ELSEVIER

European Journal of Pharmacology 314 (1996) 339–345

ejp

Sex differences in the effects of 17β-estradiol on vascular adrenergic responses

Angel L. García-Villalón [a], John N. Buchholz [b], Diana N. Krause [c], Sue P. Duckles [c,*]

[a] *Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma, 28029 Madrid, Spain*
[b] *Department of Pharmacology, School of Medicine, Loma Linda, CA 92350, USA*
[c] *Department of Pharmacology, College of Medicine, University of California, Irvine, CA 92717, USA*

Received 11 January 1996; revised 1 May 1996; accepted 16 July 1996

Abstract

The in vitro effects of 17β-estradiol on vascular responses to adrenergic nerve stimulation were studied in perfused tail arteries from age-matched male and female rats. Nerve stimulation resulted in vasoconstriction that was greater in male arteries. Addition of 17β-estradiol (3×10^{-5} M) reduced the vasoconstrictor responses in both male and female arteries, but the reduction was significantly greater in the females. Gonadectomy of the animals for 1 month prior to the experiment did not alter the in vitro responses to 17β-estradiol in either males or females. 17β-Estradiol (10^{-6}–3×10^{-5} M) also relaxed perfused tail arteries precontracted with KCl (50 mM); however the relaxation was not different between males and females, either intact or gonadectomized. Stimulation-evoked release of noradrenaline from adrenergic nerves of perfused tail arteries was measured, but no differences were found between males and females, nor was release modified by in vitro exposure to 17β-estradiol (10^{-5} M). These results suggest that 17β-estradiol acts directly on postjunctional mechanisms to relax tail arteries of either sex. The effect of the hormone on arteries constricted by adrenergic nerve stimulation, however, is greater in females compared to males.

Keywords: Tail artery, rat; Adrenergic nerve stimulation; Noradrenaline release; Gonadectomy

1. Introduction

There is ample evidence supporting a difference in cardiovascular function between males and females. Males suffer from circulatory disorders to a greater degree than females (Caplan et al., 1986; Lerner and Kannel, 1986), and women have a lower probability of developing coronary heart disease, primarily prior to menopause (Dicken, 1978). Differences in adrenergic function may be involved in these sex differences, in that it has been found that the maximal response to phenylephrine is greater in aorta from male rats (Stallone et al., 1991). The contractile response of rat tail artery to adrenergic nerve stimulation is also greater in males than in females (Li and Duckles, 1994). Mesenteric arterioles from male rats, however, show a lower vasoconstrictor response to noradrenaline (Altura, 1975) and a lower affinity of α-adrenoceptors (Colucci et al., 1982) as compared to female arterioles.

Ovarian hormones may play a role in these vascular differences, as the sex difference in phenylephrine response can be abolished by gonadectomy (Stallone, 1994). In vivo treatment with 17β-estradiol increases the in vitro contraction to noradrenaline in rat mesenteric vasculature (Colucci et al., 1982), rat aorta (Cheng and Gruetter, 1992) and rabbit aorta (Miller and Vanhoutte, 1990), but this treatment depresses contraction in rabbit femoral artery (Gisclard et al., 1987). Estradiol in vitro has been reported to increase the contraction of the rat mesenteric bed to exogenous noradrenaline, without modifying the response to perivascular nerve stimulation (Vargas et al., 1995). In contrast, estradiol produces relaxation of precontracted rat tail (Shan et al., 1994) or rabbit coronary (Jiang et al., 1991) arteries. Sex differences could account for some of these discrepancies in that the effects of 17β-estradiol have been studied either in males (Colucci et al., 1982; Shan et al., 1994; Vargas et al., 1995) or in females (Gisclard et al., 1987; Miller and Vanhoutte, 1990; Cheng and Gruetter, 1992), but few studies have compared the vascular effects of 17β-estradiol between males and females. This

* Corresponding author. Tel.: (1-714) 824-4265; Fax: (1-714) 824-4855.

PII S0014-2999(96)00565-1

comparison is physiologically relevant, as circulating estrogens are present in males as well as in females (Guyton and Hall, 1995).

