The arterial depressor response to chronic low-dose angiotensin II infusion in female rats is estrogen dependent

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1Department of Physiology and 2Department of Pharmacology, Monash University, Clayton, Victoria; 3Department of Biological Sciences, University of Queensland, Brisbane, Queensland; and 4Baker International Diabetes Institute Heart and Diabetes Institute, Melbourne, Victoria, Australia

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Sampson AK, Hilliard LM, Moritz KM, Thomas MC, Tikellis C, Widdop RF, Denton KM. The arterial depressor response to chronic low-dose angiotensin II infusion in female rats is estrogen dependent. Am J Physiol Regul Integr Comp Physiol 302: R159–R165, 2012. First published October 26, 2011; doi:10.1152/ajpregu.00256.2011.—The complex role of the renin-angiotensin-system (RAS) in arterial pressure regulation has been well documented. Recently, we demonstrated that chronic low-dose angiotensin II (ANG II) infusion decreases arterial pressure in female rats via an AT2R-mediated mechanism. Estrogen can differentially regulate components of the RAS and is known to influence arterial pressure regulation. We hypothesized that AT-R-mediated depressor effects evident in females were estrogen dependent and thus would be abolished by ovariectomy and restored by estrogen replacement. Female Sprague-Dawley rats underwent ovariectomy or sham surgery and were treated with 17β-estradiol or placebo. Mean arterial pressure (MAP) was measured via telemetry in response to a 2-wk infusion of ANG II (50 ng·kg⁻¹·min⁻¹ sc) or saline. MAP significantly decreased in females treated with ANG II (−10 ± 2 mmHg), a response that was abolished by ovariectomy (+4 ± 2 mmHg) and restored with estrogen replacement (−6 ± 2 mmHg). Cardiac and renal gene expression of components of the RAS was differentially regulated by estrogen, such that overall, estrogen shifted the balance of the RAS toward the vasodilatory axis. In conclusion, estrogen-dependent mechanisms offset the vasopressor actions of ANG II by enhancing RAS vasodilator pathways in females. This highlights the potential for these vasodilator pathways as therapeutic targets, particularly in women.

basic science; sex hormones; angiotensin receptors

THE NEWLY REVEALED VASODILATORY arm of the renin-angiotensin system (RAS) has stimulated considerable interest. It is now clear that angiotensin-converting enzyme 2 (ACE2) cleaves ANG II to form ANG (1–7) (38, 39), a peptide that acts via the Mas receptor (masR) but has also been reported to act via the type 2 angiotensin II receptor (AT2R), resulting in vasodilation and antiproliferation (19, 27, 40). This vasodilatory pathway directly opposes the classical actions of ANG II, such as vasoconstriction, growth, and sodium reabsorption, mediated by the type 1 receptor (AT1R) (4). We have previously demonstrated that chronic low-dose ANG II decreases arterial pressure in female rats by ~10 mmHg, a response that was inhibited by the AT1R blocker PD 123319, suggesting a role for the AT2R (30). Arterial pressure was reduced in females at a dose that had no significant effect on arterial pressure in males (30). This is compatible with previous work reporting an attenuated pressor response to ANG II infusion in females compared with males (32, 36, 43). We have also previously shown sex-specific alterations in the vasoconstrictor to vasodilator balance of the RAS, with a greater vasodilatory component of the RAS in females compared with males (30). That is, females have greater renal AT1R and ACE2 gene expression than males, shifting the balance of RAS stimulation toward vasodilation.

Estrogen is well recognized to directly interact with the RAS, downregulating renin and ACE activity and AT1R expression, as well as upregulating AT2R expression (1, 23, 34). Thus, we hypothesize that the increased expression of renal AT2R and ACE2, and thus the AT2R-mediated depressor response to low-dose ANG II infusion in females, is estrogen dependent and would be abolished by ovariectomy (OVX). This study investigated the response to chronic low-dose ANG II in intact, ovariectomized, and ovariectomized plus estrogen-replaced rats, on arterial pressure and gene expression of components of the RAS.

