Effects of estrogen replacement on stress-induced cardiovascular responses via renin-angiotensin system in ovariectomized rats

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Tazumi S, Yokota N, Kawakami M, Omoto S, Takamata A, Morimoto K. Effects of estrogen replacement on stress-induced cardiovascular responses via renin-angiotensin system in ovariectomized rats. Am J Physiol Regul Integr Comp Physiol 311: R898 –R905, 2016. First published August 10, 2016; doi:10.1152/ajpregu.00415.2015.—The purpose of this study was to determine whether chronic estrogen replacement in ovariectomized rats inhibits the pressor response to psychological stress by attenuating the activation of the renin-angiotensin system. Female Wistar rats aged 9 wk were ovariectomized. After 4 wk, the rats were randomly assigned to be implanted subcutaneously with pellets containing either 17β-estradiol (E2) or placebo (Pla). After 4 wk of treatment, the rats underwent cage-switch stress and, in a separate experiment, a subset received an infusion of angiotensin II. The cage-switch stress rapidly elevated blood pressure (BP) and heart rate (HR) as measured by radiotelemetry in both groups. However, the BP and HR responses to the stress were significantly attenuated in the E2 group compared with the Pla group. An angiotensin II type 1 receptor blocker, losartan, given in drinking water, abolished the difference in the pressor response to stress between the two groups. Moreover, the stress-induced elevation in plasma renin activity and angiotensin II concentration was significant in the Pla group, but not in the E2 group. In addition, the expression of renin mRNA in the kidney was lower in the E2 group relative to the Pla group. Finally, we found that intravenous angiotensin II infusion increased BP and decreased HR to a similar degree in both groups. These results suggest that the inhibitory effects of estrogen on psychological stress-induced activation of the renin-angiotensin system could be at least partially responsible for the suppression of the pressor responses to psychological stress seen in estrogen-replaced ovariectomized rats.

estradiol; renin-angiotensin system; psychological stress; blood pressure; ovariectomized rat

CARDIOVASCULAR REACTIVITY, characterized by an acute increase in blood pressure (BP) and heart rate (HR) in response to psychological stress, is thought to be a risk factor for hypertension and heart disease (12, 26, 28, 29). The stressful stimulus causes activation of the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal axis. Activation of the SNS increases BP through α-adrenergic receptors in vasculature and β-adrenergic receptors in the heart. Stimulation of renin secretion and the subsequent increase in angiotensin II (ANG II) production are mediated by an activation of SNS via β-adrenergic receptors, and a decrease in chloride concentration in the tubular fluid at the macula densa which reflects renal arterial flow and renal filtration (15, 17, 24). The renin-angiotensin system (RAS) is an important regulator of BP and body fluid balance through the central, systemic, and the intrarenal actions of ANG II (14, 33, 37). The role of ANG II in BP regulation and the pathogenesis of cardiovascular disease has been well characterized. Acute systemic administration of ANG II increases BP, largely by constricting vascular smooth muscle and increasing peripheral resistance. It is well known that RAS activity is enhanced during the stress responses to physiological/psychological stressors (hemorrhage, extremes of temperature, pain, immobilization, and novel environments, etc.), and that this activation helps to maintain hemodynamic homeostasis (11). However, it is surprising to find relatively few studies on the role of RAS in the pressor response induced by acute psychological stress (18). In a study of male mice, the increase in telemetry-measured mean arterial pressure (MAP) following cage-switch stress was broken into a rapid α1-receptor-dependent phase and a sustained β1-receptor-dependent phase. The β1-effect on BP appears to be due almost exclusively to the activity of the RAS (18). However, ANG II is increasingly recognized as an important modulator of cardiovascular reactivity to stress at several central nervous system (CNS) levels. The functional importance of brain ANG II has been demonstrated in studies showing that intracerebroventricular injection of losartan, an angiotensin II type 1 receptor (AT1R) specific antagonist, suppresses the increases in BP, HR, and plasma catecholamines caused by immobilization (13, 16, 30), restraint (10), and footshock (5) stress. Moreover, modulation of the pressor response to emotional stress by direct microinjections of ANG II receptor antagonists into the anterior and dorsomedial hypothalamic nuclei (7) and the rostral ventrolateral medulla (RVLM) (19), suggests that ANG II mediates this response at least in part through acting on sites in the CNS.

