed in either female or male SHRs in response to vehicle treatment (Figure 3). However, each of these variables increased significantly in response to C21 in female SHRs (each $P_{Group}<0.05$; Figure 3). Importantly, these responses in female SHRs to C21 were completely abolished by coinfusion of C21 with PD123319. On the contrary, no significant effect of C21 was observed on UF, $U_{Na+}V$, or FE_{Na+} in male SHRs.

Renal angiotensin type 1a receptor, angiotensin type 1b receptor, and AT₂R mRNA Expression

Renal angiotensin type 1a receptor expression was significantly greater (≈1.4-fold) in female SHRs as compared with male SHRs ($P<0.05$; Figure 4A). There was no significant difference in renal angiotensin type 1b receptor expression

between male and female SHRs (Figure 4B). Renal AT_2R expression was also significantly greater (≈4.3-fold) in female SHRs as compared with male SHRs ($P<0.05$; Figure 4C).
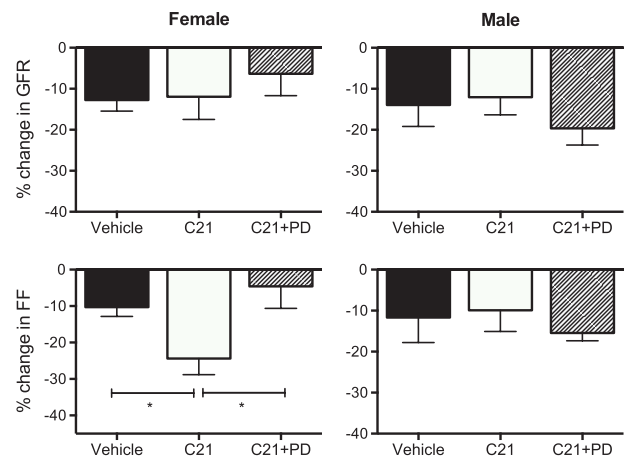

Figure 2. Percent change from baseline for glomerular filtration rate (GFR) and filtration fraction (FF) in female and male spontaneously hypertensive rats (SHRs) in response to 300 ng/kg per min of compound 21 (C21). Data are presented as mean±SEM and were analyzed using an unpaired *t* test to compare differences between vehicle-treated and C21-treated SHR and between C21-treated and C21 plus PD123319-treated SHR; n=6 to 9 per group. All values are expressed per g of left kidney wet weight.
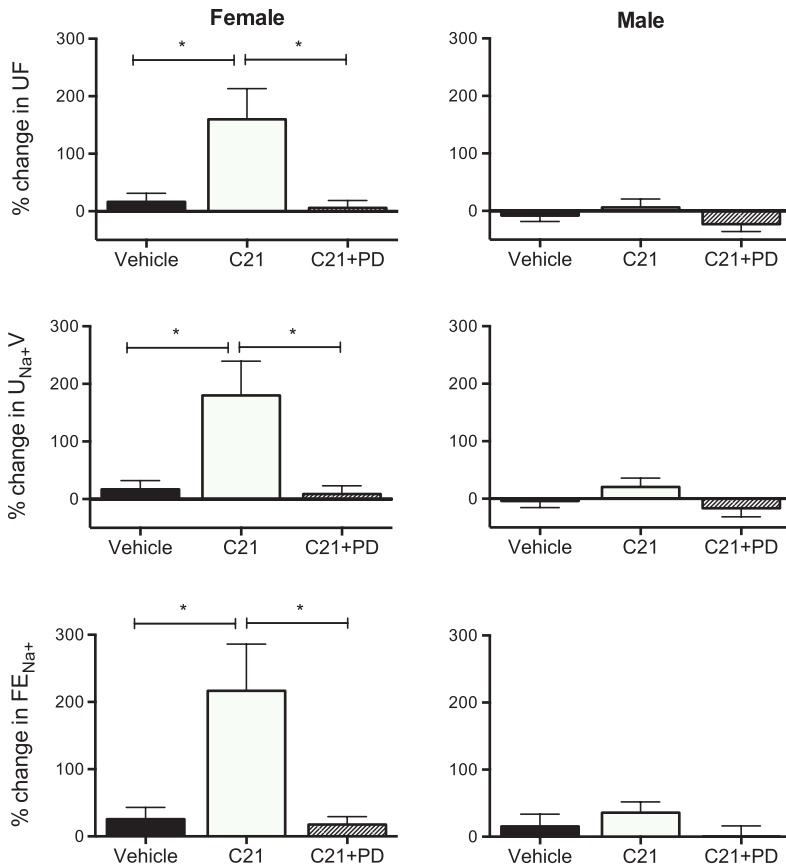
Figure 3. Percent change from baseline for urine flow (UF), sodium excretion (U$_{Na+}$V), and fractional sodium excretion (FE$_{Na+}$) in female and male spontaneously hypertensive rats (SHRs) in response to 300 ng/kg per min of compound 21 (C21). Data are presented as mean±SEM and were analyzed using an unpaired *t* test to compare differences between vehicle-treated and C21-treated SHRs and between C21-treated and C21 plus PD123319-treated SHRs. *$P<0.05$; n=6 to 9 per group. All values are expressed per g of left kidney wet weight.