The objective of this study was to compare the in vitro effect of 17β-estradiol on male and female rat tail arteries by measuring vasoconstrictor responses to either adrenergic nerve stimulation or non-specific depolarization by high K^+ as well as stimulation-evoked release of noradrenaline. Arteries were studied from both intact and gonadectomized male and female animals to assess possible sexual dimorphism and the influence of chronic exposure to gonadal steroid hormones on the vascular effects of estrogen.

2. Materials and methods

Fourteen female and thirteen male 4-month-old Fisher 344 rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN, USA) and maintained for 1 week in the University of California, Irvine, College of Medicine, animal facility before use. Food and water were continuously available.

Orchiectomy or ovariectomy were performed in male or female rats, respectively, under ketamine (Ketasel, Fort Dodge Laboratories, IA, USA, 100 mg/kg) and xylazine (Rompun, Miles, KS, 10 mg/kg) anaesthesia, using aseptic surgical techniques. After gonadectomy, the animals were maintained for 4 weeks in the animal facility before being killed. Control (intact) male and female rats were also maintained in the facility for a similar period of time. At the time of the experiment, both control and gonadectomized animals were 5 months old.

2.1. Contractile responses

Rats were killed by decapitation, and two 1.5 cm segments of tail artery were dissected from each animal. Tissues were cannulated at both ends and placed in a low volume perfusion/superfusion system. The tissue was perfused and superfused with Krebs' solution (mM: NaCl, 118; KCl, 4.8; $CaCl_2$; 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 2.5; $MgSO_4$, 1.2; disodium ethylenediaminetetraacetate, 0.1; dextrose, 11.5), bubbled with 95% O_2 and 5% CO_2, at a rate of 1 ml/min, and the entire assembly was kept at 37°C in a circulating water bath. The perfusion pressure was monitored and recorded by a pressure transducer, and resulting signals were digitized by a Mac Lab analog to digital converter and recorded by a Macintosh SE computer. Basal perfusion pressure was in the range 50–70 mmHg, and did not change throughout the duration of the experiment.

In this preparation, vascular contraction to adrenergic nerve stimulation (1–8 Hz, 60 V, 0.3 ms pulse duration and 100 pulses) was recorded before and after addition of 17β-estradiol (10^{-7}–3×10^{-5} M). Electrical stimulation was applied with two platinum electrodes placed at either end of the artery and connected to a Grass S48 stimulator. To one of the segments, five series of stimulation trains (1, 2, 4 and 8 Hz) were applied, the first series in the absence and the following four series in the presence of various concentrations of 17β-estradiol (10^{-7}–3×10^{-5} M). 17β-Estradiol was added cumulatively, and the tissue was exposed to each concentration for 15 min prior to stimulation. The same stimulation series were applied to the other vascular segment, but only the vehicle (ethanol 1:10000) was added, in order to test for reproducibility of the contractile response and possible effects of the vehicle. The duration of the experiment (about 3 h) and the number of electrical stimulation series applied were similar in the control and experimental segments. Contractile responses to adrenergic nerve stimulation were well maintained throughout the course of the experiment, and the ethanol vehicle had no significant effect.

The relaxation to 17β-estradiol was also studied in arteries precontracted with KCl (50 mM). After the contraction to K^+ had reached a stable plateau, 17β-estradiol (10^{-6}–3×10^{-5} M) was added cumulatively.

2.2. Noradrenaline release

To study the effects of 17β-estradiol on noradrenaline release, 3 cm segments of tail arteries from male and female, control or gonadectomized rats, were dissected, perfused and electrically stimulated as described above. For the noradrenaline release experiments, electrical stimulation was at 2 Hz, 60 V and 1 ms pulse duration during 3 min (a total of 360 pulses). The entire experimental protocol was performed in both vascular segments in the presence of cocaine (10^{-5} M) and deoxycorticosterone (10^{-5} M) to block neuronal and extraneuronal noradrenaline uptake, and, in one of the segments, the α_2-adrenoceptor antagonist idazoxan (10^{-6} M) was also added to eliminate the influence of prejunctional α_2-adrenoceptors. Perfusate samples were collected for 5 min immediately prior to each stimulation and also were collected at the initiation of each stimulation period and continuing for 5 min, giving total volumes of 5 ml for each perfusate sample. Both segments were stimulated first in control conditions, then 17β-estradiol (10^{-5} M) was added to the bath, and after a 25-min equilibration period a second stimulation train was applied.

