ORIGINAL RESEARCH

Blood Pressure, Sex, and Female Sex Hormones Influence Renal Inner Medullary Nitric Oxide Synthase Activity and Expression in Spontaneously Hypertensive Rats

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***Background*—**We previously reported that sexually mature female spontaneously hypertensive rats (SHRs) have greater nitric oxide (NO) synthase (NOS) enzymatic activity in the renal inner medulla (IM), compared to age-matched males. However, the mechanisms responsible for this sexual dimorphism are unknown. The current study tested the hypothesis that sex differences in renal IM NOS activity and NOS1 expression in adult SHRs develop with sexual maturation and increases in blood pressure (BP) in a female sex hormone-dependent manner.

***Methods and Results*—**Renal IM were isolated from sexually immature 5-week-old and sexually mature 13-week-old male and female SHRs. Whereas NOS activity and NOS1 expression were comparable in 5- and 13-week-old male SHRs and 5-week-old female SHRs, 13-week-old females had greater NOS activity and NOS1 expression, compared to 5-week-old female SHRs and age-matched males. NOS3 expression was greater in 5-week-old than 13-week-old SHRs regardless of sex. Treatment with antihypertensive therapy (hydrochlorothiazide and reserpine) from 6 to 12 weeks of age to attenuate age-related increases in BP abolished the sex difference in NOS activity and NOS1 expression between sexually mature SHR males and females. To assess the role of female sex hormones in age-related increases in NOS, additional females were ovariectomized (OVX), and NOS activity was studied 8 weeks post-OVX. OVX decreased NOS activity and NOS1 expression.

***Conclusions*—**The sex difference in renal IM NOS in SHR is mediated by a sex hormone- and BP-dependent increase in NOS1 expression and NOS activity exclusively in females. **(*J Am Heart Assoc*. 2015;4:e001738 doi: 10.1161/JAHA.114.001738)**

Key Words: hypertension • kidney • NOS • ovariectomy • sex difference

Nitric oxide (NO) is a potent vasodilator produced by a family of NO synthase (NOS) enzymes. Both clinical studies and animal experiments have demonstrated that NO production is greater in females than in males.[1–4] Within the kidney, the renal inner medulla (IM) has the highest amount of NOS protein expression and enzymatic activity,[5,6] and NO regulates inner medullary blood flow and inhibits transport of sodium chloride along the nephron.[7] We previously published that the renal IM is the only section of the kidney to exhibit sex differences in NOS enzymatic activity in young adult (13 weeks old) spontaneously hypertensive rats (SHRs) with greater total NOS enzymatic activity in female SHRs, compared to males.[5] Given that both renal function and NO play critical roles in blood pressure (BP) control and are differentially regulated between the sexes, elucidating the molecular mechanism(s) driving the sexual dimorphism in renal IM NOS activity may provide insight into sex differences in not only NO action in the kidney, but also BP regulation.

Experimental studies showing that the sex of the animal can impact NOS activity and expression have primarily been conducted using young, sexually mature adults.[8,9] Little is known about NO/NOS levels in sexually immature animals; therefore, it remains unknown whether sex differences in NOS in adult SHRs are the result of inherent differences that are established early in life or whether it is a consequence of sexual maturation and increases in BP.

Sex hormones influence NO bioavailability.[10–12] Female sex hormones, in particular, have been shown in vitro to increase endothelial NOS (NOS3) in pulmonary arteries and thoracic aorta of rats[13] and both NO and NOS3 in cultured human endothelial cells.[14,15] However, less is known regarding the impact of female sex hormones on NOS1, and in vivo