METHODS

Animals. Nine-week-old female Sprague-Dawley rats (200–250 g; Australian Research Centre, Perth, Western Australia) were used in this study. The rats were fed a sodium-controlled diet (0.25% wt/wt sodium chloride; Glen Forrest Stockfeeders, Western Australia) and water ad libitum. The rats were individually housed with a 12:12-h light-dark cycle at a temperature of 22–25°C. Rats were acclimatized to these conditions for 1 wk prior to entering the experimental protocol. Experiments were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Monash University Standing Committee on Ethics in Animal Experimentation.

Experimental protocol. Female rats were divided into six treatment groups: Intact + vehicle (Intact + Veh; n = 6), Intact + ANG II (n = 9), OVX + vehicle (OVX + Veh; n = 6), OVX + ANG II (n = 6), OVX plus estrogen replacement (OVX + E2 + Veh; n = 6), and OVX + E2 + ANG II (n = 9). At 10 wk of age, rats were anesthetized (isoflurane 2–4% in O2), and OVX or sham surgery (i.e., Intact) was performed via an abdominal incision, with either a 17β-estradiol (E2: 1.5 mg released over 60 days; Innovative Research of America, Sarasota, FL) or a placebo pellet implanted subcutaneously. In addition, a telemetry transmitter (TA11-PAC40; Data Sciences International, St. Paul, MN) was implanted into the abdominal aorta. Diastolic pressure, systolic pressure, locomotor activity, and heart rate (HR) recording began after a 10-day recovery period, as previously described (30, 31). Following 3 days of basal recordings, either ANG II (50 ng·kg⁻¹·min⁻¹) or vehicle (0.9% saline) was administered subcutaneously for 14 days via osmotic minipump (model 2ML2, Alzet). The data were analyzed as 24-h averages before

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and during drug infusion and are represented as change from baseline for each group. At the end of the infusion period, the rats were euthanized (pentobarbital sodium; Virbac Pty Ltd, Milperra, Australia), arterial blood-samples (2 × 1 ml) were collected for estrogen (1–7) (in an endopeptidase inhibitor cocktail containing 0.2 mol N-ethylmaleimide, 50 mmol Na2EDTA, and 2 TIU/ml aprotinin) assays. The left ventricle and kidneys were collected, weighed, and snap frozen. Plasma estrogen concentration was determined via estradiol radioimmunoassay (Ultraselect Estradiol Radioimmunoassay, Diagnostic Systems Laboratories, NSW, Australia). Plasma ANG (1–7) concentration was determined via radioimmunoassay (Prosearch International, Malvern, Australia).

AT1aR, AT1bR, AT2R, masR, and ACE2 gene expression. Total RNA was extracted from the left ventricle and kidney using RNeasy extraction kits (Qiagen). As previously described (7), 1 µg of extracted RNA was reverse transcribed into cDNA. Gene expression for previously described (7), AT1aR, AT1bR, AT2R, masR, and ACE2 (KID/BW), and uterine weight as a percentage of body weight (UTERUS/BW) are represented as means ± SE. For vehicle groups, rats received 0.9% saline; for chronic ANG II groups, rats received 50 ng·kg⁻¹·min⁻¹ ANG II. Veh, vehicle; OVX, ovariectomized; OVX+E2, ovariectomized and estrogen replaced.

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>HR, bpm</th>
<th>Activity, units</th>
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<tbody>
<tr>
<td>Intact+Veh (n = 6)</td>
<td>88 ± 2</td>
<td>401 ± 7</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Intact+ANG II (n = 9)</td>
<td>91 ± 3</td>
<td>410 ± 7</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>OVX+E2 (n = 6)</td>
<td>84 ± 3</td>
<td>411 ± 12</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>OVX+ANG II (n = 6)</td>
<td>90 ± 4</td>
<td>421 ± 6</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>OVX+E2+Veh (n = 6)</td>
<td>93 ± 4</td>
<td>381 ± 5</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>OVX+E2+ANG II (n = 9)</td>
<td>94 ± 4</td>
<td>394 ± 7</td>
<td>4 ± 1</td>
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### RESULTS

**Baseline data.** Baseline MAP, HR, and locomotor activity were not different between any of the treatment groups (Table 1). Body weight was not different between the groups prior to surgery (data not shown). However, there was a significant difference in weight gain with OVX groups gaining the most weight post-OVX (40 ± 6 g, P < 0.05 compared with Intact) and OVX+E2 rats gaining the least (8 ± 4 g; P < 0.05) compared with Intact rats (20 ± 4 g). At the conclusion of the study, OVX rats were significantly heavier than Intact and OVX+E2-treated rats, irrespective of ANG II or vehicle treatment (Table 2).

