

Estrogen protects transgenic hypertensive rats by shifting the vasoconstrictor-vasodilator balance of RAS

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Brosnihan, K. Bridget, Ping Li, Detlev Ganten, and Carlos M. Ferrario. Estrogen protects transgenic hypertensive rats by shifting the vasoconstrictor-vasodilator balance of RAS. *Am. J. Physiol.* 273 (Regulatory Integrative Comp. Physiol. 42): R1908–R1915, 1997.—In pursuit of the hypothesis that estrogen shifts the vasoconstrictor-vasodilator balance of the renin-angiotensin system, we investigated the cardiovascular responses to administration of angiotensin-(1–7) [ANG-(1–7)] and angiotensin II (ANG II) in female transgenic (mRen2)27-positive [Tg(+)] and -negative [Tg(-)] rats in the presence and absence of 3 wk of estrogen replacement therapy. Fifty-three female Tg(-) and Tg(+) rats were oophorectomized and received either 17 β -estradiol (1.5 mg/rat sc for 3 wk) or vehicle. At the end of 3 wk of estrogen treatment, mean blood pressure was lowered in freely moving chronically cannulated Tg(+) (159 \pm 4 vs. 145 \pm 5 mmHg, P < 0.05) and Tg(-) (119 \pm 4 vs. 108 \pm 2 mmHg, P < 0.05) rats. Moreover, the magnitude of the depressor component of the biphasic response to ANG-(1–7) was significantly enhanced in estrogen-treated Tg(+) rats, whereas the pressor component to ANG-(1–7) was attenuated in both Tg(+) and Tg(-) rats. Estrogen replacement significantly attenuated the pressor response to ANG II in both Tg(+) and Tg(-) rats. In addition, estrogen replacement therapy significantly reduced plasma ANG-converting enzyme activity in association with a reduction in circulating levels of ANG II. Tissue levels (kidney and aorta) of ANG-converting enzyme were also reduced with chronic estrogen replacement therapy. On the other hand, estrogen augmented the levels of plasma ANG-(1–7) in Tg(+) animals. Plasma renin activity was unchanged with estrogen treatment. These findings provide the first evidence demonstrating that estrogen is protective against hypertension, possibly by amplifying the vasodilator contributions of ANG-(1–7), while reducing the formation and vasoconstrictor actions of ANG II.

renin gene; transgenic rats; postmenopausal; hormone replacement; vascular reactivity; angiotensin peptides; angiotensin-converting enzyme

A MODEL OF TRANSGENE hypertension, devised by insertion of the mouse DBA/Ren-2^d gene into the genome of the normotensive rat, has become an important tool in deciphering the mechanisms by which excess production of angiotensin II (ANG II) may cause high blood pressure (43). The presence of the extra renin gene in the genome of these rats is associated with the development of severe hypertension and enhanced local expression of renin in the adrenal gland, brain, heart, reproductive organs, and blood vessels (3, 62).

Interestingly, both heterozygous and homozygous animals express a marked degree of sexual dimorphism in that the female transgenic hypertensive rat is manifested by blood pressures significantly lower than those obtained in males of the same age (2). To date, there is

no conclusive explanation to account for the sexual dimorphism of the blood pressure in this model of renin-dependent hypertension. Although there is no denying that the gender differences in blood pressure are related to estrogen (24, 30, 35, 39, 40), the site(s) at which the reproductive hormones exert their influence are unclear. Previous studies from this laboratory demonstrated that the NH₂-terminal heptapeptide angiotensin-(1–7) [ANG-(1–7)] counterregulates the pressor, antinatriuretic, and proliferative actions of ANG II. This concept was generated from the observation that ANG-(1–7) is a vasodilator of coronary (7), cerebral (38), and hindlimb and mesentery vessels (48); elicits long-lasting vasodepressor effects in the pithed rat (4); and lowers the blood pressure in the spontaneously hypertensive rat (SHR) (5). Neutralization of the endogenous activity of ANG-(1–7) by means of a specific antibody raises the blood pressure in transgenic hypertensive rats (42) and partially reverses the antihypertensive response produced by chronic administration of an ANG-converting enzyme (ACE) inhibitor (18). These findings suggest that the net action of the renin ANG system (RAS) in the long-term regulation of blood pressure is the product of a balance between the opposing actions of ANG II and ANG-(1–7) on pressor and depressor mechanisms, respectively. Because the vasodilator actions of ANG-(1–7) may be mediated in part by the release of nitric oxide from the vascular endothelium (7, 51), tissue prostacyclin (27), or even kinins (7, 33), there is a potential that this novel ANG peptide may be linked to the influence that gender has on the magnitude of the blood pressure elevation in the Ren-2 transgenic hypertensive rats. With this in mind, the current experiments determined whether chronic 17 β -estradiol (E₂) replacement therapy in oophorectomized transgenic hypertensive rats modified endogenous concentrations and actions of ANG-(1–7).

