**Endothelin and Intrauterine Growth Restriction**

**Sex-Specific Effect of Endothelin in the Blood Pressure Response to Acute Angiotensin II in Growth-Restricted Rats**

Suttira Intapad,* Norma B. Ojeda,* Elliott Varney, Thomas P. Royals, Barbara T. Alexander

*Abstract*—The renal endothelin system contributes to sex differences in blood pressure with males demonstrating greater endothelin type-A receptor-mediated responses relative to females. Intrauterine growth restriction programs hypertension and enhance renal sensitivity to acute angiotensin II in male growth-restricted rats. Endothelin is reported to work synergistically with angiotensin II. Thus, this study tested the hypothesis that endothelin augments the blood pressure response to acute angiotensin II in male growth-restricted rats. Systemic and renal hemodynamics were determined in response to acute angiotensin II (100 mg/kg per minute for 30 minutes) with and without the endothelin type-A receptor antagonist, Atrasentan (ABT-627; 10 ng/kg per minute for 30 minutes), in rats pretreated with enalapril (250 mg/L for 1 week) to normalize the endogenous renin–angiotensin system. Endothelin type-A receptor blockade reduced angiotensin II–mediated increases in blood pressure in male control and male growth-restricted rats. Endothelin type-A receptor blockade also abolished hyper-responsiveness to acute angiotensin II in male growth-restricted rats. Yet, blood pressure remained significantly elevated above baseline after endothelin type-A receptor blockade, suggesting that factors in addition to endothelin contribute to the basic angiotensin II–induced pressor response in male rats. We also determined sex-specific effects of endothelin on acute angiotensin II–mediated hemodynamic responses. Endothelin type-A receptor blockade did not reduce acute angiotensin II–mediated increases in blood pressure in female control or growth-restricted rats, intact or ovariectomized. Thus, these data suggest that endothelin type-A receptor blockade contributes to hypersensitivity to acute angiotensin II in male growth-restricted rats and further supports the sex-specific effect of endothelin on blood pressure. *(Hypertension. 2015;66:1260-1266. DOI: 10.1161/HYPERTENSIONAHA.115.06257.)*

**Online Data Supplement**

**Key Words:** angiotensin II ■ blood pressure ■ endothelins ■ renin-angiotensin system ■ sex characteristics

Offspring of pregnancies complicated by placental ischemia, hypertension, and preeclampsia exhibit a greater risk for the development of increased blood pressure (BP) and cardiovascular disease in later life.1,3 A mechanical reduction in uteroplacental perfusion in the rat results in placental ischemia leading to hypertension in the mother and intrauterine growth restriction (IUGR) in the offspring.4 Male growth-restricted offspring exhibit a marked increase in BP that initiates in early life and persists in adulthood.5 Male growth-restricted offspring also exhibit an enhanced sensitivity to acute angiotensin II (Ang II), further implicating that fetal exposure to pregnancies complicated by placental ischemia programs increased cardiovascular risk in the offspring. The mechanisms that contribute to the cause of increased BP that has its origins in fetal life are multifactorial and have not been fully elucidated. However, programmed increases in BP7 and the enhanced BP response to acute Ang II8 programmed by fetal exposure to placental ischemia in male growth-restricted offspring are testosterone dependent implicating a role for sex steroids. Androgens can potentiate renal responsiveness to Ang II via the Rho kinase signaling pathway.4 Previously, we reported that the enhanced BP response to acute Ang II in the male growth-restricted rats is independent of the Rho kinase pathway.9 Thus, 1 goal of this study was to investigate another potential mechanism that mediates hypersensitivity to acute Ang II in male growth-restricted rats.

Numerous experimental and human studies suggest that the endothelin system contributes to the development and progression of hypertension through its effects on vascular and renal function.10,11 Endothelin is a powerful vasoconstrictor, which exerts its effects on vascular smooth muscle via its endothelin type-A (ET\(_A\)) and endothelin type-B (ET\(_B\)) receptors.11 Hypertension induced by chronic Ang II infusion in the male rat can be blocked by an ET\(_A\) receptor antagonist,12 and endothelin is reported to enhance the pressor response to Ang II,13 implicating a synergistic effect of endothelin and Ang II on BP. Numerous studies also indicate that the endothelin system contributes to sex differences in BP with ET\(_A\)
receptor–dependent increases in BP more prevalent in males relative to females.\textsuperscript{14} Although male growth-restricted rats are hypertensive in early adulthood, female growth-restricted rats are normotensive.\textsuperscript{5} Ovariectomy induces hypertension in female growth-restricted rats in young adulthood\textsuperscript{15} and also enhances the BP response to acute Ang II.\textsuperscript{16} Thus, the aims of this study were to test the hypothesis that endothelin contributes to the enhanced BP response to acute Ang II in male growth-restricted rats and to determine whether the effect of endothelin on acute Ang II–mediated pressor responses in growth-restricted offspring is sex specific.