We previously demonstrated that estrogen replacement suppresses stress-induced cardiovascular responses through enhanced expression of peripheral endothelial nitric oxide synthase (22) and through improvement of nitric oxide bioavailability by suppression of oxidative stress in ovariectomized rats (23). However, estrogen may also have altered the responses of the RAS during psychological stress. Female sex hormones, especially estrogen, have been demonstrated to modulate the renin-angiotensin-aldosterone (RAA) system and to have beneficial effects on cardiovascular function through actions not just on the kidney, heart and vasculature, but also in the CNS (36). It has been reported that chronic low-dose ANG II infusion decreased MAP in intact female and estradiol-replaced ovariectomized rats through upregulation of renal and cardiac angiotensin II type 2 receptor (AT2R) and Mas receptor. However, this effect was not seen in ovariectomized rats (32). In addition, it has been reported that ovariectomy led to an increase in basal systolic BP and plasma renin activity, but a decrease in plasma ANG II levels 9 and 13 wk after

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ovariectomy. This effect was reversed by replacement of estrogen (35). These results suggest that it is likely that estrogen is necessary to maintain a basal level of BP and RAS. However, no study has shown whether estrogen attenuates the psychological stress-induced activation of RAS. In this study, we used cage-switch stress as an experimental model of psychological stress evoked by a mild change in environment in which rats are transferred from their home cage to a cage with a layer of water at 36°C at the bottom (20–23).

The aim of this study was to determine whether chronic estrogen replacement attenuates the pressor responses to mild psychological stress by suppressing the activation of RAS in ovariectomized rats. To clarify the mechanism of estrogen’s action, we examined the effect of estrogen replacement on plasma renin activity, ANG II, and aldosterone concentrations during the stress, on the pressor responses to intravenous ANG II infusion and on gene expression of renin in the kidney.

MATeRiALS AND METHODS

Animals

The Nara Women’s University committee on animal experiments approved the experimental protocol. A total of 78 female Wistar rats were used in this study. The rats were housed in standard rat cages [length (L): 40 cm, width (W): 25 cm, depth (D): 25 cm] under controlled temperature and light conditions (26 ± 1°C, a 12:12-h light-dark cycle, with lights on at 7:00 AM). Tap water and rodent chow (Oriental Yeast, Tokyo, Japan) were provided ad libitum.

Preparation for Experiments

Ovariectomy and estrogen (or placebo) replacement: experiments 1, 2, 3, and 4. Nine-week-old female rats were ovariectomized (22). After a 4-wk recovery period, the rats were randomly assigned to either the 17β-estradiol (E2; n = 40)– or the placebo (Pla; n = 38)–treated group and were subcutaneously implanted with either E2 (2.5 mg/90-day release) or Pla pellets (Innovative Research of America, Sarasota, FL). At 15 wk of age, the rats were implanted with radiotelemetry devices for BP and HR measurements. All surgeries were performed while the rats were under anesthesia (pentobarbital sodium: 25–40 mg/kg ip; or isoflurane: 1.5–2.0% in oxygen). We used a set of 16 E2-treated and 15 Pla-treated rats in experiments 1 and 2. A separate set of 24 E2-treated and 23 Pla-treated rats were used in experiments 3 and 4.