MATERIALS AND METHODS

Surgical procedures. Following approval by the Institutional Animal Care and Use Committee, 53 heterozygous female transgenic-negative [Tg(-)] and hypertensive transgenic positive [Tg(+)] rats (body wt: 220–250 g) from the Hypertension Center Transgenic Rat Colony of Bowman Gray School of Medicine underwent bilateral oophorectomy at age 12 wk under general anesthesia with ketamine (30 mg/kg im) and xylazine (5 mg/kg im). Pellets containing either E₂ (1.5 mg/rat, for 3 wk release; Innovative Research of America, Toledo, OH) or vehicle were implanted in the subcutaneous tissue. The pellets yielded physiologically relevant concentrations of E₂ as measured in rats during the estrous cycle (10) or as found after hormone replacement therapy (61). After recovery, rats were allowed free access to water and were fed normal powder chow, providing 17 meq of Na⁺ and 28 meq of

K⁺ per 100 g of solid weight (Rodent Laboratory Chow 5001, Purina Mills, Richmond, IN). Animals were housed individually in plastic cages in a room maintained at 22°C and lighted for 12 h.

Experimental protocol. At the end of 3 wk of hormone replacement, rats were again anesthetized with ketamine and xylazine, and a polyethylene catheter (PE-50, Clay Adams, Becton-Dickinson, Franklin Lakes, NJ) was implanted into the abdominal aorta via a femoral artery. Another plastic catheter was placed into the inferior vena cava through a femoral vein. The free end of both catheters was tunneled to the back of neck as described previously (41). All procedures were performed under sterile conditions and were followed by an intramuscular injection of 30,000 U of Penicillin G. Forty-eight hours later, the rats were brought to the laboratory, and arterial pressure was measured with a solid strain-gauge microtransducer (MP-150, Micron Instruments, Los Angeles, CA), the output of which was connected to a polygraph (model 7, Grass Instruments, Quincy, MA) and simultaneously fed into a PC-based data acquisition program developed in our laboratory (5) for the digital computation of systolic, diastolic, mean arterial pressures, and heart rate at successive 2-s intervals. After a 1-h stabilization period, baseline blood pressure was recorded, and dose-dependent phasic pressor/depressor responses to intravenous bolus injection of ANG II (10, 20, and 50 pmol) or ANG-(1–7) (100, 300, and 600 nmol) were conducted in conscious freely moving animals.

On the day following blood pressure measurements, rats were killed by decapitation, and trunk blood was collected into prechilled tubes for measurements of plasma concentrations of ANG II and ANG-(1–7) and determinations of plasma renin activity (PRA) and ANG-converting enzyme (ACE) activity. In addition, the kidneys and the thoracic aorta were removed rapidly and dissected free of connective tissue on ice and stored at –80°C until assayed for measurements of ACE activity.

Biochemical assays. Blood for the assay of peptides by radioimmunoassay (RIA) was collected in a cocktail of protease inhibitors described by us previously (32). Plasma was extracted using Sep-Pak columns (44). The eluted and reconstituted sample was split for two RIAs. For ANG II measurements, samples were reconstituted in assay buffer, whereas those processed for ANG-(1–7) were reconstituted in a tris(hydroxymethyl)aminomethane buffer in 0.1% bovine serum albumin (BSA). Recoveries of radiolabeled ANG added to the sample and followed through the extraction were 92% ($n = 23$). ANG II was measured using the Nichols Institute RIA (San Juan Capistrano, CA), and ANG-(1–7) was measured using the antibody produced and characterized by us previously (32). The cross-reactivity of the ANG II antibody was 100% for ANG II and 65% for ANG III, whereas there was less than 0.01% cross-reactivity with ANG-(1–7). The ANG-(1–7) antibody cross-reacted 100% with ANG-(1–7) and ANG-(2–7), but showed less than 0.01% cross-reactivity with ANG I or ANG II. The minimum detectable levels of the assays were 2.5 pg/tube for ANG-(1–7) and 1.4 pg/tube for ANG II. The intra-assay coefficient of variation was 8% for ANG-(1–7) and 12% for ANG II.

