Endothelin, sex, and pregnancy: unique considerations for blood pressure control in females

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Gillis EE, Sasser JM, Sullivan JC. Endothelin, sex, and pregnancy: unique considerations for blood pressure control in females. Am J Physiol Regul Integr Comp Physiol 310: R691–R696, 2016. First published March 2, 2016; doi:10.1152/ajpregu.00427.2015.—Endothelin-1 (ET-1) is a potent vasoconstrictor, and dysregulation of the endothelin (ET) system has been implicated in the development of hypertension. Sex differences in the ET system have been identified in ET receptor expression and activation, levels of ET-1, and downstream mediators of the ET system. More specifically, males have greater ET-1/ETA receptor activation, whereas females exhibit greater ETB receptor activation. These differences have been suggested to contribute to the sex differences observed in blood pressure control, with greater ETB receptor activation in females potentially acting as an important pathway contributing to the lower prevalence of hypertension in young females compared with age-matched males. This hypothesis is further supported by studies in pregnancy; the role of the ET system is enhanced during pregnancy, with dysregulation of the ET system resulting in preeclampsia. Further research is necessary to elucidate the relative roles of the ET system in blood pressure control in both sexes and to further explore the potential benefits of pharmacological ET blockade in women.

Hypertension; sex differences; preeclampsia

Hypertension is a complex and multifaceted disease, and there are well-established sex differences in many aspects of blood pressure (BP) control (34, 66). Sex differences in BP have been recognized since the 1940s (7), where college-aged men were found to have higher BP than college-aged women. It is now well established that males have higher BP relative to age-matched females until the sixth decade of life, although sex differences in BP extend beyond absolute BP readings alone. There are also sex differences in the relationship between elevations in BP with the impact of hypertension on end-organ damage (6, 19), which may be particularly relevant given the recent early findings of the Systolic Blood Pressure Intervention Trial (SPRINT) trial indicating that reductions in BP below the commonly recommended target result in improved cardiovascular outcomes in adults 50 yr and older with hypertension (64). There is also an ever-expanding basic science literature documenting sex differences in the molecular mechanisms regulating BP. The goal of this short review is to highlight recent work examining sex differences in the contribution of the endothelin (ET) system to BP control.

Endothelin-1 (ET-1) has been described as the most potent vasoconstrictor substance identified to date, and overactivation or dysfunction of the ET system has been implicated in the development and progression of hypertension in both clinical and experimental studies (5, 27, 28, 46, 53). ET-1 acts via two G protein-coupled receptors, ETA and ETB. ETA receptors are found predominately on vascular smooth muscle cells, and activation of ETA receptors induces vasoconstriction and promotes increases in inflammation and oxidative stress. ETA receptors are also found in the renal proximal tubule and collecting duct and likely contribute to ET-1-induced sodium retention, all of which will act in a prohypertensive fashion to increase BP. Indeed, ETA receptor antagonism decreases BP in patients with resistant hypertension (4, 63). In contrast, ETB receptors are predominately found on the vascular endothelium and renal collecting duct cells, and activation leads to vasodilation through enhanced nitric oxide (NO) production, natriuresis, and diuresis resulting in decreases in BP. Consistent with these data, inhibition of the ETB receptor, either via pharmacological blockade or genetic modification, results in salt-sensitive hypertension (47, 52).

Sex Differences in the ET System

There are numerous sex differences in the ET system and these differences have been suggested to contribute to sex differences in BP control (5, 26, 53, 61). The major papers focused on sex differences in the endothelin system are summarized in Table 1. Plasma ET-1 levels are increased in
hypertension, and males have higher ET-1 levels than females, both in humans and in experimental animal models (11, 37, 45). Although the mechanism(s) responsible for sex differences in the ET system are still being investigated, sex hormones have been implicated. Female sex hormones suppress ET-1 levels by inhibiting ET-1 mRNA expression and endothelin-converting enzyme activity and decrease ETB receptor expression (61). Recent studies have further shown that estrogen replacement following ovariectomy of female Sprague-Dawley rats decreases ETA receptor expression in the lung and ETB expression in the renal inner medulla (17). In contrast, testosterone has been suggested to increase ET-1 (61), although a recent paper reported that orchidectomy increases ET-1, ETA, and ETB receptors in the rat portal vein, suggesting that testosterone may also suppress the ET system (49). Regardless, these data suggest tissue-specific effects of sex hormones on the ET system and underscore the importance of further defining the impact of both male and female sex hormones on the ET system.