Materials and Methods
Detailed Materials and Methods are available in the online-only Data Supplement.

Results
Birth Weight, Body Weight, and Kidney Weight
Birth weight was significantly reduced in growth-restricted rats when compared with same-sex control counterparts (Table). Males were heavier than females, but body weight did not differ relative to same-sex counterparts at 16 weeks of age (Table); kidney weight (Table) and kidney to body weight (data not shown) did not differ in same sex groups. Kidney weight was greater in males relative to females, and ovariectomy had no significant effect on kidney weight among groups; however, body weight was increased in ovariectomized female rats (Table).

Effect of an ET\textsubscript{A} Receptor Antagonist on Mean Arterial Pressure
BP was increased to a greater degree by acute infusion of Ang II in male growth-restricted relative to male control (P<0.05; Figure 1A). However, female growth-restricted did not demonstrate a similar response (Figure 1B). Blockade of the ET\textsubscript{A} receptor significantly reduced the BP response to acute Ang II in male rats (Figure 1A), but BP remained significantly elevated relative to baseline (P<0.05; Figure 1A). However, blockade of the ET\textsubscript{A} receptor abolished the differential response to acute Ang II in male growth-restricted rats relative to male control. Blockade of the ET\textsubscript{A} receptor with Atrasentan (ABT-627) had no effect on BP in intact Ang II–treated female control or growth-restricted rats (P<0.05 versus baseline female control or IUGR; Figure 1B). Blockade of the ET\textsubscript{A} receptor with ABT-627 also did not significantly alter BP in ovariec-}

tomized female control or growth-restricted rats (Figure S1 in the online-only Data Supplement).

Effect of an ET\textsubscript{A} Receptor Antagonist on Renal Hemodynamics
Acute Ang II induced a significant decrease in glomerular filtration rate (GFR) in male control and male growth-restricted rats, which was greater in male growth-restricted than in male control (P<0.05; Figure 2A). Blockade of the ET\textsubscript{A} receptor with ABT-627 abolished the decline in GFR induced with acute Ang II in male control and growth-restricted rats restoring values back to baseline (Figure 2A). ET\textsubscript{A} receptor blockade was unable to restore GFR in intact (Figure 2B) or ovariec-
tomized (Figure S1) female control or growth-restricted offspring (P<0.05 versus baseline control). Inhibition of the ET\textsubscript{A} receptor attenuated the significant decline in effective renal plasma flow (eRPF) that occurred in response to acute Ang II in male growth-restricted offspring (P<0.05; Figure 3A); yet, eRPF remained significantly lower in ABT-627–treated male growth-restricted than in ABT-627–treated male control (Figure 3A). Ang II induced a decrease in eRPF that was not abolished by ET\textsubscript{A} receptor blockade in intact female growth-restricted rats (P<0.05 IUGR baseline versus IUGR Ang II and IUGR Ang II+ABT; Figure 3B) and did not significantly alter eRPF in ovariec-}
tomized rats versus their baseline treated counterpart (Figure S1). Normalizing GFR or eRPF to body weight or kidney weight did not alter outcomes (data not shown). The marked increase in renal vascular resistance in male growth-restricted rats relative to male control was abol-
ished by ET\textsubscript{A} receptor blockade (P<0.05; Figure 4A) and nor-
malized renal vascular resistance in male growth-restricted rats relative to male baseline, control, or growth restricted. However, renal vascular resistance remained significantly elevated in ABT-627–treated intact female growth-restricted rats relative to baseline intact female control and growth restricted (Figure 4B); ET\textsubscript{A} receptor blockade had no effect on renal vascular resistance in Ang II–treated female ovariec-
tomized rats (Figure S1). Filtration fraction did not differ between groups under baseline conditions, before or after acute infusion of Ang II, or in conjunction with ABT-627 (data not shown).