Serum and tissue ACE activity was measured during incubation with the radiolabeled tripeptide, [³H]Hip-Gly-Gly, at pH 8.0 for 60 min at 37°C, using a commercially available kit (Hycor, Portland, ME). The hippuric acid released by the enzyme is extracted into ethyl acetate with a 91% recovery. Aortic and kidney tissues were minced and homogenized over ice in 5 volumes (wt/vol) of ice-cold 0.05 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer containing 0.1 M

NaCl and 0.6 M Na₂SO₄ (pH 8.0) using a glass-glass tissue grinder, according to a modified method (45, 54). Homogenates were centrifuged at 1,000 *g* for 10 min at 4°C. The supernatants were assayed for enzyme activity. The ACE inhibitor enalaprilat (1 μM) was used to verify the specificity of the ACE measurement. The protein concentration was assayed using the Bradford method with BSA as standard (6). The minimum detectable level of the assay is 2.1 U (1 U = 1 nmol·min⁻¹·ml sample⁻¹). The precision of the assay was as follows: the intra-assay variability averaged 5.3% while the interassay variability was 11.9%.

Blood for determinations of PRA, defined as the rate of ANG I generation from endogenous substrate, was taken in chilled tubes containing 25 mM EDTA. PRA was measured at pH 6.5 in plasma treated with phenylmethylsulfonyl fluoride to prevent degradation of the generated peptide and incubated for 2 h at 37 and 0°C. The ANG I levels were quantitated by RIA using a renin kit (Incstar, Stillwater, MN). The interassay precision was 7.8%. Plasma 17-β-estradiol concentrations were measured using a commercially available kit (Serono Diagnostics, East Walpole, ME).

Statistical analysis. All values are means ± SE. Analysis of variance was used to compare the effects of hormone replacement on measurements, and within group comparisons were done using Tukey-Kramer multiple comparisons tests. The Student's *t*-test for unpaired observation was used when appropriate while Bartlett's test was used to test for homogeneity of variances. A $P < 0.05$ was used as the criteria for statistical significance.

RESULTS

Baseline values of mean arterial pressure in conscious freely moving transgenic hypertensive and normotensive rats at the end of 3 wk of either estrogen replacement therapy or vehicle are illustrated in Fig. 1. Chronic E₂ treatment produced a small but statistically significant decrease in the mean blood pressure of both transgenic hypertensive and normotensive rats. Heart rate was not changed with estrogen replacement therapy [382 ± 12 vs. 349 ± 9 beats/min, Tg(–) vehicle vs. Tg(–) E₂ (NS); and 367 ± 9 vs. 385 ± 21 beats/min, Tg(+) vehicle vs. Tg(+) E₂ (NS)]. Plasma E₂ concentration averaged 190 ± 20 pg/ml in E₂-treated rats and <15 pg/ml in vehicle-treated rats.

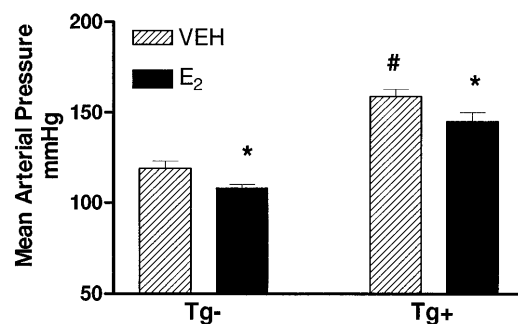


Fig. 1. Effects of 3-wk estrogen (E₂) treatment on mean blood pressure of conscious resting transgenic negative [Tg(–)] and transgenic hypertensive positive [Tg(+)] animals treated for 3 wk with vehicle (Veh) or 17β-estradiol. All animals were oophorectomized as described in MATERIALS AND METHODS. Values are means ± SE. * $P < 0.05$, Veh vs. E₂; # $P < 0.01$ Tg(+) vs. Tg(–). Each group includes 6–8 rats.

