Estrogen regulation of the brain renin-angiotensin system in protection against angiotensin II-induced sensitization of hypertension

Baojian Xue,1,* Zhongming Zhang,6* Terry G. Beltz,1 Fang Guo,1 Meredith Hay,4,5 and Alan Kim Johnson1,2,3

1Department of Psychology, University of Iowa, Iowa City, Iowa; 2Department of Pharmacology, University of Iowa, Iowa City, Iowa; 3Francois M. Abbad Cardiovascular Research Center, University of Iowa, Iowa City, Iowa; 4Department of Physiology, University of Arizona, Tucson, Arizona; 5Evelyn F. McKnight Brain Institute, University of Arizona, Tucson, Arizona; and 6Zhang Zhongjing College of Chinese Medicine, Nanyang Institute of Technology, Henan, China

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Estrogen regulation of the brain renin-angiotensin system in protection against angiotensin II-induced sensitization of hypertension. Am J Physiol Heart Circ Physiol 307: H191–H198, 2014. First published May 23, 2014; doi:10.1152/ajpheart.01012.2013.—This study investigated sex differences in the sensitization of angiotensin (ANG) II-induced hypertension and the role of central estrogen and ANG-(1–7) in this process. Male and female rats were implanted for telemetered blood pressure (BP) recording. A subcutaneous subpressor dose of ANG II was given alone or concurrently with intracerebroventricular estrogen, ANG-(1–7), an ANG-(1–7) receptor antagonist A-779 or vehicle for 1 wk (induction). After a 1-wk rest (delay), a pressor dose of ANG II was given for 2 wk (expression). In males and ovariectomized females, subpressor ANG II had no sustained effect on BP during induction, but produced an enhanced hypertensive response to the subsequent pressor dose of ANG II during expression. Central administration of estrogen or ANG-(1–7) during induction blocked ANG II-induced sensitization. In intact females, subpressor ANG II treatment produced a decrease in BP during induction and delay, and subsequent pressor ANG II treatment given during expression produced only a slight but significant increase in BP. However, central blockade of ANG-(1–7) by intracerebroventricular infusion of A-779 during induction restored the decreased BP observed in females during induction and enhanced the pressor response to the ANG II treatment during expression. RT-PCR analyses indicated that estrogen given during induction upregulated mRNA expression of the renin-angiotensin system (RAS) antihypertensive components, whereas both central estrogen and ANG-(1–7) downregulated mRNA expression of RAS hypertensive components in the lamina terminalis. The results indicate that females are protected from ANG II-induced sensitization through central estrogen and its regulation of brain RAS.

* B. Xue and Z. Zhang 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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against ANG II pretreatment-induced sensitization. To test this hypothesis, the present study investigated sex differences in the sensitizing effects of ANG II and the involvement of central estrogen and ANG-(1–7) in this sensitization process. The studies employed in vivo telemetry recording of BP and RT-PCR to assess mRNA expression of key RAS components in the LT of male rats and of intact and ovariectomized (OVX) female rats.

Methods

Animals

The present studies followed an induction-delay-expression experimental design as described previously (32, 33). During induction, a subpressor dose of ANG II (10 ng·kg⁻¹·min⁻¹) and vehicle, 17β-estradiol (E2, 20 µg·kg⁻¹·day⁻¹ dissolved in 10% DMSO in 0.9% saline), ANG-(1–7) (1.8 µg/h (Bachem) dissolved in 0.9% saline) or A-779 (60 ng·kg⁻¹·min⁻¹ (GenScript) dissolved in 0.9% saline) were delivered subcutaneously and centrally by osmotic minipump (model 2001; Alzet) for 1 wk, respectively. To assure that any exogenous drug was metabolized, the rats then rested for 1 wk (delay), after which time a second pump (model 2002; Alzet) was implanted subcutaneously to deliver ANG II (120 ng·kg⁻¹·min⁻¹) for 2 wk (expression).

