Sex Differences in Renal and Cardiovascular Function: Physiology and Pathophysiology

Discoordinate regulation of renal nitric oxide synthase isoforms in ovariectomized mRen2.Lewis rats

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Yamaleyeva LM, Gallagher PE, Vinsant S, Chappell MC. Discoordinate regulation of renal nitric oxide synthase isoforms in ovariectomized mRen2.Lewis rats. Am J Physiol Regul Integr Comp Physiol 292: R819–R826, 2007. First published October 5, 2006; doi:10.1152/ajpregu.00389.2006.—Estrogen depletion markedly exacerbates hypertension in female congenic mRen2.Lewis rats, a model of tissue renin overexpression. Because estrogen influences nitric oxide synthase (NOS) and NO may exert differential effects on blood pressure, the present study investigated the functional expression of NOS isoforms in the kidney of ovariectomized (OVX) mRen2.Lewis rats. OVX-mRen2.Lewis exhibited an increase in systolic blood pressure (SBP) of 171 ± 5 vs. 141 ± 7 mmHg (P < 0.01) for intact littermates. Renal cortical mRNA and protein levels for endothelial NOS (eNOS) were reduced 50–60% (P < 0.05) and negatively correlated with blood pressure. In contrast, cortical neuronal NOS (nNOS) mRNA and protein levels increased 100 to 300% (P < 0.05). In the OVX kidney, nNOS immunostaining was more evident in the macula densa, cortical tubules, and the medullary collecting ducts compared with the intact group. To determine whether the increase in renal nNOS expression constitutes a compensatory response to the reduction in renal eNOS, we treated both intact and OVX mRen2.Lewis rats with the selective nNOS inhibitor L-VNIO from 11 to 15 wk of age. The nNOS inhibitor reduced blood pressure in the OVX group (185 ± 3 vs. 151 ± 8 mmHg, P < 0.05), but pressure was not altered in the intact group (146 ± 4 vs. 151 ± 4 mmHg). In summary, exacerbation of blood pressure in the OVX mRen2.Lewis rats was associated with the discoordinate regulation of renal NOS isoforms. Estrogen sensitivity in this congenic strain may involve the influence of NO through the regulation of both eNOS and nNOS.

endothelial nitric oxide synthase; estrogen; hypertension; aldosterone; angiotensin II; renin

THE PROTECTIVE ROLE OF ESTROGEN in cardiovascular disease remains a highly controversial and complex area of study, particularly given the recent findings of the Women’s Health Initiative (WHI) study. Although experimental and clinical data support the beneficial actions of estrogen, both the WHI and the Heart and Estrogen Replacement Study follow-up (HERS II) studies concluded that there was no cardioprotective benefit for estrogen replacement in older women with underlying cardiovascular disease (19, 39). Indeed, the incidence for ischemic stroke and dementia were higher for this population of postmenopausal women receiving either estrogen or combined hormone replacement therapy (40, 41). The results of WHI and HERS were surprising, in part, due to the generally accepted view that estrogen has beneficial actions on nitric oxide (NO), as well as an inhibitory influence on the components of the renin-angiotensin-aldosterone system (RAAS), including ACE and the angiotensin type 1 (AT1) receptor (6, 12, 17, 26, 33, 45).

Our studies in the congenic mRen2.Lewis strain of hypertensive rats support the beneficial role of estrogen since ovariectomy exacerbates the development of hypertension in this model of tissue renin overexpression (10). Early estrogen depletion almost abolishes the gender difference in blood pressure evident between male and female mRen2.Lewis rats (10), as well as in other hypertensive models (33). Moreover, estradiol replacement or treatment with an AT1 receptor antagonist normalized the blood pressure in the ovariectomized (OVX)-mRen2.Lewis rats; these data further support an important role for estrogen in the regulation of the RAAS. In addition to the steroids’ influence on the RAAS, the NO system is an important target for the effects of estrogen. The stimulatory actions on endothelial nitric oxide synthase (NOS III or eNOS) are considered to be one of the primary mechanisms that contribute to the protective effects of estrogen (9). However, the influence of estrogen on the expression of the other isoforms (neuronal and inducible NOS) and whether they contribute to the cardioprotective actions of estrogen in female hypertensive mRen2.Lewis animals are not known. Although we hypothesize that compensatory expression of other NOS isoforms after estrogen depletion and the ensuing hypertension would be beneficial, there is evidence that NO activity may facilitate an increase in blood pressure (28, 29, 32, 42). Therefore, we assessed the expression of the three NOS isoforms in the kidney of intact and ovariectomized mRen2.Lewis rats, as well as the contribution of these isoforms to the increase in blood pressure following early estrogen depletion.

