

Bi-directional actions of estrogen on the renin-angiotensin system

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Abstract

Estrogen stimulates the renin-angiotensin system by augmenting both tissue and circulating levels of angiotensinogen and renin. We show, however, that angiotensin converting enzyme (ACE) activity in the circulation and in tissues is reduced in two animal models of post-menopausal chronic hormone replacement. We observed a reduction of ACE activity in association with a significant increase in plasma angiotensin I (Ang I) and hyperreninemia in ovariectomized monkeys treated with Premarin (conjugated equine estrogen) replacement for 30 months. Plasma angiotensin II (Ang II) levels were not increased in monkeys treated with estrogen, suggesting that the decrease in ACE curtailed the formation of the peptide. The Ang II/Ang I ratio, an *in vivo* index of ACE activity, was significantly reduced by estrogen treatment, further supporting the biochemical significance of estrogen's inhibition of ACE. In ovariectomized transgenic hypertensive (mRen2)27 rats submitted to estrogen replacement treatment for 3 weeks, ACE activity in plasma and tissue (aorta and kidney) and circulating Ang II levels were reduced, whereas circulating levels of angiotensin-(1-7) (Ang-(1-7)) were increased. Ang-(1-7), the N-terminal fragment of Ang II, is a novel vasodilator and antihypertensive peptide. Thus, the net balance of these effects of estrogen on the renin-angiotensin vasoconstrictor/vasodilator system is to promote the antihypertensive effect.

Key words

- Renin
- Angiotensin converting enzyme
- Angiotensin peptides
- Angiotensin receptors
- Bradykinin
- Nitric oxide
- Kinins
- Vasodilation
- Hormone replacement

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Introduction

Cardiovascular disease is the leading cause of female mortality, resulting in more deaths in women over 50 than all cancers (1). The incidence of cardiovascular disease in women rises steadily, approaching the incidence in men during the fifth to seventh decade of life (2). Estrogen protects women from cardiovascular disease by inhibiting atherosclerosis (3) and through effects on

lipid metabolism (4-6), carbohydrate metabolism (7,8), body composition (8), and vasomotor reactivity (9-12). Administration of estrogen to healthy women after menopause is associated with a lower rate of coronary disease (13-15). Barrett-Connor and Bush (13) estimated that the long-term use of post-menopausal estrogen decreased cardiovascular disease by 50%. However, changes in plasma lipids and atherosclerosis accounted for only 25-50% of the cardioprotective ef-

fect (1,15), suggesting that estrogen maintains vascular health through additional processes. One potential mechanism is the effect of estrogen on the renin-angiotensin system (RAS).

In this review we discuss the effects of estrogen on components of the RAS and suggest that estrogen shifts the generation of angiotensin peptides away from angiotensin II (Ang II) and towards the N-terminal heptapeptide, angiotensin-(1-7) (Ang-(1-7)). This fragment has little or none of the vasoconstrictor properties of Ang II (16,17), but instead releases prostaglandins (PGs) (18-22) and nitric oxide (NO) (23,24) and augments the vasodilator action of bradykinin (BK) (25,26).

Estrogen regulation of RAS components

The RAS is the primary regulator of blood pressure and fluid and sodium balance. Enhanced activation of the RAS contributes to the evolution of hypertension (17-29), salt retention (30,31), and hyperaldosteronism (32-34). Angiotensinogen (Aogen), the precursor of angiotensin peptides, is converted to angiotensin I (Ang I) by renin, an aspartyl protease. Angiotensin converting enzyme (ACE; EC 3.4.15.1) is the major enzyme responsible for the formation of the vasoconstrictor peptide Ang II from Ang I. This enzyme, also called kininase II, plays a pivotal role both in the RAS and the kinin system. ACE is a metalloprotease that releases C-terminal dipeptides from substrates such as Ang I and BK (35) and degrades BK, a vasodilator, to its inactive metabolite. ACE inhibition was associated with a 5-50-fold increase in Ang-(1-7) and BK in tissues and the circulation (36-38). Ang-(1-7), a heptapeptide with novel vasodilator and antihypertensive properties (39-42), is generated from either Ang I or Ang II by specific peptidases (43,44). In bovine, porcine, and human aortic and venous endothelial cells,

Ang I is primarily processed to Ang-(1-7) by neutral endopeptidase 24.11 (40-50%) and prolylendopeptidase (45). In vascular smooth muscle cells, Ang-(1-7) was the major product generated from Ang I, and its production was dependent upon metalloendopeptidase 24.15 (46). Further metabolism of Ang-(1-7) or Ang II by aminopeptidases and dipeptidases leads to the formation of smaller fragments, Ang-(3-7) and Ang IV, which may also have a biological function (47,48). Importantly, Ang-(1-7) is both a substrate (49,50) and an inhibitor of ACE (23,50).