ANG II antagonist in drinking water: experiments 1 and 2. Half of the rats in each group were randomly assigned to be given either plain tap water or losartan, an AT1R-specific antagonist (20 mg·kg–1·day–1) (LKT Laboratories, St. Paul, MN) dissolved in their drinking water for 3 days before the experimental intervention (cage-switch stress in experiment 1 or ANG II infusion in experiment 2). After the intervention, they were given plain tap water for a 2-day recovery period and were then given the alternative type of water (i.e., the animals that had been given losartan were given tap water, and the animals that had been given tap water were given losartan) for 3 days, and then the intervention was repeated.

Measurement of physiological variables: experiments 1 and 2. BP, HR, and locomotor activities were measured using a radiotelemetry system (Dataquest ART version 4.3/Silver, Data Sciences) as previously described (22). In brief, the rat was implanted with a telemetry device (model no. TA11PA-C40; Data Sciences), with the catheter inserted into the abdominal aorta and the transmitter body implanted intraperitoneally. After 2 wk of recovery, recordings of systolic BP, diastolic BP, calculated MAP, calculated HR, and locomotor activity were measured continuously. BP and locomotor activity data were collected for 15 s and 60 s per minute, respectively, before and during the stress, and then were averaged in 5-min intervals.

Placement of intravenous cannulae for ANG II infusion protocol (experiment 2) or blood sampling (experiment 3). A subset of eight rats in each group were implanted with intravenous catheters for the intravenous infusion of ANG II in experiment 2. A separate set of 16 E2-treated and 14 Pla-treated rats aged 16 wk were catheterized with cannulae into the jugular vein for the blood sampling in experiment 3 as previously described (23). The rats were then given a 5-day recovery period.

Experimental Protocols

Experiment 1: cage-switch stress. This experiment was designed to study the role of ANG II in the cage-switch stress response of ovariectomized rats that had been given estrogen replacement, compared with those that received Pla. Half of the rats were given losartan, an ANG II antagonist, in their drinking water, and half continued to be given plain tap water. On the experimental day, the basal BP, HR, and locomotor activity of the freely moving rats were monitored for 60 min just before the cage-switch stress exposure. Cage-switch stress was evoked by removing a rat from its home cage to another identical plastic cage (L: 40 cm, W: 25 cm, D: 25 cm) containing water at 36°C at a depth of 1 cm. To minimize the confounding physiological effects of the circadian rhythm, the stress exposure was performed between 10:00 AM and 4:00 PM. The same physiologic variables (BP, HR, and locomotor activity) were then monitored continuously during the 60-min period of stress exposure.

The experiment was then repeated with each rat being given the alternate type of water (losartan or plain water) as described above.

Experiment 2: ANG II infusion. This study was designed to test the effect of estrogen replacement on the physiologic response to infusion of ANG II. As in the stress protocol above, a subset of 8 rats in each group were given losartan for 3 days before the start of the experiment, or plain water.

In preparation for the experiment, the rats were housed individually in experimental plastic cages (L: 26 cm, W: 17 cm, D: 30 cm) and were acclimated to the experimental procedures the day before the experiment. Each rat’s catheter was connected to an infusion pump by an extension tube, which allowed free movement. On the experimental day, the rats were connected to the catheter and then given a 60-min rest period. Each rat then received an infusion of ANG II (30 ng·kg–1·min–1) for 60 min. ANG II was infused into rats from both pretreatment groups (losartan-pretreated or control), and BP, HR, and locomotor activity were monitored. The experiment was then repeated with each rat being given the alternate type of water (losartan or plain water) as described above.

Experiment 3: plasma renin activity, ANG II, and aldosterone concentrations in cage-switched rats. A separate set of 16 E2-treated and 14 Pla-treated rats aged 16 wk were catheterized with venous cannulae to sample blood for measuring plasma renin activity, ANG II, and aldosterone concentrations. On the experimental day, rats in each group were assigned randomly to be exposed to cage-switch stress, as described above, for 60 min, or to remain in their cages (unstressed). Approximately 1.0 ml of blood was withdrawn through the cannula at two time points: rest (time 0) and at 60 min after the start of the stress. To prevent activation of RAA system due to the decrease in circulating blood volume by blood sampling, we returned red blood cells with saline to the rats after each blood sampling. The blood was harvested into plastic tubes containing 50 mg/ml ethylene-diamine tetraacetic acid, and the plasma was stored at −45°C until it was later assayed for renin activity, ANG II, and aldosterone concentrations, using a commercial RIA (SRL, Tokyo, Japan).