Collected perfusate fractions were placed on ice and acidified with 200 μl of 1 M acetic acid. After adjustment of pH to 8.6 with 2.5 ml of Tris-hydroxymethyl aminomethane buffer, acid-washed alumina (50 mg) and 300 pg of dihydroxybenzylamine internal standard were added. Noradrenaline and dihydroxybenzylamine bound to the alumina were eluted with 300 μl of 0.1 M perchloric acid. One hundred microliter samples of eluted amines were injected into a high pressure liquid chromatograph with electrochemical detection (ESA; Bedford, MA, USA), and

a 100 pg noradrenaline standard was measured in each experiment. Noradrenaline was quantitated with the following formula:

pg of noradrenaline = (noradrenaline peak height of sample/noradrenaline peak height of standard) × (dihydroxybenzylamine peak height of standard/dihydroxybenzylamine peak height of sample) × 100 pg of noradrenaline standard.

This value was multiplied by 3, to give the total noradrenaline content in the 300 μl sample. At the end of the stimulation experiment, the arteries were homogenized in 3 ml of 0.1 M perchloric acid. 0.3 ml of the tissue homogenate were processed for measurement of noradrenaline as described above. The results were multiplied by 10 to obtain the total noradrenaline content of the artery.

The basal release of noradrenaline is expressed as pg of noradrenaline/pg of noradrenaline tissue content, and the release during stimulation is expressed as (pg of noradrenaline)/(pg of noradrenaline tissue content × number of stimulation pulses). The noradrenaline content in the tissue is expressed as pg of noradrenaline/mg of tissue wet weight.

2.3. Statistical analysis

Data for the different experimental groups were analyzed by analysis of variance (ANOVA), followed by individual comparisons between each experimental group and its control by Dunnett's *t*-test.

2.4. Drugs used

The following drugs were used: 17β-estradiol (Sigma), deoxycorticosterone (Sigma), cocaine hydrochloride (Sigma), 3,4-dihydroxybenzylamine hydrochloride (Sigma), idazoxan hydrochloride (Research Biochemicals).