MATERIALS AND METHODS

Experimental animals. All animals were obtained from the congenic colony of the Hypertension and Vascular Disease Center of Wake Forest University. Female mRen2.Lewis rats weighing 120–
130 g at the age of 4 wk were housed in a temperature-controlled room with a 12:12-h light-dark cycle (lights on 6:00 AM to 6:00 PM) in an American Association for Accreditation of Laboratory Animal Care-approved facility. Animals were randomly assigned to study groups, six or seven animals per group. Female rats were OVX at 4 wk of age. Bilateral ovariectomy was performed under ketamine/xylazine (80/12 mg/kg) anesthesia, as described previously (10). The neuronal NOS (nNOS) inhibitor N5-(1-imino-3-butenyl)-L-ornithine (L-VNIO, Alexis Biomedicals, San Diego, CA) was diluted in saline and administrated via an osmotic minipump (28 day, 2.5 μl/h; model 2ML4, ALZA, Palo Alto, CA) intraperitoneally to separate groups of 11-wk-old OVX and intact groups to achieve a target dose of 0.5 mg/kg·day. At 15 wk of age, rats were decapitated, and the trunk blood was collected for plasma and serum. Kidneys were collected for analysis of NOS mRNA, protein expression, and immunostaining. All procedures were approved by the Wake Forest University School of Medicine Institutional Animal Care and Use Committee.

**Blood pressures.** Systolic blood pressure (SBP) was measured at weekly intervals in trained rats (mean of 5 determinations per data point) using the automated tail-cuff system (Narco Bio-systems, Houston, TX) after warming the rats to 35°C under slight restraint. The heart rate was determined by a discrete Fourier transform on the pulse wave signal, yielding a frequency with highest incidence over the sampled region (heart rate in Hz). Five samples were collected and averaged for each animal at 15 wk of age for heart rate.

