Protective actions of estrogen on angiotensin II-induced hypertension: role of central nitric oxide

Baojian Xue,1* Minati Singh,1* Fang Guo,1 Meredith Hay,5 and Alan Kim Johnson1,2,3,4

Departments of 1Psychology, 2Integrative Physiology, and 3Pharmacology and 4Cardiovascular Center, University of Iowa, Iowa City, Iowa; and 5Department of Physiology, University of Arizona, Tucson, Arizona

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Xue B, Singh M, Guo F, Hay M, Johnson AK. Protective actions of estrogen on angiotensin II-induced hypertension: role of central nitric oxide. Am J Physiol Heart Circ Physiol 297: H1638–H1646, 2009. First published September 4, 2009; doi:10.1152/ajpheart.00502.2009.—The present study tested the hypotheses that 1) nitric oxide (NO) is involved in attenuated responses to ANG II in female mice, and 2) there is differential expression of neuronal NO synthase (nNOS) in the subfornical organ (SFO) and paraventricular nucleus (PVN) in response to systemic infusions of ANG II in males vs. females. Aortic blood pressure (BP) was measured in conscious mice with telemetry implants. N^3-nitro-l-arginine methyl ester (l-NNAME; 100 μg·kg^-1·day^-1), an inhibitor of NOS, was administered into the lateral cerebral ventricle for 14 days before and during ANG II pump implantation. Central infusion of l-NNAME augmented the pressor effects of systemic ANG II in females (Δ21.5 ± 2.2 vs. Δ9.2 ± 1.5 mmHg) but not in males (Δ29.4 ± 2.5 vs. Δ30.1 ± 2.5 mmHg). Central administration of N^6-(1-imino-3-butenyl)-l-ornithine (l-VNIO), a selective NOS inhibitor, also significantly potentiated the increase in BP induced by ANG II in females (Δ17.5 ± 3.2 vs. Δ9.2 ± 1.5 mmHg). In gonadectomized mice, central l-NNAME did not affect the pressor response to ANG II in either males or females. Ganglionic blockade after ANG II infusion resulted in a greater reduction in BP in central l-NNAME- or l-VNIO-treated females compared with control females. Western blot analysis of nNOS protein expression indicated that levels were ~12-fold higher in both the SFO and PVN of intact females compared with those in intact males. Seven days of ANG II treatment resulted in a further increase in nNOS protein expression only in intact females (PVN, ~51-fold). Immunohistochemical studies revealed colocalization of nNOS and estrogen receptors in the SFO and PVN. These results suggest that NO attenuates the increase in BP induced by ANG II through reduced sympathetic outflow in females and that increased nNOS protein expression associated with the presence of female sex hormones plays a protective role against ANG II-induced hypertension in female mice.

sex hormone; nitric oxide/nitric oxide synthase; blood pressure

NITRIC OXIDE (NO) and angiotensin II (ANG II) are important agents that regulate arterial blood pressure (BP). Vasoconstriction produced by sympathoexcitatory effects contributes to ANG II-induced pressor responses (13, 17). Conversely, NO has a hypotensive action via vasodilation and sympathoinhibition (36, 39). It has been shown that interactions between ANG II and NO occur in a variety of tissues, including the central nervous system (CNS) (40, 58). For example, microinjection of either an NO synthase (NOS) inhibitor or ANG II into the lateral ventricle or the paraventricular nucleus (PVN) increases the discharge of renal sympathetic nerves and elevates arterial BP and heart rate (HR) (25, 28, 52). Central or peripheral blockade of NOS potentiates or prolongs the pressor response to ANG II (9, 31). Conversely, overexpression of neuronal NOS (nNOS) within the PVN by adenoviral gene transfer significantly attenuates ANG II pressor responses (28).

There is evidence from human and animal studies that hypertension is delayed and attenuated in females compared with males (11). Previous studies from our laboratory (54) have shown that systemic infusions of ANG II are less effective in inducing hypertension in female mice compared with males. Central estrogen acts to inhibit the generation of reactive oxygen species and to attenuate increases in intracellular Ca2+ concentration induced by ANG II (29, 34, 55, 56). Although these actions of estrogen may contribute to a reduced BP response to ANG II in females, additional downstream signaling mechanisms underlying the sex differences found in ANG II-induced hypertension are not well understood.

