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Chronic estrogen alters contractile responsiveness to angiotensin II and norepinephrine in female rat aorta

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Sex hormones have been postulated to play an important role in the modulation of vascular responsiveness to endogenous vasoactive substances. This study was designed to establish the effects of chronic treatment with estrogen in vivo on subsequent contractile responsiveness of aortic rings to angiotensin II or norepinephrine in vitro. Concentration-response curves for angiotensin II were compared in aortic rings with or without endothelium isolated from ovariectomized rats (275–299 g) pretreated with 17β-estradiol (≈ 1 mg/kg per day) or placebo for 14 days and from normal prepubertal female rats (125–149 g) pretreated with 17β-estradiol (≈ 5 mg/kg per day) or placebo for 14 days. Whether or not endothelium was present, angiotensin II-induced contraction was significantly depressed in rings from 17β-estradiol-treated ovariectomized or prepubertal rats when compared to controls, and the pattern of the effects of 17β-estradiol-treatment on the concentration–response curves for angiotensin II was similar in the two models. In contrast to angiotensin II-induced contraction, norepinephrine-induced contraction was significantly enhanced in aortic rings with or without endothelium from ovariectomized rats pretreated with 17β-estradiol (≈ 1 mg/kg per day, 14 days) and from normal prepubertal female rats pretreated with 17β-estradiol (≈ 5 mg/kg per day, 14 days) when compared to controls. The results demonstrate that chronic treatment with 17β-estradiol selectively reduced and enhanced contractile responsiveness of aortic rings to angiotensin II and norepinephrine, respectively. Further, the results indicate that the normal prepubertal female rat is an efficient model to investigate modulation by estrogen of aortic responsiveness to vasoactive agents in vitro.

Estrogen; Angiotensin II; Aorta (rat); Norepinephrine; Contraction

1. Introduction

During human pregnancy, contractile responsiveness of vascular tissues to angiotensin II is decreased (Abdul-Karim and Assali, 1961). This observation has been subsequently confirmed in a variety of animal species, including rat (Paller, 1987). Although a variety of hormonal changes occur during pregnancy which could influence vascular reactivity to angiotensin II, most early studies focused on the potential role of estrogen and progesterone in influencing responses to angiotensin II. Early studies failed to demonstrate an effect of acute estrogen administration in vascular responsiveness to angiotensin II (Chelsy and Tepper, 1967; Hittiaratchi and Pickford, 1968; Altura, 1975). However, a more recent investigation has demonstrated that acute infusion of estrogen reduced the pressor effect of angiotensin II in chronically instrumented sheep (Rosenfeld and Jackson, 1984). Thus, controversy still exists regarding the influence of estrogen on vascular responsiveness to angiotensin II.

The present study was designed to establish the capacity of chronic estrogen-treatment in vivo to alter subsequent contractile responsiveness of aortic rings to angiotensin II in vitro. Further, to evaluate if an observed effect of estrogen on angiotensin II-induced contraction was selective, the effects of estrogen-treatment on the responsiveness of aortic rings to norepinephrine were also determined.

2. Materials and methods

2.1. Drugs and solutions

Angiotensin II (human form, synthetic, acetate salt) and norepinephrine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). 17β-Estradiol pellets (5 mg/pellet) and placebo pellets

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were purchased from Innovative Research of America (Toledo, OH). Other chemicals used were reagent grade. Angiotensin II was dissolved in H_2O. The resulting angiotensin II stock solution (1.0 mM) was aliquoted. Aliquots (100 μl) were stored at -5°C. Just prior to use, individual aliquots were warmed to room temperature. Stock solutions of norepinephrine (10 mM) in H_2O were prepared daily. Stock solutions of angiotensin II and norepinephrine were further diluted with H_2O as necessary such that addition of 100 μl or 50 μl aliquots to the 10 or 5 ml bath chamber resulted in the final bath concentration desired. Krebs buffer used in all experiments consisted of (millimolar): NaCl 118; $NaHCO_3$ 25; KCl 4.7; $CaCl_2$ 1.5; $MgCl_2$ 1.1; KH_2PO_4 1.2 and glucose 5.6. The composition of the depolarizing potassium solution (HiK) was the same as the Krebs buffer used, except all the NaCl was replaced with an equimolar amount of KCl. Eighty millimolar potassium solution was made by mixing 2 volumes of HiK solution with 1 volume of Krebs buffer.