**Immunoblots.** Samples of kidney cortex and medulla were homogenized in a buffer containing 10 mM HEPES (pH 7.4), 125 mM NaCl, 1 mM EDTA, 1 mM NaF, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, and 1 mM PMSF final concentrations. Samples were centrifuged at 2,000 g, and then at 100,000 g for 60 min at 4°C. Protein concentrations in the pellet and supernatant were measured by the bicinchoninic acid assay (BCA) method using a Pierce kit. Samples (50 μg of the total protein) were separated by gel electrophoresis, and then proteins were eluted from the gels to Hybond PVDF membranes (Bio-Rad, Hercules CA) for 3 h at 100 V. Nonspecific binding was blocked in 5% nonfat dried milk in 0.1% Tween 20 in TBS for 60 min at room temperature. The blots were reacted with a monoclonal anti-nNOS (1:2,000), monoclonal anti-eNOS (1:500), monoclonal anti-inducible NOS (iNOS; 1:2,000), all from Transduction Laboratories (Lexington, KY) or a monoclonal anti-β-actin (1:2,000) from Sigma (St. Louis, MO). After washings in TBS and Tween-TBS, the blots were incubated with horseradish peroxidase-labeled secondary goat anti-mouse (Amersham Biosciences, Piscataway, NJ) at a 1:3,000 dilution, for 60 min at room temperature. After the final washings, the blots were incubated with Pierce Super Signal West Pico chemiluminescent substrate and exposed to Hyperfilm ECL (Amersham Biosciences). The corresponding bands were quantified using densitometry (MCID software); the results were expressed as arbitrary units of intensity and normalized to the β-actin protein intensities. As positive controls, we used human endothelial cell lysate for eNOS, rat cerebrum cell lysate for nNOS, and mouse macrophage IFN-β/LPS lysate for iNOS.
RNA isolation and real-time reverse-transcriptase polymerase chain reaction assay. RNA was isolated from kidney tissue, using the Trizol reagent (GIBCO Invitrogen, Carlsbad, CA). The RNA concentration and integrity were assessed using an Agilent 2100 Bioanalyzer with an RNA 6000 Nano LabChip (Agilent Technologies, Palo Alto, CA). Approximately 1 μg of total RNA was reverse transcribed using AMV reverse transcriptase in a 20-μl reaction mixture containing deoxyribonucleotides, random hexamers and RNase inhibitor in reverse transcriptase buffer. Heating the reverse transcriptase reaction product at 95°C terminated the reaction. The resultant cDNA (2 μl) was added to TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA) with the appropriate gene-specific primer/probe set and amplification was performed on an ABI 7000 Sequence Detection System. The primer/probe sets for rat eNOS, iNOS, and nNOS were purchased from Applied Biosystems. The mixtures were heated at 50°C for 2 min, at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. All reactions were performed in triplicate and 18S ribosomal RNA, amplified using the TaqMan Ribosomal RNA Control Kit (Applied Biosystems), served as an internal control. The results were quantified as Ct values, where Ct is defined as the threshold cycle of PCR, at which amplified product is first detected, and expressed as the ratio of target/control.

Biochemical assays. The concentration of ANG II was determined in the plasma as described by Allred et al. (2). Plasma was obtained from trunk blood collected directly into chilled Vacutainer tubes containing a mixture of peptide inhibitors (25 mM EDTA, 0.44 mM o-phenanthroline, 1 mM PCMB, 10 μM lisinopril, 10 μM SCH39370, 2 μM amastatin, and 10 μM bestatin). After 20 min on ice, blood samples were centrifuged at 2,000 g for 20 min, and plasma was drawn without disturbing the packed cells. Aliquots of plasma were stored at −80°C before extraction on Sep-Pak C18 columns (200 mg, Waters, Milford, MA). The sensitivity and specificity for the ANG II radioimmunoassay were previously described (2). Plasma aldosterone levels were measured by an RIA kit (DPC, Los Angeles, CA) and expressed as nanograms per deciliter. Nitrate and nitrite concentrations in the renal cortex and medulla of the control and L-VNIO-treated intact and OVX groups were measured in the supernatant fraction using a Griess assay (nitrate/nitrite colorimetric assay kit; Alexis Biomedicals, San Diego, CA). Tissues were homogenized in 10 mM PBS (pH 7.4), 10 μg/ml leupeptin, 10 μg/ml pepstatin A, and 1 mM PMSF and centrifuged at 100,000 g for 30 min at 4°C. Protein concentrations were measured by the BCA method using a Pierce kit. Then the supernatants were tested for the nitrate/nitrite content using the manufacturer’s instructions. Rat plasma renin concentrations (PRCs) were determined at pH 6.5 by addition of exogenous angiotensinogen (from nephrectomized rat plasma) and expressed as ng ANG 1·ml⁻¹·h⁻¹.