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data regarding the impact of female sex hormones on NOS activity and expression are controversial. Removal of female sex hormones by ovariectomy (OVX) has been shown to either decrease,[3] increase,[8,16] or not change renal NOS protein expression.[8,17] We previously published that renal IM NOS activity and expression of NOS1 and NOS3 were not altered 3 weeks after OVX when OVX was performed when rats were 10 weeks of age.[18] In contrast, studies that reported that female sex hormones increase renal NOS activity in vivo were performed in adult Sprague-Dawley and mRen2.Lewis rats that were ovariectomized before sexual maturation.[8,16] Ovariectomy reduced NOS3 and lipopolysaccharide-stimulated NOS2 expression in the renal medulla from Sprague-Dawley rats and reduced renal NOS3 gene expression in the mRen2.Lewis rat. However, in the mRen2.Lewis rat, OVX was associated with increased BP and an increase in renal NOS1 expression, highlighting the need for a greater understanding of regulation of NOS activity and expression of the NOS isoforms in the setting of hypertension (HTN). Therefore, the current study was designed to test the hypothesis that sex differences in renal IM NOS activity in adult SHRs develop with sexual maturation and increases in BP in a female sex hormone-dependent manner.

Methods

Animals

Five- and 13-week-old male and female SHRs were used in this study (Georgia Regents University colony, 44 male rats and 92 female rats). The 5-week time point was chosen because, at this age, rats have not yet reached sexual maturity, as defined by vaginal opening in females and balanopreputial separation in males.[7,19] In addition, 13-week-old male and female WKY rats were obtained from Harlan (n=6/group; Harlan Laboratories, Indianapolis, IN). All experiments were conducted in accord with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and use was approved and monitored by the Georgia Regents University Institutional Animal Care and Use Committee (Augusta, GA). Animals were housed under conditions of constant temperature and humidity and exposed to a 12:12-hour light-dark cycle. All rats were given free access to rat chow and tap water.

To elucidate the relative contribution of increases in BP versus maturation on NOS, IM were isolated from a subset of rats (n=5 to 7/group) randomized to receive vehicle or titrated doses of hydrochlorothiazide (HCTZ; 10 to 55 mg/kg/day) and reserpine (0.6 to 4.5 mg/kg/day) in drinking water beginning at 6 weeks of age until 12 weeks of age to attenuate age-related increases in BP.[20] Rats were individually housed throughout the study. Water intake and body weights were measured every 3 days and doses of drugs were adjusted as needed to maintain consistent BP lowering. BP was measured weekly in HCTZ/reserpine-treated SHRs by tail-cuff plethysmography (IITC Life Sciences, Inc, Woodland Hills, CA), as previously described.[21]

A subset of female SHRs (n=8 to 12/group) underwent OVX as previously described[18] at either 5 or 10 weeks of age, and OVX was confirmed as previously described.[22] OVX was performed at 2 ages to determine whether the timing of OVX, before or after sexual maturation, impacted the experimental outcome. In both cases, animals were allowed to age 8 additional weeks; therefore, tissues were collected at 13 and 18 weeks of age, respectively.

At the end of all studies, rats were anesthetized with ketamine/xylazine (50 and 6 mg/kg intraperitoneally, respectively; Phoenix Pharmaceuticals, St. Joseph, MO), kidneys were removed, and the renal IM was dissected from each kidney and snap-frozen in liquid nitrogen.

Measurement of Total NOS Activity and Expression

Renal IM was homogenized in ice-cold homogenization buffer (50 mmol/L of Tris HCl [pH 7.4] 0.1 mmol/L of EDTA, 0.1 mmol/L of EGTA, 250 mmol/L of sucrose, and 10% glycerol) in the presence of protease inhibitors (1 mmol/L of PMSF, 1 μmol/L of pepstatin A, 2 μmol/L of leupeptin, and 0.1% aprotinin), and the homogenate was used for NOS activity assays and Western blot protocols. NOS activity was determined as previously described[5] and is based on the rate of L-[^{3}H]citrulline formation from L-[^{3}H]arginine and defined as L-[^{3}H]arginine to L-[^{3}H]citrulline conversion inhibited by the nonselective NOS inhibitor, N^{ω}-nitro-L-arginine (L-NNA; 1 mmol/l). NOS activity was normalized to milligrams of protein and expressed as pmol/mg protein/30 min. Cerebellum from Sprague-Dawley rats was assayed as a positive control.[5,18] Three separate experiments were conducted to measure NOS enzymatic activity using distinct sets of rats to directly compare the effects of sex and maturation, HTN, and female sex hormones on NOS activity. Activity assays were not run simultaneously in all 3 experimental protocols owing to the small amount of inner medullary tissue available in each study; therefore, the results do have assay-to-assay variability among the 3 study groups.