Recent studies performed in both peripheral and brain tissues indicate that estrogen affects NO (15, 35, 43). García-Durán et al. (14) reported using a Western blot analysis of protein isolated from neutrophils to show that levels of nNOS were greater in cells acquired from premenopausal women during the ovulatory phase of the menstrual cycle, when estrogen is high, compared with the follicular phase, when circulating levels of estrogen fall. Also, estrogen upregulates the expression of nNOS protein in neutrophils derived from men (14). Positive correlations between circulating estrogen and levels of both plasma and brain NO also have been found in humans and experimental animals (7, 8, 21). These studies suggest that changes in NO production might be one of the cellular biochemical pathways that is affected by estrogen and that this plays a mechanistic role in producing the sex differences seen in experimental hypertension (43).

Circulating ANG II has access to brain sensory circumventricular organs (CVOs), such as the subfornical organ (SFO), organum vasculosum of the lamina terminalis (OVLT), and area postrema (AP), sites where the blood-brain barrier is lacking (24). The PVN lies within the blood-brain barrier and is a key integrative nucleus in the diencephalon that plays an important role in the control of sympathetic nerve activity. This hypothalamic nucleus receives direct monosynaptic input from the SFO and projects directly to areas in the medulla and spinal cord controlling the neural activity of preganglionic sympathetic neurons (51). Of particular interest regarding the role of estrogen and NO in the regulation of ANG II-induced hypertension is the finding that estrogen receptors (ER), NOS and their mRNA, and angiotensin type 1 receptors (AT1) are present in the SFO and PVN (10, 26, 32, 47). With this in mind, we hypothesized that important interactions among ANG II, estrogen, and NO in the SFO and PVN influence sympathetic tone.

* B. Xue and M. Singh contributed equally to this work.

Address for reprint requests and other correspondence: B. Xue, Dept. of Psychology, Univ. of Iowa, 11 Seashore Hall E, Iowa City, IA 52242 (e-mail: baojian-xue@uiowa.edu).

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and arterial BP. Corollaries of this hypothesis are that 1) the expression and availability of nNOS in the SFO and PVN of male and female mice is responsible for the differential response to systemic infusions of ANG II, and 2) a greater availability of central NO at these sites is responsible for the attenuated pressor response to ANG II seen in females. To test these hypotheses, we first used telemetry to study BP responses to systemic ANG II infusions in central Nω-nitro-L-arginine methyl ester (L-NAME)-treated intact and gonadectomized male and female mice. Second, we analyzed protein isolated from the SFO and PVN by performing Western blotting studies to determine the effects of the presence of sex hormones on nNOS protein expression in intact and gonadectomized male and female mice following control or ANG II treatments. Third, we used immunocytochemistry to examine the distribution of ER and nNOS in the SFO and PVN to determine whether both proteins are present in the same cells.

METHODS

Animals

Wild-type (C57BL/6) male and female mice (12–16 wk old) were obtained from Harlan (Indianapolis, IN). They were housed in temperature- and light-controlled animal quarters and provided with mouse chow (7013 NIH-31 modified mouse diet, 0.25% NaCl) ad libitum. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Iowa Animal Care and Use Committee.

Control and Experimental Groups

In in vivo experiments, mice were divided into 11 groups: intact males with central vehicle or L-NAME infusions, castrated males with central vehicle or L-NAME infusions, intact females with central vehicle, or the vehicle, L-NAME, or methyl ester (L-NAME)-treated intact and gonadectomized male and female mice. Second, we analyzed protein isolated from the SFO and PVN by performing Western blotting studies to determine the effects of the presence of sex hormones on nNOS protein expression in intact and gonadectomized male and female mice following control or ANG II treatments.