Renal Expression of the Endothelin System
Whole kidney preproendothelin mRNA expression and 24-hour urinary excretion of endothelin did not differ in male or female growth-restricted relative to same-sex control counterpart (Figure S2). Protein expression of the ET\textsubscript{A} and the ET\textsubscript{B} receptor was significantly elevated in male growth-restricted offspring within the cortex and medulla than in male control (Figure S3). In females, ET\textsubscript{A} receptor protein expression did not differ, but medullary ET\textsubscript{B} receptor protein expression was significantly decreased in female growth-restricted relative to female control (Figure S4).

| Table. Birth Weight, Body Weight, and Kidney Weight in Male and Female, Intact and Ovariectomized, Control and Growth-Restricted (IUGR) Rats at 16 Weeks of Age |
|---|---|---|---|---|---|
| Experimental Group | Birth Weight, g | Body Weight, g | Kidney Weight, g |
| Males | | | |
| Control | 6.7±0.2 | 409±13 | 2.7±0.2 |
| IUGR | 5.5±0.1* | 432±19 | 2.7±0.1 |
| Females | | | |
| Control intact | 6.1±0.1 | 256±8 | 1.6±0.2 |
| IUGR intact | 5.1±0.1* | 254±6 | 1.5±0.1 |
| Control ovariectomized | 6.0±0.1 | 315±9† | 1.6±0.1 |
| IUGR ovariectomized | 5.0±0.1* | 318±9† | 1.6±0.1 |

Values represent mean±SEM. IUGR indicates intrauterine growth restriction.

*P<0.05 vs same-sex counterpart.
†P<0.05 vs intact counterpart.
Discussion

The main findings from this study demonstrate a role for the endothelin system in the sexual dimorphic developmental programming of BP control in growth-restricted rats exposed to placental ischemia during fetal life. Male growth-restricted rats exhibited a significantly greater increase in BP in response to acute Ang II than in male control. The increased BP response to acute Ang II was significantly reduced by blockade of the ETA receptor in male control and growth-restricted rats. Blockade of the ETA receptor also abolished the exaggerated BP response to Ang II in male growth-restricted rats, suggesting that hypersensitivity to acute Ang II is mediated by the ETA receptor. However, ETA receptor blockade during acute infusion of Ang II failed to restore BP entirely back to baseline in male control and growth-restricted rats indicating that other factors in addition to endothelin may contribute to the basal acute Ang II–mediated BP response in male rats. ETA receptor blockade had no significant effect on acute Ang II–induced increases in BP in female control or growth-restricted rats regardless of ovarian status. Therefore, administration of an ET$_A$ receptor antagonist at a dose that significantly reduced Ang II–mediated increases in BP in male rats had no effect in female control or growth-restricted rats, intact or ovariectomized. These findings strongly suggest that the sexual dimorphism observed in the regulation of BP in adult rats is partially mediated by the endothelin system.

Investigation into the contribution of the endothelin system to the developmental programming of BP control is limited. Male offspring exposed to early life stress (ELS) are normotensive under basal conditions but exhibit a hyper-responsiveness to chronic Ang II that is androgen dependent in male ELS rats. Male ELS rats also exhibit an exaggerated BP response to acute air jet stress. The exaggerated response to air jet stress is abolished in male ELS rats homozygous for

Figure 1. Mean arterial pressure (MAP). A, Male control and growth-restricted (intrauterine growth restriction [IUGR]) rats. B, Female control and growth-restricted rats. MAP was measured at 16 wk of age in chronically instrumented, conscious animals pretreated with the angiotensin-converting enzyme inhibitor, enalapril (250 mg/L for 1 wk). MAP was measured at baseline during an acute infusion of angiotensin II (Ang II; 100 ng/kg per minute) for 30 min, and during a 30-min infusion of Ang II plus the ET$_A$ receptor antagonist, Atrasentan (ABT-627; 10 ng/kg per minute for 30 min). Values were allowed to return to baseline between acute treatments. $^*P<0.05$ vs baseline control. $^†P<0.05$ vs baseline IUGR. $^‡P<0.05$ vs Ang II control. $^#P<0.05$ vs Ang II IUGR. Data values represent mean±SEM.