Western blotting was performed as previously described[5] using 50 μg of total protein/lane. Following transfer onto PVDF, membranes were blocked in Object 1-P buffer (Nacalai Tesque, Kyoto, Japan) in Tris-buffered saline and Tween-20. Two-color immunoblots were performed using primary antibodies (Abs) to NOS1 or NOS3 (610309 and 610299, respectively, 1:500; BD Transduction Laboratories BD Biosciences, San Jose, CA), and a monoclonal Ab to actin

(A1978, 1:10 000; Sigma-Aldrich, St. Louis, MO). NOS1 and NOS3 protein expression were normalized to actin. Specific bands were detected using the Odyssey Infrared Imager (LI-COR Biosciences, Lincoln, NE). Protein concentrations were determined by standard Bradford assay (Bio-Rad, Hercules, CA) by using BSA as the standard.

Statistical Analysis

All data are expressed as means±SEM. Data were compared using a two-way analysis of variance, followed by a Bonferroni post-hoc test comparing sex, age, and their interaction (GraphPad Prism 5; GraphPad Software Inc., La Jolla, CA). Renal inner medullary NOS activity in male and female WKY was compared using a Student *t* test. For all comparisons, $P<0.05$ was considered statistically significant.

Results

Renal IM NOS Activity Is Increased in Female SHRs With Sexual Maturation

Total NOS enzymatic activity was measured in renal IM homogenates from 5- and 13-week-old male and female SHRs. NOS activity was comparable between 5 and 13 week old male SHR, while 13 week old female SHRs had significantly higher levels of NOS activity, compared to 5-week-old females. Consistent with our previous publication,[5] total NOS activity was greater in the renal IM of 13-week-old females, compared to male SHRs; however, NOS activity was comparable between the sexes in 5-week-old animals. Therefore, based on a significant interaction term, the effect of age on renal inner medullary NOS activity is sex dependent (Figure 1; effect of sex: $P=0.0002$; effect of age: $P=0.0001$; interaction: $P=0.0006$).

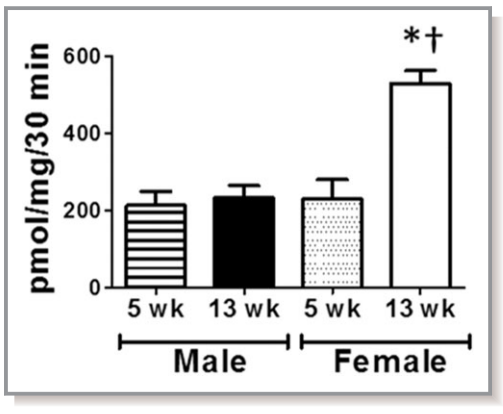

Figure 1. Total NOS enzymatic activity in the renal inner medulla of 5- and 13-week-old male and female spontaneously hypertensive rats; N=11 to 15. *$P<0.05$ versus male of same age; †$P<0.05$ versus 5-week-old of same sex. NOS indicates nitric oxide synthase.

13-Week-Old Female SHRs Have Greater Renal IM NOS1 Protein Expression

Western blot analysis of NOS1 and NOS3 was performed to determine the NOS isoform responsible for sex and age differences in NOS activity. NOS1 protein expression was comparable between 5- and 13-week-old male SHRs, whereas 13-week-old female SHRs had greater NOS1 than 5-week-old female SHRs. Though 5-week-old animals had comparable NOS1 protein expression between sexes, NOS1 protein expression was greater in 13-week-old female SHRs than age-matched males (Figure 2A; effect of sex: $P=0.05$; effect of age: $P=0.01$; interaction: $P=0.09$). NOS3 protein expression was greater in 5-week-old male and female SHRs, compared to same-sex 13-week-old SHRs. However, NOS3 protein expression was comparable between male and female SHRs regardless of age (Figure 2B; effect of sex: $P=0.66$; effect of age: $P=0.02$).