2.2. *Animals*

Female Sprague–Dawley rats (normal or ovariectomized) were obtained from Hilltop Lab Animals, Inc. (Scottsdale, PA). Normal female prepubertal rats when purchased weighed between 50 and 74 g (< 26 days of age). Ovariectomized rats when purchased weighed between 75 and 99 g (< 31 days of age) and were housed for 21 days, reaching a weight of 225–249 g prior to treatment. Animals were housed in the university animal facilities and given food and water ad libitum. Using a 10-gauge trochar, normal prepubertal and ovariectomized rats were implanted s.c. at the back of the neck with a placebo pellet or a pellet containing 5 mg of 17β-estradiol.

2.3. *Preparation of aortic rings*

Fourteen days after implantation of placebo or 17β-estradiol-containing pellets, rats were weighed (prepubertal rats, 125–149 g; ovariectomized rats, 275–299 g) and killed. Rats were administrated 100 mg/kg sodium pentobarbital. After achievement of deep anesthesia, the abdomen was opened and a mesenteric blood vessel was severed for exsanguination and the thoracic aortas were isolated. Once isolated, the aortas were placed into Krebs buffer, and fat and loosely adhering connective tissue was removed. Rings were prepared by transversely cutting the vascular segments into 5-mm rings using fine-tipped scissors. Care was taken to minimize rubbing of the intimal surfaces when preparing rings in which an intact endothelium was desired. To destroy endothelium in selected rings, the intimal surface was rubbed (20–35 s, prepubertal; 45–60 s, ovariectomized) using needles (10 gauge, prepubertal; 18 gauge, ovariectomized) with lightly scuffed external surfaces. Within experiments, rings with and without endothelium were prepared from the same aortic segment isolated from one animal.

2.4. *Measurement of isometric tension*

Aortic rings were mounted in 5 or 10 ml jacketed tissue baths by suspending them between two L-shaped stainless steel hooks. The lower hooks were attached to stationary supports such that they could be positioned in the bottom of the bath chambers. The upper hooks were attached to force-displacement transducers (FTO3C, Grass Instrument Company, Quincy, MA). The bath chambers contained Krebs buffer (37 $\pm$ 0.5°C) constantly bubbled with 95% O_2–5% CO_2. Isometric force was measured and recorded using either Beckman R-611 Dynograph (Sensormedics, Anaheim, CA) or Grass 7D or 19D Polygraphs (Grass Instrument Company, Quincy, MA). After mounting, rings were equilibrated for 1–2 h with several adjustments of length until optimal baseline force was maintained.

Optimal force for development of active tension by aortic rings from each group of rats was determined in preliminary experiments by mounting rings at an initial force of 1 g and subsequently increasing baseline force by 0.5-g intervals. At each level of baseline force, contractile responses to 80 mM potassium were measured. Optimal baseline forces were selected as those which were associated with the maximum contraction to 80 mM potassium. Optimal loads were 2 g (prepubertal) or 3 g (ovariectomized).

2.5. *Contractile experiments*

Following equilibration under optimal load, vascular rings were initially exposed to 80 mM potassium to obtain a reference response. When contractile responses to 80 mM potassium plateaued, rings were rinsed with Krebs buffer and allowed to return to baseline tension and tested for functional endothelium.

To test for endothelium, tone was induced by exposing the rings to 0.1 μM norepinephrine. When contractile responses reached maximum, the rings were exposed to 1 μM acetylcholine. Relaxation induced by 1 μM acetylcholine in norepinephrine-precontracted aortic rings, was interpreted as indicating the presence of a significant amount of functional endothelium. The absence of relaxation by exposure to the same concentration of acetylcholine in aortic rings, was interpreted as indicating the absence of a significant amount of functional endothelium. Aortic rings were rinsed several times with Krebs buffer and allowed to re-equilibrate for 45–60 min before subsequent exposure to angiotensin II and norepinephrine. To evaluate re-

sponses to angiotensin II in ovariectomized rats, each ring was exposed to only a single concentration of angiotensin II. Aortic rings from prepubertal rats were exposed to progressively increased concentrations of angiotensin II for 10 min with 30 min of washout and drug-free recovery time between exposure to each. Concentration–response curves for norepinephrine were determined cumulatively.

2.6. Data analysis

Angiotensin II- and norepinephrine-induced contraction was calculated as percentage of reference contraction to 80 mM potassium. Results from preliminary experiments using aortic rings from 17β-estradiol- and placebo-treated ovariectomized rats did not reveal any significant influence of the estrogen on contraction (dynes/mm^2) induced by 80 mM potassium. Data were calculated as the means ± S.E. Comparisons were made using analysis of variance. A P value < 0.05 was accepted as denoting statistically significant differences.