Surgical Procedures

Gonadectomy. Ten days before implantation of telemetry probes, bilateral gonadectomies were performed in female and male mice anesthetized with a mixture of ketamine and xylazine (100 mg/kg and 10 mg/kg, respectively). In the females, a single 1- to 2-cm dorsal midline incision was made in the skin and underlying muscles. The ovaries were isolated, tied off with sterile suture, and removed, and the incisions were closed. In the males, a single incision was made in the skin covering the scrotum. The testicles were exteriorized, tied off, and removed. The skin of the scrotum was then sutured.

Telemetry probe implantation. Implantable mouse BP transmitters (TA11PA-C10; Data Sciences International, St. Paul, MN) were used to chronically measure arterial BP. Mice were anesthetized with a ketamine-xylazine mixture. Through a ventral incision, the left carotid artery was accessed and isolated, and the catheter of a telemetry probe was inserted into the carotid and advanced into the aorta. Through the same ventral incision, a subcutaneous tunnel was formed that passed across the right pectoral area and extended into the right flank, where it was enlarged to form a pocket. The body of the transmitter was slipped into the pocket and secured with tissue adhesive. The ventral incision was then closed with suture.

Chronic intracerebroventricular cannula implantation. After baseline BP and HR recordings were obtained, the mice were again anesthetized with a ketamine-xylazine mixture, and an intracerebroventricular (ICV) cannula with an ALZET osmotic pump (Durect, Cupertino, CA) was implanted into the right lateral ventricle (coordinates 0.3 mm caudal, 1.0 mm lateral to bregma, and 3.0 mm below the skull surface) for chronic infusions of L-NAME (a nonselective NOS inhibitor, 100 μg·kg⁻¹·day⁻¹; Sigma-Aldrich, St. Louis, MO) or L-VNIO (a selective nNOS inhibitor, 100 μg·kg⁻¹·day⁻¹; Alexis Biomedicals, San Diego, CA). At the end of the experiment, the animals were euthanized and perfused transcardially with saline followed by fixative. The location of the lateral ventricle cannula implantation was verified histologically.

Osmotic pump implantation. Mice were anesthetized with inhalational isoflurane for implantation of osmotic pumps. Osmotic pumps (ALZET model 1002) containing ANG II (Sigma-Aldrich) at a concentration sufficient to allow an infusion rate of 800 ng·kg⁻¹·min⁻¹ were implanted subcutaneously on the left side of the back.

Western blotting analysis. Total cellular protein was isolated using a lysis buffer (Cell Signaling, Boston, MA). Protein (30–60 μg) was separated on 10% PAGE, blotted, and probed with anti-nNOS rabbit polyclonal antibody (Cell Signaling) at 1:1,000 (vol/vol) and anti-β-actin rabbit monoclonal antibody (Sigma-Aldrich) at 1:2,500 (vol/vol), respectively. This was followed by incubation in Alexa Fluor 800 anti-rabbit secondary antibody (Invitrogen, Carlsbad, CA) at 1:10,000 (vol/vol). The secondary antibody was detected directly using an Odyssey infrared imaging system in accordance with the manufacturer’s instructions (LI-COR Biosciences, Lincoln, NE). Relative nNOS protein expression in the SFO and PVN was normalized to β-actin in each sample. After normalization with β-actin, relative nNOS protein expression was plotted in relation to that of intact males. The linear range of detection for the assay was defined by quantifying serial dilutions of tissue homogenates (45).

Fluorescent immunohistochemistry. Immunohistochemical studies were performed to assess colocalization of nNOS and ER in the SFO and PVN of female mice. Brain sections were incubated with a rabbit polyclonal anti-nNOS antibody (Cell Signaling) at 1:100 and a mouse monoclonal anti-ER antibody (Invitrogen) at 1:100. Sections were postfixed with 4% paraformaldehyde, washed thoroughly with PBS, then incubated with Rhodamine Red-X-conjugated AffiniPure donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) at 1:100 and Cy2-conjugated AffiniPure donkey anti-rabbit IgG (Jackson ImmunoResearch) at 1:100 in PBS for 4 h at 4°C. Fluorescence was then identified using confocal microscopy.