Preventing Age-Related Increases in BP Attenuates Increases in NOS Activity and NOS1 Expression in Female SHRs

SHRs exhibit both an increase in BP and undergo sexual maturation from 5 to 13 weeks of age. Therefore, to elucidate the relative contribution of increases in BP on the sexual dimorphism in NOS activity and NOS1 expression, IM were isolated from male and female SHRs treated with vehicle or HCTZ/reserpine from 6 until 12 weeks of age. The increase in BP over this time and the effect of the HCTZ/reserpine treatment in these same animals was recently published.[23] HCTZ/reserpine treatment significantly attenuated the age-dependent increase in systolic BP in both male and female SHRs ($P<0.05$) and abolished the sex difference in BP observed in 12-week-old vehicle-treated SHRs (control male: 174±4 mm Hg; male HCTZ/reserpine: 137±5 mm Hg; control female: 161±1 mm Hg; female HCTZ/reserpine: 132±4 mm Hg).[20] HCTZ/reserpine treatment did not change NOS activity in males; however, HCTZ/reserpine decreased NOS activity in females to the levels observed in vehicle- and HCTZ/reserpine-treated males. Therefore, based on a significant interaction term, the effect of treatment on renal inner medullary NOS activity is sex dependent (Figure 3A; effect of sex: $P=0.01$; effect of treatment: $P=0.03$; interaction: $P=0.01$). Because IM NOS1 expression increased with age in female rats between 5 and 13 weeks of age, NOS1 expression was also assessed in the IM from SHRs treated with HCTZ/reserpine. Consistent with the observed inhibition

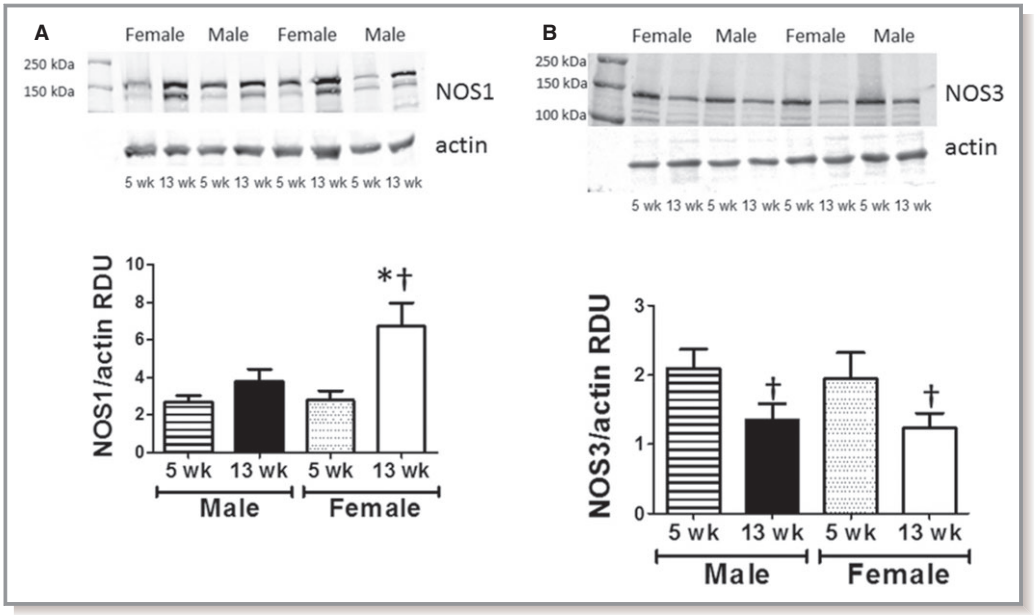

Figure 2. NOS1 (A, N=12) and NOS3 (B, N=12) protein expression in the renal inner medulla of 5- and 13-week-old control male and female spontaneously hypertensive rats. Data are expressed as relative densitometric units (RDU). **P*<0.05 versus male of same age; †*P*<0.05 versus 5-week-old of same sex. NOS indicates nitric oxide synthase.

of age-related increases in NOS activity in female SHRs treated with HCTZ/reserpine, chronic treatment with HCTZ/reserpine prevented the increase in NOS1 protein expression (Figure 3B, *P*=0.01), with no change in expression with treatment in IM isolated from male rats.

