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Influence of estradiol and progesterone on the sensitivity of rat thoracic aorta to noradrenaline

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Abstract

The aim of this study was to investigate the effects of low and high doses of estradiol, and of progesterone on the response to noradrenaline in rat thoracic aorta. Two weeks after bilateral ovariectomy, female rats received a s.c. injection of vehicle (corn oil, 0.1 mL/day), estradiol (10 μg/kg/day or 4 mg/kg/day) and/or progesterone (20 mg/kg/day), for eight days. On the ninth day, the rats were sacrificed and aortic rings, with or without endothelium, were used to generate concentration-response curves to noradrenaline. Aortic rings with intact endothelium from the high-dose (4 mg/kg/day) estradiol group were supersensitive to noradrenaline compared to the vehicle or low-dose (10 μg/kg/day) estradiol groups (pD_2 values = 7.86±0.09, 7.30±0.11 and 7.35 ± 0.04, respectively). Endothelium-intact aortic rings from high-estradiol rats were supersensitive to noradrenaline when compared to vehicle-, progesterone- and progesterone + high-estradiol-treated rats (pD2 values = 7.77±0.12, 7.21±0.13, 6.93±0.04 and 7.22±0.18, respectively). There were no significant differences among the pD2 values for noradrenaline in aortic rings without endothelium. In conclusion, at high but not low doses, estradiol increased the sensitivity to noradrenaline and this was prevented by progesterone. Both of these effects were endothelium-dependent.

Keywords: Estradiol; Progesterone; Ratl Aorta; Noradrenaline; Sensitivity

Introduction

Women suffer less from cardiovascular disorders than men during their reproductive life do [1,2] and after menopause the incidence of cardiovascular disease is similar in both sexes [3,4]. Estrogens are believed to exert a cardioprotective role in this phenomenon [5]. Al-

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though the mechanisms of estrogen action are not well understood, this hormone may induce a favorable lipid profile [6], affect advanced atherosclerosis [7], reduce blood pressure [8], and modulate coronary artery reactivity [9]. Estrogen also has a direct action on the arterial wall [10].

Female sex steroids have been reported to the increased risk of cardiovascular disease in users of oral contraceptives [1] by changing calcium homeostasis and the synthesis and/or responses to vasoactive substance [11].

The blood vessels and heart are target organs for sex hormones [11,12]. Ovarian steroids can modulate vascular function by acting on specific receptors in different vascular beds [13,14] as well as by receptor-independent mechanisms [15,16]. Ovarian hormones may change the sensitivity of blood vessels to α-adrenoceptor agonists [17], although this effect varies with dosage, type of hormone, species and region of the vascular system. Mesenteric arteries isolated from estrogen-treated rats are supersensitive to adrenergic stimulation [18] and treating ovariectomized or pre-puberty rats with estrogen increases the response to noradrenaline [19]. In contrast, estrogen decreases the contractile response to noradrenaline in femoral arteries from female rabbits [20].

These contradictory actions of estrogens may be related to differences in vascular beds and in the hormone doses used. Moreover, progesterone and the estrogens/progesterone ratio may influence the vascular response to vasoactive substances. Dogterom & De Jong [21] observed that high doses of progesterone decreased the contractile response to noradrenaline in tail arteries of rats. In the rat thoracic aorta, noradrenaline-induced contraction was also inhibited after treatment with progesterone or pregnanolone [22].

In non-vascular tissues, the actions of estrogen and progesterone may be synergistic or antagonistic [23]. In human breast and endometrial cancers [23], progesterone inhibited estrogen-induced tissue growth whereas in rat endometrium, treatment with progesterone amplified the actions of estrogen [23]. In chicken oviduct, progesterone synergized with estradiol to increase the number of estrogen receptors [24]. In vascular tissue, the administration of progesterone and estradiol relaxed rabbit coronary arteries pre-contracted with Bay K8644, a voltage-dependent Ca^{+2}-channel agonist [15, 16]. In contrast, in coronary arteries from ovariectomized female dogs, progesterone antagonized the stimulatory effects of estrogen on endothelium-dependent responses to acetylcholine [25].

In this study, we examined the effects of low and high estradiol doses on noradrenaline-induced contractions in rat thoracic aorta and evaluated the interaction between estradiol and progesterone in this tissue.

