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Possible mechanisms for the suppressing action of 17β-estradiol on β-adrenoceptor-mediated vasorelaxation in rat aorta

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Abstract

The mode of action of estrogen on β-adrenoceptor-mediated relaxation was investigated by using isolated ring preparations of thoracic aorta from ovariectomized rats. Administration of 17β-estradiol to ovariectomized rats significantly suppressed isoprenaline-induced relaxation of aortic rings. There was no alteration in the β-adrenoceptor binding characteristics. The suppressing action of 17β-estradiol on the N^G-nitro-L-arginine and indomethacin-resistant relaxation induced by isoprenaline disappeared after pretreatment with *N*,*N*-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525A), an inhibitor of cytochrome P450 (CYP). The levels of CYP2C11 expression were the highest of the CYP mRNAs examined in rat aorta. 17β-Estradiol replacement increased the expression of CYP2C11 mRNA in the aorta, compared with that in ovariectomized rats. These results suggest that estrogen suppresses β-adrenoceptor-mediated vasorelaxation, and that the mechanisms may be associated with alterations in CYP2C11 metabolites. © 2001 Published by Elsevier Science B.V.

Keywords: β-Adrenoceptor; Isoprenaline; 17β-Estradiol; Cytochrome P450; Aorta, rat

1. Introduction

Several observations have indicated that ovarian steroid hormones cause physiological changes in the cardiovascular system (Nabulsi et al., 1993; Miller and Vanhoutte, 1991; Skafar et al., 1997). The actions of estrogen on the cardiovascular system are mediated through the effects of lipoprotein metabolism, nitric oxide synthase (NOS), antioxidation and calcium homeostasis (Weiner et al., 1994; Ruehlmann and Mann, 1997; Mendelsohn and Karas, 1999). Such effects are known to explain the protective actions of estrogen against vascular diseases.

Pregnancy results in physiological changes in the cardiovascular system (Myatt, 1992). Maternal blood pressure remains constant, even though both cardiac output and blood volume increase. It has been reported that systemic vascular resistance and diastolic pressure both decrease during pregnancy, when estrogen levels increase (Griendling et al., 1985; Paller, 1987). The response of vascular smooth muscle to several vasoconstrictors decreases during pregnancy (Weiner et al., 1992; Kim et al., 1994; Honda et al., 1996). The endothelium-dependent relaxation induced by acetylcholine is significantly enhanced in arteries isolated from pregnant guinea pigs (Kim et al., 1994) and rats (Honda et al., 1996, 1998a), compared with the response in arteries from non-pregnant animals. It is suggested that the decreased response to vasoconstrictors and the enhanced relaxing response to acetylcholine are due to an enhancement in endothelium-derived nitric oxide (NO) activity by 17β-estradiol (Weiner et al., 1994; Honda et al., 1996; Rahimian et al., 1997). We have recently found that administration of estradiol for 3 days to diestrous female rats significantly suppresses isoprenaline-induced relaxation in aortic rings (Honda et al., 1998b). β-Adrenoceptor agonists increase cyclic AMP levels, causing relaxation of vascular smooth muscle. The relaxing response of rat aorta to isoprenaline (Honda et al., 1998b) as well as sulbutamol (Wang et al., 1993) is partially attenuated by either removal of the endothelium or treatment with an NOS inhibitor such as N^G-nitro-L-arginine (L-NOARG) or N^G-nitro-L-arginine methyl ester. Therefore, it is suggested that there is a NO-dependent component in the relaxing response to β-adrenoceptor agonists in rat aorta. The relaxing response of rat aorta to isoprenaline is also inhibited by a cytochrome P450 (CYP) inhibitor such as metyrapone, α-naphthoflavone or 8-methoxypsoralen, but metyrapone

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does not affect isoprenaline-induced relaxation in the aorta denuded of endothelium (Satake et al., 1997). These data suggest that the CYP pathway is involved in the endothelium-dependent relaxation induced by isoprenaline (Satake et al., 1997; Honda et al., 1998b). In the present study, we investigated the mode of action of estradiol and progesterone on the isoprenaline-induced relaxation in rat aorta.

2. Materials and methods

2.1. Animals and tissues isolation

All procedures were carried out in accordance with the institutional guidelines for animal research of Tokyo University of Pharmacy and Life Science. Seven-week-old female Wistar–Imamichi rats (150–180 g), which were supplied by the Institute for Animal Reproduction (Ibaraki, Japan), were ovariectomized under ether anesthesia. Two weeks after ovariectomy, the animals (9 weeks old) were subcutaneously treated with estradiol (50 μg/kg), progesterone (5 mg/kg), estradiol + progesterone or sesame oil for 3 days. The rats were anesthetized with ether and euthanized by exsanguination. Blood samples were collected from the femoral artery and the serum was frozen (−80 °C) for analysis of the estradiol levels. The thoracic aorta was isolated and cleaned of adherent fat and connective tissue in modified Krebs–Henseleit solution of the following composition (mM); NaCl, 118.0; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.0 at pH 7.4. The tissue bath solution was maintained at 37 °C and bubbled with a 95% O_2 and 5% CO_2 mixture. The preparations were used immediately in the functional studies or stored at −80 °C for the radioligand binding assay.