Additional studies measured NOS enzymatic activity in the renal IM of 13-week-old male and female WKY (n=6). Consistent with a BP-dependent increase in NOS activity in female SHRs, total NOS activity was comparable in male and female WKY (pmol/mg per 30 min: 396±46 versus 304±35, respectively; *P*=0.14).

OVX Decreases Renal IM NOS Activity and NOS1 Protein Expression

To determine the relative contribution of sexual maturation and female sex hormones on the sexual dimorphism in NOS activity and NOS1 expression, NOS enzymatic activity and NOS1 protein expression were measured in renal IM homogenates from 13-week-old gonad-intact female SHRs, 13-week-old female SHRs ovariectomized at 5 weeks of age, 18-week-old gonad-intact female SHRs, and 18-week-old female SHRs ovariectomized at 10 weeks of age. OVX decreased total NOS activity regardless of the age at which rats were ovariectomized (Figure 4A; effect of age: *P*=0.92; effect of ovariectomy: *P*=0.01; interaction: *P*=0.84). Western blot analysis of NOS1 was also performed. Consistent with decreases in NOS activity after OVX, NOS1 protein expression was also decreased by OVX. NOS1 protein expression was comparable in 13- and 18-week-old gonad-intact female SHRs and in SHRs ovariectomized at 5 and 10 weeks (Figure 4B; effect of age: *P*=0.67; effect of ovariectomy: *P*=0.0002; interaction: *P*=0.87).

Discussion

Results of the current study verified our previous observation that young, adult male SHRs have less IM NOS enzymatic activity than age-matched female SHRs and extended these findings by investigating mechanisms responsible for the increase in NOS activity. The novel findings of the current study are that (1) there is no difference in NOS expression or activity in sexually immature (5-week-old) male and female SHRs, and (2) increased NOS activity/NOS1 expression in female SHRs is dependent on sex hormones and increases in BP. Elucidating the mechanism(s) by which female SHRs increase NOS activity may provide insight into sex differences in not only NO bioavailability, but also BP regulation.

NO plays a crucial role in regulation of sodium chloride transport in the distal nephron and maintenance of inner medullary blood flow by regulation of the tone of the vasa recta.[20,24] Both NOS1 and NOS3 are highly expressed within the renal IM, with expression observed in the vasa recta, the inner medullary thin limb of the loop of Henle, and the inner medullary collecting duct.[25–27] Previous studies by our group examined NOS localization in kidneys of male and female

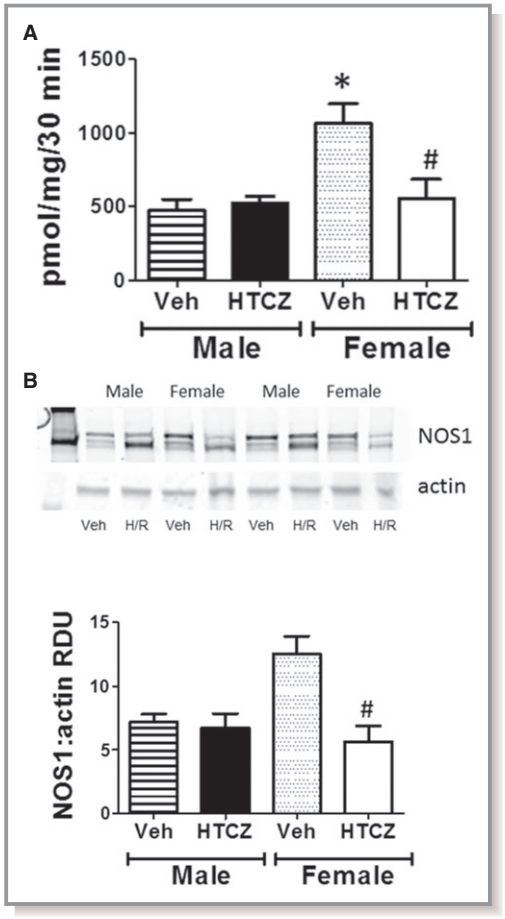

Figure 3. Total NOS enzymatic activity (A) in the inner medulla (IM) of control and HCTZ/reserpine-treated male and female spontaneously hypertensive rats (SHRs); N=5 to 7. NOS1 protein expression (B) in the renal IM of control and HCTZ/reserpine-treated male and female SHRs; n=4 to 6. Data are expressed as relative densitometric units (RDU). *P<0.05 versus male; #P<0.05 versus vehicle-treated female. HCTZ indicates hydrochlorothiazide; NOS, nitric oxide synthase; Veh, vehicle.