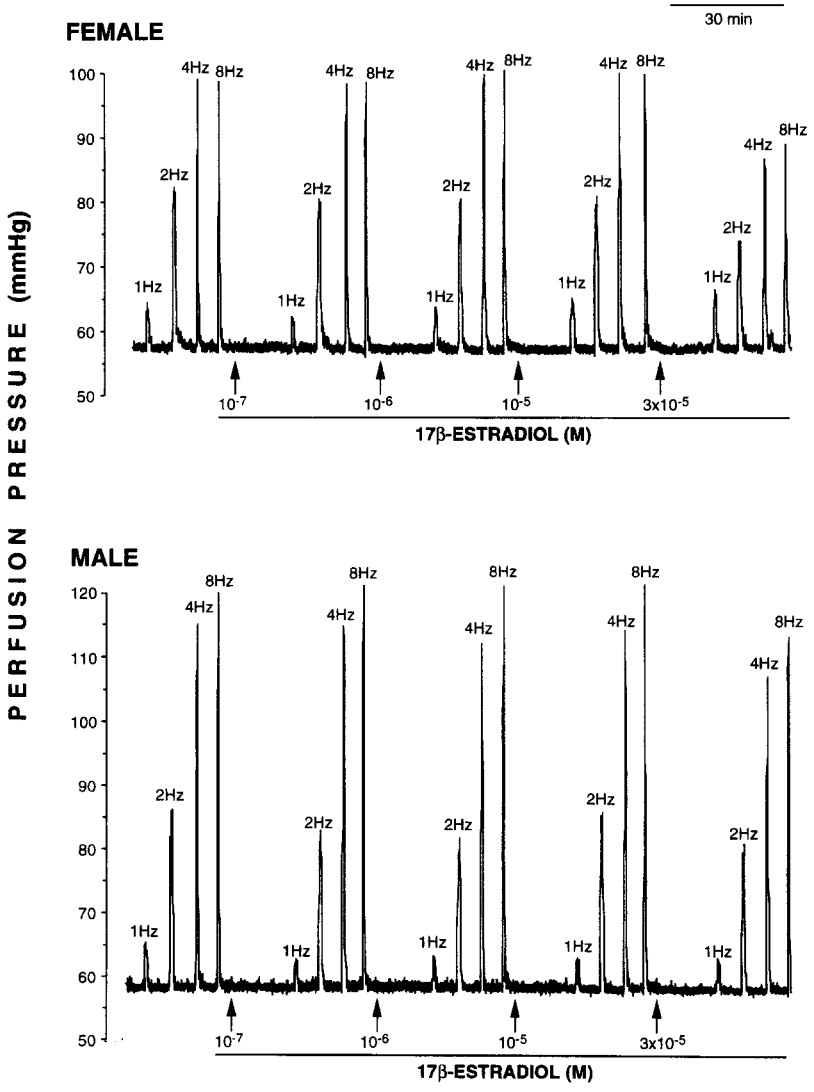

Fig. 1. Experimental recordings of the effects of 17β-estradiol (10^{-7}–3×10^{-5} M) on the contraction to transmural adrenergic nerve stimulation (1–8 Hz, 0.3 ms, 60 V, 100 pulses) in a perfused tail artery from a female and a male rat.

3. Results

3.1. Contractile responses

As shown in Fig. 1 and Fig. 2, adrenergic nerve stimulation produced frequency-dependent contraction of the perfused tail artery, which was higher in male than in female rats ($P < 0.005$, by ANOVA). Contractile responses to adrenergic nerve stimulation were not significantly different between orchiectomized and control males or between ovariectomized and control females.

Addition of 17β-estradiol (10^{-7}–10^{-5} M) to the perfusate did not modify significantly the vascular contraction to nerve stimulation, except at the highest concentration of 17β-estradiol tested, 3×10^{-5} M, which reduced significantly the contraction of arteries from control or ovariectomized females and from control or orchiectomized male rats. The magnitude of reduction was significantly greater ($P < 0.05$, by ANOVA) in arteries from control or ovariectomized female rats than in arteries from control or orchiectomized male rats. Thus 17β-estradiol reduced contractile responses at 4 Hz by $46 \pm 8\%$ in female tail arteries compared to $23 \pm 7\%$ in male arteries. No differences were observed in the effects of 17β-estradiol between arteries from control female rats compared to ovariectomized rats, nor in control male rats compared to orchiectomized rats. The ethanol vehicle used for 17β-estradiol did not produce any effect by itself, and the contraction to each frequency was reproducible over the course of the experiment.

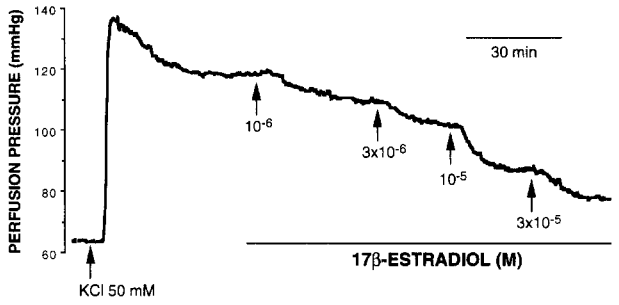

Fig. 3. Experimental recording of the relaxing effect of 17β-estradiol (10^{-6}–3×10^{-5} M) on a perfused rat tail artery precontracted with KCl (50 mM).

K^+ (50 mM) produced contraction of tail artery segments which was not significantly different in any of the experimental groups (42 ± 7 and 43 ± 3 mmHg in control and ovariectomized females, and 51 ± 4 and 43 ± 4 mmHg in control and orchiectomized males, respectively). In these precontracted segments, 17β-estradiol produced concentration-dependent relaxation (Fig. 3), and in this case no differences were observed in the effects of 17β-estradiol between arteries of male and female rats, or when comparing orchiectomized or ovariectomized groups to control male or female arteries, respectively (Fig. 4).