2.2. Measurement of isometric tension

The aortic preparation was cut into rings about 5 mm long, and five ring preparations were obtained from each animal. The contractile and relaxing responses were measured by suspending the rings between two stainless-steel hooks, one of which was attached to the end of a bathing tube and the other to a force transducer (45196A NEC Sanei Instruments, Tokyo, Japan). The isometric tension changes were recorded on a polygraph (LECT-HORIZ-8K NEC Sanei). Each preparation was equilibrated in the bath solution for 90–120 min before the experiment. The resting tension was 0.7 g, which was the optimal preload for force development in these blood vessels, as previously described (Honda et al., 1999). For the relaxing response studies, submaximal tone was induced with 0.6 μM norepinephrine, and then isoprenaline was added in a cumulative fashion or at a single concentration of 3 μM. After the preparations were washed three times within 60 min, the effects of the various inhibitors on the isoprenaline-induced vasorelaxation were tested by adding them 10 min before the contraction with norepinephrine. Responses are expressed as the percentage contraction of norepinephrine-induced tone and the contraction in the absence of the drugs was taken to be 100%.

2.3. Radioligand binding assay for β-adrenoceptor

Assays were performed as described by Shaul et al. (1990) with a minor modification. Briefly, the aorta was homogenized in 0.25 M sucrose buffer at pH 7.4 containing 10 mM Tris–HCl, 0.1 mM phenylmethylsulfonyl fluoride, 0.01 mM leupeptin and 5 μg/ml aprotinin using a Polytron, and centrifuged at $1500 \times g$ for 10 min at 4 °C. The supernatant was filtered through Kodak lens cleaning paper, and then centrifuged at 100,000 × g for 30 min at 4 °C. The pellet was resuspended in 5 mM HEPES buffer at pH 7.4 containing 1.0 mM $MgSO_4$. Protein concentration was determined colorimetrically using a Bio-Rad protein assay kit. Membrane preparations (4 μg of protein) were incubated for 60 min at 37 °C with 1.0 pM [^{125}I]iodocyanopindolol in the presence of increasing concentrations (0–10 ng/ml) of unlabeled alprenolol. Binding was terminated by rapid vacuum filtration through a Whatman GF/C filter. The filters were rinsed two times with 5 ml of 5 mM HEPES buffer. The receptor density and affinity were determined by Scatchard analysis.

2.4. Analysis of cytochrome P450 mRNAs by reverse transcription-polymerase chain reaction (RT-PCR) analysis

Poly (A) RNA was extracted using a QuickPrep Micro mRNA Purification Kit (Amersham Pharmacia Biotech, Buckinghamshire, UK). RT-PCR was performed using an RNA LA PCR™ Kit (AMV) Ver. 1.1 (TaKaRa, Japan) and a rat cytochrome P450 Competitive RT-PCR Set (TaKaRa) for detecting 9 CYP isoforms (1A1, 1A2, 2B1/2B2, 2C11, 2E1, 3A1, 3A2 and 4A1) according to the manufacturer's instructions. PCR reactions included 2 min predenaturation at 94 °C and then 36 cycles of 45 s at 94 °C, 45 s at 55 °C and 2 min at 72 °C. RT-PCR data for cyclophilin were used for standardization as an internal control. Parallel samples from ovariectomized animals with no RNA competitor served as a negative control, and the same samples with the RNA competitor for CYP2C11 (expected size: 300 bp) were reverse-transcribed and amplified as a positive control. The PCR products were electrophoresed on 3% agarose gels containing ethidium bromide and were photographed under UV-transillumination. The abundance of the PCR products was quantified as pixel intensity, using a densitometer, and normalized to that of cyclophilin.

2.5. Chemicals

[^{125}I]Iodocyanopindolol (2000 Ci/mmol) was obtained from Amersham Pharmacia Biotech. Isoprenaline, norepinephrine, 17β-estradiol, progesterone, protease inhibitors, L-NOARG, indomethacin and *N,N*-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF 525A) were obtained from Sigma (St. Louis, MO, USA). Atenolol and (±)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol (ICI-118,551) were obtained from Research Biochemicals International (Natick, MA, USA). The other chemicals were of analytical grade and obtained from Wako (Osaka, Japan).

2.6. Statistical analysis

Values are expressed or plotted as means ± S.E.M. Analysis of variance (ANOVA) was used to compare the concentration–response relaxation curves. Individual points were compared using a multiple Tukey's test, Dunnett's test, or Student's *t*-test, and differences were considered significant at $P < 0.05$.