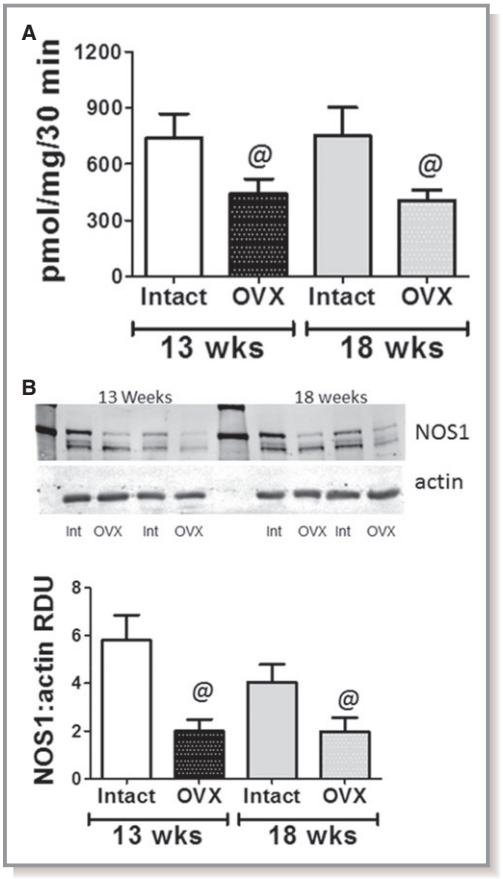

Figure 4. Total NOS enzymatic activity (A) and NOS1 protein expression (B) in the inner medulla (IM) of 13-week-old gonad-intact female spontaneously hypertensive rats (SHRs) (13 weeks; N=12), 13-week-old female SHRs ovariectomized at 5 weeks of age (13 weeks OVX; N=12), 18-week-old gonad-intact female SHRs (18 weeks; N=8), and 18-week-old female SHRs ovariectomized at 10 weeks of age (18 weeks OVX; N=12). Protein expression data are expressed as relative densitometric units (RDU). @P<0.05 versus intact female of same age. NOS indicates nitric oxide synthase; OVX, ovariectomized.

SHRs, and there are no apparent sex differences in renal NOS distribution and localization.[5] Notably, the inner medullary collecting duct is the site of the highest total NOS activity in the kidney with expression of both NOS1 and NOS3.[28] Renal medullary interstitial infusion of the NOS inhibitor, L-NA, decreases inner medullary blood flow and sodium excretion and increases in BP.[20,24] A specific role for NOS1 on cardiovascular physiology is supported by studies in which NOS1 has been blocked with antisense oligonucleotides or the NOS1 inhibitor, 7-NI, and, more recently, with mice that have a tissue-specific knockout of NOS1 in the collecting duct. Blockade of NOS1 resulted in ~15 mm Hg increase in BP,[29] and NOS1 deficiency in the collecting duct is associated with a rightward shift in the pressure natriuresis relationship and salt-sensitive hypertension.[30] Therefore, the current findings expand on our understanding of NOS1 in particular, and suggest that the increase in NOS1 expression and NOS activity in female SHRs may be a protective mechanism to protect against further increases in BP.

Consistent with our previous publication, sexually mature female SHRs have greater IM NOS activity than males,[5] suggesting that increased renal NOS activity could contribute to the lower BP observed in female SHRs, compared to male SHRs. Interestingly, NOS activity was comparable in 5-week-old sexually immature male and female SHRs. Although aging from adulthood into senescence is known to differentially impact renal NO expression in males and females,[31,32] little is