3. Results

3.1. Angiotensin II-induced responses in 17β-estradiol- and placebo-treated ovariectomized rats

Concentration–response curves for angiotensin II-induced contraction in aortic rings with or without endothelium isolated from ovariectomized rats which were pretreated with 17β-estradiol or placebo are compared in fig. 1. To minimize a potential influence of tachyphylaxis, concentration–response curves for angiotensin II-induced contraction were determined by exposure of individual aortic rings to a single concentration of angiotensin II. The presence of endothelium markedly suppressed angiotensin II-induced contraction in aortic rings from both 17β-estradiol-pretreated and placebo-pretreated ovariectomized rats. Angiotensin II-induced contraction, normalized as percentage of reference contraction induced by 80 mM potassium, was significantly reduced in both endothelium-intact and -denuded aortic rings from ovariectomized rats pretreated with 17β-estradiol when compared to rings from placebo-treated rats (two-way ANOVA, $P < 0.05$).

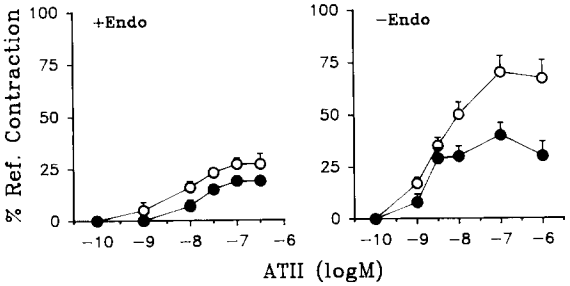

Fig. 1. Effects of 17β-estradiol (≈ 1 mg/kg per day for 14 days) on angiotensin II-induced contraction in aortic rings with or without endothelium isolated from ovariectomized female rats which were treated with 17β-estradiol (•) or treated with placebo (○). Each aortic ring was exposed to a single concentration of angiotensin II. Data were calculated as percentage reference contraction to 80 mM potassium. Each point represents mean data obtained using five to six aortic rings. Brackets indicated S.E.

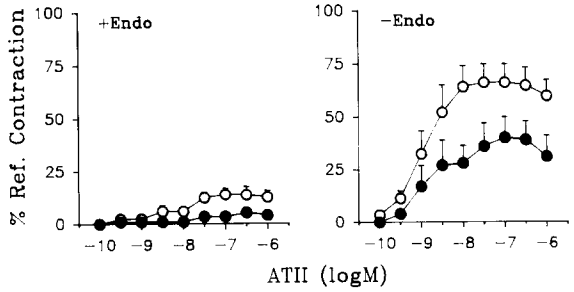

Fig. 2. Effects of 17β-estradiol (≈ 5 mg/kg per day) contraction induced by angiotensin II in aortic rings with or without endothelium isolated from normal prepubertal female rats which were treated with 17β-estradiol (•) or placebo (○) for 14 days. Each aortic ring was exposed to single, progressively increasing concentrations of angiotensin II for 10 min with 30 min of washout and drug-free recovery time between each exposure. Data were calculated as percentage reference contraction to 80 mM potassium. Each point represents mean data obtained using five to six aortic rings. Brackets indicated S.E.

3.2. Angiotensin II-induced responses in 17β-estradiol- and placebo-treated normal prepubertal rats

Concentration–response curves for angiotensin II-induced contraction in aortic rings with or without endothelium isolated from normal prepubertal female rats which were pretreated with 17β-estradiol or placebo are compared in fig. 2. Rings were exposed to single, progressively increasing concentrations of angiotensin II for 10 min with 30 min of washout and drug-free recovery time between each exposure. The presence of endothelium markedly suppressed angiotensin II-induced contraction in aortic rings from both 17β-estradiol-pretreated and placebo-pretreated prepubertal rats. Angiotensin II-induced contraction, normalized as percentage of reference contraction induced by 80 mM potassium, was significantly reduced in both endothelium-intact and -denuded aortic rings from prepubertal rats pretreated with 17β-estradiol when compared to rings from placebo-treated rats (two-way ANOVA, $P < 0.05$).