Figure 2. Glomerular filtration rate (GFR). A, Male control and growth-restricted (intrauterine growth restriction [IUGR]) rats. B, Female control and growth-restricted rats. GFR was measured at 16 wk of age in chronically instrumented, conscious animals pretreated with the angiotensin-converting enzyme inhibitor, enalapril (250 mg/L for 1 wk). Renal function was measured at baseline during an acute infusion of angiotensin II (Ang II; 100 ng/kg per minute) for 30 min, and during a 30-min infusion of Ang II plus the endothelin type-A receptor antagonist, Atrasentan (ABT-627; 10 ng/kg per minute for 30 min). Values were allowed to return to baseline between acute treatments. $^*P<0.05$ vs baseline control. $^†P<0.05$ vs baseline IUGR. $^‡P<0.05$ vs Ang II control. $^#P<0.05$ vs Ang II IUGR. Data values represent mean±SEM.
ET_B receptor deficiency, suggesting a role for the endothelin system in the cause of increased cardiovascular risk in ELS rats. Whether the endothelin system contributes to the enhanced BP response to chronic Ang II in male or female ELS rats has not been examined. Male offspring exposed to hypoxia during fetal life develop IUGR and a significant increase in BP with age that is not observed in their female IUGR counterparts. Blockade of the ET_A/B receptor attenuates the increase in BP in male IUGR rats programmed by fetal exposure to hypoxia but has no effect on baseline BP in female rats, control, or IUGR. Thus, these studies suggest that the endothelin system contributes to the developmental programming of BP in male rats and indicates a sex-specific effect of endothelin on BP in the model of IUGR induced via prenatal hypoxia.

Numerous models of developmental programming exhibit a sex difference in BP control with females protected relative to their male counterparts. We previously reported that female growth-restricted rats are normotensive during young adulthood, whereas male growth-restricted rats exhibit a significant increase in BP that is testosterone dependent. Hyper responsiveness to acute Ang II is also testosterone dependent in male growth-restricted rats, whereas BP is increased and hypersensitivity to acute Ang II is exacerbated by ovariectomy in female growth-restricted rats in young adulthood. Many experimental models that mimic essential hypertension also exhibit sex differences in the importance of the endothelin system in BP regulation. Females are protected against the development of Ang II–induced hypertension and exhibit a delay in the development of hypertension induced...
via deoxycorticosterone acetate-salt that is also attenuated relative to their male deoxycorticosterone acetate-counterparts. The renal endothelin system contributes to sex differences in the regulation of BP in these experimental models with males exhibiting greater ET$_A$-mediated responses, whereas the ET$_A$ receptor is protective against increased BP in the female. ET$_A$ receptor blockade reduces BP in male but not in female salt-loaded stroke-prone spontaneously hypertensive rats (SHR), an experimental model of more severe hypertension. However, ET$_A$ receptor blockade has no effect on BP in female SHRs in young adulthood but attenuates the hypertension that develops in postcycling SHR, a model of postmenopausal hypertension. Endothelin levels increase with age, and circulating endothelin-1 levels are positively associated with increased testosterone levels in postmenopausal women. Circulating endothelin-1 levels are also positively associated with increased testosterone in women with polycystic ovary syndrome. Testosterone upregulates endothelin-1 mRNA and induces an increase in secretion of endothelin-1 from endothelial cells. Thus, elevated testosterone levels that occur in menopause or women with polycystic ovary syndrome could contribute to increase in circulating levels of endothelin in female-specific conditions that are associated with increased cardiovascular risk and involve a role for the endothelin system in their cause of elevated BP. Elevations in circulating levels of testosterone may also contribute to increased activation of the endothelin system in male growth-restricted rats. Hypertension and enhanced sensitivity to acute Ang II in male growth-restricted rats are testosterone dependent and are also associated with a significant increase in circulating testosterone. However, estradiol also mediates an influence on endothelin production. In vitro estradiol reduces the increase in endothelin-1 production stimulated by Ang II. Whether estradiol exerts similar actions in vivo is not clear. Nonetheless, these studies indicate that modulation of the endothelin system by sex steroids may contribute to sex differences in Ang II sensitivity in growth-restricted offspring.