known regarding the impact of sexual maturation on NOS activity or expression either within each sex or between the sexes. Whereas studies have compared renal NOS expression in male SHRs to the normotensive WKY, NOS protein expression has been shown to be greater,[33] comparable,[34] or lower[33] in 3- to 5-week-old male SHRs, compared to age-matched WKY, whereas whole-kidney NO levels are lower in 2-week-old female SHRs, compared to WKY.[35] We are not aware of any other studies that have compared the impact of maturation on renal NOS between the two sexes. Our current data suggest that sexual maturation is involved in establishing sex differences in renal IM NOS activity and support the hypothesis that female SHRs have a compensatory increase in NOS activity, compared to males, with maturation. This hypothesis is consistent with findings that female SHRs maintain a lower BP, compared to male SHRs, at 13 weeks of age[36] and that maintenance of BP is more dependent on NOS in female SHRs than in males.[37]

The increase in NOS activity in female SHRs with maturation was associated with an increase in NOS1 protein expression. We previously published that young adult female SHRs have significantly greater NOS1 enzymatic activity, compared to male SHRs, and NOS1 is abundantly expressed in the renal IM.[5] SHRs not only undergo sexual maturation from 5 to 13 weeks of age, but they also exhibit a progressive increase in BP. To elucidate the relative contribution of increases in BP versus maturation on NOS, additional animals were treated with a combination of HCTZ/reserpine to maintain BP at baseline levels. Preventing the rise in BP that occurs during maturation from 5 to 12 weeks of age using HCTZ/reserpine prevented the increase in renal IM NOS1 expression and abolished the sex difference in NOS activity by attenuating the age-related increase observed in female SHRs. These data suggest that female SHR exhibit a compensatory increase in NOS activity as BP increases independent of sexual maturation, and this may account for the sex difference in BP observed between 13-week-old male and female SHRs. This conclusion is supported by the finding that NOS activity is comparable in the renal IM of normotensive male and female WKY. HCTZ/reserpine were selected as nonspecific BP-lowering agents allowing us to examine the role of BP on NOS in rats that were genetically identical to SHRs. Though typically used in combination with hydralazine in "triple therapy,"[38] one endpoint for alternative studies in these rats was to assess immune cells,[23] and hydralazine has been shown to reduce the number of adherent and migrating leukocytes in vasculature of male SHRs independent on an effect on BP.[39] We chose not to use inhibitors of the renin angiotensin system owing to well-known and described sex differences in the renin angiotensin system.[40,41] However, it should be noted that reserpine has been suggested to impact estrogen secretion,[42] act as a weak agonist on the estrogen receptor when given subcutaneously,[43] enhance the actions of estrogen,[44] and increase plasma levels of estrone, although not estradiol.[45] Additional studies need to be done to better define the potential complex relationship between reserpine and estrogens in our animal model.

Interestingly, sexual maturation from 6 to 12 weeks of age in both male and female SHRs was associated with a decrease in total NOS3 protein expression despite no change in NOS activity in males and an increase in females, suggesting that (1) females stimulate NOS activation independent of alterations in total NOS3 protein abundance and (2) that although NOS protein expression is lower at 13 weeks than 5 weeks of age, females still achieve sufficient activation of NOS. It should be noted that NOS enzymatic activity was performed using optimal concentrations of cofactors and reflects the potential of the enzyme to make NO and is not a measure of in vivo NO production. In addition, female sex hormones have been shown to increase NOS3 activity, primarily by enhanced phosphorylation on serine residue 1177.[46] As a result, the finding that total NOS3 protein expression is comparable between 13-week-old male and female SHRs does not preclude sex hormone-dependent effects on the post-translation modifications of NOS3 contributing to the observed sex differences in total NOS activity.