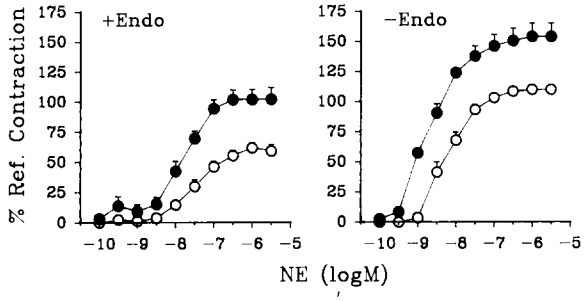

Fig. 3. Effects of 17β-estradiol (≈ 1 mg/kg per day) on contraction induced by norepinephrine in aortic rings with or without endothelium isolated from ovariectomized rats which were treated with 17β-estradiol (●) or placebo (○) for 14 days. Each aortic ring was exposed to cumulatively increasing concentration of norepinephrine. Data were calculated as percentage reference contraction to 80 mM potassium. Each point represents mean data obtained using five to six aortic rings. Brackets indicate S.E.

3.3. Norepinephrine-induced responses in 17β-estradiol- and placebo-treated ovariectomized rats

Cumulative concentration–response curves for norepinephrine-induced contraction in aortic rings with or without endothelium isolated from ovariectomized rats which were pretreated with 17β-estradiol or placebo are compared in fig. 3. Norepinephrine-induced contraction, normalized as percentage of reference contraction induced by 80 mM potassium, was significantly enhanced in aortic rings with or without endothelium from ovariectomized rats pretreated with 17β-estradiol for 14 days when compared to rings from placebo-treated rats (two-way ANOVA, $P < 0.05$). The presence of endothelium suppressed norepinephrine-induced contraction in aortic rings from both 17β-estradiol-pretreated and placebo-pretreated ovariectomized rats.

3.4. Norepinephrine-induced responses in 17β-estradiol- and placebo-treated normal prepubertal rats

Cumulative concentration–response curves for norepinephrine-induced contraction in aortic rings with or without endothelium isolated from normal prepubertal female rats which were pretreated with 17β-estradiol or placebo are compared in fig. 4. Norepinephrine-induced contraction, normalized as percentage of reference contraction induced by 80 mM potassium, was significantly enhanced in aortic rings with or without endothelium from prepubertal rats pretreated with 17β-estradiol for 14 days when compared to rings from placebo-treated rats (two-way ANOVA, $P < 0.05$). The presence of endothelium suppressed norepinephrine-induced contraction in aortic rings from both 17β-estradiol-pretreated and placebo-pretreated prepubertal rats.

4. Discussion

Results from the present study demonstrate that chronic in vivo treatment of ovariectomized adult or prepubertal female rats with 17β-estradiol markedly depresses contractile responses of aortic rings to angiotensin II determined in vitro. The depression of angiotensin II-induced contractions by 17β-estradiol was apparently selective in that treatment with 17β-estradiol enhanced, rather than depressed, norepinephrine-induced contraction in aortic rings from the ovariectomized adult or prepubertal female rats.

The observed depression of angiotensin II-induced contraction following chronic treatment with 17β-estradiol is consistent with a previous study demonstrating reduced pressor effects of angiotensin II in sheep following acute estrogen infusion (Rosenfeld and Jackson, 1984), but contrasts with earlier studies which failed to demonstrate an acute effect of estrogen on vascular responsiveness to angiotensin II (Chelsy and Tepper, 1967; Hittiaratchi and Pickford, 1968; Altura, 1975). The differences in the results of the various studies may be related to differences in: (1) modes of treatment; (2) durations of treatment; and (3) treatment doses. Chronic treatment with high doses of estrogen, as was employed in the present study, may be necessary, to consistently observe estrogen-induced depression of angiotensin II-induced vascular contraction.

Previous studies have suggested that estrogen may increase release of endothelium-derived relaxing factor (Miller et al., 1988) and decrease release of endothelium-derived constricting factors (Vanhoutte, 1987; Miller et al., 1988). As the presence of endothelium markedly depresses angiotensin II-induced contraction in rat aorta (Gruetter et al., 1988), an effect of estrogen to depress angiotensin II-induced contraction through an effect on the secretion of endothelium-de-