In addition to sex difference in the levels of expression of the ET system, males are also more sensitive to ET-1-induced vasoconstriction than females, and this has been linked to sex differences in ET receptor expression in the renal medulla and saphenous veins; males have greater ETA receptor expression compared with females (12, 25, 61). As a result, males have greater ETA-mediated vasoconstriction than females in both clinical and basic science studies (12, 13, 55, 60). In contrast, females exhibit enhanced ETB-mediated dilation that antagonizes ETA-mediated increases in vascular tone (55). In additional studies, treatment of men and women with an ETB receptor antagonist to determine the contribution of the ETB receptor to resting vascular tone found that in men, ETB receptors mediate vasoconstriction, whereas, in women, ETB receptors mediate vasodilation (24). The authors concluded that this response was most likely mediated by greater ETB receptor expression in vascular smooth muscle cells in cutaneous vessels of men to induce constriction, whereas in women there was greater ETB receptor expression in the endothelium mediating dilation. These data further suggest a more prominent role for ETB receptor function in females compared with males, which may contribute to more advantageous cardiovascular outcomes in females relative to males.

There are also sex differences in the impact of ET receptor activation on renal function. ET-1 is critical in the regulation of salt and water excretion by the kidney with ETB receptor activation inducing diuretic and natriuretic responses via a NO synthase (NOS) 1, cGMP, and protein kinase G pathway in male Sprague-Dawley rats (41). Followup studies by the same group expanded this work to determine the impact of sex of the animal on medullary ETB function and found that renal medullary infusion of the ETB receptor antagonist S6c increases urine flow rate and sodium excretion in both sexes; however, intramedullary infusion of ET-1 caused significant diuretic and natriuretic responses in female but not male rats (40). Additionally, studies found that female rats exhibit an ETA-dependent diuresis and natriuresis that are dependent on activation of NOS1, whereas male rats exhibit an ETB-mediated reduction in medullary blood flow not observed in females. NOS1 has also been shown to contribute to ETB-dependent shear stress-mediated NO production in the renal collecting duct (20), although if there are sex differences in ETB-NOS1, mediated NO production in isolated collecting duct cells has not been directly examined. Separate studies following chronic ANG II infusion in male and female rats found that renal medullary ETB receptor function remains intact in female rats but is lost in male rats after ANG II infusion (25). Male rats exhibit a decrease in ETB receptor expression after ANG II infusion, and as a result, medullary infusion of S6c has no effect on water or sodium excretion. In contrast, female rats maintain ETB expression and ETB agonist-induced diuresis and natriuresis with ANG II hypertension, although urine flow rate and sodium excretion decrease in hypertensive females compared with normotensive controls. Together, these studies suggest 1) sex-specific actions of ETA receptor activation in the renal medulla, and 2) sex differences in the contribution of ETB receptor activation to modulate renal function. Moreover, both of these findings remain consistent with females having less ET-mediated cardiovascular and renal injury relative to their male counterparts.