Immunohistochemistry. Kidney paraffin-embedded 5-μm sections were stained using a standard avidin-biotin method. The staining required antigen retrieval treatment—sections were autoclaved in sodium citrate buffer at pH 6.0 for 10 min. The endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxide in 10% methanol for 10 min. Nonspecific binding was blocked in a buffer containing 2% normal goat serum, 0.1% Triton-X in PBS for

Fig. 2. Effect of ovariectomy on renal neuronal NOS (nNOS) in female mRen2.Lewis rats. nNOS mRNA data for renal cortex (A) and medulla (D) of the intact and OVX mRen2.Lewis rats. The mRNA data were expressed as the ratio of nNOS to 18 S rRNA levels (n = 7; *P < 0.05 compared with intact group). Scatterplots for renal cortical (B) and medullary (E) nNOS mRNA and SBP in the intact and OVX groups, nNOS protein levels in the renal cortex (C) and medulla (F) of 15-wk-old intact and OVX rats. Protein data were expressed as the ratio of nNOS to β-actin (n = 5; *P < 0.05 compared with intact group). Inset: immunoreactive nNOS bands at 125 kDa.
60 min. We used the affinity purified nNOS rabbit polyclonal antibody (1:400 dilution, BD Transduction) and a biotinylated goat anti-rabbit antibody (Vector Laboratories, Burlingame, CA). Antibody binding was detected using Vectastain Elite avidin-biotin complex kit and incubated with 0.1% diaminobenzene solution.

Statistical analysis. All measurements are expressed as the means ± SE computed from an average of five determinations per rat for SBP or duplicate values for the biochemical data from each rat. Comparisons between groups were evaluated using one-way ANOVA with the appropriate post hoc tests (Tukey’s) or by unpaired t-test (GraphPad Prism IV plotting and statistical software, San Diego, CA). The associated scatterplots and the linear regression line with 95% confidence limits were constructed with the Prism IV software.

RESULTS

Consistent with our previous study (10), OVX at 4 wk of age significantly increased SBP in the female mRen2.Lewis rats at 15 wk of age (OVX: 171 ± 5 mmHg vs. intact: 141 ± 7 mmHg, P < 0.01). As shown in Fig. 1, ovariectomy in the mRen2.Lewis rats was associated with 60% lower mRNA levels for eNOS in both the cortex and medulla (A and D) of the kidney. Correlation analysis revealed a negative association between SBP and both cortical (Fig. 1B) and medullary (Fig. 1E) mRNA for eNOS in the intact and ovariectomized groups. Immunoblots for eNOS protein confirmed the reduction in cortical mRNA levels (Fig. 1C), but we observed no change in medullary protein content (Fig. 1F). Although not shown as a scatterplot, we also find a negative correlation for cortical eNOS protein and blood pressure (r = −0.62; P = 0.047).

In contrast to eNOS, the mRNA for nNOS increased 300–400% in the renal cortex and medulla following ovariectomy (Fig. 2, A and D, respectively). Correlation analysis revealed a positive association between the SBP and both cortical and medullary mRNA levels for nNOS (Fig. 2, B and E, respectively). The protein analysis also revealed an approximate two-fold increase for nNOS expression in the renal cortex of the OVX-mRen2.Lewis rats (Fig. 2C) but no difference in protein in the renal medulla (Fig. 2F). We further assessed the distribution of nNOS in the kidneys of the mRen2.Lewis rats by immunocytochemical methods. Staining for nNOS in the intact tissue was predominantly localized to the macula densa region of the renal cortex (Fig. 3, A and B), with very light staining of the proximal tubules in cortex (Fig. 3B) and the collecting ducts of the medullary region (Fig. 3C). In the kidneys from the ovariectomized mRen2.Lewis rats, the intensity of nNOS staining appeared greater throughout the tubular elements and macula densa of the renal cortex, as well as the collecting ducts in the medulla. Regarding iNOS, we did not detect this isoform in renal cortex or medulla of the OVX group by either protein immunoblot or RT-PCR methods (data not shown).