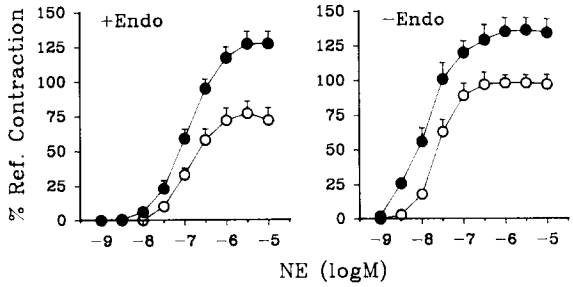

Fig. 4. Effects of 17β-estradiol (≈ 5 mg/kg per day) on contraction induced by norepinephrine in aortic rings with or without endothelium isolated from normal prepubertal female rats which were treated with 17β-estradiol (●) or placebo (○) for 14 days. Each aortic ring was exposed to cumulatively increasing concentration of norepinephrine. Data were calculated as percentage reference contraction to 80 mM potassium. Each point represents mean data obtained using five to six aortic rings. Brackets indicate S.E.

rived vasoactive factors is possible. However, the marked depression by 17β-estradiol of angiotensin II-induced contraction in endothelium-denuded vessels in the present study demonstrates an effect of estrogen which persists following removal of the endothelium, indicative of an effect on vascular smooth muscle function.

A potential action of estrogen directly on vascular smooth muscle is supported by the recent evidence for the presence of estrogen receptors in aortic smooth muscle from baboon (Lin et al., 1986). Functionality of these receptors was suggested by redistribution of the receptors from the cytoplasmic to the nuclear fraction in the presence of estrogen. Further, down-regulation of angiotensin II-receptors in response to estrogen has been reported to occur in the anterior pituitary and adrenal cortex (Carriere et al., 1986). The functional data observed in the present study taken together with these previous findings raise the possibility that estrogen may depress angiotensin II-induced contraction through a direct action to down-regulate vascular smooth muscle angiotensin II receptors. However, the mechanism involved in the depression by estrogen of angiotensin II-induced contraction observed in the present study may also involve other direct and/or indirect mechanisms.

An indirect action of estrogen to alter angiotensin II receptors has been suggested previously. Douglas (1987) demonstrated that the effects of estrogen to alter angiotensin II receptors in adrenal glomerulosa, glomerular capillary loops and uterine myometrium did not occur in hypophysectomized animals. In addition, Drouva et al. (1990) has demonstrated that the activity of protein kinase C, which is responsible for the synthesis or release of pituitary hormones (Sihag et al., 1988; Beretta et al., 1989), is modulated by estradiol in the rat pituitary in vivo and in vitro. This suggests that pituitary-derived factors released in the presence of estrogen have the potential to modulate angiotensin II receptor function in other tissues, possibly in vascular smooth muscle.

In contrast to its effects on aortic responsiveness to angiotensin II, 17β-estradiol treatment in the present study enhanced responsiveness to norepinephrine in aortic rings from prepubertal and ovariectomized rats pretreated with 17β-estradiol. This finding is consistent with results from previous studies. Pretreatment of dogs with estradiol for 6–8 days enhanced pressor responses to i.v. administered norepinephrine (Boxill and Brown, 1955). Additional studies have also demonstrated that administration of either natural or synthetic estrogenic hormones enhances vascular responses to norepinephrine and epinephrine using intact animals (Altura, 1972, 1975) and isolated or perfused vessels (Kalsner, 1969; Graham and Sani, 1971; Nicol and Rae, 1972; Vanhoutte, 1977).

Although the mechanism for enhancement of norepinephrine-induced vascular contraction remains unresolved, previous studies suggest several possibilities. Regulatory effects of estrogen on tyrosine hydroxylase (Kohler et al., 1975) and monoamine oxidase (Holzbauer and Youdium, 1973) have been reported to occur in rat tissues. Inhibitory effects of estrogen on neuronal uptake of norepinephrine and epinephrine in perfused rabbit ear artery have also been reported (Nicol and Rae, 1972). Enhancement of tyrosine hydroxylase activity or reduction of monoamine oxidase activity or neuronal uptake could, at least theoretically, result in an increased level or a longer duration of action of norepinephrine at receptor sites on the vascular smooth muscle cells, resulting in enhanced contraction. However, rat thoracic aorta contains little adrenergic innervation (Patil et al., 1972). Thus, it seems unlikely that alterations by estrogen of tyrosine hydroxylase activity and/or neuronal uptake of norepinephrine are involved in the enhancement by estrogen of norepinephrine-induced contraction in this tissue.

In summary, the present study describes the development of a rat model which demonstrates differential modulation by estrogen of vasoactive responses to angiotensin II and norepinephrine. Additional investigation is required to determine the physiological importance of the modulation of responsiveness to vasoactive hormones by estrogen and the mechanisms involved.

Acknowledgements

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