Sex differences in the ET system further extend to vasoactive mediators downstream of receptor activation. ET-1 stimulates the generation of oxidative stress, modulates NO levels, and induces inflammation. Moreover, each of these individual pathways have been shown to contribute to the development of hypertension, and all exhibit sex differences (66). The mechanism(s) responsible for causing sex differences in oxidative stress, NO, and inflammation are still under investigation; however, the ET system may be a prime candidate. There is indirect evidence supporting the hypothesis that ETB receptor activation is critical in the ability of female rats to limit ET-1-induced increases in oxidative stress relative to males based on BP measurements (56); however, males may be more dependent on the ETB receptor to limit increases in vascular
endothelin (ET) system is arguably more idiosyncratic than most BP control systems and many of these subtleties could provide important mechanistic insights to BP control (for a complete review on the role of ET system see Ref. 27), yet they remain understudied with regard to sex. For example, ET receptors are important for the clearance of circulating ET-1, and the rise in BP following antagonism of ET receptors in vivo likely reflects increased ET activation due to increased circulating levels of ET-1 as opposed to a loss of ET-mediated vasodilation. The ET system has also been suggested to contribute to sex-specific alterations in vascular function with age (30, 32, 55). More studies are needed to better understand the impact of sex on the local regulation of the ET system across the lifespan to provide greater insight as to the regulation of cardiovascular and renal health at all ages.

Sex Differences in ET Control of BP

Despite numerous reported sex differences in the ET system as outlined above, the vast majority of the studies examining the impact of ET on BP have been conducted exclusively using male experimental animals. Based on the potential of greater ET receptor activation to be an important mechanism regulating cardiovascular health in females and the fact that approximately half of the hypertensive population is female, understanding the role of ET in hypertensive females is an important, although understudied, area of research. Moreover, additional studies are needed to establish if sex differences in the ET system are physiologically relevant.

Studies of ET receptor-deficient rats have been used as a tool to explore the role of the ET system in BP control as well as sex differences in BP; we are unaware of any studies that have directly compared BP responses to chronic ET-1 infusion. Male rats lacking functional ET receptors have a higher BP compared with females when maintained on a normal-salt (NS) diet (57), despite comparable ET-1 levels between the sexes. In response to a high-salt (HS) diet, BP increases in both sexes, although female ET-deficient rats exhibit a greater increase in BP than males (56, 57). More specifically, systolic BP increased from ~130 to ~165 mmHg in male ET-deficient rats, and from ~125 to ~175 mmHg in females; resulting in a 15-mmHg greater increase in BP in females. Since even modest decreases in BP result in significant cardiovascular protection, this would suggest a physiologically relevant difference in the impact of the ET system on overall cardiovascular health between the sexes. Moreover, the exaggerated BP response to HS in female rats is attenuated by treatment with the antioxidant Tempol, suggesting that females are more sensitive to loss of ET receptor function and that the ET system typically acts in the female to limit ET-mediated increases in oxidative stress, and in turn, BP (56). However, a recent study potentially challenges this conclusion, BP in both of the above studies was measured by tail-cuff analysis. When BP is instead measured by telemetry in male and female ET receptor-deficient rats on NS and HS, there are no apparent sex differences in BP with mean arterial pressure in both sexes increasing from ~130 mmHg at the beginning of HS treatment to ~160 mmHg after 4 wk (52). The authors then examined acute BP responses to air-jet stress in male and female ET receptor-deficient rats and found that females maintained on both normal-salt (NS) and HS diets were more sensitive to acute stress-induced increases in BP versus males, which could explain the greater increase in BP reported when BP was measured by tail-cuff. The total mean arterial pressure response to acute air-jet stress, expressed as area under the curve, was ~35 mmHg x 3 min in males versus ~60 mmHg x 3 min in females following 3 wk on HS. The greater acute increase in BP was not mediated by increased vascular reactivity to ET-1, since male ET receptor deficient rats exhibited a greater increase in BP with intravenous infusion of ET-1 compared with females on NS, and both sexes exhibited comparable increases in BP with infusion of an ET agonist. HS exacerbated the acute increase in BP with ET-1 infusion, but there were no apparent sex differences. The authors concluded that female rats have reduced ET receptor activity when on a HS diet compared with males, and may still support the hypothesis that females in particular are dependent on ET receptor activation to limit increases in BP in response to stressful stimuli.