Estrogen causes over-expression of the RAS by augmenting both tissue and circulating levels of Aogen (51,52) and renin (53-56). Plasma renin activity (PRA) increases after estrogen treatment in nephrectomized rats (53). In addition, tissue renin is increased in the ovary, submaxillary gland, uterus, and adrenal gland after estrogen treatment (54). Ovariectomy of spontaneously hypertensive female rats is associated with a fall in kidney renin mRNA; this decrease is not observed in tissue from rats receiving estrogen supplement (53). In association with increased circulating estrogen, renin is increased in the mother during pregnancy (57). Plasma Aogen also increases in normotensive and hypertensive menopausal women placed on estrogen replacement therapy (58). However, no change in plasma Ang II was observed. The use of higher doses of estrogen in oral contraceptives increases hepatic and plasma Aogen and blood pressure (59,60), especially for the higher dose estrogens found in early contraceptives. However, lower doses of estrogen in modern contraceptives also result in an increase in plasma Aogen, without the complication of hypertension (59,60). During pregnancy, there is a large increase of plasma Aogen due to stimulation of hepatic Aogen synthesis by estrogen (57,59). During the normal menstrual cycle, plasma Aogen and prorenin, a precursor of renin, increase during the follicular phase, but active plasma renin does not change until the

luteal phase when both estrogen and progesterone are elevated (61). Thus, Aogen and renin are elevated in response to increased plasma estrogen due to normal cycling events or pharmacological hormone replacement.

If estrogen treatment activates the RAS, why do the hyper-reninemia and hyperaogenemia states not result in significant increases in blood pressure? The effects of hormone replacement on blood pressure are conflicting, with findings of either no change or a decrease in blood pressure (12,62-64). In investigating this question, we found that estrogen shifts the pattern of angiotensin peptide formation in a tissue-specific manner, reducing production of Ang II, while augmenting the production of the N-terminal Ang II fragment, Ang-(1-7). This shift in the pattern of peptides arises in part due to estrogen decreasing the activity of ACE.

Chronic hormone replacement in cynomolgus monkeys

In the first study we evaluated the effects of chronic hormone replacement in a model of post-menopausal hormone replacement, i.e., ovariectomized monkeys receiving premarin (conjugated equine estrogens; CEE) orally. At the time the study was initiated 29 feral adult female cynomolgus monkeys (*Macaca fascicularis*) ranging in age from 5 to 13 years had been fed a moderately atherogenic diet for 22 months to induce atherosclerosis. Bilateral ovariectomies were performed to initiate surgical menopause on all animals 4 months after the diet started. At the end of 22 months of continuous feeding of the atherosclerotic diet, all animals received a lipid-lowering diet and were randomly assigned to a replacement therapy protocol lasting 30 months as indicated: group 1 (placebo, N = 14) and group 2 (CEE, N = 15). Groups 2 received 7.2 µg/day of CEE (Premarin®, Wyeth-Ayerst, Radnor, PA, USA) for the first eight months and 166 µg/day of CEE for the remaining 22 months.

The latter dose of CEE was used to raise the levels of circulating 17 β-estradiol to approximately 150 pg/ml, a concentration equivalent to the therapeutic concentrations achieved in women (~0.625 mg/day) (65). Hormones were administered twice daily in the diet. Blood samples characterizing the levels of sex hormones were taken 4 h after administration of the oral drug (peak level) under ketamine hydrochloride sedation (10 to 15 mg/kg body weight, *im*) (Fort Dodge Laboratories, Inc., Fort Dodge, IA, USA) 5 months before necropsy.