One-hundred-six Sprague-Dawley male and female rats (10–12 wk old; Harlan) were used. They were housed in a temperature- and humidity-controlled facility. Rats were maintained on a 12:12-h light-dark cycle (6:00 A.M. to 6:00 P.M.) and were provided with rat chow (7013 NIH-31 modified rat diet, 0.25% NaCl) ad libitum. Rats were randomly assigned to one of 11 groups. Groups 1–4: Males: induction (I) with saline (S) plus expression (E) with ANG II (I-S + E-ANG II, n = 7); I with ANG II and intracerebroventricular (icv) vehicle (V), plus E with ANG II (I-ANG II/icv V + E-ANG II, n = 5); I with ANG II and icv E2, plus E with ANG II (I-ANG II/icv E2 + E-ANG II, n = 5); I with ANG II and icv ANG-(1–7), plus E with ANG II [I-ANG II/icv ANG-(1–7) + E-ANG II, n = 5]. Groups 5–7: Intact females: I with S, plus E with ANG II (I-S + E-ANG II, n = 7); I with ANG II and icv V, plus E with ANG II (I-ANG II/icv V + E-ANG II, n = 6); I with ANG II and icv A-779, plus E with ANG II (I-ANG II/icv A-779 + E-ANG II, n = 5). Groups 8–11: OVX females: I with S, plus E with ANG II (OVX I-S + E-ANG II, n = 6); I with ANG II and icv V, plus E with ANG II (OVX I-ANG II/icv V + E-ANG II, n = 6); I with ANG II and icv E2, plus E with ANG II (OVX I-ANG II/icv E2 + E-ANG II, n = 5); I with ANG II and icv ANG-(1–7), plus E with ANG II (OVX I-ANG II/icv ANG-(1–7) + E-ANG II, n = 5). Eleven additional control (saline, n = 4) and experimental [subpressor dose of ANG II with E2, ANG-(1–7), or A-779; n = 4 each group] groups received identical induction and delay procedures but had their brains collected at the end of delay for analysis of mRNA expression. The microdissected tissue samples for mRNA expression contained the set of structures lying along the LT (i.e., SFO, MnPO, and OVLT).

All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Iowa Animal Care and Use Committee.

Surgical Procedures

Ovariectomy and telemetry probe implantation. Bilateral OVX was performed in female rats. A single 2- to 3-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. Ten days later, OVX female rats and intact males and females were chronically instrumented with telemetry probes (TA11-PA40; DSI) inserted through the femoral arteria for continuous monitoring of mean arterial pressure (MAP) and heart rate (HR).

Chronic intracerebroventricular cannula and osmotic pump implantation. After baseline BP and HR recordings were made, the rats were again anesthetized with a ketamine-xylazine mixture, and an intracerebroventricular cannula with an osmotic pump (model 2001; Alzet Brain Infusion Kits) was implanted into the right lateral ventricle (coordinates 1.0 mm caudal, 1.5 mm lateral to bregma, and 4.5 mm below the skull surface) for chronic infusion of E₂, A-779, or ANG-(1–7) for 1 wk. At the same time, osmotic pumps (model 2001; Alzet) containing a subpressor dose of ANG II were implanted subcutaneously in the back.

Measurement of BP and HR

All rats were allowed 7 days to recover from transmitter implantation surgery before any measurements were made. Thereafter, BP and HR were telemetrically recorded 5 min every hour and stored with the Dataaart ART data acquisition system (DSI). BP and HR were collected for 5 baseline days and then for 28 consecutive days beginning with pump implantation for induction and continuing through delay and expression.

Measurement of mRNA Expression in the LT

Total RNA was isolated from the LT using the TRIzol method (Invitrogen) and treated with DNase I (Invitrogen). RNA integrity was checked by gel electrophoresis. Total RNA was reverse transcribed using random hexamers following the manufacturer’s instructions (Applied Biosystems). Real-time PCR was conducted using 200–300 ng of cDNA and 500 nM of each primer in a 20-µl reaction with iQ SYBR Green Supermix (Bio-Rad). Amplification cycles were conducted at 95°C for 3 min, followed by 40 cycles at 95°C for 15 s, and 60°C for 1 min. SYBR Green Supermix (Bio-Rad). Amplification cycles were conducted at 95°C for 3 min, followed by 40 cycles at 95°C for 15 s, and 60°C for 1 min. Changes in mRNA expression levels were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels and calculated using the ΔΔCt method. Results are expressed as relative fold change or mean fold change ± SE. Primers were purchased from Integrated DNA Technologies (Coralville, IA). The sequences of the primers are shown in Table 1.