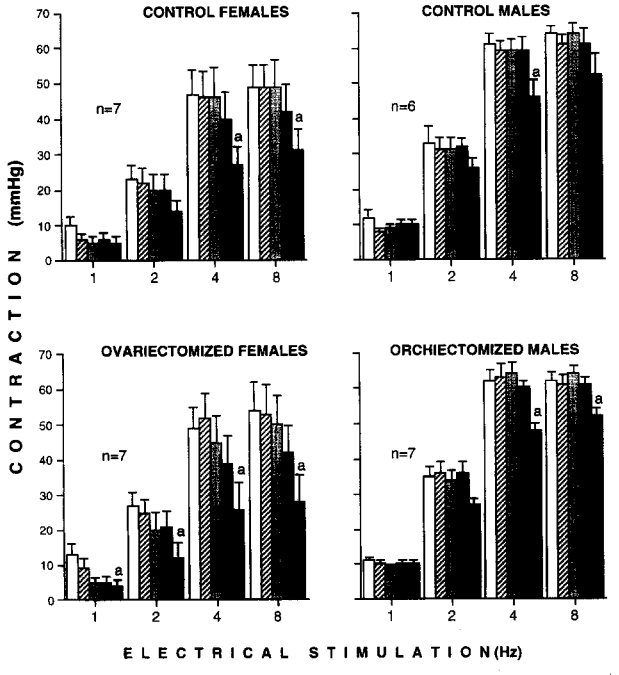

Fig. 2. Contractile responses of perfused rat tail arteries to transmural adrenergic nerve stimulation (1–8 Hz, 0.3 ms, 60 V, 100 pulses) in the absence (empty bars) or in the presence of 17β-estradiol (10^{-7} M, dashed bars; 10^{-6} M, light grey; 10^{-5} M, dark grey; 3×10^{-5} M, black). Values are means ± S.E.M. n = number of animals. [a] Statistically significant ($P > 0.05$) difference from control by Dunnett's t-test.

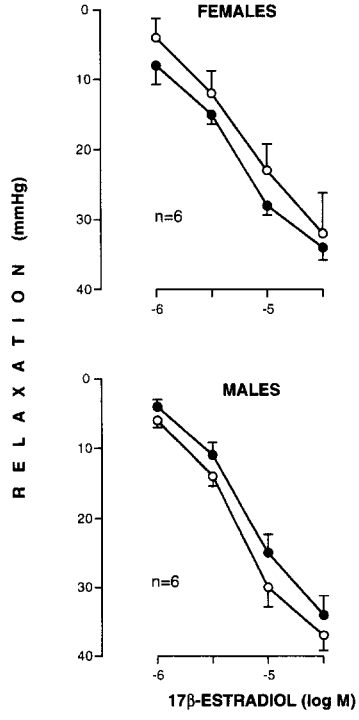

Fig. 4. Summary of the relaxation to 17β-estradiol (10^{-6}–3×10^{-5} M) in perfused rat tail arteries precontracted with KCl (50 mM) in control (○) or gonadectomized (●) animals. Values are means ± S.E.M. n = number of animals.

Table 1
Rat tail artery noradrenaline content, basal noradrenaline efflux and noradenaline release during transmural electrical stimulation

	Noradrenaline content (ng/mg wet weight)	Basal noradrenaline efflux (pg/pg content × 10^{-4})	Stimulation-evoked fractional noradrenaline release (pg/pg content/pulse × 10^{-6})			
			Control	Estradiol 10^{-5} M	Idazoxan 10^{-6} M	Idazoxan 10^{-6} M + estradiol 10^{-5} M
Control female	38 ± 3	3.0 ± 1.2	19 ± 3	18 ± 2	68 ± 10 [b]	76 ± 11
Ovariectomized female	37 ± 4	8.3 ± 3.4	22 ± 3	25 ± 3	58 ± 13 [b]	56 ± 9
Control male	26 ± 3 [a]	3.2 ± 0.8	18 ± 2	20 ± 3	78 ± 12 [b]	74 ± 11
Orchiectomized male	23 ± 2 [a]	4.8 ± 1.8	23 ± 3	25 ± 3	77 ± 3 [b]	72 ± 2

Values are means ± S.E.M. from 5 experiments.
[a] Statistically significant difference compared to control females ($P > 0.01$).
[b] Statistically significant difference compared to control ($P < 0.01$).