There was no significant effect of hormone replacement on mean arterial blood pressure or body weight. As expected, plasma 17-β-estradiol levels were near the minimum detectable level of the assay in untreated control animals (2.4 ± 1.1 pg/ml) but rose significantly (148.4 ± 13.4 pg/ml, $P < 0.05$) in CEE-treated animals. Figure 1 shows that long-term replacement therapy with CEE produced significant increases in PRA. These changes were accompanied by a significant reduction in plasma ACE activity in CEE-treated animals. Replacement of estrogen resulted in a significant, three-fold increase in plasma Ang I in animals given CEE (Figure 2A). On the other hand, there

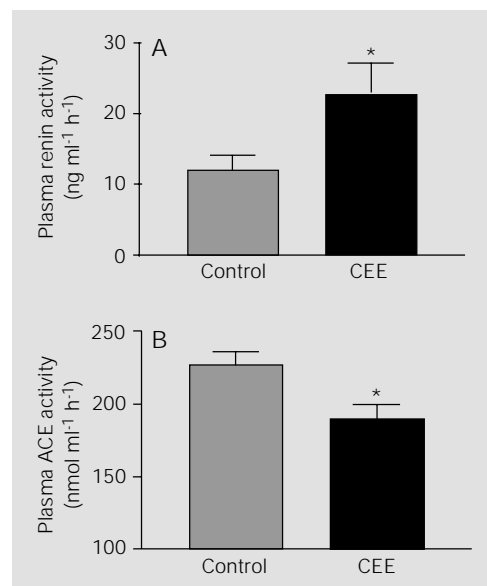


Figure 1 - Effects of estrogen on plasma renin activity (A) and angiotensin converting enzyme activity (B). Values are reported as mean ± SEM. * $P < 0.05$ vs control (Student t-test). All cynomolgus monkeys were ovariectomized and left either untreated (control, N = 14) or treated with conjugated equine estrogens (CEE; N = 15). (Reproduced from Ref. 72 with permission).

was no significant effect of CEE replacement on the circulating levels of Ang II or Ang-(1-7) (Figure 2B and C). The Ang II/Ang I ratio (1.22 ± 0.56 vs 0.38 ± 0.08 , $P < 0.05$; control vs CEE), an *in vivo* estimate of ACE activity (66), was significantly reduced in hormone-treated animals. Thus, in cynomolgus monkeys with chronic experimental atherosclerosis, replacement therapy with estrogen for 30 months in addition to a lipid-lowering diet resulted in significant and reciprocal changes in the activity of the two enzymes that account for the generation of angiotensin peptides. In our study estrogen replacement therapy augmented renin activity and reduced ACE activity. The increase in PRA was sufficient to be reflected in

higher plasma Ang I concentrations in the CEE-treated group. By curtailing the formation of the vasoconstrictor end-product Ang II, the effect of estrogen on ACE activity prevented an otherwise expected increase in the production of Ang II. These experiments provided new information on the potential mechanisms that may contribute to the described cardio-protective action of estrogen replacement therapy on post-menopausal women.

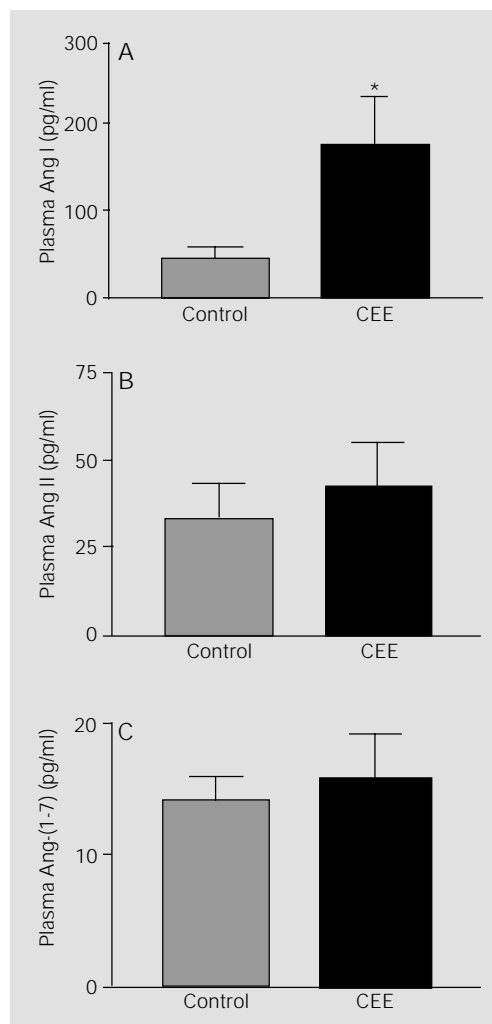
Estrogen replacement in transgenic hypertensive (mRen-2)27 rats