Data Analysis

MAP and HR are presented as mean daily values. Differences scores for MAP and HR were calculated for each animal on the basis of the

Table 1. Primer sequences for real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Product Size (bp)</th>
<th>Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
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<td>ACGCATACGTAGCAAGAAGTGATCA</td>
<td>141</td>
<td>XM_001062726.2</td>
</tr>
<tr>
<td>AT1R</td>
<td>GTCAGCTGCTGGTCTGCAAGAATGAG</td>
<td>GTCAGTGTGCTTCTTGCTGCT</td>
<td>188</td>
<td>NM_030985.4</td>
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<tr>
<td>ACE1</td>
<td>GCTGACGTTAGAGAAATGGCG</td>
<td>CATTCTTTATATTGAGCTTGA</td>
<td>187</td>
<td>AF539425.1</td>
</tr>
<tr>
<td>ACE2</td>
<td>TTAAATGCTACAGAGGCTTC</td>
<td>GCCAATCTGTTAGGTCAT</td>
<td>170</td>
<td>GP26788.1</td>
</tr>
<tr>
<td>MasR</td>
<td>TGGCTGTTGCGCTGTTACATT</td>
<td>GGCGCCTGACATGAGAAGAT</td>
<td>159</td>
<td>NM_012752.2</td>
</tr>
</tbody>
</table>

AT1R, angiotensin type 1 receptor; ACE, angiotensin converting enzyme; bp, base pairs; MasR, ANG-(1–7) receptor.
mean of the 5-day baseline subtracted from the mean of the final 5 days of treatment. Two-way ANOVAs for the experimental groups were then conducted on the means of calculated difference scores. [The factors were sex (male, intact female, and OVX female) and treatment (effects of pressor dose of ANG II during expression with or without a low dose of ANG II pretreatment during induction.)] After establishing a significant ANOVA, post hoc analyses were performed with Tukey’s multiple comparison tests between groups.

RESULTS

Sex Differences in Baseline, Pressor Dose of ANG II-Induced Hypertension, and Subpressor Dose of ANG II-Induced Sensitization

Baseline values for MAP (n = 22; 100.2 ± 2.1 mmHg; P < 0.05) in intact female rats were lower than in males (n = 18; 106.0 ± 1.2 mmHg) and OVX females (n = 22; 104.6 ± 1.2 mmHg), whereas baseline HR in females (intact, 366.8 ± 8.7; OVX, 359.1 ± 5.0 beats/min) were higher than in males (342.6 ± 6.8 beats/min; P < 0.05). For male rats and intact and OVX female rats given saline during induction, the pressor dose of ANG II given during expression resulted in a greater increase in BP in males (Δ22.3 ± 3.3 mmHg) and in OVX females (Δ23.9 ± 4.1 mmHg) compared with intact females (Δ9.9 ± 3.4 mmHg; P < 0.05 vs. males and OVX females). Moreover, a subpressor dose of ANG II had no sustained effect on BP during induction and delay, but enhanced the pressor response to ANG II during expression in males and OVX females (males, Δ41.8 ± 6.2 mmHg; OVX, Δ41.2 ± 4.3 mmHg; P < 0.05 vs. saline pretreatment during induction; Fig. 1, A and B; Fig. 2, B and C). Notably, a subpressor dose of ANG II actually induced a small decrease in BP during induction and delay in intact females (Δ−6.6 ± 2.3 mmHg; P < 0.05 vs. baseline). The subsequent pressor dose of ANG II given during expression did not result in an enhanced increase in BP in intact females (Δ12.9 ± 2.4 mmHg; P > 0.05 vs. saline pretreatment during induction in intact females, but P < 0.05 vs. the subpressor dose of ANG II pretreatment during induction in male and OVX females; Fig. 2, A and C). Systemic ANG II infusions also produced slight, comparable decreases in HR in all groups (Figs. 1C and 2D).

Effect of Intracerebroventricular Infusions of E2 or ANG-(1–7) During Induction on ANG II-Induced Sensitization of Hypertension in Males

Following 14 days of treatment with a pressor dose of ANG II during expression, MAP was significantly enhanced in males with a subpressor dose of ANG II given during induction. Intracerebroventricular infusion of either E2 or ANG-(1–7) during induction blocked ANG II-induced sensitization [E2, Δ23.6 ± 2.4 mmHg; ANG-(1–7), Δ16.4 ± 4.0 mmHg; P < 0.05; Fig. 1, A and B]. Systemic ANG II infusions produced slight, comparable decreases in HR in all groups (Fig. 1C).