3.2. Noradrenaline content

Noradrenaline content (Table 1), expressed in relation to tissue wet weight of the arterial segments, was significantly higher in arteries from female rats, compared to males. However, noradrenaline content in arteries from orchiectomized males or ovariectomized females was not different from that in control male or female rats, respectively.

3.3. Noradrenaline release

In the rat tail arteries incubated with cocaine and deoxycorticosterone, a small release of noradrenaline was observed under basal conditions, which was not different in any of the experimental groups (Table 1). Electrical stimulation increased the release of noradrenaline, but the amount of release was not significantly different between male or female, control or gonadectomized rats. Idazoxan significantly increased stimulation-evoked release in all groups ($P < 0.01$). Addition of 17β-estradiol did not modify the basal release or the stimulation-evoked release of noradrenaline, in any of the experimental groups, either in the absence or presence of idazoxan (Table 1).

4. Discussion

The present results suggest that ovarian steroid hormones may have a modulatory role on vascular responses to adrenergic stimulation, a role which may be more important in females. Previous investigation has supported the concept that sex differences exist in adrenergic responses. For example, contraction of rat tail artery to adrenergic nerve stimulation was found to be greater in males than in females (Li and Duckles, 1994). However, the possible role of ovarian hormones in these differences has not yet been determined. Little information is available about the effect of estrogen on responsiveness to sympathetic nerve stimulation.

In the present study we have found that 17β-estradiol reduced the contraction of rat tail artery to adrenergic nerve stimulation. To test whether 17β-estradiol had any prejunctional effect on vascular nerve terminals, we measured the amount of noradrenaline released by the arteries during nerve stimulation in a perfusion-superfusion system. In these conditions, nerve stimulation induced the release of noradrenaline, and this release was greater when prejunctional α_2-adrenoceptors were blocked. There were no significant differences in fractional noradrenaline release between male and female arteries. However 17β-estradiol failed to modify the release of noradrenaline, either in control conditions, or in the presence of α_2-adrenergic blockade when tested on arteries from intact or gonadectomized male or female rats. This suggests that the reduction by 17β-estradiol of contraction to adrenergic nerve stimulation is probably not due to a prejunctional effect. Rather it is most likely that 17β-estradiol reduced the vasoconstrictor effect of noradrenaline released from nerve terminals by a postjunctional action. Indeed, we have also shown that 17β-estradiol applied in vitro reduces contractile responses of the rat tail artery to exogenous noradrenaline (McNeill et al., 1996).

Estradiol has been shown previously to have direct vasodilator effects on vascular smooth muscle. For example, estradiol induces relaxation of rabbit coronary artery precontracted with prostaglandin $F_{2\alpha}$ or high K^+ (Jiang et al., 1991) and it has been shown to relax rat tail arteries precontracted with KCl, arginine vasopressin or noradrenaline (Shan et al., 1994). The relaxing effect of 17β-estradiol was not dependent on the endothelium (Jiang et al., 1991) and has been suggested to be due to Ca^{2+} antagonistic properties of 17β-estradiol (Jiang et al., 1991; Shan et al., 1994). More recently estrogen has been shown to open BK_{Ca} channels in isolated pig coronary arteries (White et al., 1995). Thus our results essentially confirm previous findings in the literature that 17β-estradiol relaxes vascular contractile responses produced by a wide variety of vasoconstrictors.

However, the primary objective of our study was to compare the effects of 17β-estradiol in arteries from male and female rats and to determine whether these effects are

altered by gonadectomy. We found that 17β-estradiol reduced the contraction to adrenergic nerve stimulation in both male and female tail arteries, but the reduction by estradiol was greater in female arteries. In contrast, the relaxation to 17β-estradiol in arteries precontracted with K^+ was not different between males and females. It has previously been reported that relaxations to 17β-estradiol in rabbit coronary arteries precontracted with K +, prostaglandin $F_{2\alpha}$ or the Ca^{2+} channel agonist methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyrydine-5-carboxylate (Bay K 8644), were not different when males and females were compared (Jiang et al., 1991). Our findings, together with these previous observations, suggest that the mechanism by which estradiol causes vasodilation may depend, to some degree, on the vasoconstrictor agent.