Experimental Protocol

Measurement of BP and HR. All mice were allowed 7 days to recover from transmitter implantation surgery before any measurements were made. Thereafter, BP and HR were telemetrically recorded and stored with the Dataquest ART data acquisition system (Data Sciences International).

To determine the effects of central blockade of NO on ANG II-induced hypertension in male and female mice, ICV cannulas with osmotic pumps were implanted into the right lateral ventricle for chronic infusions of vehicle, L-NAME, or L-VNIO for 14 days. On day 7, osmotic pumps filled with ANG II were implanted subcutaneously.

Determination of BP fall after autonomic blockade. BP was measured in the presence of the ganglionic blocker hexamethonium (30 mg/kg ip). Ganglionic blockade was repeated in each animal, once during baseline and once after 7 days of ANG II infusion. On experimental days to determine the effects of ganglionic blockade, mice were allowed to adapt for at least 60 min, after which time BP
was recorded for 20 min before and 20 min after hexamethonium injection.

**nNOS protein expression measurements.** Both intact and gonadectomized male and female mice were either treated with ANG II or vehicle. After 7 days of ANG II infusion, mice were deeply anesthetized with isoflurane. The brains were removed and stored at −80°C until assay. Brains were cut into consecutive 100-µm sections in a cryostat at −20°C, and micropunches were made to collect the SFO and the PVN. Total cellular protein was isolated from tissue punches of the PVN and SFO and analyzed for the protein expression of nNOS by Western blotting.

**Data Analysis**

Mean arterial blood pressure (MAP) and HR were collected and plotted as mean values for 5 and 14 consecutive days before and during NOS inhibition and ANG II pump implantation, respectively. All data are means ± SE. Statistical analyses of the effects of central administration of l-NAME or l-VNIO on BP before and after ANG II infusion were performed with two-way ANOVAs for repeated measures (SigmaStat version 2.06). Post hoc analysis was performed with Fisher’s least significant difference multiple comparison test where appropriate. A one-way ANOVA was used for comparing changes in BP and nNOS protein expression. Statistical significance was accepted at P < 0.05.

**RESULTS**

**Effects of ICV Infusion of l-NAME on ANG II-Induced Hypertension in Female Mice**

In intact females, baseline MAP was unaltered during central infusion of vehicle, l-NAME (Δ2.7 ± 0.2 mmHg, n = 5; Fig. 1A) or l-VNIO (Fig. 1A). Central l-NAME (n = 6; Fig. 1, A and B) or l-VNIO (n = 6, Fig. 1, A and B) significantly augmented the increase in MAP induced by ANG II (Δ21.5 ± 2.2 and Δ17.5 ± 3.2 mmHg, respectively; P < 0.05) compared with that seen in mice with central vehicle plus systemic ANG II (Δ9.2 ± 1.5 mmHg, n = 6).

In OVX females, central l-NAME did not significantly potentiate the increase in MAP induced by ANG II (Δ23.2 ± 3.1 mmHg, n = 5; Fig. 2, A and B) compared with that seen in OVX females with central vehicle plus systemic ANG II (Δ22.5 ± 2.5 mmHg, n = 5; Fig. 2, A and B) or in intact females with central l-NAME plus systemic ANG II (Δ21.5 ± 2.2 mmHg, n = 6; Fig. 1, A and B). ANG II infusion did not significantly change HR in any group (data not shown).

**Effects of ICV Infusion of l-NAME on ANG II-Induced Hypertension in Male Mice**

In intact males, baseline MAP was unaltered during central infusion of either vehicle or l-NAME (Δ3.5 ± 0.6 mmHg, n = 5; Fig. 3A). Central l-NAME (n = 6; Fig. 3, A and B) did not result in a further increase in MAP induced by ANG II (Δ29.4 ± 2.5; P > 0.05) compared with that in mice with central vehicle plus systemic ANG II (Δ30.1 ± 2.5 mmHg, n = 6). Although castration attenuated ANG II-induced hypertension in males (intact: Δ30.1 ± 2.5 mmHg vs. castrated: Δ12.6 ± 2.7 mmHg; see Fig. 3A vs. Fig. 4A), neither central l-NAME (n = 5; Fig. 4, A and B) nor vehicle treatment (n = 5; Fig. 4, A and B) affected the pressor effect of ANG II in castrated males (Δ13.4 ± 2.6 and Δ12.6 ± 2.7 mmHg, respectively).