**Chronic ANG II infusion.** In Intact+ANG II rats, chronic low-dose ANG II treatment significantly decreased MAP by 10 ± 2 mmHg (P < 0.0001) compared with Intact+Veh-treated rats (Fig. 1, A and D). In OVX+ANG II animals, conversely, there was a significant increase in MAP in response to ANG II treatment compared with the OVX+Veh-treated group (4 ± 2 mmHg, P = 0.04, Fig. 1, B and D). However, in OVX+E2+ANG II rats, treatment with ANG II significantly decreased MAP (–6 ± 2 mmHg, P < 0.03) compared with OVX+E2+Veh rats (Fig. 1, C and D). Vehicle infusion had no effect on MAP in the Intact, OVX, or OVX+E2-treated rats (Fig. 1). The response of arterial pressure to all treatments was similar during day and night periods (data not shown). There was also no significant difference in HR or locomotor activity between any of the treatment groups (24 h or day/night; data not shown).

**Organ weights.** There was no significant difference in left ventricular weight or left ventricular weight corrected for body weight between any group. Kidney weight (P < 0.03) and kidney-to-body weight ratio (P < 0.001) were significantly greater in the OVX+E2 groups, irrespective of vehicle or ANG II treatment, compared with the Intact and OVX groups (Table 2). Uterine-to-body weight ratio was significantly less in the

### Table 2. Body and organ weights after 2 wk chronic ANG II or vehicle infusion in intact, OVX, or OVX+E2 female rats

<table>
<thead>
<tr>
<th></th>
<th>BW, g</th>
<th>LV, g</th>
<th>LV/BW, %</th>
<th>KID, g</th>
<th>KID/BW, %</th>
<th>Uterus/BW, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact+Veh</td>
<td>270 ± 6</td>
<td>0.76 ± 0.04</td>
<td>0.28 ± 0.01</td>
<td>1.74 ± 0.03</td>
<td>0.65 ± 0.01</td>
<td>0.26 ± 0.02</td>
</tr>
<tr>
<td>Intact+ANG II</td>
<td>282 ± 12</td>
<td>0.83 ± 0.05</td>
<td>0.29 ± 0.02</td>
<td>1.86 ± 0.1</td>
<td>0.66 ± 0.02</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>OVX+Veh</td>
<td>337 ± 20***</td>
<td>0.90 ± 0.07</td>
<td>0.27 ± 0.01</td>
<td>2.05 ± 0.10</td>
<td>0.61 ± 0.03</td>
<td>0.13 ± 0.02*</td>
</tr>
<tr>
<td>OVX+ANG II</td>
<td>319 ± 18*</td>
<td>0.82 ± 0.03</td>
<td>0.26 ± 0.02</td>
<td>1.84 ± 0.03</td>
<td>0.58 ± 0.03</td>
<td>0.12 ± 0.02**</td>
</tr>
<tr>
<td>OVX+E2+Veh</td>
<td>256 ± 7</td>
<td>0.79 ± 0.05</td>
<td>0.31 ± 0.02</td>
<td>2.00 ± 0.10*</td>
<td>0.79 ± 0.05***</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>OVX+E2+ANG II</td>
<td>256 ± 16</td>
<td>0.78 ± 0.02</td>
<td>0.31 ± 0.02</td>
<td>2.13 ± 0.10*</td>
<td>0.83 ± 0.04***</td>
<td>0.28 ± 0.03</td>
</tr>
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</table>

Body weight (BW), left ventricle (LV), left ventricle as a percentage of body weight (LV/BW), total kidney (KID), total kidney as a percentage of body weight (KID/BW), and uterine weight as a percentage of body weight (UTERUS/BW) are represented as means ± SE. For vehicle groups, rats received 0.9% saline; for chronic ANG II groups, rats received 50 ng·kg⁻¹·min⁻¹ ANG II. *P < 0.05, **P < 0.01, ***P < 0.005 as compared to Intact+Veh group.