In the next study, we evaluated hormone replacement in hypertensive and normotensive rats. Fifty-three female transgenic negative Tg(-) and heterozygous hypertensive Tg(+) rats (body weight: 220~250 g) from the Hypertension Center Transgenic Rat Colony of Wake Forest University School of Medicine underwent bilateral ovariectomy at age 12 weeks under general anesthesia with ketamine (30 mg/kg, *im*) and xylazine (5 mg/kg, *im*). Pellets containing either 17 β -estradiol (E₂) (1.5 mg/rat, for 3-week release; Innovative Research of America, Toledo, OH, USA) or vehicle (VEH) were implanted into the subcutaneous tissue.

Chronic 17 β -estradiol treatment produced a small but statistically significant decrease in the mean blood pressure of both transgenic hypertensive (159 ± 4 vs 145 ± 5 mmHg, $P < 0.05$) and normotensive (119 ± 4 vs 108 ± 2 mmHg, $P < 0.05$) rats. There was no change in heart rate with estrogen treatment. Plasma 17 β -estradiol concentration averaged 190 ± 20 pg/ml in E₂-treated rats and < 15 pg/ml in VEH-treated rats.

The magnitude of the pressor response produced by the injection of three doses of Ang II was similar in VEH-treated Tg(+) and Tg(-) rats. In contrast, chronic 17 β -estradiol treatment significantly attenuated the pressor responses to intravenous injection of Ang II in both strains at all doses tested

Figure 2 - Effects of estrogen on plasma Ang I (A), Ang II (B), and Ang-(1-7) (C). Values are reported as mean \pm SEM. * $P < 0.05$ vs control (Student t-test). All cynomolgus monkeys were ovariectomized and left either untreated (control) or treated with conjugated equine estrogens (CEE). (Reproduced from Ref. 72 with permission).



(Figure 3A and B). Intravenous injections of Ang-(1-7) in normotensive and 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. 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 normotensive rats (Figure 4A and C). In Tg(+) rats treated with estrogen, the blunting of the pressor component of the Ang-(1-7) response was comparable to that obtained in Tg(-) rats (Figure 4A and B). In hypertensive rats, estrogen potentiated the magnitude of the fall in blood pressure produced by Ang-(1-7) (Figure 4D).

Replacement with estrogen resulted in a nearly 2-fold reduction in the circulating levels of Ang II in Tg(+) rats while it had no effect on plasma Ang II in Tg(-) rats (Figure 5A). In contrast, estrogen replacement significantly increased the levels of plasma Ang-(1-7) in Tg(+) animals (Figure 5B). In accordance with the reduction in plasma Ang II levels in Tg(+) rats, estrogen significantly reduced plasma ACE activity in Tg(+) animals (Figure 6A). A similar reduction in plasma ACE was observed in Tg(-) rats. There was no difference in plasma, kidney, or aorta ACE activity levels between Tg(-) and Tg(+) animals on similar treatment (Figure 6A-C), whereas chronic estrogen replacement therapy significantly decreased both kidney and aorta ACE activity in Tg(+) but not in Tg(-) rats.

In summary, this study combined a monogenetic model of renin-dependent hypertension with a surgically induced postmenopausal model. Hormone replacement in this model attenuates hypertension in association with reduced generation of Ang II and increased production of Ang-(1-7). In spite of the overall impression in the literature that estrogen activates the RAS (51,52,67) by increasing the levels of angiotensinogen and renin, estrogen acts downstream in relation