Effect of Intracerebroventricular Infusions of the MasR Antagonist A-779 During Induction on ANG II-Induced Sensitization of Hypertension in Intact Females

The subpressor dose of ANG II reduced baseline BP (Δ−6.6 ± 2.3 mmHg; P < 0.05) and did not sensitize the hypertensive response to the subsequent pressor dose of ANG II in intact females. However, central blockade of ANG-(1–7) by intracerebroventricular infusion of A-779 during induction abolished the decreased BP induced by the subpressor dose of ANG II during induction, and further enhanced the pressor effects of the subsequent high dose of ANG II during expression (Δ26.9 ± 2.1 mmHg; P < 0.05; Fig. 2, A and C). Systemic ANG II infusions produced slight, comparable decreases in HR in all groups (Fig. 2D).
Central Estrogen and ANG II-Induced Sensitization

Effect of Intracerebroventricular Infusions of E2 or ANG-(1–7) During Induction on Subpressor ANG II-Induced Sensitization in OVX Females

The subpressor dose of ANG II during induction resulted in an enhanced pressor response to the subsequent pressor dose of ANG II given during expression compared with those rats receiving vehicle during induction. Central administration of either E2 or ANG-(1–7) during induction abolished the subpressor dose ANG II-induced sensitizing effect on the pressor response to subsequent ANG II [E2, Δ20.1 ± 1.7 mmHg; ANG-(1–7), Δ20.7 ± 2.2 mmHg; P < 0.05; Fig. 2, B and C]. Systemic ANG II infusions produced slight, comparable decreases in HR in all groups (Fig. 2D).

Effect of Sensitizing ANG II Infusions on the mRNA Expression of RAS Components in the LT of Male Rats

In LT tissue collected from males, the subpressor dose of ANG II given during induction induced a significant increase in the mRNA expression of AT1R, ACE1, and ACE2 when compared with controls (P < 0.05). The expression of MasR in the LT was not changed (P > 0.05). Central infusion of either E2 or ANG-(1–7) normalized the increased mRNA expression of AT1R and ACE1 produced by ANG II given during induction (P < 0.05; Fig. 3), whereas the mRNA expression of ACE2 and MasR remained high or showed no change, respectively. These results suggest that in male rats, central infusion of E2 or ANG-(1–7) resulted in decreased mRNA expression of AT1R and ACE1 in the LT, and that this may be responsible for the attenuation of the sensitizing effects induced by subpressor ANG II.

Effect of Sensitizing ANG II Infusions on the mRNA Expression of RAS Components in the LT of Female Rats

In LT tissue collected from intact females at the end of delay, the subpressor dose of ANG II given during induction produced a significant increase in mRNA expression of ACE2, MasR, and ACE1 (P < 0.05), but had no effect on the mRNA expression of AT1R when compared with controls. Central infusion of A-779 resulted in an enhanced increase in mRNA expression of ACE1 after the sensitizing ANG II infusion. In contrast, mRNA expression of ACE2 and MasR was decreased (P < 0.05; Fig. 4). These results suggest that in intact female rats with central blockade of ANG-(1–7), increased mRNA expression of ACE1 and decreased ACE2 and MasR expression in the LT may be collectively responsible for the augmentation of sensitizing effects induced by subpressor ANG II treatment.

Ovariectomy alone induced a significant increase in mRNA expression of ACE1 and MasR in the LT. In OVX females, a subpressor dose of ANG II resulted in a significant increase in mRNA expression of AT1R and ACE1 in the LT, whereas ACE2 was decreased (P < 0.05). Central infusion of ANG-(1–7) reversed the changes in mRNA expression of these genes.
Central Ang II infusion (P < 0.05; Fig. 4). Central infusion of E2 not only normalized the increased mRNA expression of AT1R and ACE1, but also enhanced ACE2 and MasR expression during ANG II infusion (P < 0.05; Fig. 4). These results indicate that in OVX female rats receiving central infusions of ANG-(1–7), a decrease in mRNA expression of AT1R and ACE1 in the LT may play a protective role against the ANG II-induced sensitization. Whereas central E2 exerted a protective effect through both decreased mRNA expression of AT1R and ACE1, and increased mRNA expression of ACE2 and the MasR.