### Table 1. Baseline hemodynamics prior to vehicle or chronic ANG II infusion in intact, ovariectomized, or estrogen-replaced female rats

Values for mean arterial pressure (MAP), heart rate (HR), and locomotor activity (activity) are represented as means ± SE. For vehicle groups, rats received 0.9% saline; for chronic ANG II groups, rats received 50 ng·kg⁻¹·min⁻¹ ANG II. Veh, vehicle; OVX, ovariectomized; OVX+E2, ovariectomized and estrogen replaced.

### Statistical analysis.** All data are presented as means ± SE. Changes in MAP, HR, and locomotor activity from baseline measurements were analyzed via a repeated-measures one-way ANOVA with factors treatment (ANG II or vehicle) and time. Body weights, organ weights, plasma estrogen concentration, plasma ANG(1–7) concentration, mRNA gene expression, and renal ACE2 activity were analyzed using a two-way ANOVA with factors treatment (P_treatment, vehicle, or ANG II) and hormonal status (P_hormonal status; Intact, OVX, or OVX+E2) and the interaction of treatment and hormonal status (P_treatment×Hormonal status) followed by a Tukey post hoc test. The statistical package SYSTAT version 9.0 was used, with statistical significance accepted at P < 0.05.
OVX groups ($P < 0.0001$) and not different in the OVX+E2 groups ($P = 0.44$ compared with Intact groups) (Table 2). ANG II treatment did not alter uterine-to-body weight ratio between groups.

**Plasma estrogen and ANG (1–7) concentration.** Intact female rats had a plasma estrogen concentration of 38 ± 2 pg/ml, which was significantly higher than that of the OVX females with a concentration of 3 ± 1 pg/ml ($P = 0.001$). OVX+E2 rats had a plasma estrogen concentration of 131 ± 8 pg/ml, significantly higher than both the intact and OVX groups (both $P = 0.001$). ANG II infusion did not significantly affect plasma estrogen concentration in any group. Plasma ANG (1–7) was not different between any of the treatment groups (Intact+Veh was 116 ± 22 pg/ml, Intact+ANG II was 79 ± 33 pg/ml, OVX+Veh was 86 ± 19 pg/ml, OVX+ANG II was 85 ± 25 pg/ml, OVX+E2+Veh was 73 ± 9 pg/ml, and OVX+E2+ANG II was 47 ± 11 pg/ml).

**Renal gene expression.** In the vehicle-treated groups, renal AT$_{1a}$R mRNA expression levels were not different between the Intact, OVX, and OVX+E2 groups (Fig. 2). However, AT$_{1a}$R gene expression was affected by estrogen status ($P_{\text{Status}} = 0.011$) being greater in the OVX groups, although this only reached significance following post hoc analysis in the OVX+ANG II ($P < 0.05$), and this effect was abolished by estrogen replacement (Fig. 2). There was no significant difference in renal AT$_{1b}$R expression between any treatment groups (Fig. 2). AT$_{1b}$R expression was not affected by OVX but was significantly greater in the OVX+E2 group treated with ANG II compared with Intact+Veh (approximately twofold, $P < 0.05$; Fig. 2). Renal masR expression was greatest in the OVX+E2+ANG II-treated group (Fig. 2). In
the presence of ANG II, renal ACE2 mRNA expression levels were greater in the Intact/ANG II group compared with Intact/Veh group ($P < 0.05$, Fig. 3). This effect was abolished in the OVX+ANG II group and restored in OVX+E2+ANG II group (Fig. 3). Renal ACE2 expression was not different between Intact+ANG II and OVX+E2+ANG II groups (Fig. 3).

Renal ACE2 activity. Similar to the mRNA expression data, renal ACE2 activity in Intact groups was greater in the presence of ANG II compared with Veh ($P < 0.05$, Fig. 3). In the OVX groups, there was no difference between renal ACE2 activity in response to either vehicle or ANG II treatment (Fig. 3). Renal ACE2 activity in the OVX+E2 groups followed a similar pattern to the Intact groups, with significantly greater activity in the OVX+E2+ANG II compared with the OVX+E2+Veh (Fig. 3).