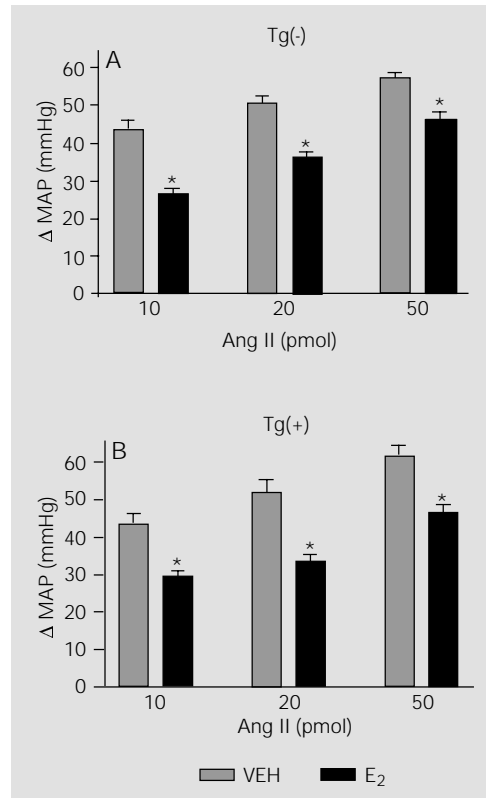


Figure 3 - Effects of 3-week estrogen (E₂) replacement on Ang II pressor responses of conscious, resting Tg(-) (A) and Tg(+) (B) rats. E₂ attenuates the Ang II-induced pressor responses. Values are reported as mean ± SEM. *P<0.05, VEH vs E₂ (ANOVA). Each group included 6–8 rats. VEH, Vehicle. (Reproduced from Ref. 62 with permission).

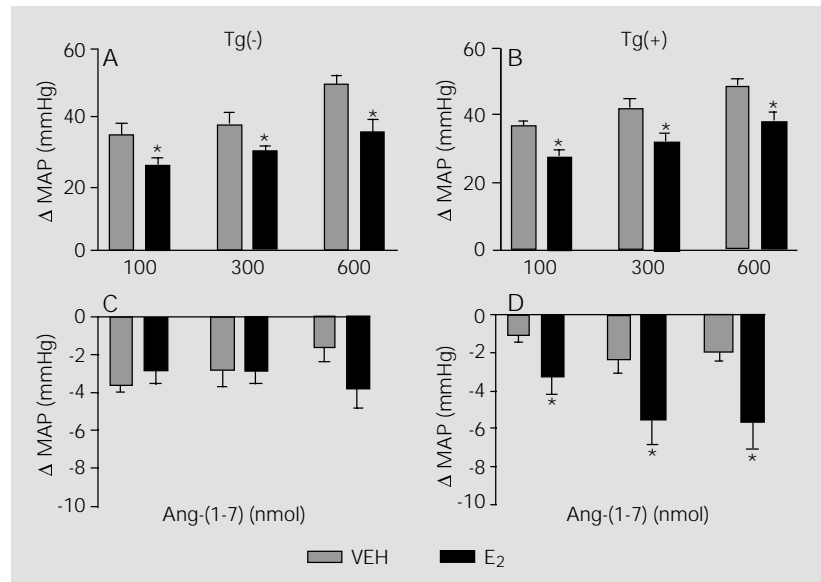


Figure 4 - Effects of 3-week estrogen (E₂) replacement on Ang-(1-7) pressor/depressor responses of conscious, resting Tg(-) (A, C) and Tg(+) (B, D) rats. E₂ attenuates the Ang-(1-7)-induced pressor responses of both Tg(-) (A) and Tg(+) (B) animals, but augments the depressor responses of Tg(+) rats (D). Values are reported as mean ± SEM. *P<0.05, VEH vs E₂ (ANOVA). VEH = Vehicle. (Reproduced from Ref. 62 with permission).

Figure 5 - A, Effects of estrogen replacement (E_2) on plasma levels of Ang II. Estrogen reduces the circulating levels of plasma Ang II in Tg(+) animals. B, Effects of estrogen replacement (E_2) on plasma levels of Ang-(1-7). Estrogen increases the circulating levels of plasma Ang-(1-7) in Tg(+) animals. Values are reported as mean \pm SEM. * $P < 0.05$, VEH vs E_2 (Tukey-Kramer test); # $P < 0.01$ Tg(+) vs Tg(-) (Tukey-Kramer test). Each group included 6–8 rats. VEH = Vehicle. (Reproduced from Ref. 62 with permission).