Female sex hormones consistently increase NO production in vitro,[13–15] although in vivo studies have yielded conflicting results.[3,8,16,17] We previously published that manipulation of female sex hormones in SHRs does not alter NOS enzymatic activity or expression in the renal IM[5] or cortex[36] when rats were ovariectomized at 10 weeks of age and studied at 13 weeks. In contrast to our previous findings in SHRs, renal cortical and medullary NOS3 protein expression decreased in hypertensive congenic female mRen.2 Lewis rats and normotensive Sprague-Dawley rats when ovariectomized at 4 weeks and studied at 15 and 10 weeks of age, respectively.[8,16] Based on these findings, the current study was designed to determine whether age of OVX was a critical determinant of impact on NOS. We found that OVX decreased NOS activity and NOS1 expression when rats were studied 8 weeks post-OVX, regardless of the age at which the OVX was performed. Therefore, despite the fact that 3 weeks is sufficient to allow sex hormone levels to decrease[47] and the uterus to atrophy,[48,49] the effects of sex hormone deprivation on NOS are delayed in female SHRs. In contrast, OVX of Sprague-Dawley rats at 4 weeks,[8] Dahl salt-sensitive rats (on 0.1% NaCl) at 3 months[17] or Fischer-344 rats at 3 to 4 months[31] does not alter cortical or medullary total NOS expression when studied 6 weeks to 6 months post-OVX. Based on the dependence on the NOS system to limit increases in BP in female SHRs,[37,50] we speculate that they have a greater sensitivity to estrogen-mediated loss of NOS. Indeed, female SHRs have much greater NOS expression than Dahl salt-sensitive rats; as a result, it is not surprising that the effect of

OVX is more pronounced in SHRs.[50] Regardless, our results indicate that timing may be a critical determinant of post-OVX outcomes on the renal NOS system. The decrease in NOS activity with OVX was likely independent of BP given that we and others have reported that OVX does not alter BP in female SHRs.[11,12,36,51] A limitation of the current study is the lack of sex hormone replacement studies to confirm and identify the sex hormone responsible for the changes in NOS following gonadectomy. Future studies will be designed to determine the mechanism(s) by which female sex hormones increase NOS activity in responses to an increase in BP.

The goal of the current study was to gain additional mechanistic insight into sex differences in NOS1 protein expression in young-adult SHRs. A limitation of the current study is that we did not examine the impact of HCTZ/reserpine or OVX on NOS3 expression. The selective increase in total NOS activity in female SHRs between 5 and 13 weeks of age may reflect increases in NOS3 activity as well as NOS1, despite no change in total NOS3 protein expression, as discussed above. Similarly, we would predict that the decrease in total NOS activity following OVX would be accompanied by a decrease in the activity of NOS3, with or without a change in total NOS3 protein expression. The impact of high BP on NOS3 expression in SHRs is controversial; NOS3 protein expression in male SHRs has been shown to be greater, comparable, and less than expression in WKY. Aortic NOS3 protein expression decreases with maturation from 4 to 14 to 17 weeks of age in male SHRs, but not WKY,[52] raising the possibility that SHRs exhibit a BP-dependent decrease in NOS3 expression. In contrast, additional studies have shown the aortic and renal NOS3 expression are greater in male SHRs, compared to male WKY, at 12 weeks of age[33]; however, there were no strain differences in the kidney at 3 weeks of age, suggesting an age-related increase in NOS3 in SHRs. Using immunohistochemistry, the distribution of NOS3 was shown to be similar in kidney of male SHRs and WKY.[53] We recently published that although total NOS enzymatic activity was comparable among 12-week-old male and female SHRs and WKY, male SHRs have greater aortic NOS3 protein expression than male WKY with no strain differences in females.[54] Whereas WKY are often used as normotensive controls for SHRs, they are not a genetic match. For this reason, the current studies employed a pharmacological approach to lower BP in SHRs and remove genetic background as a variable. Future studies will be necessary to more fully understand the impact of BP and sex hormones on NOS3 expression and regulation in male and female SHRs.

Perspectives

Although numerous studies have indicated a correlation between sex hormones, NOS/NO, and BP, the molecular mechanisms connecting these factors remain unknown. This may, in part, reflect a lack of consistency in the design of experiments and differences in the time points examined following loss of sex hormones. The role of female sex hormones changes over the life span of a female, and overlooking this fact may impact the endpoints examined. Studies that are conducted to examine the protective effects of female sex hormones should consider how much time has elapsed post-OVX/menopause and that the effects of female sex hormones on NOS are long lasting. Moreover, examining the effects of sex hormones and the NO pathway at varying ages across a life span will likely provide a clearer picture of how these factors come together to regulate BP and may provide for age- and sex-specific factors to target to improve BP control rates in HTN.

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Disclosures

None.

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