**Effects of Autonomic Blockade on BP**

Figure 5 shows the decreases in BP with acute ganglionic blockade in all groups of males and females. The averaged reduction in the BP response to hexamethonium injection before infusion of l-NAME and ANG II was −22.6 ± 1.2 mmHg in females and −23.4 ± 0.9 mmHg in males (Fig. 5, A and B). Fourteen days of central l-NAME alone tended to induce a greater reduction in BP, but this did not reach statistical significance for either females (−26.9 ± 2.5 mmHg; Fig. 5A) or males (−27.5 ± 4.5 mmHg; Fig. 5B) compared with control mice.

In female mice (Fig. 5A), after 7 days of ANG II infusion, acute hexamethonium injection resulted in a greater reduction in BP in central l-NAME-treated (−60.1 ± 5.2 mmHg) and central l-VNIO-treated females (−65.1 ± 6.6 mmHg) compared with central vehicle-treated females (−39.3 ± 3.6 mmHg; P < 0.05). Central blockade of NO production in intact females augmented reductions in the BP response to acute hexamethonium, which were similar to those seen in OVX females with or without central l-NAME treatment (−65.6 ± 6.2 and −58.8 ± 4.6 mmHg, respectively).

In male mice (Fig. 5B), after 7 days of ANG II infusion, acute hexamethonium injection resulted in a greater reduction in BP in central l-NAME-treated (−62.5 ± 6.5 mmHg) and
central vehicle-treated intact males (−60.0 ± 5.9 mmHg) compared with central vehicle-treated (−38.5 ± 4.1 mmHg; P < 0.05) and central l-NAME-treated castrated males (−41.1 ± 4.9 mmHg; P < 0.05). Central l-NAME treatment did not potentiate the depressor response to acute hexamethonium in both intact and castrated males after ANG II infusion.

nNOS Protein Expression in the SFO and PVN

Figure 6A presents a set of representative Western blots showing nNOS protein expression and β-actin in intact females with or without ANG II treatment. The Western blot analysis data are expressed as the changes in relative nNOS protein expression, normalized to intact males. Basal nNOS protein expression indicated that levels were 12-fold higher in both the SFO (Fig. 6B) and the PVN (Fig. 6C) in intact females (n = 8) compared with intact males (n = 8; P < 0.05). nNOS protein was reduced in females (to 5-fold in the SFO; to 1.2-fold in the PVN, n = 8; P < 0.05) by gonadectomy. Seven days of ANG II treatment resulted in a further increase in nNOS protein expression only in intact females (to 51 fold in the PVN, n = 8; P < 0.05).

Colocalization of ER and nNOS in the SFO and PVN

Fluorescence immunohistochemical studies indicated that the SFO and PVN contained high levels of ER and nNOS immunoreactivity in female mice. Approximately 70% of the nNOS-positive cells showed ER immunocytochemical staining (Fig. 7).

DISCUSSION

The main findings of this study are 1) central nonselective blockade of NOS or, more specifically, nNOS, augments the pressor effects of systemic ANG II in female but not in male mice; 2) at least a portion of the enhanced BP effect on ANG II-induced hypertension in intact females with central NOS blockade is a result of increased sympathetic outflow; 3) nNOS protein expression in both the SFO and PVN is higher in females with or without ANG II treatment compared with males; and 4) gonadectomy eliminates the augmented effects of NOS blockade on the pressor effect of ANG II in females. Together, these results implicate NO-related mechanisms in the attenuated hypertensive response to ANG II in females and suggest that increased nNOS protein expression associated with the presence of female sex hormones plays a protective role against sympathetically mediated ANG II-induced hypertension in female mice.