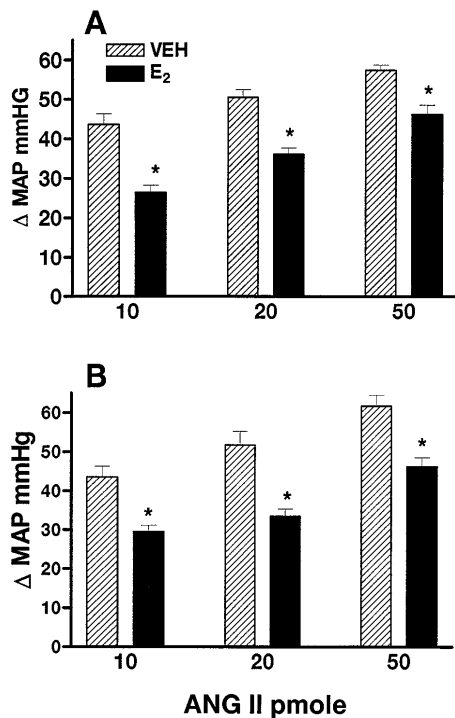


Fig. 2. Effect of 3-wk estrogen (E₂) replacement on ANG II pressor responses of conscious resting Tg(-) (A) and Tg(+) (B) rats. E₂ attenuates the ANG II-induced pressor responses. Values are mean ± SE. * *P* < 0.05, Veh vs. E₂. Each group includes 6–8 rats. MAP, mean arterial pressure.

Effects of estrogen replacement therapy on vascular reactivity. The magnitude of the pressor response produced by the injection of three doses of ANG II was similar in vehicle-treated Tg(+) and Tg(-) rats. In contrast, chronic E₂ treatment significantly attenuated the pressor responses to intravenous injection of ANG II in both strains at all doses tested (Fig. 2, A and B). Intravenous injections of ANG-(1–7) in transgenic rats elicited a biphasic response consisting of a rapid (55.6 s) pressor component followed by a longer-lasting (138.9 s) depressor component (Fig. 3), as was previously described in pithed Sprague-Dawley rats (4, 5) and

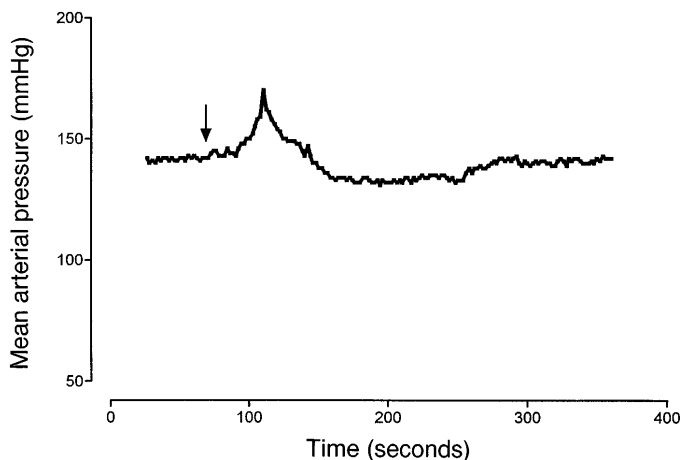


Fig. 3. A typical recording of a biphasic response to intravenous injection of 300 nmol of ANG-(1–7) in a conscious freely moving Tg(+) rat.

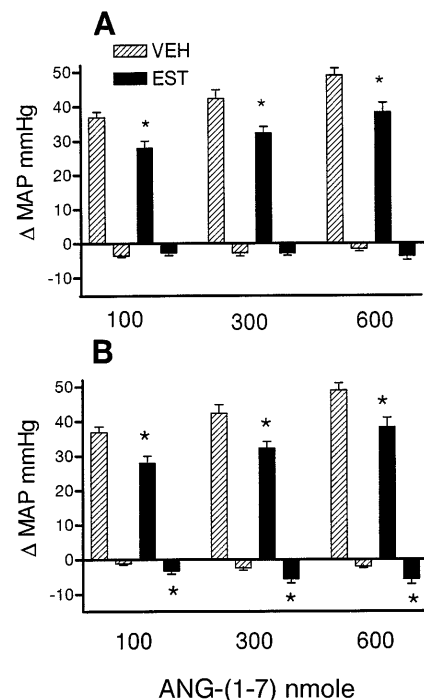


Fig. 4. Effect of 3-wk E₂ replacement on ANG-(1–7) pressor-depressor responses of conscious resting Tg(-) (A) and Tg(+) rats (B). E₂ attenuates the ANG-(1–7)-induced pressor responses of both Tg(-) and Tg(+) animals, but it augments the depressor responses of Tg(+) rats only. Values are means ± SE. * *P* < 0.05, Veh vs. E₂. Est, estrogen.