**DISCUSSION**

The main findings of this study are as follows: 1) A subpressor dose of ANG II given during induction did not have a sensitizing effect in intact females. However, either the removal of female sex hormone by OVX or the central blockade of ANG-(1–7) restored sensitization so that the hypertensive response was similar to the effect observed in males. 2) Central infusion of either E2 or ANG-(1–7) abolished ANG II-induced sensitization of hypertension in males and in OVX females. 3) There were marked changes in expression of RAS components in the LT that were affected as a function of the sex of the rats, ovariectomy, central administration of estrogen, and antagonism of the MasR. The sensitizing subpressor dose of ANG II induced greater increases in mRNA expression of ACE2 and MasR in the LT of intact females. In contrast, mRNA expression of AT1R and ACE1 was increased after ANG II infusion in male and OVX rats. Central ANG-(1–7) induced a decrease in mRNA expression of AT1R and ACE. Whereas central E2 resulted in both decreased mRNA expression of AT1R and ACE1, and increased mRNA expression of ACE2 and MasR. Taken together, these results indicate that a sex difference exists in the sensitizing effects of ANG II, and that the interactions between central estrogen and the RAS are involved in this sensitization process. Estrogen appears to upregulate brain ACE2/ANG-(1–7)/MasR to offset the sensitizing effects induced by a low, nonpressor dose of ANG II. The E2-mediated increase in activity of the antihypertensive axis of the RAS probably plays a protective role against the development of ANG II-induced hypertension.

The biological process of response sensitization to stimuli involves two temporally distinct phases: first, an induction phase comprising an initial exposure to a stimulus; and second, an expression phase, which occurs later in time as an aug-

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**Fig. 3.** Changes of mRNA expression at the end of the delay period of renin-angiotensin system components in the lamina terminalis (LT) of male rats after subpressor ANG II alone or concurrent central infusion of E2 or ANG-(1–7). *P < 0.05 vs. I-S; #P < 0.05 vs. I-ANG II/icv vehicle.

**Fig. 4.** Changes in mRNA expression at the end of the delay period in renin-angiotensin system components in the LT of intact and OVX female rats after subpressor ANG II alone or concurrent central infusion of A-779, E2, or ANG-(1–7). *P < 0.05 vs. intact I-S; †P < 0.05 vs. intact I-ANG II/icv vehicle; #P < 0.05 vs. OVX I-ANG II/icv vehicle.
mented response to a stimulus or challenge (27). Previous studies from our laboratory using a similar induction-delay-expression experimental design demonstrated that subpressor ANG II pretreatment altered the brain RAS to produce a sensitized hypertensive response to subsequent ANG II in male rats (32). Numerous studies have also demonstrated that sex and estrogen are involved in regulation of sensitization in response to drugs, neurotransmitters, and hormones (3, 22). For example, Hu and Becker reported sex differences in behavioral sensitization, and that estrogen affected sensitization to cocaine in rats (14). In the present study, we found a sex difference in the subpressor ANG II sensitizing effect on ANG II-induced hypertension. Subpressor ANG II given during induction did not have a sensitizing effect in intact females. However, removal of female sex hormones by OVX restored sensitization so that the effect was similar to that of subpressor ANG II observed in males. These results suggest that female sex hormones, especially central estrogen, play a protective role in the sensitization process because central E2 supplementation abolished ANG II-induced sensitization in both male and OVX rats. This is consistent with our previous studies showing a clear role of the CNS in estrogen protection against hypertension produced by ANG II (30, 34). In addition, we also found that the enhanced hypertensive response to ANG II in both males and OVX females was greater than that observed in intact females with restored sensitization produced by central blockade of ANG-(1–7) (male, Δ41.8 ± 6.2 mmHg; OVX, Δ41.2 ± 4.3 mmHg vs. intact females, Δ26.9 ± 2.1 mmHg). This result might reflect an additional protective effect of estrogen against the full effect of sensitization on the hypertensive response.

Denton and colleagues previously demonstrated that infusion with a subpressor dose of ANG II produces a decrease in BP in intact female rats and mice (2, 23). This fall in BP was mediated by an enhanced renal AT2R mechanism that in part offset ANG II-induced sensitization of the tubuloglomerular feedback (2). In the present study, we observed a similar decrease in BP in intact females with a subpressor dose of ANG II pretreatment. However, central blockade of ANG-(1–7) abolished this decrease in BP. Our results indicate that there may be an important central mechanism by which subpressor levels of ANG II can upregulate central ACE2/ANG-(1–7)/MasR to reduce the BP and provide further protection against hypertension in females. This mechanism could involve activation of the antihypertensive axis of the RAS and brain components in the LT.