Recent studies provide convincing evidence that central ANG II and NO are important components of cellular transduction pathways in neural systems that regulate BP and sympathetic outflow (4, 5). The inhibition of the NO synthesis results in an imbalance between the renin-angiotensin system (RAS) and the NO system in favor of the RAS; thus the effects of ANG II may prevail (20, 30). A substantial body of work has shown that central or peripheral blockade of NO synthesis potentiates or prolongs the pressor response to ANG II (9, 28, 31, 33), upregulates AT1 expression, and activates cardiovascular angiotensin-converting enzyme (49). On the other hand, ANG II has been found to regulate NOS activity and NO.
release (27, 57). Li et al. (28) reported that push-pull administration of ANG II into the PVN induced an increase in NO release. Gene expression of NOS is also increased in autonomic centers, particularly the PVN, in animals with increased sympathetic activity (37). These findings indicate that during episodes of increased ANG II and sympathetic activity, NO production is increased to inhibit ANG II-mediated effects. This negative feedback mechanism within the CNS may play an important role in maintaining the overall balance and tone of sympathetic outflow.

In the present study it is interesting that central blockade of NOS by L-NAME did not augment basal BP, ANG II-induced increases in BP, and sympathetic activity in either intact or gonadectomized males. This could be because, in males, the increase in BP induced by ANG II is greater compared with that in females, and this already potentiated increase in BP could not be augmented further by inhibiting NOS activity. Another possibility is that NO production in the male might not be increased during ANG II infusions so that NO has little effect against the ANG II-induced increase in BP. This notion is supported by showing that nNOS protein expression was lower in the basal condition and not increased after ANG II treatment in males in the present study. It has been shown that central ANG II infusion reduces nNOS mRNA and NO release in the posterior hypothalamic nuclei and the PVN and that this decrease in NO expression caused by ANG II results in greater sympathetic activity (4, 5). Finally, it is possible that the prohypertensive effect of testosterone in ANG II-induced hypertension may not involve the NO system in the CNS of males. It has previously been shown that castration increases nNOS activity and that androgen treatment decreases nNOS activity, nNOS mRNA expression, and the number of nNOS-positive neurons in the brain (46). However, in the present study after castration, nNOS protein expression did not appear to increase in parallel with the reduced pressor response to ANG II. The discrepancy between results of the present study and previous findings could be due to different experimental interventions and parameters, chronic vs. acute administration of ANG II, nNOS protein expression vs. nNOS mRNA expression, or nNOS activity.

In the present study, in contrast to males, the pressor effects of systemic ANG II in female mice were augmented by central blockade of NOS. Previous studies from our laboratory (54–56) have demonstrated that sex differences in ANG II-induced hypertension can be at least partially attributed to the protective effects of estrogen in the brain. The present investigation provides further insight into the central actions of estrogen in BP regulation. In humans, estrogen enhances basal NO release in the forearm vasculature in perimenopausal women (48). Likewise, hormone replacement therapy increases plasma levels of NO in postmenopausal women (8, 21). In animals, estrogen replacement in OVX rats reduces BP responses to psychological stress and increases NO levels in the hypothalamus and brain stem (7). Inhibition of NO production in the brain augments the response in restrained OVX/estrogen-treated rats but not in OVX/vehicle-treated animals (7).
nNOS isoform over either the endothelial NOS or the inducible NOS isoforms (1). Similar to l-NAME, central administration of L-VNIO significantly augmented the increase in BP induced by ANG II in female mice. These results suggest that brain nNOS probably contributes to an attenuated BP increase to ANG II in mice with available estrogen.

It has been shown that estrogen acting on ER upregulates nNOS in the brain and that this effect appears to be regionally and/or receptor specific (6, 18, 22, 23). To confirm the important role of estrogen, Sica et al. (44) observed a significant decrease of NOS immunoreactive cells in the medial preoptic area of aromatase knockout male mice. A moderate decrease in immunoreactivity was also detected in the PVN. In male mice with ERα knocked out, the nNOS-expressing population of cells was markedly reduced in specific brain regions, including the PVN (35). By using a double mutant mouse in which males lacked functional ERα, androgen receptors, or both, Scordalakes et al. (43) demonstrated that the presence of functional ERα is correlated with more nNOS-immunoreactive cells after testosterone treatment and greater immunoreactive staining in the preoptic area under both testosterone and 17β-estradiol (E2) treatment conditions.