Methods

Female Wistar rats (*Rattus norvergicus*), 4–5 months old were used. The animals were housed in standard cages in a temperature controlled room (22°C), on a 12 h light/12 h dark cycle with lights on at 6:00 a.m. Standard laboratory chow and tap water were available *ad libitum*. The rats were bilaterally ovariectomized under i.m. Xylazine (25mg/kg) - Ketamine (50mg/kg) anesthesia. The effects of different doses of estradiol on the reactivity of rat thoracic aorta to noradrenaline were assessed two weeks after surgery. For this, rats were assigned to one of three groups as follows: vehicle (corn oil, 0.1 mL/day, s.c.), low 17β-estradiol (10 μg/kg/day, s.c.) and high 17β-estradiol (4 mg/kg/day, s.c.). The rats received one injection/day

for eight days. The interaction between estradiol and progesterone in rat thoracic aorta was examined by using four groups of rats: vehicle (corn oil, 0.1 mL/day, s.c.), high 17β-estradiol (HE, 4 mg/kg/day, s.c.), progesterone (P, 20 mg/kg/day, s.c.) and HE+P (4 and 20 mg/kg/day, s.c.). The Institute of Biology/UNICAMP Ethical Committee approved all procedures for Animal Research in accordance with the guidelines of the Brazilian Council for Animal Experimentation (Protocol n° 011-2).

One day after the last injection of hormone or vehicle the rats were sacrificed by a blow to the back of the head. The thoracic aorta was removed and dissected free of fatty and connective tissue before being cut into rings 3–5 mm long. Two rings were obtained from the middle portion of each aorta: the first of these was manipulated carefully to avoid damaging the endothelium, and the intimal surface of the second was scraped gently with a scalpel to remove the endothelial layer. The rings were suspended in organ baths containing 20 mL of Krebs-Henseleit solution of the following composition (mmol/L): NaCl 115.0, KCl 4.6, $CaCl_2$ $2H_2O$ 2.5, KH_2PO_4 1.2, $MgSO_4$. $7H_2O$ 2.5, $NaHCO_3$ 25.0, glucose 11.0 and ascorbic acid 0.1, in presence of propranolol (0.1 μmol/L, to block beta-adrenoceptors) at 37°C, and continuously aerated with a mixture of 95% O_2-5%CO_2. After 60 min for stabilization, the intactness of the endothelium was assessed by determining the vasodilating response to 10 μmol/L acetylcholine (ACh) in rings contracted with noradrenaline (0.1 μmol/L). Only aortic rings in which >70–80% relaxation by ACh was induced were used as preparations with intact endothelium. The absence of relaxation, or relaxation below 70–80%, by exposure to ACh was interpreted as indicating the absence of a significant amount of functional endothelium (rings without endothelium). Aortic rings were rinsed three times with Krebs buffer and allowed to re-equilibrate for 45–60 min. Concentration-response curves to noradrenaline were obtained by cumulative addition of molar concentration of the drug, increasing by one-half log intervals. The maximum contractile response and potency of noradrenaline (pD_2 values - expressed as the negative of the logarithm of the agonist concentration, producing 50% (EC_{50}) of its maximum effect) were determined. Each ring was subsequently processed for histological analysis.

17 β-estradiol, progesterone, acetylcholine (ACh), noradrenaline and propranolol hydrochloride were purchased from Sigma. All stock solutions and their dilutions were prepared in deionized water, except for steroid hormones, which were diluted in corn oil.

Curves were fitted to the data by non-linear regression, using the software Prism (GraphPAD software San Diego, CA, USA) according to the equation $E = E_{max} / ((1+(10^c/10^x)^n) + \Phi)$, where E was the increase in the contractile response induced by the agonist; Emax was the maximum effect of the agonist; c was the logarithm of the agonist concentration, producing 50% of the E_{max}; n was the angular coefficient and Φ was the effect in the absence of the agonist. Maximum responses to noradrenaline and pD_2 values (negative logarithms of EC_{50}) were expressed as the mean ± SEM. Comparisons of the means were done using one-way analysis of variance and the Tukey test for multiple comparisons. P values < 0.05 were considered to indicate significance.

Results

Histological examination confirmed the intactness of the endothelium in aortic rings relaxed by ACh and the lack of endothelium in rings unresponsive to ACh (results not shown).

Table 1
Concentration-response parameters for the contractile effect of noradrenaline on aortic rings from rats treated with vehicle, low and high estradiol doses, progesterone and estradiol + progesterone

Treatment	N	pD_2	Rmax (mg/100 mg tissue)	Slope parameter
EXPERIMENT 1				
Vehicle	7	7.30 ± 0.11	28.00 ± 6.46	0.79 ± 0.06
High estradiol	6	7.86 ± 0.09*	27.40 ± 5.14	0.80 ± 0.10
Low estradiol	5	7.35 ± 0.04	30.37 ± 6.04	0.83 ± 0.04
EXPERIMENT 2				
Vehicle	8	7.21 ± 0.13	26.15 ± 5.60	0.77 ± 0.06
High estradiol	7	7.77 ± 0.12*	25.11 ± 4.90	0.78 ± 0.09
Progesterone	5	6.93 ± 0.04	31.50 ± 5.40	0.81 ± 0.04
High estradiol + progesterone	5	7.22 ± 0.18	29.81 ± 3.10	0.84 ± 0.06

N = number of rats/group. The values in parentheses indicate mean ± SEM of the pD2, maximum response to noradrenaline and slope parameters. * $P<0.05$ compared to the other groups in the same experiment.