Experiment 4: RNA isolation and quantitative real-time RT-PCR. The rats were euthanized by pentobarbital sodium overdose 5 days after the previous experiment. The kidneys and mesenteric arteries were harvested from a subset of 8 E2 control and 8 Pla control rats used in experiment 3 and a separate set of 8 E2-treated and 9 Pla-treated rats. The tissues were stored in RNA stabilization solution.
until real-time RT-PCR analysis for renin or ANG II type 1a receptor (AT_{1a}R) mRNA was performed. Total RNA was extracted by using TRI Reagent Solution (Ambion, Austin, TX), according to the manufacturer’s protocol. The amount of total RNA extracted was determined and its purity (OD_{260}/OD_{280} absorption ratio >1.9) was verified spectrophotometrically using a Nanodrop 2000 (Thermo Scientific, Wilmington, DE). cDNA was synthesized using the High-Capacity RNA-to-cDNA kit (Applied Biosystems, Carlsbad, CA). Real-time RT-PCR was performed using StepOne software (version 2.1; Applied Biosystems). The commercially available TaqMan Gene Expression Assay (Applied Biosystems): renin (Rn00561847_m1), AT_{1a}R (Rn02758772_s1) and β-actin (Rn00667669_m1) were used in this study. For the analysis, gene expression levels of renin and AT_{1a}R were normalized using β-actin as a housekeeping gene and are expressed with respect to the average value for the Pla group. All reactions were done in duplicate. The thermal cycling conditions were as follows: 95°C for 20 s, followed by 40 cycles at 95°C for 1 s, and 60°C for 20 s. No amplification of fragments occurred in control samples without reverse transcriptase. Quantity of mRNA was calculated using the relative standard curve method.

Measurement of Plasma Estradiol

At the end of experiment 1, blood was sampled from the cardiac ventricle while the rats were under deep anesthesia (pentobarbital sodium: 60 mg/kg ip). The plasma estradiol concentration was determined via electrochemiluminescence immunoassay (Elecsys E2 III, Roche Diagnostics) commercially (SRL).

Statistical Analysis

All values are expressed as means ± SE. Two-way repeated-measures ANOVA followed by Tukey HSD post hoc analysis was used to analyze the effects of estrogen or losartan pretreatment on MAP and HR responses to cage-switch stress or injection of ANG II, and on average MAP and HR responses to the stress over 60 min. Similarly, the effects of estrogen or the cage-switch stress on plasma RAS were analyzed using two-way repeated-measures ANOVA followed by Tukey HSD post hoc test. To analyze the differences between the two groups for renin or AT_{1a}R mRNA expression, an unpaired t-test was used. We considered a value of P < 0.05 to be statistically significant.

RESULTS

Body Weight

At 17 wk of age, which was 4 wk after the rats were implanted with E2 or Pla pellets, the body weight of the rats in the E2 group was significantly lower (252.1 ± 4.7 g) than that of the rats in the Pla group (289.5 ± 3.9 g).

Plasma Estradiol Concentration

The plasma estradiol concentrations were significantly higher in the E2 group than in the Pla group (137.7 ± 17.2 vs. 14.5 ± 2.0 pg/ml). The rats in the E2 group had estrogen levels within the accepted range for rats in proestrus, close to the levels reported in previous studies (3, 32).