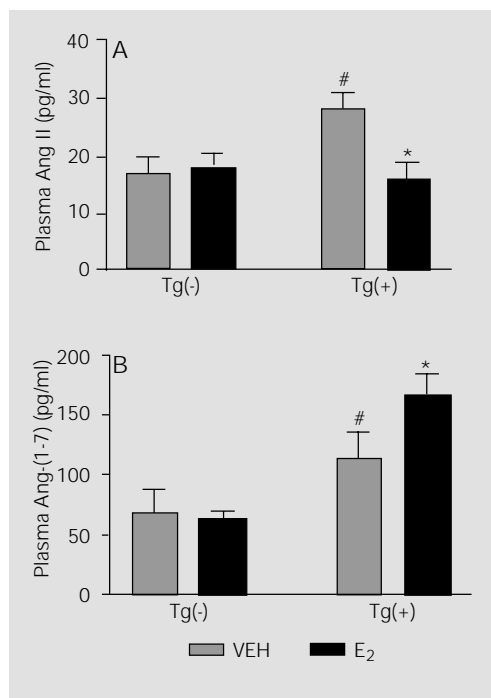
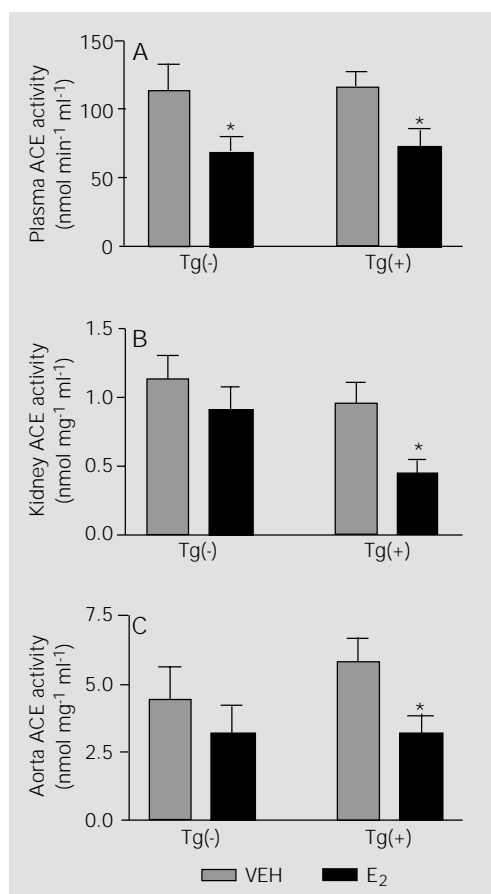


Figure 6 - Effect of estrogen replacement (E_2) on plasma (A), kidney (B) and thoracic aorta (C) levels of angiotensin converting enzyme. Estrogen reduces the plasma angiotensin converting enzyme activity in Tg(+) and Tg(-) animals, whereas it only reduces the tissue levels of kidney and thoracic aorta in Tg(+) rats. Values are reported as mean \pm SEM. * $P < 0.05$, VEH vs E_2 (Tukey-Kramer test). Each group included 6–8 rats. VEH = Vehicle. (Reproduced from Ref. 62 with permission).



to these two proteins by reducing ACE activity and shifting the profile of the circulating angiotensin peptides. Thus, estrogen acts as a fulcrum reducing the magnitude of the response to and levels of Ang II, while increasing the formation and vasodilator effect of Ang-(1-7). These studies provide new information on the potential mechanisms that may contribute to the therapeutic action of estrogen replacement therapy in postmenopausal women who are at an increased risk of cardiovascular morbidity.

Estrous cycle influence on angiotensin peptides

A third study was conducted to evaluate if local regional angiotensin peptide levels are modulated by the estrous cycle. Ten-week-old normotensive (N = 15) and hypertensive (mRen2)27 (N = 15) rats were sacrificed by decapitation without prior anesthesia. Vaginal smears were obtained from each rat to determine the stage of the estrous cycle at sacrifice, as described by Long and Evans (68). Immunoreactive (Ir) peptide levels were measured using three different radioimmunoassays, as previously described (69). Table 1A shows the influence of the estrous cycle on angiotensin peptides in the hypothalamus. There was no influence of the estrous cycle on hypothalamic peptide levels of normotensive rats. Ir-Ang I was significantly elevated during proestrus as compared to diestrus in hypertensive rats. Ir-Ang II levels were unchanged with the estrous cycle. Ir-Ang-(1-7) levels were significantly elevated during both proestrus and estrus as compared to diestrus. Table 1B illustrates that the peptide levels in the lower brain stem of normotensive or hypertensive rats did not change with the estrous cycle. In summary, the estrous cycle exerts an influence on hypothalamic angiotensin peptides, enhancing the expression of Ang I and Ang-(1-7) in hypertensive rats.