conscious dogs (44). Estrogen replacement therapy had a small but significant effect on the pressor but not the depressor component of the response to intravenous injections of ANG-(1–7) in Tg(-) rats (Fig. 4A), whereas it markedly potentiated the magnitude of the fall in blood pressure produced by ANG-(1–7) in Tg(+) rats (Fig. 4B). In Tg(+) rats treated with E₂, the blunting of the pressor component of the ANG-(1–7) response was comparable to that obtained in Tg(-) rats (Fig. 4, A and B).

Reciprocal effects of estrogen replacement on plasma levels of ANG II and ANG-(1–7). Plasma renin activity averaged 12.3 ± 3.2 and 14.3 ± 3.5 ng·ml⁻¹·h⁻¹ in vehicle-treated Tg(-) and Tg(+) rats (*P* > 0.05), respectively. Estrogen replacement therapy had no effect on PRA (14.5 ± 2.3 and 12.4 ± 3.5 ng·ml⁻¹·h⁻¹) in Tg(-) and Tg(+) rats. In confirmation of previous studies (58), plasma ANG II levels were significantly higher in Tg(+) compared with Tg(-) rats (Fig. 5A). Replacement with estrogen resulted in a nearly twofold reduction in the circulating levels of ANG II in Tg(+) rats, whereas it had no effect on plasma ANG II in Tg(-) rats. In contrast, estrogen replacement significantly increased the levels of plasma ANG-(1–7) in Tg(+) animals (Fig. 5B). In accordance with the reduction in plasma ANG II levels in Tg(+) rats, estrogen significantly reduced plasma ACE activity in Tg(+) animals (Fig. 6A). A similar reduction in plasma ACE was observed in Tg(-) rats. There was no difference in ACE activity levels between Tg(-) and Tg(+) animals on similar treatment. There were no differences between

kidney and aorta ACE activity of Tg(-) and Tg(+) vehicle-treated animals (Fig. 6, B and C), whereas chronic estrogen replacement therapy significantly decreased both kidney and aorta ACE activity in Tg(+) but not in Tg(-) rats.

DISCUSSION

The present study demonstrates for the first time that estrogen acts to shift the vasoconstrictor-vasodilator balance of the renin-ANG system by enhancing the formation of the NH₂-terminal heptapeptide ANG-(1-7) and augmenting the vasodepressor actions of ANG-(1-7) in female oophorectomized transgenic hypertensive animals expressing the mouse (Ren2) gene. This augmented vasodepressor response was observed in the presence of reduced formation of circulating ANG II, blunting of the vasoconstrictor responses to ANG II, and decreased levels of plasma, kidney, and thoracic aorta ACE activity. These effects of estrogen replacement therapy on the circulating peptides and an intermediate enzyme of the renin-ANG system were associated with a moderation of the hypertension in Tg(+) rats and a lowering of the blood pressure in Tg(-) rats. In the altered endocrine milieu of the hypertensive rat, a sustained elevation in estrogen levels elicited a response of the RAS that is totally concordant with the hypothesis that ANG-(1-7) functions as an antihypertensive hormone. Although the associated decrease in plasma and tissue ACE activity may explain the presence of lower circulating levels of ANG II, this finding also illustrates clearly the intertwining nature of the mechanisms regulating the production of ANG-(1-7) relative to ANG II. Numerous studies from this and