Baseline Data

Resting values of MAP and HR during the 10 min just before the exposure to the cage-switch stress were not statistically different between Pla (n = 15) and E2 (n = 16) groups of control rats being given plain water (96.7 ± 2.7 vs. 98.0 ± 2.5 mmHg, 303.6 ± 4.5 vs. 304.9 ± 7.8 beats/min in Pla and E2 groups, respectively). Losartan had no effects on the basal BP (90.1 ± 2.6 vs. 87.8 ± 2.7 mmHg in Pla and E2 groups, respectively) or the basal HR (311.1 ± 6.2 vs. 302.5 ± 6.8 beats/min in Pla and E2 groups, respectively) in the two groups.

Effect of Estrogen Replacement on the Cage-Switch Stress-Induced Cardiovascular and Locomotor Activity Responses in Ovariectomized Rats

Figure 1A shows that both the Pla and E2 groups of rats had significant and sustained elevations of the MAP during the 60 min following the cage-switch stress. The elevation of MAP was significantly attenuated in the E2 group compared with Pla group. Pretreatment with losartan abolished the difference in MAP elevation between the two groups. The bar graph of the average MAP response to the stress over 60 min shows that losartan pretreatment significantly attenuated the MAP response in the Pla group, but not in the E2 group.

As shown in Fig. 1B, the cage-switch stress evoked significant and sustained elevations of the HR in both of the groups, and this HR elevation was significantly attenuated in the E2 group compared with the Pla group. The pretreatment of losartan had no effect on the HR responses in either group. Therefore, the difference in HR elevation between the Pla and E2 groups persisted in the animals that had received losartan as well as the control groups.

The locomotor activity was not different between the Pla and E2 groups under either the losartan-treated or untreated conditions.

Effect of Estrogen Replacement on Cardiovascular Responses to ANG II in Ovariectomized Rats

As shown in Fig. 2, intravenous infusion of ANG II for 60 min significantly increased MAP, but tended to decrease HR in the Pla and E2 groups. However, there were no significant differences in MAP responses to the ANG II between the Pla and E2 groups. Pretreatment with losartan suppressed these cardiovascular responses in the Pla and E2 groups, to a similar degree.

Plasma Renin Activity, ANG II, and Aldosterone Concentrations

The cage-switch stress significantly increased plasma renin activity (Fig. 3A) and plasma ANG II concentration (Fig. 3B) only in the Pla group, but not in the E2 group. Therefore, the response of plasma renin activity to the cage-switch stress was significantly greater in the Pla group than that in the E2 group (P < 0.05) (Fig. 3A). In contrast, the cage-switch stress significantly elevated plasma aldosterone levels in both the groups (Fig. 3C).

In the control experiment (no cage-switch stress), we found no change in plasma renin activity, plasma ANG II, or plasma aldosterone concentrations at 60 min relative to the resting levels at 0 min in either the Pla/control group or the E2/control group (Fig. 3).

Renin mRNA Levels in the Kidney and AT_{1a}R Levels in the Mesenteric Artery

The levels of renin mRNA in the kidney and AT_{1a}R in the mesenteric artery were determined by real-time RT-PCR. As
shown in Fig. 4A, the expression of renin mRNA in the kidney was lower in the E2 group than the Pla group. In contrast, the expression of AT1aR mRNA in the mesenteric artery was not significantly different between the two groups. These findings suggest that the RAS contributes to the stress-induced pressor response by suppressing activation of RAS.

The RAS is a well-characterized systemic endocrine pathway that regulates BP and salt-water metabolism. In addition to the systemic RAS, there is a local tissue RAS found in a variety of tissues including brain, kidney, and vasculature. A number of studies have shown that psychological stress can activate systemic RAS (4, 18) and brain RAS (6, 13, 30). A previous study reported that immobilization stress increased BP and plasma levels of ANG II in rats via enhancement of angiotensin-converting enzyme (ACE) activity, thereby provoking vascular oxidative stress (4). Davern et al. (6) have suggested that AT1R in the hypothalamus and RVLM plays a key role in autonomic cardiovascular reactions to acute aversive stress from a study using genetically AT1R-deficient mice.