Discussion

The major findings of this study were that pharmacological stimulation of AT$_2$R induced renal vasodilatory and natriuretic effects in female SHRs. In contrast, no significant renal vasodilatory or natriuretic response to C21 was observed in male SHRs. In addition, we confirmed that renal AT$_2$R mRNA expression was significantly greater in female than in male SHRs. Together, this knowledge provides a strong rationale for subsequent studies to investigate the long-term and sex-specific renal functional effects of pharmacological AT$_2$R stimulation to establish whether AT$_2$R agonist therapy represents a novel therapeutic approach for hypertension, particularly in women.

Given the dominant role of kidneys in the long-term regulation of arterial pressure, the preservation of renal function is a major goal in the identification of novel therapeutic targets for hypertension. In the current study, C21 induced significant renal vasodilatory effects in female SHRs, in the absence of any significant change in arterial pressure. These responses were completely abolished by AT$_2$R blockade, thus confirming the role of AT$_2$R in mediating this response while providing further support for the selectivity of this dose range of C21 for AT$_2$R. The fact that C21 had no significant effect on arterial pressure yet induced renal vasodilatation in female SHRs might reflect greater AT$_2$R expression (or a greater AT$_2$R-to-AT$_1$R ratio) in the renal vasculature as compared with the systemic circulation in female SHRs. Certainly, studies in both humans and experimental models have demonstrated a functional role for AT$_2$R in numerous vascular territories. This includes resistance vessels from the renal, mesenteric, uterine, adrenal, coronary, and peripheral circulations, as well as large capacitance vessels such as the aorta.[7]

This observation of a sex-specific renal vascular response to C21 in the present study is consistent with our previous observations in rodents that AT$_2$R plays a greater functional role in female than in male renal vasculature, providing protection against the vasoconstrictor effects of AngII.[5,6,8] Furthermore, our data support the notion that differences in the renal response to C21 between male and female SHRs is attributable to sex differences in renal AT$_2$R expression. We observed significantly greater basal AT$_2$R mRNA expression in the kidneys of female SHRs as compared with male SHRs, in agreement with the previous findings of Silva-Antonialli et al.[2] In addition, although we observed significantly greater renal angiotensin type 1a receptor expression in female SHRs as compared with their male counterparts, the relative AT$_2$R-to-AT$_1$R ratio was much greater in female than in male SHRs, although such sex differences in basal renal AT$_1$R or AT$_2$R mRNA expression are not always reported.[9] Overall, it is highly plausible that the lack of response to C21 we observed in male SHRs is attributable to significantly lower renal expression of AT$_2$R, as compared with their female counterparts. Subsequently, the dominant AT$_1$R-mediated effects of endogenous AngII may mask any AT$_2$R-mediated vasodilatory responses induced by C21 in male sex. Certainly, we have previously shown in conscious male SHRs that C21 alone administered >4 hours did not elicit any significant effect, at least on arterial pressure. However, when given in combination with the AT$_1$R antagonist, candesartan, C21 lowered arterial pressure in SHRs,[10] which fits with this hypothesis.
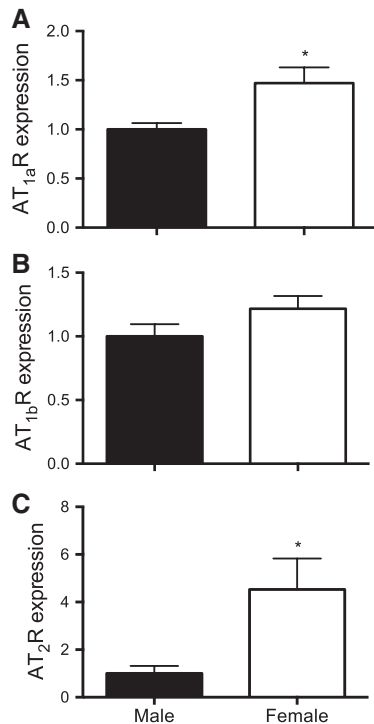
Figure 4. Relative renal mRNA expression of (**A**) angiotensin type 1a receptor ($AT_{1a}R$), (**B**) angiotensin type 1b receptor ($AT_{1b}R$), and (**C**) angiotensin type 2 receptor (AT_2R) in female and male spontaneously hypertensive rats (SHRs). Data are presented as mean±SEM and are expressed relative to the male SHR group; n=7 to 8 per group. Data were analyzed using an unpaired t test. $*P<0.05$ vs male SHRs.