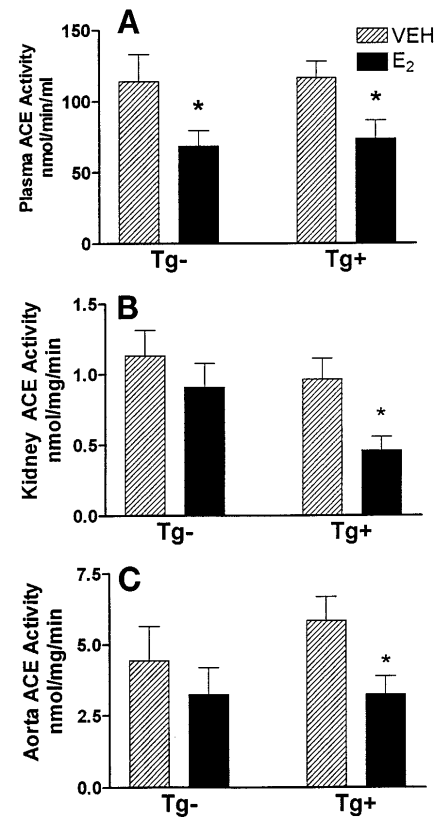


Fig. 6. Effect of E₂ replacement on plasma (A), kidney (B), and thoracic aorta (C) levels of ANG-converting enzyme (ACE). E₂ reduces the plasma ACE activity in Tg(+) and Tg(-) animals, whereas it only reduces tissue levels of kidney and thoracic aorta in Tg(+) rats. Values are mean \pm SE. * $P < 0.05$, Veh vs. E₂. Each group includes 6-8 rats.

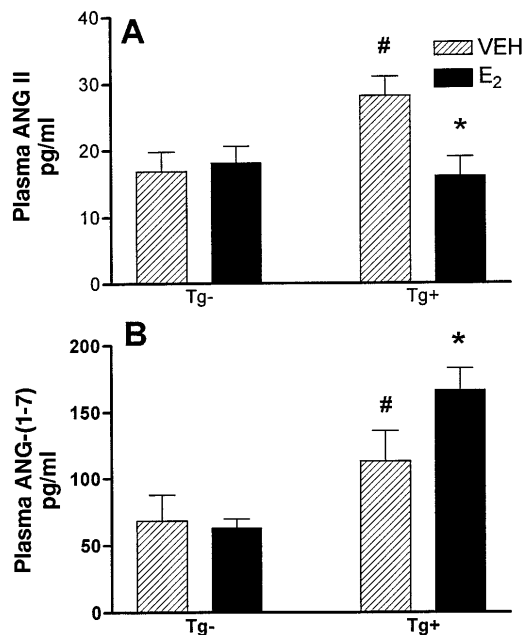


Fig. 5. A: effect of E₂ replacement on plasma levels of ANG II. E₂ reduces circulating levels of plasma ANG II in Tg(+) animals. B: effect of E₂ replacement on plasma levels of ANG-(1-7). Estrogen increases circulating levels of plasma ANG-(1-7) in Tg(+) animals. Values are means \pm SE. * $P < 0.05$, Veh vs. E₂; # $P < 0.01$ Tg(+) vs. Tg(-). Each group includes 6-8 rats.

other laboratories in animals (11, 31) and human subjects (20) showed that pharmacological inhibition of ACE with a consequent rise in plasma ANG I concentration are associated with increased formation of ANG-(1-7). The physiological action of estrogen as an endogenous inhibitor of ACE, first demonstrated by us in nonhuman primates (9), now duplicates the conclusions that were derived from pharmacological studies. In this context, the current experiments give credence to the concept (22) that ACE may be a site at which the renin-ANG system has a built-in feedback control mechanism to regulate the pressor and proliferative actions of ANG II through the counterbalancing production of ANG-(1-7).

Removal of the carboxyl-terminal phenylalanine from ANG II imparts selective properties to the heptapeptide ANG-(1-7) (19). ANG-(1-7) produced a long-lasting depressor response in the pithed rat and vasodilation of piglet pial arterioles (4, 38). The depressor response was blocked by indomethacin, but not by losartan (4). In perfused mesenteric and hindquarter vascular beds, Osei et al. (48) reported that ANG-(1-7) produced vasodilation because of the release of nitric oxide. Infusion of ANG-(1-7) in SHR lowers their elevated blood pressure (5). In Tg(+) hypertensive rats, cerebroventricular administration of antibodies to ANG-(1-7) caused significant elevation of blood pressure,

whereas administration of a monoclonal antibody to ANG II reduced blood pressure (42). In the canine coronary circulation, ANG-(1–7) acts as a vasodilator, whereas ANG II administered at equivalent doses constricted the coronary vessels (7). Furthermore, Mahon et al. (37) showed that ANG-(1–7) antagonized ANG II-induced vasoconstriction. Thus these studies demonstrate that ANG-(1–7) may be a counterregulator of the cardiovascular effects of ANG II by acting as a local modulator of vascular tone. Our studies supplement these studies by demonstrating for the first time that estrogen amplifies the vasodepressor responses to ANG-(1–7), while diminishing the responses to ANG II.