Gender differences in cardiovascular dis-

ease incidence have been established (70). After menopause, the prevalence of hypertension and coronary heart disease increases markedly in women (59,70). Hormone replacement with estrogen provides cardioprotective effects. However, estrogen also increases the production of Aogen and renin, two components of the RAS. An elevation of Aogen with unchanged or increased levels of renin would appear to favor the development of hypertension and increase cardiovascular risk. Yet women taking estrogen replacement have either unchanged or slightly lower blood pressure (8,71). The literature reviewed above and our studies offer a new hypothesis for the effects of estrogen on the RAS and its potential relevance to hypertension and local vasoreactivity. Although renin and Aogen are increased, we show that ACE activity is reduced by estrogen. The reduction in ACE activity acts to curb the activated RAS by shifting the amount of the peptides which are formed, i.e., increasing Ang I, reducing the production of Ang II and enhancing formation of Ang-(1-7). Our findings that estrogen reduces ACE activity require a re-evaluation of the long held tenet that estrogen's activation of the RAS increases circulating Ang II. Estrogen's reduction of ACE activity leads to increased levels of Ang-(1-7) due to the diversion of the processing pathway from Ang II to increased Ang I. Thus, we schematically propose in Figure 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 effect of Ang-(1-7). In addition, new studies carried out by Dr. Chappell (49) of our group showed that ACE metabolizes Ang-(1-7). By reducing ACE activity, estrogen would decrease the breakdown of Ang-(1-7) and add further to the increased levels of Ang-(1-7), potentially resulting in an enhancement of vasodilation. Because ACE reduction also leads to less degradation of bradykinin, another aspect of estrogen's action would be to enhance

Table 1 - Influence of estrous cycle on immunoreactive (Ir) angiotensin peptides in hypothalamus (A) and lower brain stem (B).

Values are reported as mean \pm SEM. *P<0.05 vs proestrus; +P<0.05 vs estrus. n.d., Not detectable.

	N	Ir-Ang I (fmol/mg protein)	Ir-Ang II (fmol/mg protein)	Ir-Ang-(1-7) (fmol/mg protein)
A. Hypothalamus				
Proestrus				
Tg(-)	5	n.d.	3.0 \pm 0.7	6.1 \pm 0.6
Tg(+)	5	37.8 \pm 12.0	48.0 \pm 4.0	225 \pm 52
Estrus				
Tg(-)	5	n.d.	3.6 \pm 0.7	6.6 \pm 3.0
Tg(+)	5	4.6 \pm 3.0	50.0 \pm 13.0	115 \pm 24
Diestrus				
Tg(-)	5	n.d.	2.8 \pm 0.9	8.5 \pm 3.4
Tg(+)	5	4.6 \pm 2.0*	50.0 \pm 4.0	5.7 \pm 0.5**
B. Lower brain stem				
Proestrus				
Tg(-)	5	26.5 \pm 10	6.5 \pm 2.2	11.4 \pm 1.0
Tg(+)	5	20.1 \pm 3.1	29.4 \pm 3.0	16.3 \pm 1.5
Estrus				
Tg(-)	5	15.7 \pm 5.5	4.5 \pm 0.6	12.7 \pm 2.0
Tg(+)	5	22.1 \pm 4.1	27.6 \pm 2.5	11.3 \pm 1.1
Diestrus				
Tg(-)	5	13.6 \pm 1.5	4.2 \pm 0.5	12.5 \pm 0.7
Tg(+)	5	14.5 \pm 1.5	17.7 \pm 2.7	11.4 \pm 0.8

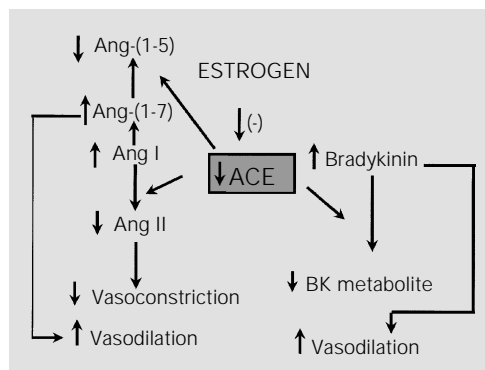


Figure 7 - Schematic diagram demonstrating the effects of estrogen on the renin-angiotensin system. ACE, Angiotensin converting enzyme; BK, bradykinin.

vasodilation by both Ang-(1-7) and BK. Our studies enhance the understanding of the role of estrogen in the regulation of the RAS and may provide a new rationale for the use of estrogen to prevent cardiovascular disease in postmenopausal women who are at increased cardiovascular risk.

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