The possibility also exists that high levels of endogenous AngII may result in significant AT_2R stimulation and thus limit an additional vasodilator effect of C21 administration, as suggested recently by Brouwers et al.[11] Similar to the present study, this group did not identify any significant effect of intravenous bolus doses of C21 on RBF in male SHRs,[11] which may reflect the presence of anesthesia, unlike other studies,[10] although AT_2R-mediated vasodilatation occurred in anesthetized female SHRs in the current study. Interestingly, during combined angiotensin-converting-enzyme inhibition with captopril to inhibit the endogenous production of AngII, Brouwers et al[11] reported that C21 induced dose-dependent renal vasodilatation in male SHRs in the absence of any change in arterial pressure, thus supporting their proposition that the inhibition of endogenous AngII is required in male SHRs for AT_2R-mediated vasodilatory responses to be manifest.

In the current study, we also observed significant increases in both UF and $U_{Na+}V$ in female SHRs in response to acute C21 administration. This was observed in the absence of a concomitant increase in GFR, suggesting that AT_2R-mediated natriuresis induced by C21 in female SHRs was more than likely because of changes in the renal tubular reabsorption of sodium, rather than intrarenal hemodynamic changes. This ability of C21 to increase RBF in the absence of any major change in GFR is similar to what we observed previously in male and female normotensive rats.[6] As discussed in this previous study, there are several possible mechanisms that could explain no change in GFR while RBF increased. First, similar vasodilatory effects of C21 at the pre- and postglomerular vessels occurred such that RBF can increase in the absence of any change in GFR. Second, given

the reports that AT_2R expression on podocytes contributes to the maintenance and function of the glomerular filtration barrier,[12] it is also possible that C21 is able to induce the relaxation of podocytes to reduce the surface area available for filtration, thus leading to a reduction in GFR. Subsequently, a reduction in glomerular capillary ultrafiltration coefficient could counteract any AT_2R-mediated increases in glomerular capillary pressure, thus preventing any significant change in GFR.

On the contrary, we did not observe any significant effect of C21 administration on $U_{Na+}V$ in male SHRs. This finding is somewhat surprising given that we and others have previously reported significant natriuretic effects of AT_2R stimulation alone in numerous male rodent models, including normotensive Sprague–Dawley rats, uninephrectomized rats, and obese Zucker rats.[6,13–15] This finding suggests that the lesser renal AT_2R expression we and others have identified in male versus female SHRs may be responsible for the lack of natriuretic response of male SHRs to C21 in the present study.[2] Moreover, renal AT_2R expression is likely lesser in male SHRs as compared with these other male rodent models in which C21-mediated natriuretic effects have been observed. Overall, concomitant blockade of AT_1R or angiotensin-converting enzyme inhibition may, therefore, also be required for AT_2R-mediated natriuresis in male SHRs to manifest and should be addressed in future studies.

Although our studies demonstrate the ability of acute AT_2R stimulation to modulate renal function, at least, in female SHRs, caution should be applied when interpreting these findings given that they were performed in anesthetized and renal denervated animals. Prospective studies should investigate the chronic effects of pharmacological AT_2R stimulation on renal function and arterial pressure control in the hypertensive setting to confirm the physiological significance of this work. In addition, in light of the knowledge that the enhancement of the depressor RAS in women is modulated by estrogen, it will also be important to take into account how aging and age-related changes in sex hormone balance influence the potential of AT_2R as a therapeutic target in female and male sexes. This is of course essential to the clinical translation of this work given that the population most frequently in need of clinical treatment for hypertension and associated disease is the elderly subjects.[4] Certainly, it is highly plausible that the protective role of AT_2R in the female kidneys is lost with age in association with a reduction in estrogen levels, such that menopausal status may be a critical factor when it comes to establishing the suitability of AT_2R as a therapeutic target in women.