It has been previously described that estrogen diminishes the vasoconstrictor actions of ANG II in oophorectomized nonpregnant and pregnant sheep (36, 52), rats (15, 50, 59), and human females (1, 16). The contractile responsiveness of aortic rings to ANG II was also demonstrated to be reduced in both endothelium intact and denuded aortic vessels obtained from oophorectomized animals receiving chronic treatment with E_2 (15). The persistence of the attenuated response to ANG II in the presence of estrogen in denuded vessels suggests an effect of estrogen on vascular smooth muscle ANG II receptors. Downregulation of ANG II receptors in response to estrogen has been reported to occur in the adrenal cortex (13), the kidney (17), and pituitary gland (13, 25). In studies using oophorectomized ewes treated chronically with estradiol, decreased pressor responses occurred when ANG II levels had returned to basal levels, suggesting that the downregulation was not related to elevated circulating levels of ANG II (36). These studies, taken together with the current results, suggest that estrogen may depress ANG II-induced pressor responses through a direct action to downregulate vascular smooth muscle ANG II receptors. However, indirect mechanisms may also participate, since previous studies have shown that chronic estrogen treatment increases endothelium-derived relaxing factors, such as nitric oxide (15, 24, 34, 39) and reduces endothelium-derived constricting factors (39).

The two components of the systemic response to ANG-(1–7) have been characterized previously to be mediated by separate mechanisms and receptors. The pressor component of the ANG-(1–7) response was blocked by losartan, an AT_1 receptor antagonist, but not by an AT_2 receptor subtype since the selective AT_2 receptor antagonists CGP-42112A or PD-123319, were not effective in blocking the ANG-(1–7) response (4). On the other hand, none of these three receptor antagonists prevented the vasodepressor effect of ANG-(1–7). This latter finding, together with the observation that [Sar¹Thr⁸]ANG II prevented both the pressor and depressor component (4), suggested the presence of a novel ANG receptor responsible for the vasodepressor actions of ANG-(1–7). In addition, Benter et al. (4) showed that the depressor response to systemic ANG-(1–7) was blocked by indomethacin, suggesting that estrogen may augment ANG-(1–7)-mediated release of prostaglandins. Furthermore, the presence of the re-

sponse to ANG-(1–7) in the pithed rat suggests its independence from a reflex response (4). Further studies are required to evaluate specifically the effects of estrogen on the vasodepressor mechanism of the actions of ANG-(1–7).

Three weeks of estrogen replacement was associated with a significant reduction in plasma ACE activity in both Tg(–) and Tg(+) rats and in kidney and aorta ACE activity in the Tg(+) rat. The reduction in plasma ACE activity agrees with our recent report describing the decrease in ACE activity during long-term estrogen replacement in surgically induced postmenopausal cynomolgus monkeys (9). In those studies, the decrease in ACE activity was found in conjunction with significant decreases in the ratio of ANG II/ANG I, an *in vivo* index of ANG-peptide-related ACE activity (28). Our findings of a reduction of kidney and aorta ACE agree with the report by Seltzer et al. (57) who demonstrated that addition of estrogen to oophorectomized rats reduced the levels of ACE activity in the anterior pituitary. The additional effect of estrogen on local tissue ACE activity in the Tg(+) rats, but not Tg(–) rats, may contribute to the differences in circulating profile of ANG peptides observed in our study.

Consistent with the reduction in ACE activity was the observation that estrogen decreased circulating levels of ANG II in hypertensive animals, confirming a previous report that found that following 2 wk of estradiol treatment circulating levels of ANG II were decreased by 27% (13). These results, however, contrast with the overall consensus that estrogen activates the RAS. Mainly, it is well known that estrogen augments both liver and plasma levels of angiotensinogen, the renin substrate that is a biochemically rate-limiting step of the system (46, 60). The reports of estrogen's effects on renin are more variable. During ovulation of the normal menstrual cycle, Sealey et al. (56) reported that estrogen increased plasma renin substrate and plasma prorenin, but active plasma renin did not change. A hormonal influence on plasma renin was observed during the luteal phase when both estrogen and progesterone were elevated. In oophorectomized ewes, Magness et al. (36) demonstrated that acute (1–3 days) estrogen treatment increases PRA, but with more chronic treatment (14 days) compensatory influences, probably related to the volume status of the animal, arise to return PRA back to baseline. These latter findings are consistent with the findings of normal PRA values in our study. Others, however, have reported that tissue and circulating levels of renin are increased after chronic estrogen treatment (14, 23, 26, 36, 53). In accordance with this latter observation, tissue renin in