Accumulating evidence shows that ANG-(1–7) functions as a counterregulatory peptide for ANG II-hypertension-inducing effects. The protective effects of ANG-(1–7) are mediated by actions on both the brain and peripheral tissues (11, 28). Feng et al. reported that brain overexpression of ACE2 attenuated ANG II-induced hypertension, at least partly, by increasing AT2R, MasR, and nitric oxide synthase (NOS) mRNA expression and nitric oxide production in the brain of male mice (9). Intracerebroventricular infusion of ANG-(1–7) reduced the pressor effects of deoxycorticosterone acetate/salt and of Aldo/salt in male and OVX female rats (12, 31). Microinjection of ANG-(1–7) into the anterior hypothalamic area also resulted in a significant decrease in BP and an increase in NOS expression in the hypothalamus in SHRs (4). These previous studies demonstrated the protective effects of ANG-(1–7), especially in the CNS, in the development of hypertension. In the present study, central blockade of ANG-(1–7) restored subpressor ANG II-induced sensitization in intact females, whereas central infusion of ANG-(1–7) abolished the ANG II-induced sensitization in both male and OVX female rats. The present results indicate that ANG-(1–7) can also play a pivotal role in protecting against ANG II-induced sensitization.

Our previous studies have shown that both endogenous and exogenous ANG-(1–7) play a protective role against Aldo-induced hypertension in intact and OVX females, respectively (31). Endogenous ANG-(1–7) in the presence of estrogen and exogenous ANG-(1–7) in the absence of estrogen act via different mechanisms to attenuate an Aldo-induced increase in BP. In the first condition, the antihypertensive axis of the brain RAS is upregulated, whereas in the second, the hypertensive axis is downregulated (31). In the present study, increased ACE2 and MasR mRNA expression were attenuated by central MasR antagonist in intact females given a sensitizing dose of ANG II. In contrast, exogenous ANG-(1–7) infusion decreased AT1R and ACE1 expression and had no effects on ACE2 or MasR mRNA expression in male and OVX female rats. These data are consistent with those of previously described studies showing a similar pattern of changes in ACE2 and MasR mRNA expression in the renal cortex, and in adipose tissue and brain LT of ANG II-, obesity- or Aldo/NaCl-hypertensive females (13, 24, 31).

Hypertension is associated with augmented activation of the classic RAS hypertensive axis in both the periphery and brain. Previous studies have shown that the sensitizing effect of ANG II pretreatment in male rats was mainly due to an upregulation of RAS hypertensive components such as AT1R and ACE1 in the LT (32). Estrogen has been shown to downregulate components of the RAS hypertensive axis (i.e., ACE1/ANG II/AT1R) (17). Nevertheless, it is important to determine whether there is also a protective effect conferred by estrogen regulation of the ACE2/ANG-(1–7)/MasR antihypertensive axis. In the present study, OVX itself increased mRNA expression of ACE1. ACE1 has been shown to participate in the generation of ANG-(1–7) by metabolizing ANG-(1–9) or by degrading ANG-(1–7) to inactive metabolites (5). Thus the OVX-induced increase in ACE1 may result in a decreased level of ANG-(1–7). Conversely, estrogen downregulates ACE1 expression, which leads not only to an attenuation of ANG II production, but also to an increase in ANG-(1–7).

Recent studies from two groups have reported that ACE2/ANG-(1–7) contributes to the sex differences in the development of ANG II- or obesity-induced hypertension, because both OVX and the MasR antagonist abolished the sex differences in these forms of hypertension (13, 24). We and others also have shown that female sex hormones upregulate renal and brain ACE2/ANG-(1–7) mRNA expression to provide a protective role against the development of renal wrap- and Aldo/NaCl-induced hypertension (15, 31). In the present study, subpressor ANG II treatment mainly increased LT mRNA expression of ACE2 and MasR in intact females. In contrast, the same treatment resulted in an increase in LT mRNA expression of ACE1 and AT1R in males and OVX females, and a decrease in ACE2 expression in OVX females. These results suggest that upregulation of ACE2/ANG-(1–7)/MasR in the presence of estrogen is responsible for the diminished sensitizing effect of subpressor ANG II, whereas subpressor ANG II...
mainly upregulated ACE1/AT1R to cause increased sensitization in the absence of estrogen. Thus it is conceivable that a loss of estrogen would change the pattern of RAS component expression responding to ANG II stimulation, thereby shifting the balance between the hypertensive vs. the antihypertensive axes of the RAS in the LT, which collectively is an ANG II-sensing, and body fluid and BP information-related processing region of the brain (16).