Left ventricular gene expression. There was no significant difference in left ventricular AT1aR expression between any groups (Fig. 4). Left ventricular AT1bR expression was not different between the vehicle-treated groups. However, AT1bR expression was significantly greater following ANG II infusion in the OVX-treated group ($P < 0.001$), but not the vehicle or OVX+E2 groups (Fig. 4). OVX reduced left ventricular AT2R expression in vehicle-treated rats ($P < 0.05$; Fig. 4). ANG II-treated OVX rats showed significantly higher AT2R expression in the Intact+Veh-treated group compared with Intact+ANG II treated rats ($P < 0.01$, Fig. 4). However, in the OVX group, no change in masR expression was observed following ANG II treatment. Left ventricle ACE2 gene expression was similar in all treatment groups.

FIG. 3. Renal ACE2 mRNA expression and activity in response to ANG II treatment in intact, OVX, and OVX+E2 rats following 14 days of vehicle (Veh, solid bars) or ANG II (50 ng·kg$^{-1}$·min$^{-1}$; open bars) treatment are represented as means ± SE. Renal ACE2 mRNA expression is expressed relative to the Intact+Veh-treated group. All data were analyzed using a two-way ANOVA using the factors treatment ($P_{\text{Treat}}$; Veh or ANG II) and status ($P_{\text{Status}}$; intact, OVX or OVX+E2) and the interaction of treatment and status ($P_{\text{Treat} \times \text{Status}}$) with a post hoc Tukey test. *$P < 0.05$ compared with Intact+Veh-treated group, # $P < 0.05$ compared with OVX+E2+Veh-treated group.

FIG. 4. The relative left ventricular gene expression of renin-angiotensin system components in response to ANG II treatment in intact, OVX, and OVX+E2 rats. Left ventricular expression of AT1aR, AT1bR, AT2R, masR, and ACE2 following 14 days of vehicle (solid bars) or ANG II (50 ng·kg$^{-1}$·min$^{-1}$; open bars) treatment are represented as means ± SE. Data are expressed relative to the Intact+Veh-treated group and were analyzed using a two-way ANOVA using the factors treatment ($P_{\text{Treat}}$; Veh or ANG II) and status ($P_{\text{Status}}$; Intact, OVX, or OVX+E2) and the interaction of treatment and status ($P_{\text{Treat} \times \text{Status}}$) with a post hoc Tukey test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ compared with Intact+Veh-treated group. # $P < 0.05$ compared with OVX+E2+Veh-treated group.
DISCUSSION

We have shown that the depressor response to ANG II, mediated by the AT_{2}R, is estrogen dependent. This study demonstrates that chronic low-dose ANG II infusion decreases arterial pressure in intact females and that this arterial depressor response is abolished by OVX and restored by estrogen replacement, demonstrating that this response is estrogen dependent. Supporting this finding, we have shown that both cardiac and renal gene expression of components of the RAS are differentially regulated by estrogen with the renal RAS balance shifted toward vasodilation in the presence of estrogen. While the actions of estrogen are multifaceted, this study provides evidence for a clear antihypertensive role of estrogen in response to RAS stimulation in females.

Previously, we reported that chronic low-dose ANG II in females decreased arterial pressure in two separate cohorts (30). Here, we confirm this remarkable decrease in arterial pressure in a third cohort showing a reduction in arterial pressure of around 10 mmHg in response to chronic ANG II administration in intact females (30). This finding is in accord with other reports of an attenuated pressor response to higher doses of ANG II in females compared with males, supporting our hypothesis that there is a greater vasodilatory component of the RAS in females compared with males (10, 36, 43). The present study found that this depressor response to chronic ANG II infusion was abolished by OVX and, in fact, the infusion of ANG II caused a significant increase in arterial pressure (~4 mmHg) in the ovariectomized group, similar to the response reported at this dose of ANG II in males in our previous study (30). Of particular significance was the fact that estrogen replacement in ovariectomized animals restored the depressor response to chronic low-dose ANG II. Similarly, the attenuated pressor response to higher doses of ANG II in females compared with males has been shown to be estrogen dependent (44, 45). Thus, our data provide clear evidence that this ANG II-induced reduction in arterial pressure in females is estrogen dependent. We observed a delayed response to ANG II in the OVX and the OVX+E_{2}-treated groups compared with the intact group with little to no change in blood pressure during the 1st wk of ANG II infusion. The ANG II treatment period began 2 wk after OVX or OVX+E_{2} surgery, and this delayed response suggests that in our model, the effects of OVX may not reach equilibrium until 3 wk after surgery, as the responses to chronic ANG II in the 1st wk were not different between OVX and OVX+E_{2}.