Fig. 3. Immunohistochemical staining of nNOS in the intact (A–C) and OVX (D–F) mRen2.Lewis rats. A and D: nNOS staining through all kidney regions in intact and OVX rats, respectively. B and E: nNOS expression in the renal cortex of intact and OVX, respectively. C and F: nNOS in the renal medulla of intact and OVX rats, respectively. Results shown are typical of 3 or 4 sections per kidney from 2 or 3 rats per group. CD, collecting ducts; CTX, cortex; GLM, glomerulus; IM, inner medulla; MD, macula densa; OM, outer medulla; PT, proximal tubule.
To evaluate the role of nNOS in the development of hypertension after estrogen depletion in the mRen2.Lewis rats, we administered the selective nNOS inhibitor, L-VNIO for a 4-wk period by osmotic minipumps to both intact and OVX groups. L-VNIO is an irreversible and potent nNOS inhibitor (Kᵢ of 100 nM) exhibiting over 100-fold lower affinity for eNOS and iNOS isoforms (Kᵢ of 12 μM and 60 μM, respectively) (3). In Fig. 4A, we show the time course of 11 to 15 wk for SBP of the L-VNIO-treated intact and OVX-mRen2.Lewis, as well as untreated intact and OVX-mRen2.Lewis rats. At 11 wk of age before L-VNIO treatment, the systolic blood pressures were markedly higher in the ovariectomized vs. the intact group (185 ± 3 mmHg vs. 146 ± 4 mmHg; P < 0.01). L-VNIO treatment significantly reduced blood pressure by week 12, and pressure remained lower than the control OVX group through week 15 (151 ± 8 mmHg vs. 171 ± 5 mmHg, P < 0.05) but was not different than either the treated (151 ± 4 mmHg) or the control intact groups (141 ± 7 mmHg) at 15 wk of age. The heart rate data for all four groups at 15 wk of age were comparable to one another (Fig. 4B). In Fig. 5, we determined the nitrate/nitrite (NOₓ) content in the renal cortex (A) and medulla (B). In the cortex, NOₓ levels were substantially lower in the 15-wk OVX group compared with the intact group and tended to decline with treatment; however, the treated intact group exhibited the highest NOₓ content. In contrast, medullary NOₓ levels were higher in the OVX group. Similar to the cortex, NOₓ increased in the intact treated group but remained practically unchanged in OVX rats.

The substantial reduction in systolic blood pressure after treatment with the nNOS inhibitor was unexpected. As evidence suggests that nNOS may influence renin expression (5, 7), we determined whether circulating components of the RAAS were altered to an extent that may contribute to the reduction in blood pressure. As shown in Fig. 6, rat PRC (A) was not suppressed by L-VNIO treatment nor was circulating ANG II (B), although the high SE for the untreated OVX group reduces our ability to detect differences for this group. Plasma aldosterone levels were significantly increased by ovariectomy consistent with our earlier study (11) and tended to decline in the OVX group treated with the nNOS inhibitor such that aldosterone was not different compared with the intact groups

Fig. 4. Effects of the nNOS inhibitor N5-(1-imino-3-butenyl)-L-ornithine (L-VNIO) on systolic blood pressure and heart rate in intact and OVX mRen2.Lewis rats. A: time course for SBP (mmHg) in L-VNIO-treated intact and OVX-mRen2.Lewis rats, as well as untreated intact and OVX-mRen2.Lewis rats. Treatment began at 11 wk of age for 4 wk (n = 5 or 6; *P < 0.05 vs. 11 wk OVX-treated group; #P < 0.05 vs. 11 wk intact group; δP < 0.05 vs. intact treated group at 15 wk; δδP < 0.01 vs. age-matched treated intact or OVX-Ren2.Lewis rats). B: heart rate (HR, beats/min) in the L-VNIO-treated rats compared with age-matched 15-wk-old rats (n = 6 or 7).

Fig. 5. Effects of the nNOS inhibitor L-VNIO on nitrate/nitrite content. Cortical (A) and medullary (B) content of nitrate/nitrite was expressed as nanomoles per milligram protein in untreated and treated 15-wk-old mRen2.Lewis rats (n = 4–6; *P < 0.05 vs. untreated intact group; #P < 0.05 vs. treated intact).
Correlation analysis of the untreated and treated OVX groups revealed a positive association between plasma aldosterone and blood pressure (Fig. 6D).