Additional support for this hypothesis is found in DOCA-salt hypertension. A bolus injection of ET-1 results in a transient decrease in BP followed by a prolonged increase in BP in both sexes; however, female DOCA-salt rats exhibit a greater decrease in BP compared with males (59). Injection of an ET receptor agonist also results in a greater depressor response in females (~7 mmHg decrease in males vs. ~18 mmHg in females), although females also exhibit an attenuated increase in BP following the transient dip compared with males (~14 mmHg increase in males vs. ~6 mmHg in females). These data suggest greater ET function in DOCA-salt females and greater ET receptor function in males, a conclusion that was further supported by additional studies showing that male DOCA rats exhibit a greater decrease in basal BP in response to an ET receptor blocker versus females (~43 mmHg decrease in males vs. ~14 mmHg decrease in females; 58). Separate studies directly assessed the role of the ET receptor
in BP control in DOCA-salt hypertension using wild-type and ETB receptor-deficient rats (23). Of interest, the sex difference in BP typically exhibited in wild-type rats with DOCA-salt, where males have greater increases in BP than females (16), was abolished in ETB receptor-deficient rats despite the finding that ETB-deficient rats of both sexes display significantly greater increases in BP with DOCA-salt compared with wild-type rats. These findings indicate that ETB receptor activation typically functions to limit DOCA-salt-induced increases in BP in the female and further support a critical role for the ET-1/ETB system in regulating BP in females in response to a stressor. Consistent with these results, blockade of ETB receptors has been reported to result in greater increases in BP to angiotensin II infusion in female rats versus males (53).

**ET System in Normal Pregnancy**

Additional support for a prominent role for the ETB receptor in the regulation of BP in females is found in pregnancy. During normal pregnancy, there is a significant peripheral and renal vasodilation to accommodate the plasma volume expansion required for optimal pregnancy outcomes. Previous studies have demonstrated a critical role of adaptations in the ET system in mediating the vascular and renal hemodynamic response to healthy pregnancy. Pharmacological inhibition of the ETB receptor prevents the renal vasodilation and renal hyperfiltration that occurs in normal pregnancy (9), and ETB antagonism increases BP and reduces fetal growth during pregnancy providing further evidence that activation of the ETB receptor is necessary for optimal pregnancy outcomes in the rat (33). Recent work by Khalil and colleagues (35) indicates an increase in ETB receptor expression during a normotensive, healthy pregnancy, with corresponding increases in ETB receptor-mediated vasorelaxation and microvascular activity. Activation of the ET/ETB pathway in healthy pregnancy is thought to be stimulated by the pregnancy hormone relaxin (10, 42), and other factors including estrogen, vascular endothelial growth factor, and placental growth factor may play a role in this response as well (31, 43, 44). In addition, ET-1 contributes to the contractile tone of the uteroplacental vasculature, and this tone diminishes near term (2). These studies demonstrate that activation of the ET system is critical in maintaining a normal cardiovascular response to pregnancy.

**ET System in Preeclampsia**

Dysregulation of the ET system during pregnancy has been implicated in the pathogenesis of preeclampsia, a syndrome of new onset hypertension and proteinuria during pregnancy that is associated with significant morbidity and mortality of the mother and fetus. Many groups have demonstrated increases in circulating ET-1 in the plasma of preeclamptic women compared with normotensive pregnant controls (15). Increases in ET-1 levels positively correlate with sFlt-1 levels (38, 39) or the AT1-AA (8). In all of these models, blockade of the ETB receptor attenuates the rise in BP. Injection of IgG from women with preeclampsia also induces increases in ET-1 levels positively correlates with sFlt-1 levels in preeclamptic women compared to normotensive controls. Circulating ET-1 is elevated in the RUPP model of placental ischemia. AT1-AA infusion increases circulating ET-1 in a rodent model of preeclampsia. Circulating ET-1 is elevated in an animal model of HELLP syndrome. Infusion of sFlt-1 in a pregnant rat increases circulating ET-1. Pharmacological blockade of the ETA receptor attenuates BP in the RUPP model of HELLP syndrome. Mice injected with IgG from preeclamptic women have elevated plasma ET-1 levels and develop the maternal syndrome of preeclampsia. ETB receptor is downregulated in the RUPP rat, with a significant decrease in ETB-mediated nitric oxide production. ETB deficient rats exhibit elevations in BP during late pregnancy. RUPP, reduced uteroplacental perfusion pressure; HELLP, hemolysis, elevated liver enzymes, low platelet count; AT1-AA, angiotensin II type 1 receptor autoantibody; TNF-α, tumor necrosis factor-α; BII, blood pressure.