The mechanism that mediates endothelin-induced amplification of acute Ang II–mediated systemic and renal hemodynamic responses is unknown. Rigggleman et al demonstrated that endothelin acting via its ET$_A$ receptor contributes to the acute pressor response to acute Ang II. Endothelin also contributes to the enhanced pressor response to acute Ang II in the SHR relative to WKY rats, implicating that endothelin amplifies the actions of acute Ang II. Ang II receptor density and ligand affinity are increased in the SHR, suggesting that differences in the binding and distribution of the Ang II receptors may be a contributory factor in the hyper-responsiveness to acute Ang II observed in the SHR. Renal AT$_1$ receptor expression and glomerular 125I-Ang II binding are increased in male offspring exposed to maternal protein restriction. A greater reduction in GFR after acute Ang II is noted in male offspring exposed to a maternal low protein diet relative to control, suggesting that differences in Ang II receptor expression and binding may be a contributory factor in the development of impaired renal function. However, renal AT$_1$ receptor mRNA expression and density are not elevated in male growth-restricted rats programmed by exposure to placent al ischemia. Oriji and Keiser demonstrated that Ang II stimulation of rat aortic rings results in the rapid release of endothelin mediated via protein kinase C. Thus, the enhanced actions of endothelin on Ang II–mediated responses could also involve the rapid release of endothelin from the vasculature.

Renal preproendothelin mRNA expression and urinary excretion of endothelin-1 were not significantly different in female or male growth-restricted rats relative to their control counterpart. Urinary endothelin-1 is reported not to differ in male versus female rats. Whether expression of the endothelin system was altered in males relative to females was not directly compared in this study. Nevertheless, renal protein expression of the ET$_A$ receptor was upregulated in the renal cortex and medulla of male but not of female growth-restricted rats relative to same-sex control. The endothelin system including type A and type B receptors is highly expressed in the collecting duct with males rats reported to exhibit greater ET$_A$ receptor expression relative to females. Activation of the ET$_A$ receptor is also enhanced in male rats relative to females, suggesting that differences in signaling of the endothelin receptor may contribute to sex differences in IUGR-induced hypertension. Endothelin is tightly linked to the nitric oxide (NO) system within the medulla and renal endothelin can differentially regulate nitric oxide synthase (NOS) activity within the kidney. Sex steroids can also modulate NO availability, and renal levels of endothelial NOS are higher in female rats than in males. Yet, male rats are more susceptible to NOS inhibition than females, indicating that a loss of NO may uncover the actions of a vasoconstrictor such as endothelin, which is more prominent in male versus female rats. Whether NOS activity is increased in a compensatory manner in female growth-restricted rats or whether enhanced activation of the ET$_A$ receptor mediates enhanced sensitivity to acute Ang II in male growth-restricted rats is not known. Thus, the mechanisms that contribute to enhanced renal sensitivity to acute Ang II–mediated responses in growth-restricted rats are not known. Additional studies are required to fully elucidate the mechanisms that mediate sex differences in the BP response to acute Ang II after IUGR.

Clinical Perspectives

Hypertension is more common in men than in women before menopause. Birth weight is inversely associated with BP in both men and women, and low birth weight men exhibit a greater cardiovascular risk in young adulthood relative to their female low birth weight counterparts. The mechanisms that contribute to sex difference in BP control are multifactorial and within the low birth weight population, not clearly elucidated. This study supports an important role for the endothelin system in the cause of increased cardiovascular risk that develops in male growth-restricted rats and also implicates the endothelin system as a contributor to sex differences in the developmental programming of BP.

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Disclosures
None.

References
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**Novelty and Significance**

**What Is New?**

- Our study demonstrates that the endothelin type-A (ET$_A$) receptor contributes to the pressor response to acute angiotensin II (Ang II) in male rats, and further reveals that the ET$_A$ receptor mediates hypersensitivity to acute Ang II in male growth-restricted rats.

- Our study also shows that blockade of the ET$_A$ receptor at a dose that significantly reduces Ang II–mediated increases in blood pressure (BP) in male rats has no effect on Ang II–mediated increases in BP in female rats regardless of their birth weight or ovarian hormone status.

**What Is Relevant?**

- Currently, birth weight is not a consideration in the management of BP within the general population. Demonstration that the ET$_A$ receptor contributes to the developmental programming of high BP in male animals, and that the effect of ET$_A$ receptor blockade on BP is sex specific should encourage the development of endothelin antagonists for the treatment of high BP with the caveat that their use may be limited by sex yet, their viability as an antihypertensive agent may be enhanced in low birth weight men.

**Summary**

Additional studies are necessary to discern the impact of sex and birth weight on effectiveness of an antihypertensive regimen in low birth weight individuals.
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SUPPLEMENTAL MATERIALS

Sex-specific effect of endothelin in the blood pressure response to acute angiotensin II in growth-restricted rats.