In female animals, many components of the RAS have been shown to be regulated by estrogen (8, 25, 36). Aortic AT1R mRNA was downregulated after estrogen replacement in ovariectomized female rats (25). Xu et al. (35) reported that ovariectomy led to an increase in BP and renin activity, but a decrease in plasma ANG II levels; this effect could be reversed by subcutaneous estrogen treatment in female rats. In this
study, the resting MAP, plasma renin activity, and ANG II concentration were not different between the Pla and E2 groups. Our findings showed that estrogen replacement had inhibitory effects on the AT1R activation-induced MAP elevation during the cage-switch stress. In other words, ANG II induced the difference in the pressor response by causing an additional BP elevation due to AT1R in the Pla group.

Taken together, the RAS appears to contribute to the psychological stress-induced pressor response seen in estradiol-deficient female rats. Estradiol nearly completely suppresses this limited portion of the stress-induced pressor response. In this study, the inhibitory effect of losartan on the stress-induced pressor responses was limited because of the low dose of the drug, which was used out of concern that a higher dose...
expressed as means and central autonomic nuclei and inhibits the renal sympathetic in male rats. It is tempting to speculate that estrogen acts on renergic receptor. Saleh et al. (31) reported that injection of macula densa, or by sympathetic nerve stimulation via pressure and a small decrease in the delivery of NaCl to the released from juxtaglomerular cells. It is tightly regulated and stress-induced elevation of plasma renin activity. Renin is concentration. We found that estrogen replacement inhibited the mesenteric arteries between the two groups.

In this study, plasma aldosterone concentration was increased by cage-switch stress in both E2 and Pla groups. Aldosterone secretion is primarily regulated by ANG II or serum potassium, as well as ACTH. Previous studies have reported that the estrogen replacement prolongs the recovery from the footshock stress-induced ACTH responses due to impaired glucocorticoid receptor-mediated slow negative feedback in ovariectomized female rats (2, 34). Taken together, it is possible that the stress-induced elevation in glucocorticoid masked the attenuation of ANG II-induced aldosterone secretion in the E2 group, resulting in the similar increase in plasma aldosterone level in both groups. Moreover, although plasma aldosterone was increased to the same degree by the stress in both the Pla and E2 groups, the pressor response was attenuated in the E2 group. These findings suggest that aldosterone was not involved in the difference in the stress-induced pressor response between the two groups. In this study, we returned red blood cells with saline to rats to prevent an activation of the RAA system due to a decrease of circulating blood volume by blood sampling. As a result, we observed no change in plasma RAA components in control rats under the stress-free conditions (Fig. 3). Therefore, we demonstrated that the stress elicited the elevation of RAA components, independent of the influence of the blood sampling.

In this study, the Pla and E2 groups were compared without reference to an intact female group of rats. In our study design, rats in the E2 group were given 17β-estradiol replacement beginning 4 wk after ovariectomy, to allow time for plasma estradiol levels to stabilize at a low level. After 4 wk of 17β-estradiol replacement, when the plasma levels of estradiol were at a high stable level, they were used in the experiments. Therefore, the rats in the Pla group may correspond to a postmenopausal animal model, despite their young age. The rats in the E2 group have sustained high levels of estradiol, as would be seen in a postmenopausal model with estradiol replacement, or in proestrus rats. These findings suggest that estradiol replacement attenuates the exaggerated pressor response to the stress in postmenopausal model of rats. However, there is a possibility that the long periods after ovariectomy and then after E2 treatment influenced group differences due to an indirect effect of estrogen (e.g., differences in body weight).

Similarly, estradiol may have an inhibitory effect on the stress-induced response in intact females. However, it is well known that proper function of estradiol requires pulsatile release mechanisms. Therefore, we cannot rule out the possibility that estradiol at noncyclic high concentrations may have different effects from that seen in intact female rats. We did not include a sham ovariectomized group in our experiment, since the use of these animals would introduce other variables that would complicate the interpretation of the stress response data. The variation in estradiol concentration during estrous cycle may affect the stress-induced pressor response, but it was

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**Fig. 4.** A: relative values of renin mRNA in the kidney of the placebo (Pla, n = 8)- and the 17β-estradiol (E2, n = 8)-treated groups. B: relative values of angiotensin II type 1a receptor mRNA in the mesenteric arteries of the placebo (Pla, n = 9)- and the 17β-estradiol (E2, n = 8)-treated groups. Data are expressed as means ± SE *P < 0.05, difference between Pla and E2 groups.