**DISCUSSION**

The influence of estrogen on NOS expression and subsequent NO production is an important aspect of the complex regulatory events associated with this steroid hormone (9). In the present study, we characterized the expression of NOS isoforms in the kidneys of the female mRen2.Lewis rat after ovariectomy. Estrogen depletion markedly exacerbated the extent of hypertension in the congenic rats but did not increase blood pressure in the normotensive Lewis controls (10). We demonstrated that the increased blood pressure in the estrogen-depleted mRen2.Lewis rat was associated with the differential expression of eNOS and nNOS isoforms in the kidney. Both mRNA and protein levels of eNOS in the renal cortex were markedly attenuated, but the expression of cortical nNOS (mRNA and protein) was higher in the estrogen-depleted mRen2.Lewis rats. The enhanced expression of nNOS does not appear to be a compensatory response to the reduction in renal eNOS, as chronic administration of the nNOS inhibitor L-VNIO produced a sustained reduction in blood pressure in the ovariectomized but not intact mRen2.Lewis rats. Although the mechanisms for the blood pressure-lowering effects of the nNOS inhibitor are as yet unresolved, the circulating levels of aldosterone in the OVX-mRen2.Lewis rats tend to be reduced after treatment with the nNOS inhibitor.

The increase in blood pressure following ovariectomy in the mRen2.Lewis rat is consistent with the presence of an estrogen-sensitive response element on the eNOS gene (9, 25), although estrogen per se may not solely influence eNOS, as cortical eNOS protein was not diminished in the OVX-Lewis rats (Yamaleyeva L and Chappell M, unpublished results). Estrogen depletion is also associated with an activation of the RAAS in the mRen2.Lewis, as well as other strains, and the increased expression of ANG II or the AT1 receptor subtype may contribute to the reduction in the renal levels of eNOS. The extent that reduced cortical eNOS contributes to the sustained increase in blood pressure in the mRen2.Lewis rat is not presently known. Certainly, a reduction in vascular NO due to lower eNOS expression may underlie an enhanced vascular tone in the kidney and possibly other vascular beds in the mRen2.Lewis rat. Studies are in progress to assess whether renal blood flow or other renal parameters are reduced in the ovariectomized mRen2.Lewis rat.

In contrast to the reduction in eNOS expression, mRNA and protein levels of nNOS were increased in the cortical areas of the kidney following ovariectomy in the mRen2.Lewis rats. In the intact rat, immunoreactive nNOS was primarily localized to the macula densa cells of the renal cortex. After ovariectomy, nNOS staining was more evident throughout both the cortical and medullary regions of the kidney. The influence of estrogen on the expression of nNOS is not as well characterized as that for eNOS, and there is no clear consensus on the regulatory role for estrogen, particularly within the kidney (14, 16, 20, 27, 29, 38). To our knowledge, the present study is the first to assess the effects of chronic treatment with a nNOS inhibitor in female hypertensive rats. The increase in nNOS expression does not appear to be a compensatory response to the reduced levels of renal eNOS, as the nNOS inhibitor L-VNIO produced a sustained reduction in blood pressure to a
level not different than that of the intact mRen2.Lewis rats. Moreover, the nNOS inhibitor did not reduce blood pressure in the intact mRen2.Lewis strain by the end of the treatment period. Indeed, the role of nNOS in the regulation of blood pressure is far from established, as studies report either increased (4, 28, 31), decreased (21, 22) or no change (1) in blood pressure after nNOS inhibition. In the rat, this may reflect the type, site, and duration of administration of the nNOS inhibitor (18). We used the nNOS inhibitor L-VNIO rather than the more commonly used agent 7-nitroindazole given the greater selectivity and solubility of L-VNIO (3). Indeed, Mattson and colleagues (21) reported that L-VNIO administration acutely reduced blood pressure and attenuated NO levels (30–40%) in both cortical and medullary regions of the kidney from normotensive male Sprague-Dawley rats. Importantly, their studies reveal that nNOS contributes to a significant extent in the renal production of NO, although it should be noted that the acute reduction in NO production by L-VNIO did not affect cortical or medullary renal blood flow (21). The current study did not directly measure NOS-dependent production of NO in the kidney; however, cortical nitrate/nitrite levels were lower in the ovarietomized group and tended to further decline following L-VNIO administration. Whether the greater nitrate/nitrite levels in the treated intact group reflect greater eNOS activity is not known; however, eNOS null mice exhibit increased expression of eNOS (22), and the OVX-treated group may not be capable of upregulating eNOS. Although medullary mRNA levels of nNOS and NOx content increased after ovariectomy, protein levels were not altered. It is possible that membrane-associated forms of nNOS are present in the medullary tissue and the current protocol using the cytosolic fraction may not have detected these forms.