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Running title: Endothelin, Sex, Ang II, and IUGR
MATERIALS AND METHODS
All experimental procedures were in accordance with National Institutes of Health guidelines with approval by the Animal Care and Use Committee at the University of Mississippi Medical Center. Offspring from 15 control pregnant and 17 reduced uterine perfusion pregnant litters were used to determine systemic and renal hemodynamics responses to acute Ang II plus and minus ABT 627 in male control (n=8) and growth-restricted (n=9) rats; and in female intact control (n=7) and female ovariectomized control (n=7), and female intact growth-restricted (n=9) and female ovariectomized growth-restricted (n=8) rats. A subgroup of males and female rats were analyzed simultaneously to measure concurrent actions of ET\textsubscript{A} receptor blockade on blood pressure.

**Animals.** Rats were housed in a temperature-controlled room (23°C) with a 12:12-hour light/dark cycle with food and water available *ad libitum*. Timed pregnant Sprague Dawley (SD) rats were purchased from Harlan Inc (Indianapolis, IN). At day 14 of gestation rats destined for reduced uterine perfusion were clipped as described below. All dams were allowed to deliver at term with birth weight recorded within 12 hours of delivery. At this time the number of pups in the control and reduced uterine perfusion litter were culled to 8 pups per dam to ensure equal nutrient access for all offspring. Animals were weighed twice weekly. Pups were weaned at 3 weeks of age.

**Reduced uterine perfusion in the pregnant rat.** In brief and as previously described (1), at day 14 of gestation a silver clip (0.203-mm ID) was placed around the lower abdominal aorta above the iliac bifurcation and around each branch of the ovarian arteries (0.100-mm ID).

**Ovariectomy in Female Offspring.** Ovariectomy was performed at 10 weeks of age as previously described (2) with a sham operation involving visualization of the ovaries but no removal.

**Drug administration.** The ACE inhibitor, enalapril (40 mg/kg/day) (Sigma Aldrich, San Louis, Missouri, USA), was administered in the drinking water from 15 to 16 weeks of age at a dose previously shown to block endogenous production of angiotensin II (Ang II) in the rat (3). Water consumption was monitored daily for the duration of the treatment period and average daily water intake did not differ between same-sex animals during treatment with enalapril (data not shown). Prior to study all animals were pretreated with the angiotensin converter enzyme (ACE) inhibitor, enalapril, to investigate the blood pressure (BP) response to acute Ang II without participation of endogenous Ang II. Chronic enalapril resulted with all groups initiating with a similar baseline BP. At 16 weeks of age systemic and renal hemodynamics were measured in the conscious state during an acute infusion (30 minutes) of: a) 0.9% saline solution, b) Ang II (100ng/kg/min) in 0.9% saline solution, and c) Ang II (100ng/kg/min) plus or minus the ET\textsubscript{A} receptor antagonist, ABT-627 (10 mg/kg/min in 0.9% saline solution) at a dose previously shown to reduce BP in the male (4) and pregnant female SD rat (5). The order of infusion of Ang II and Ang II + ABT-627 was interchanged in a subset of rats to ensure that pre-administration of Ang II did not alter the pressor response to co-administration of Ang II + ABT-627. Systemic and renal hemodynamic parameters were measured during each 30 minute infusion period. BP values were allowed to return to baseline between each infusion during 0.9% saline solution infusion. A subgroup of males and female rats were analyzed simultaneously to measure concurrent actions of ET\textsubscript{A} receptor blockade on BP.

**Measurement of systemic and renal hemodynamics.** As previously described (1) animals were instrumented with flexible catheters (PE 50 tubing) in the right jugular vein for infusion and in the right carotid artery for measurement of BP and collection of blood; the bladder was also
instrumented with a flexible catheter (PE 90 tubing) for collection of urine. After a 24-hour recovery, renal function and arterial pressure measurements were performed in the conscious state with glomerular filtration rate (GFR) and effective renal plasma flow (eRPF) calculated from radioactivity of 125I-iothalamate and concentration of para-aminohippuric acid (PAH), respectively, in plasma and urine. Renal vascular resistance (RVR) and filtration fraction (FF) were calculated: RVR = (MAP / ERPF) X (1 - hematocrit) and FF = (GFR / ERPF), respectively.