It will also be of interest to determine the ability of pharmacological AT_2R stimulation to improve the efficacy and utility of existing RAS-targeted therapies. Certainly, there is significant evidence in literature of a biological cross-talk between the angiotensin receptors. Human and animal studies have shown that AT_2R expression is increased during AT_1R blockade[16–18] and that AT_2R contributes, at least in part, to the antihypertensive effects of angiotensin receptor blockers. For example, Savoia et al[18] reported that peripheral resistance arteries from diabetic hypertensive patients treated with an AT_1R antagonist exhibit enhanced AT_2R expression and associated AT_2R-mediated vasodilatation in response to AngII. In addition, studies have shown that AT_2R activation reduces AT_1R expression and function.[16,19] Taken together, these findings support the postulate that AT_2R stimulation in the

presence of AT$_1$R blockade would likely provide complementary therapeutic benefit and potentiate the long-term antihypertensive effects of existing angiotensin receptor blockers. Moreover, in the context of the present study, it is likely that greater renal vasodilatory and natriuretic effects may be observed in response to C21 treatment against a background of AT$_1$R blockade. As previously mentioned, we have already demonstrated the ability of acute C21 administration to reduce arterial pressure in male SHRs during simultaneous AT$_1$R blockade, and this was observed to a significantly greater extent compared with AT$_1$R blockade alone.[10] However, we are yet to establish the chronic renal functional and antihypertensive effects of this combination therapy and whether or not these effects are enhanced in females and/or males.

Perspectives

In summary, the present study demonstrates that AT$_2$R stimulation enhances renal vasodilatation and natriuresis in female SHRs, without concomitant alterations in GFR. Chronic studies of AT$_2$R agonist therapy on renal function and arterial pressure in hypertensive states are now required to establish whether pharmacological stimulation of AT$_2$R with highly selective AT$_2$R agonists such as C21 could, therefore, represent a suitable therapeutic target for the treatment of hypertension and associated renal disease, at least in women. This includes investigations into whether AT$_2$R agonist therapy can improve the efficacy and utility of existing antihypertensive therapies.