Reports of the effects of estrogen on NOS expression in the PVN are conflicting. Accounts of no changes in nNOS mRNA (6), increases in the numbers of NADPH-diaphorase neurons (42), and decreases in nNOS-positive neurons in the PVN after estrogen treatment (15) have all been described. Although Gingerich and Krukoff (15) demonstrated a decrease in nNOS-positive neurons in cultured PVN slices after estrogen treatment that is ERβ dependent, they showed more recently in cultured hypothalamic neurons that activation of ERβ rapidly increases phosphorylation of nNOS and increases NO production (16). They interpret this as suggesting that estrogen increases NO production by a posttranslational modification of nNOS.

A recent study from García-Durán et al. (14) using a Western blot analysis of protein showed that estrogen induced significant increases in nNOS protein expression in a time- and dose-dependent manner in neutrophils from male and female subjects. In the present study, using a Western blot analysis for relative nNOS protein expression, we found that relative nNOS protein expression in both the SFO and PVN was higher in males under basal conditions and that it was further enhanced by ANG II treatment in females compared with males. OVX results in a significant decrease in relative nNOS expression in females with or without ANG II treatment. These results provide new evidence that there are differences in NO availability and expression in key brain cardiovascular control areas between males and females. Increased expression of nNOS within the SFO and PVN in intact females with or without ANG II treatment is related to the presence of estrogen, and increased production of NO in the brain may be responsible for the reduced pressor effect and sympathetic outflow produced by ANG II.

Compared with the large number of studies that have examined the effects of estrogen on nNOS expression, relatively few have focused on the role of progesterone. Fadel et al. (12) reported that skeletal muscle nNOS is reduced in estrogen-deficient female rats and is restored by E2, but not progesterone, replacement. Skeletal muscle nNOS expression is highly correlated with plasma E2, suggesting that progesterone is
unlikely to be a key factor mediating increased nNOS expression.

There also are conflicting reports regarding the expression of nNOS and nNOS activity in near-term pregnant rats, which is a state where estrogen and progesterone are high. Plasma progesterone levels increase early in pregnancy and remain elevated until ~24 h before parturition, at which time they decrease to nonpregnant levels (3). Heesch et al. (19) reported that nNOS activity and expression are decreased in the PVN of pregnant rats. In their study, brain tissue was harvested on the morning of day 21, which would be 1.5–2 days before parturition. At this time, plasma progesterone levels would likely be elevated above that of nonpregnant rats. In contrast, in pregnant rats on the day of parturition after progesterone falls and is low or in rats where progesterone is withdrawn after hormone treatment to mimic the time of parturition, nNOS activity in both the SON and PVN is increased (38, 53). Together, these data suggest that decreased nNOS expression in the PVN during pregnancy may be attributed to high progesterone levels rather than high estrogen levels.

It has been established that sensory CVOs are primary sites of the central action of circulating ANG II and that the PVN is an important site of integration for the neural control of sympathetic outflow. Immunohistochemistry from others (41) and in the present studies indicate a high degree of the colocalization of ER, nNOS, and AT1 in the PVN and SFO (41). In view of the well-established projection from the SFO to the PVN, such colocalization provides an anatomical basis for the interaction between NO and estrogen in ANG II-induced hypertension. Bains and Ferguson (2) have reported that the pressor response to stimulation of the SFO is enhanced following administration of L-NAME, which is mediated via an angiotensin-NO interaction in the PVN. Another recent study also showed that estrogen attenuates the drinking response induced by ANG II activation of the SFO projections to the PVN (50). These results, combined with ours, suggest that ANG II activates PVN neurons to increase sympathetic outflow and BP via projections from the SFO and that the neuronal activities in the SFO and/or PVN are attenuated by NO production enhanced by estrogen.

In summary, the central actions of estrogen on NO production play an important protective role in the development of ANG II-induced hypertension. This protective effect, at least in part, seems to involve actions to decrease sympathetic outflow.
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