In contrast, the sex differences in relaxation induced by 17β-estradiol when arteries are contracted by adrenergic nerve stimulation suggest that this effect of estradiol may involve a distinct mechanism of action. 17β-Estradiol does not appear to modulate the function of the adrenergic nerves themselves, as we have shown that 17β-estradiol does not influence basal or stimulation-evoked noradrenaline overflow. Thus we hypothesize that the effect of estradiol to inhibit contractile responses to adrenergic nerve stimulation depends on a postjunctional action of the steroid. Indeed, we have recently demonstrated that estradiol relaxes rat tail arteries preconstricted with noradrenaline, and the effects of estradiol in this case are greater in arteries from female as compared to male rats (McNeill et al., 1996). Furthermore, these effects of 17β-estradiol are not observed when the endothelium is removed, underscoring a distinct mechanism of action of 17β-estradiol in arteries contracted by adrenergic nerve stimulation or noradrenaline as compared to arteries precontracted by other vasoconstrictors. Indeed, relaxation to 17β-estradiol of rabbit coronary arteries precontracted with high K^+ or prostaglandin $F_{2\alpha}$ has been shown to be endothelium-independent (Jiang et al., 1991).

Our findings suggest a greater effectiveness of 17β-estradiol to inhibit contractile responses to noradrenaline in female arteries as compared to male. These vascular effects of 17β-estradiol, in contrast to the non-specific inhibition of Ca^{2+} channels, may be mediated by an action on specific estrogen receptors (Vargas et al., 1995). However, the relatively rapid time-course suggests this effect is not mediated by classical steroid receptors which enter the nucleus and influence gene transcription. Rather, it is more likely that these effects of estrogen depend on non-genomic mechanisms mediated by plasma membrane binding sites for estrogen. Our results could be explained if the arteries from females have a greater concentration of these specific estrogen receptors. In fact, a greater concentration of estrogen receptors has been found in the hypothalamus of female as compared to male rats (Brown et al., 1988). However, to our knowledge, possible gender differences in vascular estrogen receptors have not been studied, although specific estrogen receptors have been detected in arteries (Stumpf, 1990).

We have also observed that the inhibitory effect of 17β-estradiol on contractile responses to adrenergic nerve stimulation was not influenced by gonadectomy. The greater effect of 17β-estradiol on adrenergic responses in female, compared to male, arteries persisted 4 weeks after either orchiectomy or ovariectomy. These findings are in contrast to previous studies demonstrating that some gender-related differences in the vasculature are influenced by gonadectomy. For example, gender-related differences in affinity of α-adrenoceptors in rat mesenteric artery disappear after gonadectomy (Colucci et al., 1982), and differences in contractile responses of rat aorta to phenylephrine or vasopressin are also modified by gonadectomy (Stallone, 1994). The lack of influence of gonadectomy on gender differences in response to 17β-estradiol in our studies suggests that the greater sensitivity may be constitutive in female vessels and may be determined during development.

The present results may have significant relevance to understanding differences in physiological control of the cardiovascular system between males and females. Previous studies indicate that arteries from females are less sensitive to adrenergic stimulation (Stallone et al., 1991; Li and Duckles, 1994). Although concentrations of 17β-estradiol used in our study are higher than circulating plasma levels, the greater sensitivity of female arteries suggests a fundamental gender-related difference in vascular function, perhaps involving differences between males and females in the complement of vascular estrogen receptors. These findings underscore that cardiovascular effects of endogenous or exogenous estrogen may be greater in females and might contribute to the reduced adrenergic response and lower incidence of cardiovascular disease in females.

Acknowledgements

This work was supported, in part, by NIH Grant # HL50775 and by DGICYT (PR95-065).