Disclosures

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References

1. Go AS, Mozaffarian D, Roger VL, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics–2013 update: a report from the American Heart Association. *Circulation*. 2013;127:e6–e245.
2. Silva-Antonialli MM, Tostes RC, Fernandes L, Fior-Chadi DR, Akamine EH, Carvalho MH, Fortes ZB, Nigro D. A lower ratio of AT1/AT2 receptors of angiotensin II is found in female than in male spontaneously hypertensive rats. *Cardiovasc Res*. 2004;62:587–593.
3. Hilliard LM, Sampson AK, Brown RD, Denton KM. The "his and hers" of the renin-angiotensin system. *Curr Hypertens Rep*. 2013;15:71–79.
4. Hilliard LM, Mirabito KM, Denton KM. Unmasking the potential of the angiotensin AT2 receptor as a therapeutic target in hypertension in men and women: what we know and what we still need to find out. *Clin Exp Pharmacol Physiol*. 2013;40:542–550.
5. Hilliard LM, Nematbakhsh M, Kett MM, Teichman E, Sampson AK, Widdop RE, Evans RG, Denton KM. Gender differences in pressure-natriuresis and renal autoregulation: role of the Angiotensin type 2 receptor. *Hypertension*. 2011;57:275–282.
6. Hilliard LM, Jones ES, Steckelings UM, Unger T, Widdop RE, Denton KM. Sex-specific influence of angiotensin type 2 receptor stimulation on renal function: a novel therapeutic target for hypertension. *Hypertension*. 2012;59:409–414.
7. Padia SH, Carey RM. AT2 receptors: beneficial counter-regulatory role in cardiovascular and renal function. *Pflugers Arch*. 2013;465:99–110.
8. Brown RD, Hilliard LM, Head GA, Jones ES, Widdop RE, Denton KM. Sex differences in the pressor and tubuloglomerular feedback response to angiotensin II. *Hypertension*. 2012;59:129–135.
9. Sullivan JC, Bhatia K, Yamamoto T, Elmarakby AA. Angiotensin (1-7) receptor antagonism equalizes angiotensin II-induced hypertension in male and female spontaneously hypertensive rats. *Hypertension*. 2010;56:658–666.
10. Bosnyak S, Welungoda IK, Hallberg A, Alterman M, Widdop RE, Jones ES. Stimulation of angiotensin AT2 receptors by the non-peptide agonist, Compound 21, evokes vasodepressor effects in conscious spontaneously hypertensive rats. *Br J Pharmacol*. 2010;159:709–716.
11. Brouwers S, Smolders I, Massie A, Dupont AG. Angiotensin II type 2 receptor-mediated and nitric oxide-dependent renal vasodilator response to compound 21 unmasked by angiotensin-converting enzyme inhibition in spontaneously hypertensive rats in vivo. *Hypertension*. 2013;62:920–926.
12. Suzuki K, Han GD, Miyauchi N, Hashimoto T, Nakatsue T, Fujioka Y, Koike H, Shimizu F, Kawachi H. Angiotensin II type 1 and type 2 receptors play opposite roles in regulating the barrier function of kidney glomerular capillary wall. *Am J Pathol*. 2007;170:1841–1853.
13. Ali Q, Hussain T. AT2 receptor non-peptide agonist C21 promotes natriuresis in obese Zucker rats. *Hypertens Res*. 2012;35:654–660.
14. Hakam AC, Hussain T. Angiotensin II AT2 receptors inhibit proximal tubular Na+-K+-ATPase activity via a NO/cGMP-dependent pathway. *Am J Physiol Renal Physiol*. 2006;290:F1430–F1436.
15. Kemp BA, Bell JF, Rottkamp DM, Howell NL, Shao W, Navar LG, Padia SH, Carey RM. Intrarenal angiotensin III is the predominant agonist for proximal tubule angiotensin type 2 receptors. *Hypertension*. 2012;60:387–395.
16. Savoia C, Tabet F, Yao G, Schiffrin EL, Touyz RM. Negative regulation of RhoA/Rho kinase by angiotensin II type 2 receptor in vascular smooth muscle cells: role in angiotensin II-induced vasodilation in stroke-prone spontaneously hypertensive rats. *J Hypertens*. 2005;23:1037–1045.
17. Cosentino F, Savoia C, De Paolis P, Francia P, Russo A, Maffei A, Venturelli V, Schiavoni M, Lembo G, Volpe M. Angiotensin II type 2 receptors contribute to vascular responses in spontaneously hypertensive rats treated with angiotensin II type 1 receptor antagonists. *Am J Hypertens*. 2005;18(4 Pt 1):493–499.
18. Savoia C, Touyz RM, Volpe M, Schiffrin EL. Angiotensin type 2 receptor in resistance arteries of type 2 diabetic hypertensive patients. *Hypertension*. 2007;49:341–346.
19. Yang J, Chen C, Ren H, Han Y, He D, Zhou L, Hopfer U, Jose PA, Zeng C. Angiotensin II AT(2) receptor decreases AT(1) receptor expression and function via nitric oxide/cGMP/Sp1 in renal proximal tubule cells from Wistar-Kyoto rats. *J Hypertens*. 2012;30:1176–1184.

Novelty and Significance

What Is New?

- This is the first study to demonstrate the sex-specific ability of pharmacological stimulation of angiotensin type 2 receptor (AT$_2$R) to modulate renal function in hypertension.

What Is Relevant?

- Acute AT$_2$R stimulation induced renal vasodilatation and increased natriuresis in the female spontaneously hypertensive rat kidney. This was observed in the absence of any significant change in arterial pressure or glomerular filtration rate.
- No significant effect of acute AT2R stimulation on renal hemodynamic or excretory function was observed in male spontaneously hypertensive rats.

- The sex difference in renal response to C21 may be attributable to significantly greater renal AT$_2$R mRNA expression in the kidneys of female versus male spontaneously hypertensive rats.

Summary

AT$_2$R stimulation enhances renal vasodilatation and sodium excretion without concomitant alterations in glomerular filtration rate in female spontaneously hypertensive rats. Chronic studies of the sex-specific effects of AT$_2$R agonist therapy in hypertensive states are now required to establish the potential of AT$_2$R stimulation as a novel therapeutic target for the treatment of cardiovascular disease, at least in women.