**Measurement of PRA.** PRA was measured by radioimmunoassay as previously described to confirm blockade of endogenous RAS (6).

**Measurement of urinary endothelin.** Urine was collected via 24 metabolism cages with quantitation of ET-1 determined using an Endothelin-1 Quantiglo ELISA Kit (R&D Systems).

**Western blot analysis for protein expression.** As previously described (7) isolation of proteins and determination of protein expression was performed with ETA and ETB receptors antibodies purchased from Abcam, Cambridge, MA and the beta-Actin antibody was purchased from Sigma, St. Louis, MO. Secondary anti-rabbit and anti-mouse antibodies were purchased from GE Healthcare Bio-Science, PA. All ET receptor bands were normalized to beta-Actin control as described (8) and a molecular weight marker (BioRad, Precision Plus Protein ™ Dual color Standards) was used to ensure identification of the correct band.

**Determination of renal preproendothelin mRNA levels.** Kidneys were snap frozen in liquid nitrogen and stored at −80°C. Total RNA was extracted using the ToTALLY RNA kit (Ambion). cDNA was synthesized from 1 μg of RNA with Bio-Rad Iscript cDNA reverse transcriptase and real-time PCR was performed using the Bio-Rad Sybre Green Supermix and iCycler. The following primer sequences were used for analysis of ppET: forward 1, ctggtagctcttgctgcttg, and reverse 1, tctttgtctgttggc (Life Technologies). Level of mRNA expression was calculated using the mathematical formula for delta/delta CT recommended by Applied Biosystems (Applied Biosystems User Bulletin, No. 2, 1997).

**Statistics.** Statistical analysis was performed using unpaired Student’s t test or 1 way ANOVA and Bonferoni’s post hoc test was used to utilize for multiple comparisons. Graphpad PRISM version 5 was utilized for all statistical analysis. Statistical significance of interaction was set with $P<0.05$. All values are given as mean ± SEM.
REFERENCES
Figure S1. Difference in systemic and renal hemodynamic responses to acute Ang II versus Ang II plus ET_A receptor blockade in female ovariectomized offspring at 16 weeks of age. a) Change in mean arterial pressure (MAP). b) Change in glomerular filtration rate (GFR). c) Change in effective renal plasma flow (eRPF). d) Change in renal vascular resistance (RVR). Parameters were measured at 16 weeks of age in chronically instrumented, conscious animals pretreated with the angiotensin convertor enzyme inhibitor, enalapril (250mg/L for 1 week). MAP and renal hemodynamics were measured during an acute infusion of ANG II (100 ng/kg/min) for 30 min, and then during a 30 minute infusion of ANG II plus the ET_A receptor antagonist, ABT-627 (10 ng/kg/min for 30min). Values represent the difference between Ang II and Ang II plus ET_A receptor blockade in female control or growth-restricted (IUGR) ovariectomized rats. Data values represent mean±SEM.
Figure S2. Renal preproendothelin (ppET) expression and urinary excretion of endothelin-1 (ET-1) in male and female control and intrauterine growth restricted (IUGR) offspring at 16 weeks of age. a) Renal preproendothelin expression b) Urinary endothelin-1 excretion. Values represent the difference between same-sex control and growth-restricted rats. Data values represent mean±SEM.
Figure S3. Renal protein expression of the endothelin receptor type A and B receptors (ET<sub>A</sub> and ET<sub>B</sub>) in male growth-restricted (IUGR) offspring at 16 weeks of age. Representative western blot for (a) quantification of renal cortex ET<sub>A</sub>, (b) renal cortex ET<sub>B</sub>, (c) renal medullary ET<sub>A</sub>, (d) and renal medullary ET<sub>B</sub> Values were normalized to beta-actin. *P<0.05 versus control. Data values represent mean±SEM. n=4 in each group.
Figure S4. Renal protein expression of the endothelin receptor type A and B receptors (ET\textsubscript{A} and ET\textsubscript{B}) in female growth-restricted IUGR offspring at 16 weeks of age. Representative western blot for (a) quantification of renal cortex ET\textsubscript{A}, (b) renal cortex ET\textsubscript{B}, (c) renal medullary ET\textsubscript{A}, (d) and renal medullary ET\textsubscript{B}. Values were normalized to beta-actin. *$P<0.05$ versus control. Data values represent mean±SEM. n=4 in each group.