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Table 2. Select papers from this review demonstrating a role for ET-1 in the pathogenesis of preeclampsia
ET-1 expression and increases BP in mice, and the BP response is again attenuated by ET\textsubscript{A} receptor blockade (65). Together, these studies suggest that ET-1 and activation of the ET\textsubscript{A} receptor may be serving as a common target for multiple vasoactive factors in the pathogenesis of preeclampsia.

In addition to increased activation of the ET\textsubscript{A} receptor during preeclampsia, recent studies suggest that dysregulation of the ET\textsubscript{B} receptor in pregnancy may also contribute to the development of pregnancy-induced hypertension. Support for this notion is found in the RUPP rat model, expression of the ET\textsubscript{B} receptor decreases in the aorta and the mesenteric microvessels compared with normal pregnant controls (35). Additionally, this study also found that the RUPP rats had significantly decreased ET\textsubscript{B} receptor-mediated NO production compared with normotensive pregnant controls (36). The role of the ET\textsubscript{B} receptor in preeclampsia has been directly assessed using the RUPP model in ET\textsubscript{B}-deficient rats. The typical decrease in renal vascular myogenic tone that is observed during normal pregnancy is absent in ET\textsubscript{B}-receptor-deficient rats (21), and recent work has demonstrated that these rats have a higher BP during late pregnancy compared with wild-type counterparts (54). Not only does the RUPP procedure result in an exacerbated increase in BP in ET\textsubscript{B}-deficient rats compared with the transgenic control RUPP rats, fetal outcomes are also affected in the ET\textsubscript{B}-deficient RUPP rats (54). The pups of the ET\textsubscript{B}-deficient rats are significantly smaller than the transgenic controls following the RUPP procedure, and there is a significant decrease in placental sufficiency, a marker for placental function.

**Clinical Considerations**

ET-receptor antagonists are not approved to treat hypertension based, in large part, on the failure of clinical trials to reach the primary endpoint as well as the availability of safe alternative BP-lowering agents (28). Although the ET\textsubscript{A} receptor antagonist darusentan significantly reduced ambulatory BP in patients with resistant hypertension, the primary end point of the trial, clinic BP, was not significantly different from placebo on the final day of the trial. As a result, the development of the drug was discontinued (4, 63). However, the possibility exists that women in particular may benefit from pharmacological targeting of the ET pathway, specifically agents that would increase ET\textsubscript{B} receptor activation, to lower BP. Indeed, ET receptor antagonists are used in the treatment of pulmonary arterial hypertension (PAH), and women with PAH exhibit a greater increase in 6-min walk distance over 12 wk than men (14), suggesting a sex difference in functional responses to ET receptor antagonists. Currently, ET\textsubscript{A} receptor antagonists are contraindicated during pregnancy due to teratogenic effects during the first trimester, although, these antagonists may be useful in the third trimester when the dangers of preeclampsia are the greatest. The development of newer agents that do not cross the placental barrier or delayed treatment in pregnancy could provide systemic benefit to the mother to improve the symptoms of preeclampsia and prolong pregnancy to improve fetal outcomes.

**REFERENCES**


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