Subcutaneous infusion of E2 at a dose of 25 μg/day has been shown to result in plasma E2 levels within the normal range during the estrous cycle of the rat (21). In the present study, a similar dose of E2 (20 μg·kg⁻¹·day⁻¹) was infused into the brain lateral ventricle. Although this might be considered to be initially a pharmacological concentration, this is diluted in the cerebrospinal fluid and in the brain extracellular space. As with all in vivo intracerebroventricular injection studies, it is difficult if not impossible to estimate the concentration of the agent at the receptors that initiate the response. In addition, it should be noted that the changes in one or several components of the RAS may not be as meaningful as observing the whole picture of what is happening to the RAS before and after the subpressor dose of ANG II pretreatment. Moreover, mRNA gene expression results do not always reflect protein expression or activity levels. In future studies the activity levels and protein expression of the RAS components after treatment with a subpressor dose of ANG II in males and females should be examined to further clarify the precise effector(s) responsible for the interaction between E2 and the RAS in the sensitization process.

**Perspectives**

There are many differences in the way that males and females respond to stimulation and inhibition of the RAS (13, 15, 23, 24) in relation to its role in the generation of hypertension. The present studies provide further evidence of a role for E2 in protection against the induction of the increased vulnerability to and enhanced expression of hypertension in response to pressor challenges. In this case, these studies indicate that the process of the induction of CNS-related neuroplasticity and sensitization of the hypertensive response induced by exposure to an earlier stimulus can be abrogated by the presence of E2.

In previous work (6, 32, 33) we demonstrated that earlier administration of nonpressor doses of ANG II or aldosterone induce sensitization of hypertensive responses and sustained changes in components of the brain RAS as well as upregulation of some key canonical markers of neuroplasticity in LT structures. Because estrogen receptors are present in components of the LT, it seems reasonable to propose that one of the key regions in the CNS upon which E2 acts to suppress activity of the hypertensive axis and to enhance the actions of the antihypertensive axis of the brain RAS is within the structures along the LT.

There is increasing recognition that many human diseases as complex as hypertension are likely to be the result of gene × environment interactions (7). From this perspective, epigenetic mechanisms initiated by earlier life conditions (i.e., exogenous and endogenous stressors and the mediators they trigger) are proposed to influence the expression of pressor and antipressor genes to influence the outcome when challenged at a later time with hypertension-eliciting conditions. The present experiments and our other studies (6, 32, 33) that demonstrate that the hypertensive response can be sensitized by earlier exposure to sensitization-inducing stimuli provide useful models for studying the role of epigenetic mechanisms in hypertension. Further study of the role of the CNS in mediating the sustained effects of early experience on the hypertensive response and the protective effects of E2 against such sensitizing challenges will in all likelihood profit from the new concepts and methods evolving with the emerging field of neuroepigenetics (25, 26).

Although the present studies demonstrate that it is likely that LT structures contain important sites where central E2 and neuroplasticity interact to play a role in the sensitization process, there are estrogen receptors, albeit of different types, located in most if not all of the structures that comprise the extensive central neural network participating in the regulation of BP. Consequently, future studies need to be conducted to identify additional brain sites where estrogen interacts with components of the brain RAS and to determine the intracellular signaling pathways and related neuroepigenetic mechanisms involved in mediating the protective actions of E2 in the long-term regulation of BP.

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Z. Zhang and B. Xue contributed equally to this study.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: B.X., M.H., and A.K.J. conception and design of research; B.X., Z.Z., T.G.B., and F.G. performed experiments; B.X., Z.Z., and T.G.B. analyzed data; B.X. and Z.Z. interpreted results of experiments; B.X. and Z.Z. prepared figures; B.X. drafted manuscript; B.X., M.H., and A.K.J. edited and revised manuscript; B.X., M.H., and A.K.J. approved final version of manuscript.

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