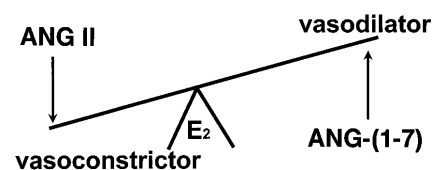


Fig. 7. A schematic representation of effects of E_2 on the renin-angiotensin system (RAS). E_2 shifts the balance of the RAS.

the ovary, submaxillary gland, uterus, and adrenal gland was shown to be increased after estrogen treatment (53). Similarly, oophorectomy is associated with a fall in kidney renin mRNA levels in female SHR that is canceled in the presence of estrogen supplement (14). Our previously published studies in cynomolgus monkeys showed that both plasma renin and ANG I were increased 5- to 10-fold after 30 mo of conjugated equine estrogen treatment. In that study, the hyperreninemia following chronic estrogen treatment was also accompanied with a reduction in ACE activity (9), in agreement with the observation made in this study. Unexpectedly, the potentially harmful effects of an estrogen-induced hyperreninemia were balanced by its actions interfering with the formation of the vasoactive product ANG II, since in that study the increased plasma ANG I levels were accompanied by no changes in plasma ANG II. Thus, from previous studies it has been shown that estrogen activates a number of components of the renin-ANG system, including renin substrate and ANG I, and possesses a variable, probably a time-dependent, influence on renin activity. In the current study, we have demonstrated that this activated system can be thwarted by estrogen acting to decrease the vasoconstrictor peptide ANG II and ACE levels and increase ANG-(1-7) levels.

ANG-(1-7) is generated from either ANG I or ANG II by specific peptidases, namely neutral endopeptidase 24.11, prolyl endopeptidase, and metalloendopeptidase 24.15 (8, 21). The formation of ANG-(1-7) occurs independently of ACE; however, it has been demonstrated that in the presence of converting enzyme inhibition a 5- to 50-fold increase in ANG-(1-7) occurs both in tissues and in the circulation (12, 31, 55). Estrogen by decreasing the activity of ACE can increase the levels of ANG I, making available more substrate for the formation of ANG-(1-7) directly from ANG I. Estrogen has been reported to increase the activity of a number of these candidate enzymes, including prolyl endopeptidase (47) and neutral endopeptidase 24.11 (49). Further studies are warranted to demonstrate whether the increase in ANG-(1-7) observed in these studies occurs in conjunction with an increase in the activity of these ANG-(1-7) processing enzymes.

PERSPECTIVES

Presently, there is a scarcity of data regarding the effects of estrogen on the natural history of hypertension. Only in the early decades of life is the prevalence of hypertension more frequent in men than women. The increased incidence of hypertension in women after the age of 50 suggests that endocrine changes associated with a decline in ovarian function play a role in the pathogenesis and clinical manifestation of hypertension. The natural history of estrogen reduction with age could carry with it an increased risk of cardiovascular disease, perhaps mediated by hypertension. Consequently, the value of estrogen replacement as prophylactic to hypertensive disease is plausible. In the current studies, we have combined a monogenetic model of renin-dependent hypertension with a surgically in-

duced postmenopausal model. We have evaluated hormone replacement in this model and have determined that hypertension can be reduced and the renin-ANG system modified. Despite the overall impression in the literature that estrogen activates the RAS (29, 46, 60) by increasing the levels of the angiotensinogen and renin, we have demonstrated that estrogen may also act downstream of these two proteins by reducing ACE and shifting the profile of the circulating peptides. Thus we schematically propose in Fig. 7 that estrogen acts as a fulcrum reducing the magnitude of the response to and levels of ANG II, while increasing the formation and vasodilator response of ANG-(1-7). These studies provide new information on the potential mechanisms that may contribute to the therapeutic action of estrogen replacement therapy on postmenopausal women who are at an increased risk of cardiovascular morbidity.

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