The concentration-response parameters for noradrenaline on isolated aortic rings obtained from rats treated with vehicle, low and high estradiol doses, progesterone and progesterone + estradiol are presented in Table 1.

In aortic rings with intact endothelium, the potency of noradrenaline was significantly greater in the high estradiol (HE) than in vehicle (V) or low estradiol (LE) treated groups (Table 1 and Figure 1; $P<0.05$). There were no significant differences in the maximum responses to noradrenaline among the groups (Table 1; $P>0.05$). In aortic rings without endothelium, there were no differences in the pD2 values of three groups (V: 8.09 ± 0.07, LE: 8.25 ± 0.21 and HE: 7.93 ± 0.12; $P>0.05$) or in the maximum responses to noradrenaline (V: 27.54 ± 5.13, LE: 39.44 ± 3.39 and HE: 24.82 ± 4.70 mg/100 mg tissue; $P>0.05$).

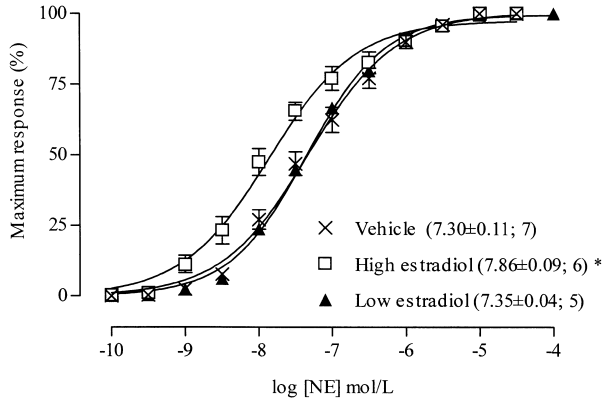

Fig. 1. Concentration-response curves to noradrenaline (NE) in endothelium-intact thoracic aortic rings from vehicle (corn oil, 0.1 mL/day), high estradiol (4 mg/kg/day) and low estradiol (10 μg/kg/day) treated ovariectomized rats. The values in parentheses indicate mean ± SEM of the pD_2 and the number of rats. * $P<0.05$ compared to the other groups.

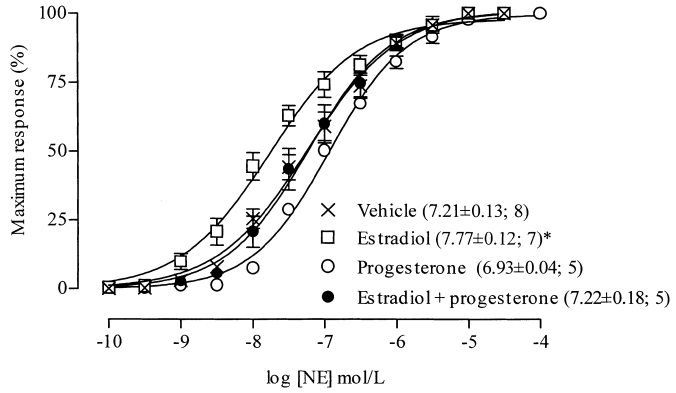

Fig. 2. Concentration-response curves to noradrenaline (NE) in endothelium-intact thoracic aortic rings from vehicle (corn oil, 0.1 mL/day), estradiol (4 mg/kg/day), progesterone (20 mg/kg/day) and estradiol+progesterone treated ovariectomized rats. The values in parentheses indicate mean ± SEM of the pD_2 and the number of rats. * $P<0.05$ compared to the other groups.

Since only the high estradiol dose changed the sensitivity to noradrenaline, we used this dose to compare the effects of estradiol and progesterone in the rat thoracic aorta in the experiment 2. In estradiol-treated rats, the concentration-response curves to noradrenaline were shifted to the left 3.6, 6.9 and 3.6 times compared to vehicle (V)-, progesterone (P)- and hormones HE+P -treated rats, respectively ($P<0.05$; Table 1 and Figure 2). In aortic rings with endothelium, there was no difference in the potency of noradrenaline among the vehicle, progesterone and HE+P -treated groups ($P>0.05$; Table 1 and Figure 2) or in the maximum responses to noradrenaline ($P>0.05$; Table 1).