*possible explanation is that estrogen replacement might suppress the stress-induced increase in plasma ANG II concentration. We found that estrogen replacement inhibited the stress-induced elevation of plasma renin activity. Renin is released from juxtaglomerular cells. It is tightly regulated and is readily stimulated by slight changes in renal perfusion pressure and a small decrease in the delivery of NaCl to the macula densa, or by sympathetic nerve stimulation via β-adrenergic receptor. Saleh et al. (31) reported that injection of estrogen into the nucleus tractus solitaries, RVLM, and the intrathecal space depressed the renal sympathetic nerve activity in male rats. It is tempting to speculate that estrogen acts on central autonomic nuclei and inhibits the renal sympathetic nerve activity during the stress. However, other possibilities exist. This study revealed that estradiol replacement decreased renin mRNA in kidney. This supports the hypothesis that estradiol suppresses renal gene expression of renin. In fact, there are some reports documenting the presence and activity of estrogen receptors (ER) on RAS components in the kidney (1, 27). The ERα was the primary receptor subtype responsible for the regulation of kidney ACE2, AT1R, and AT2R genes (1). Also, others suggested that estradiol attenuated the AT1R activity by increasing ERα expression in kidney (27).

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difficult to determine the stage of the estrous phase without introducing a stressful procedure. Daily screening by vaginal smear to evaluate the estrous phase would likely cause psychological stress in these rats. Only one report has shown that female spontaneously hypertensive rats in the diestrous stage of their estrous cycle had greater pressor response to open-field exposure compared with either female rats not in diestrous (9). In conclusion, we have shown that estrogen replacement attenuates the stress-induced elevation of MAP by inhibiting the stress-induced increase in plasma renin activity and the subsequent increase in plasma ANG II. We suggest that in the estrogen-treated rats, downregulated expression of renal renin mRNA at least partially contributes to the suppression of plasma renin activity during the stress.

**Perspectives and Significance**

Our findings suggest that the RAS contributes to the acute stress-induced pressor response, and that estradiol suppresses this response by its inhibitory effect on the RAS. It is likely that estradiol decreases the basal level of renin mRNA expression in the kidney, and inhibits renin release induced by the renal sympathetic nerve activation during the stress. We believe that this lower renin activation would then inhibit plasma ANG II elevation, which, in turn, would attenuate the increase in peripheral arterial resistance, finally leading to suppression of the pressor response. Alternatively, estradiol could cause these changes by suppressing the stress-induced activation of renal nerves. The mechanism by which estradiol attenuates the stress-induced pressor response was not fully demonstrated in this study. In a previous study, we reported that NO is a key mediator in mechanisms accounting for the inhibitory effect of estradiol on the stress-induced pressor response. NO may mediate the inhibitory effect of estradiol on pressor response in the brain or kidney. In any case, further study is needed to provide insight into mechanisms by which estradiol attenuates the stress-induced pressor response. A better understanding of the relationship between stress-induced hypertension and estrogen will be helpful in providing menopausal women with healthy lives.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

S.T. and K.M. conception and design of research; S.T., N.Y., M.K., S.O., and K.M. performed experiments; S.T., N.Y., M.K., S.O., and K.M. analyzed data; S.T., A.T., and K.M. interpreted results of experiments; S.T. and K.M. prepared figures; S.T. and K.M. drafted manuscript; S.T. and K.M. edited and revised manuscript; S.T., N.Y., M.K., S.O., A.T., and K.M. approved final version of manuscript.

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