Previously, we have demonstrated that the reduction in arterial pressure in response to low-dose ANG II infusion was abolished by AT_{1}R blockade and thus mediated by the AT_{2}R (30), consistent with the well-recognized role of AT_{2}R in vasodilation, counteracting the AT_{1}R-mediated vasoconstriction (3, 5, 9, 11, 19, 42). The components of the RAS are differentially expressed in males and females, as we and others have shown (1, 2, 30, 33–35). Certainly, females have been shown to have a greater renal gene expression of AT_{2}R and ACE2 than males (30, 34), though not all reports support this finding (33, 35), which might be explained by differences in species, diet, and age of the animals in the study. In addition, ACE2 mRNA expression is greater in the kidney and heart in females compared with males (30, 35). Thus, in females, not only is there the likelihood that ANG II has a greater effect on increased expression of the AT_{2}R, but it is also possible that ANG (1–7) generation via ACE2 is enhanced in females. In support of this hypothesis, we have previously demonstrated that ANG (1–7) can act via the AT_{2}R to reduce arterial pressure (40) with female spontaneously hypertensive rats shown to have higher renal cortical levels of ANG (1–7) than males under basal conditions, as well as after chronic ANG II infusion (35). Stimulation of the AT_{2}R via induction of a bradykinin, nitric oxide, and cGMP cascade leads to vascular dilation (26). However, estrogen can also directly stimulate eNOS activation, which enhances nitric oxide synthesis. In the current study, we are unable to determine whether the vasodilatory response to low-dose ANG II in the presence of estrogen is mediated via AT_{2}R-stimulated nitric oxide or estrogen-stimulated eNOS activation. We previously demonstrated that the depressor response to low-dose ANG II was prevented by AT_{2}R blockade (30). Furthermore, given that we observed no differences in baseline MAP between intact, OVX, or OVX+E_{2} groups, but observed a decrease in arterial pressure in response to ANG II infusion, it is likely that AT_{2}R-mediated nitric oxide release plays a greater role than the estrogen-mediated nitric oxide release in the hypotension observed, but this hypothesis requires further investigation.

Estrogen was associated with changes in both left ventricular and renal expression of components of the RAS. In both intact and estrogen-replaced animals, we observed a greater renal ACE2 mRNA expression and activity in response to ANG II treatment. It is well accepted that mRNA gene expression results do not always reflect the protein expression or activity levels. Unfortunately, we were unable to reproducibly quantify AT_{1}R or AT_{2}R protein expression, which was likely due to the difficulties well reported in the measurement of G protein-coupled receptors (such as AT_{1}R and AT_{2}R) (22). However, we observed a similar renal ACE2 activity profile as our mRNA gene expression data, suggesting that, in this instance, the renal ACE2 mRNA expression is indicative of the renal ACE2 enzyme activity. This is a particularly important finding, given that the kidney, which plays an important role in blood pressure regulation, has the greatest expression of ACE2 in the body (8, 18, 41). Therefore, we suggest that compared with males, the vasodilator arm of the RAS is enhanced in females (20, 30). The role of ACE2 in the conversion of ANG II to ANG (1–7) is supported by previous work demonstrating that ANG II infusion in male ACE2 knockout mice leads to a greater increase in arterial pressure and a 5-fold increase in renal ANG II levels, compared with male wild-type mice (13). Further evidence from Sullivan et al. (35) demonstrates that the level of renal cortical ANG (1–7) is significantly higher in female compared with male hypertensive rats before and after exogenous ANG II infusion (35). These data suggest that in female hypertensive rats, a large proportion of exogenous ANG II may be converted to ANG (1–7) (35). We did not observe any difference in plasma ANG (1–7) levels between treatment groups; however, measurement of renal tissue ANG (1–7) levels would be warranted in future studies. Ji et al. (16) observed an estrogen-dependent increase in ACE2 expression in a model of renal wrap hypertension, with a lower ACE2 activity and protein expression in the renal cortex of OVX compared with intact renal wrap female rats, which was restored with estrogen replacement. We observed no difference in renal ACE2 mRNA expression or activity between vehicle-
animals did have lower AT2R expression in the left ventricle unchanged in response to ANG II infusion; however, OVX binding (2). In our study in the OVX group, renal AT2R was RAS, by upregulating angiotensinogen, downregulating renin mRNA gene expression and activity. In the current study, ANG II infusion. In fact, ANG II infusion caused a slight increase in renal ACE2 mRNA gene expression and activity in OVX+ANG II animals compared with intact+ANG II, which was restored in the OVX+E2+ANG II group.