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ONLINE DATA SUPPLEMENT

ANGIOTENSIN TYPE 2 RECEPTOR STIMULATION INCREASES RENAL FUNCTION IN FEMALE, BUT NOT MALE, SPONTANEOUSLY HYPERTENSIVE RATS

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Running title: AT$_2$R stimulation & renal function in SHR

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Methods

Animals

Seventeen-week old male and female spontaneously hypertensive rats (SHR) were obtained from the Animal Resources Centre (Perth, WA, Australia). Rats were housed under standard laboratory conditions (12-hour light/dark cycle at a temperature of 21°C) and were fed a sodium-controlled diet (0.25% sodium chloride; Specialty Feeds, Glen Forrest, WA, Australia) ad libitum. Experiments were approved by the Monash University, School of Biomedical Sciences Animal Ethics Committee and were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Rats were allowed 1-2 weeks to acclimatize prior to the commencement of the study protocol.

Surgical Procedure

The rats were surgically prepared as described in detail previously.[1] Briefly, rats were anesthetized with Inactin (thiobutabarbital; 150 mg/kg intraperitoneal injection; Sigma Aldrich, St Louis, Missouri, USA). The left carotid artery and jugular vein were catheterized for the continuous recording of mean arterial pressure (MAP) and the intravenous infusion of fluid replacement (2% BSA ;Sigma Aldrich) and tritiated inulin ([^{3}H]-inulin; Sigma Aldrich), respectively. The left kidney was then exposed and denervated and the left ureter was catheterized for the collection of timed urine samples. Finally, a transit-time ultrasound flow probe (0.7VB; Transonic Systems, Ithaca, New York, USA) was positioned around the renal artery for the continuous recording of renal blood flow (RBF).

Experimental Procedure

Following surgery, intravenous infusion of PD123319 (1 mg/kg bolus plus 1 mg/kg/h; Sigma Aldrich) or vehicle (0.9% saline; 1 ml bolus plus 1 ml/h) began and this was continued for the duration of the experiment. After 60 minutes, intravenous infusion of constant vehicle or graded doses of C21 (0, 100, 200 and 300 ng/kg/min) then commenced. At each dose, a 10-minute equilibration period was allowed, followed by 5-minute measurements of RBF. In addition, during the baseline and 300 ng/kg/min C21 periods, urine produced by the left kidney was collected and corresponding arterial blood samples were taken from the left carotid artery at the end of both urine collection periods. Upon completion of each experiment, urinary sodium excretion ($U_{Na+}V$) and fractional sodium excretion (FE_{Na+}) were measured, as previously described.[1] Glomerular filtration rate (GFR) was measured based on [^{3}H]-inulin clearance. Finally, the left kidney was excised and weighed. In addition, the stage of the estrus cycle of each female SHR was also determined by a vaginal smear and uterine weight was measured.

Renal AT$_{1a}$R, AT$_{1b}$R and AT$_2$R expression

In a separate cohort of rats, kidneys were collected, weighed and snap frozen. RNA was extracted from the kidney using the RNeasy Mini kit (Qiagen, Doncaster, Victoria, Australia). AT$_{1a}$R, AT$_{1b}$R and AT$_2$R mRNA expression was analyzed by real-time RT-PCR Realplex software with the Applied Biosystems 7900HT Fast RT-PCR system (Applied Biosystems, Life Technologies, Australia). Samples were run in triplicate using TaqMan gene expression assays (Applied Biosystems) with 18S rRNA as the internal housekeeping gene. Reactions were setup on a 384-well PCR plate using an automated liquid handler (CAS-1200 liquid handler, Qiagen). Relative expression was calculated using the comparative cycle of threshold fluorescence ($2^{-\Delta\Delta CT}$) method.

Statistical analyses

Data are expressed as mean $\pm$ SEM. To compare differences in baseline variables, data were analyzed using an analysis of variance (ANOVA) with Tukey's post-hoc test. Mean arterial pressure, RBF and renal vascular resistance (RVR) data were analyzed using repeated-measures ANOVA. To compare differences in percent changes in variables at 300 ng/kg/min C21 from baseline between vehicle-treated and C21-treated rats and C21-treated and C21+PD123319-treated rats in each sex, and differences in renal angiotensin receptor mRNA expression, data were analyzed using an unpaired t-test. P values ≤ 0.05 were considered statistically significant.

References

1.   Hilliard LM, Nematbakhsh M, Kett MM, Teichman E, Sampson AK, Widdop RE, Evans RG, Denton KM. Gender differences in pressure-natriuresis and renal autoregulation: role of the Angiotensin type 2 receptor. *Hypertension*. 2011;57:275-282.