References

Altura, B.M., 1975, Sex and estrogens and responsiveness of terminal arterioles to neurohypophyseal hormones and catecholamines, J. Pharmacol. Exp. Ther. 193, 403.

Brown, T.J., R.B. Hochberg, J.E. Zielinski and N.J. MacLusky, 1988, Regional sex differences in cell nuclear estrogen-binding capacity in the rat hypothalamus and preoptic area, Endocrinology 123, 1761.

Caplan, L.R., P.B. Gorelick and D.B. Hier, 1986, Race, sex, and occlusive cerebrovascular disease, Stroke 17, 648.

Cheng, D.Y. and C.A. Gruetter, 1992, Chronic estrogen alters contractile responsiveness to angiotensin II and norepinephrine in female rat aorta, Eur. J. Pharmacol. 215, 171.

Colucci, W.S., M.A. Gimbrone, Jr., M.K. McLaughlin, W. Halpern and R.W. Alexander, 1982, Increased vascular catecholamine sensitivity and alpha-adrenergic receptor affinity in female and estrogen treated male rats, Circ. Res. 50, 805.

Dicken, C., 1978, Sex roles, smoking and smoking cessation, J. Health Soc. Behav. 19, 324.

Gisclard, V., N.A. Flavahan and P.M. Vanhoutte, 1987, Alpha adrenergic responses of blood vessels of rabbits after ovariectomy and administration of 17β-estradiol, J. Pharmacol. Exp. Ther. 240, 466.

Guyton, A.C. and J.E. Hall, 1995, Reproductive and hormonal functions of the male (and the pineal gland), in: Textbook of Medical Physiology (W.B. Saunders, Philadelphia) p. 1003.

Jiang, C., P.M. Sarrel, D.C. Lindsay, P.A. Poole-Wilson and P. Collins, 1991, Endothelium-independent relaxation of rabbit coronary artery by 17β-oestradiol in vitro, Br. J. Pharmacol. 104, 1033.

Lerner, D.J. and W.B. Kannel, 1986, Patterns of coronary heart disease morbidity and mortality in the sexes: A 26-year follow up of the Framingham population, Am. Heart J. 111, 383.

Li, L. and S.P. Duckles, 1994, Influence of gender on vascular reactivity in the rat, J. Pharmacol. Exp. Ther. 268, 1426.

McNeill, A.M., S.P. Duckles and D.N. Krause, 1996, Relaxant effects of 17β-estradiol in the rat tail artery are greater in females than in males, Eur. J. Pharmacol. 308, 305.

Miller, V.M. and P.M. Vanhoutte, 1990, 17β-estradiol augments endothelium-dependent contractions to arachidonic acid in rabbit aorta, Am. J. Physiol. 258, R1502.

Shan, J., L.M. Resnick, Q.Y. Liu, M. Barbagallo and P.K. Pang, 1994, Vascular effects of 17β-estradiol in male Sprague-Dawley rats, Am. J. Physiol. 266, H967.

Stallone, J.N., 1994, Sex differences in nitric oxide-mediated attenuation of vascular reactivity to vasopressin are abolished by gonadectomy, Eur. J. Pharmacol. 259, 273.

Stallone, J.N., J.T. Crofton and L. Share, 1991, Sexual dimorphism in vasopressin-induced contraction of rat aorta, Am. J. Physiol. 260, H453.

Stumpf, W.E., 1990, Steroid hormones and the cardiovascular system: direct actions of estradiol, progesterone, testosterone, gluco- and mineralcorticoids, and soltriol (vitamin D) on central nervous regulatory and peripheral systems, Experientia 46, 13.

Vargas, R., M. Delaney, M.Y. Farhat, R. Wolfe, A. Rego and P.W. Ramwell, 1995, Effect of estradiol-17β on pressor responses of rat mesenteric bed to norepinephrine, K^+ and U-46619, J. Cardiovasc. Pharmacol. 25, 200.

White, R.E., D.J. Darkow, and J.L. Falvo Lang, 1995, Estrogen relaxes coronary arteries by opening BK_{Ca} channels through a cGMP-dependent mechanism, Circ. Res. 77, 936.