OVX abolished the arterial depressor response to low-dose ANG II infusion. In fact, ANG II infusion caused a slight increase in arterial pressure, associated with an increase in the AT1R-to-AT2R ratio, and the impact of ANG II to increase ACE2 mRNA expression and activity was lost in the OVX group. Previously, it has been demonstrated in the kidney, heart, and brain that OVX increases AT1R expression and binding (2, 14, 29) and decreases AT2R expression and binding (2). In our study in the OVX group, renal AT2R was unchanged in response to ANG II infusion; however, OVX animals did have lower AT2R expression in the left ventricle compared with intact animals. OVX alone or combined with ANG II infusion, had no effect on left ventricle or renal ACE2 mRNA expression or activity, which is in contrast to both the sham and estrogen replaced ANG II-treated rats, which had a greater renal ACE2 mRNA expression and activity compared with vehicle treatment. Thus, in the absence of estrogen, the vasodilatory response to ANG II in female rats is abolished in association with no differences in renal gene expression. Given the known role of ACE2 in the kidney, the inability of the OVX+ANG II-treated animals to upregulate ACE2 expression and activity may provide one possible explanation as to the pressor response to ANG II in this group. Estrogen replacement restored both the arterial depressor response and decreased the AT1R-to-AT2R receptor ratio in the kidney in response to low-dose ANG II infusion back to the level in the intact female rats via enhanced renal AT1R, masR, and ACE2 mRNA gene expression and activity. In the current study, ANG II infusion in the sham and OVX+E2 group resulted in significantly greater renal ACE2 mRNA gene expression and activity, further demonstrating the importance of the balance of the vasodilator to vasoconstrictor components of the RAS in the response to RAS stimulation.

The evidence suggesting estrogen replacement in ovariectomized rats influences ANG II receptors has been conflicting. It has been shown that estrogen can directly interact with the RAS, by upregulating angiotensinogen, downregulating renin and AT1R and upregulating AT2R (1, 23, 24, 34); however, others have shown estrogen treatment increases AT1R expression in the heart (28), uterus (17), and the renal cortex (25) in rats. Differences in the level of estrogen given may greatly influence the response. In our study, we observed that at the time of death, plasma estrogen levels were around 3 times higher in our estrogen-replaced animals compared with the intact animals. Previously, it has been shown that female rats in estrus have a plasma estradiol concentration between 150 and 190 pg/ml, close to the levels detected in estrogen-replaced groups in the present study (23). Given that our OVX+E2 animals had estrogen levels within the accepted range for rats in estrus, we suggest that in our study, the intact treatment groups may not have been in estrus at the time of tissue collection.

Activation of the vasodilator arm of the RAS [masR, AT2R, ANG (1–7), and ACE2] has been shown to reduce ANG II-induced cardiac hypertrophy (12, 21). ANG (1–7) has been shown to bind to the masR and inhibit hypertrophy and fibrosis (15). Therefore, the decrease in masR expression, which we observed in the left ventricle in response to ANG II infusion, is surprising. Perhaps this can be attributed to the difference in the dose of ANG II used between our study and others and most notably, the difference in arterial pressure response to these ANG II doses. In the current study, we administered a very low dose of ANG II, a dose that actually decreased arterial pressure by around 10 mmHg. Given the fact that the masR has been shown to stimulate the release of nitric oxide in the heart (6), it is possible that the masR may have been downregulated in response to the decrease in arterial pressure.

**Perspectives and Significance**

We have shown that the enhanced vasodilator pathway of the RAS in females is estrogen dependent. This provides a novel insight into the in vivo role of estrogen in RAS stimulation, as well as potential targets for therapeutic interventions in the treatment of hypertension in premenopausal women.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