There were no changes in the sensitivity to noradrenaline in aortic rings without endothelium (pD2 values: V = 8.00 ± 0.10, HE = 7.75 ± 0.12, P = 7.71 ± 0.20 and P+E = 7.85 ± 0.09; $P>0.05$). Similarly, the maximum response to noradrenaline did not change with hormonal treatment (V = 25.24 ± 5.00, HE = 21.12 ± 3.60, P = 26.29 ± 3.50 and P+E = 31.80 ± 4.60 mg/100 mg tissue; $P>0.05$).

Discussion

The overall effect of noradrenaline in vascular smooth muscle represents the sum of its constrictor activity on and its relaxing action mediated by the release of endothelium-derived vasodilators [26].

A high dose of estradiol increased the sensitivity of thoracic aorta from ovariectomized rats to noradrenaline, but no changes were observed at low dose. Although progesterone did not alter the sensitivity to noradrenaline, it abolished the estradiol-induced supersensitivity to this catecholamine when both sex hormones were administered simultaneously. The estradiol-induced supersensitivity to noradrenaline and the progesterone-mediated inhibition of this estradiol effect were both endothelium-dependent responses.

Our results agree with those of other studies. Thus, estradiol increased the sensitivity to noradrenaline in thoracic aorta from ovariectomized female rats treated with estradiol for

three days [27], in mesenteric arteries from rats [18] and in chicken oviduct arteries [28]. The authors suggested that this effect of estradiol seem to be related to an increase in the affinity of the vascular α-adrenergic receptors for noradrenaline [18] and/or an increase in α_1-spare population in the tissue and increase in the amount of calcium available for contraction [27].

17β-estradiol is a potent inhibitor of the Uptake-2 [30]. In our results, the slope of the concentration-response curves were less than the unity, which suggests an interference with uptake systems on the estimation of the potency of noradrenaline. Therefore, an inhibition of the Uptake-2 by the high dose of estradiol could also be involved in the supersensitivity to noradrenaline observed in aorta from female rats treated with high dose of this hormone.

In some cases, however estradiol has been reported to inhibit the vascular reactivity to noradrenaline in rat mesenteric arteries *in vivo* [29] and in femoral arteries isolated from female rabbits [20]. In saphenous veins from ovariectomized rabbits treated with 17β-estradiol, there were no changes in the response to noradrenaline or phenylephrine [20]. Thus, the effect of estradiol on blood vessels depends on the vascular bed examined, on variations in the type and density of receptors for vasoactive substances and on changes in the synthesis of vasoconstrictor and vasodilator agents [20].

In addition to the type of tissue, the dose of estrogen is an important factor in its action on blood vessels. In the present study, estradiol at high dose (4 mg/kg) increased the sensitivity of aortic rings from ovariectomized rats to noradrenaline, but this hormone produced no such change at a low dose (10 μg/kg). Bolego et al. [31] showed that a low dose of estradiol (5 μg/kg) increased the basal release of nitric oxide (NO) from the aorta of ovariectomized rats. However at a high dose (100 μg/kg), estradiol inhibited basal NO release and increased the formation of vasoconstrictor agents. Thus, the effects seen with different protocols of hormone administration may produce divergent actions.

This divergence is also seen in epidemiological data for human cardiovascular diseases. Estrogens may protect women from coronary heart disease in midlife but after menopause, a subsequent decrease in its production may contribute to coronary heart disease [3]. Users of oral contraceptives have a higher susceptibility to diseases that involve changes in vascular function [1], including migraine headaches, preeclampsia and primary pulmonary hypertension [11]. The contradictory effects of ovarian hormones that may protect against cardiovascular diseases but can also increases the susceptibility to vascular diseases may be related to differences in the circulating levels of estrogens and to fluctuation in the ratio of different female sex hormones. In various vascular tissues, the combination of estradiol and progesterone can have very different effects on the response to vasoactive substances [23].

Although progesterone did not change the response to noradrenaline in thoracic aorta from ovariectomized rats in this study, noradrenaline-induced contractions of rat tail or thoracic arteries are inhibited after progesterone treatment [21,22].

In thoracic aorta from ovariectomized rats treated with estradiol, there was a decrease in KCl-induced contraction and an increase in the response to acetylcholine [32]. Treatment with progesterone alone or in conjunction with estradiol did not change the response to KCl or the relaxation induced by ACh [32]. Again, the effects of these hormones were not complementary.

As shown above, the combined administration of progesterone and estradiol abolished the estradiol-induced supersensitivity to noradrenaline in thoracic aorta from ovariectomized

rats. These results agree with those reported by Miller and Vanhoutte [25] who observed that in dog coronary arteries progesterone also attenuated the estrogen-induced stimulation of endothelium-dependent responses [25].

In conclusion, progesterone prevented the effect of high dose of estradiol on noradrenaline-induced contractions in rat thoracic aorta and these responses were endothelium-dependent. Further studies are required to assess the importance of these interactions in protecting against cardiovascular diseases.

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