In regard to the blood pressure-lowering actions of the nNOS inhibitor, we can only speculate on the potential mechanisms at this time. Staining for nNOS was clearly evident in the macula densa, but plasma renin activity and ANG II were not different between the control and treated groups despite a significant reduction in blood pressure. That renin did not increase in the treated OVX-mRen2.Lewis rat suggests an inhibitory influence of nNOS under stimulatory conditions for renin, and this may contribute to the maintenance of lower blood pressure with chronic administration of L-VNIO (5). Several reports implicate a role for nNOS to attenuate tubuloglomerular feedback (TGF) (36, 43); however, one would expect an increase in blood pressure due to an enhanced TGF response following nNOS inhibition. Johns and colleagues (4, 44) found that nNOS inhibition attenuated the response of renal nerve stimulation on sodium excretion, suggesting a facilitatory role for NO on adrenergic release. In this regard, Cervenka et al. (8) demonstrated increased sodium excretion following acute nNOS inhibition in intact but not denervated male mRen2/27 rats, the founder strain to the current congenic strain. We do not know the status of the adrenergic system following ovariecotomy in the mRen2.Lewis rat, although estrogen exerts an inhibitory influence on sympathetic activation (13, 15, 30, 34). Moreover, estrogen depletion exacerbated the salt-dependent increase in blood pressure and renal injury (proteinuria) in the mRen2.Lewis rat, and altered NO levels in the kidney may well contribute to the sodium-sensitive phenotype (11). Alternatively, the inhibition of nNOS in the OVX-mRen2.Lewis rat to reduce blood pressure may reflect the suppression of NO-dependent pressor metabolites, such as peroxynitrite or other reactive oxygen species (ROS). Tetrahydrobiopterin (BH4), an estrogen-sensitive cofactor for NOS (35), is essential for NO generation, and reduced BH4 may lead to enhanced levels of superoxide products (23, 24, 32, 37, 42). Clearly, additional studies are required to address whether increased NO or nNOS-derived ROS in the kidney (or other tissue sites) contributes to the sustained hypertensive response to estrogen depletion in the mRen2.Lewis strain.

In summary, ovariecotomy in the female mRen2.Lewis strain exacerbates the extent of hypertension and is associated with the discordinate regulation of renal NOS isoforms as evidenced by a reduction in eNOS and the enhanced expression of nNOS in the renal cortex. The increase in renal nNOS does not confer a protective or compensatory response to estrogen depletion in the congenic model since chronic nNOS inhibitor treatment produced a sustained reduction in blood pressure. Although the exact pathways for the NOS-dependent actions remain to be resolved, we conclude that estrogen sensitivity in the mRen2.Lewis rat may involve the influence of NO through the regulation of both eNOS and nNOS isoforms.

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REFERENCES


19. Carretero OA. Estrogen regulates adrenal angio-


21. Herra
type and renal nitric oxide synthases.


33. Wu Z, Zheng W, and Sandberg K. Estrogen regulates adenal angio-


41. Wu Z, Zheng W, and Sandberg K. Estrogen regulates adenal angio-


43. Nickenig G, Baumer AT, Grohe C, Kahlert S, Strehlow K, Rosen-


45. Nickenig G, Baumer AT, Grohe C, Kahlert S, Strehlow K, Rosen-