3. Results

3.1. Effects of ovarian steroid hormones on isoprenaline-induced relaxation in ovariectomized rats

Addition of isoprenaline (1 nM–3 μM) produced a concentration-dependent relaxation in all groups (Fig. 1).

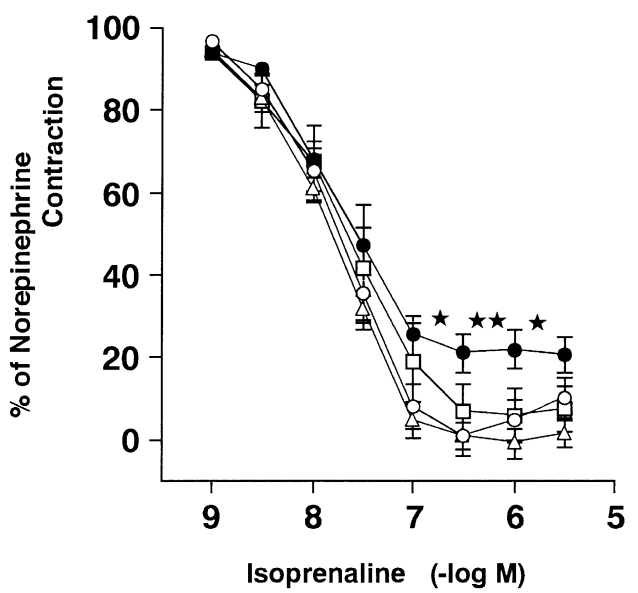

Fig. 1. Isoprenaline-induced relaxation of aortic rings precontracted with norepinephrine (0.6 μM) in vehicle (○)-, 17β-estradiol (●)-, progesterone (△)- or 17β-estradiol + progesterone (□)-treated ovariectomized rats. 17β-Estradiol (50 μg/kg/day), progesterone (5 mg/kg/day) or 17β-estradiol + progesterone was administered subcutaneously for 3 days. Each value represents mean ± S.E.M. from 4–6 rats. $^{\star}P < 0.05$, $^{\star\star}P < 0.01$ from vehicle control.

Table 1
Effects of selective β_1- and β_2-adrenoceptor antagonists on isoprenaline-induced relaxation in ovariectomized rats or 17β-estradiol-treated ovariectomized rats

Groups	pA_2 value	
	Atenolol	ICI-118,551
Ovariectomy	6.20 ± 0.13	8.23 ± 0.16[a]
Ovariectomy + 17β-Estradiol	5.82 ± 0.28	8.40 ± 0.21[a]

Atenolol and ICI-118,551 were used as selective β_1- and β_2-adrenoceptor antagonists, respectively. 17β-Estradiol (50 μg/kg/day) was administered subcutaneously for 3 days to ovariectomized rats. Each value represents mean ± S.E.M. from 5–8 rats.
[a] $P < 0.001$ from atenolol.

The isoprenaline-induced relaxation was significantly suppressed in the aortas from estradiol-treated ovariectomized rats at concentrations of 0.1–1 μM, compared with that in the aortas from vehicle-treated ovariectomized rats (maximal percent relaxation; vehicle: 100.1 ± 5.2% vs. estradiol: 81.6 ± 4.7%, $P < 0.01$). Progesterone had no influence on the isoprenaline-induced relaxation, although it inhibited the suppression of relaxation induced by estradiol (maximal percent relaxation; progesterone: 103.6 ± 3.6%, estradiol + progesterone: 99.8 ± 2.7%). The serum estradiol levels, which were measured by radioimmunoassay (Taya et al., 1985), were 24.86 ± 2.90 pg/ml in vehicle-treated ovariectomized rats and 50.33 ± 11.24 pg/ml in estradiol-treated ovariectomized rats. The levels in estradiol-treated ovariectomized rats were similar to those in proestrous rats (54.84 ± 3.35 pg/ml).

3.2. Effects of atenolol and ICI-118,551 on isoprenaline-induced relaxation

The pA_2 values for atenolol and ICI-118,551 were calculated from the concentration–response relaxation curve for isoprenaline in the presence of ICI-118,551 (0.1, 1 μM) or atenolol (1, 10 μM) (Table 1). ICI-118,551 caused a greater inhibition of the isoprenaline-induced vasorelaxation than did atenolol in preparations from both vehicle- and estradiol-treated ovariectomized rats. However, estradiol showed no significant influence on the antagonistic actions of atenolol or ICI-118,551 on the isoprenaline-induced relaxation.

3.3. Effects of ovariectomy and 17β-estradiol replacement on β-adrenoceptor binding characteristics

To address whether β-adrenoceptor binding characteristics are affected by estradiol treatment, the effects of estradiol on β-adrenoceptor density (B_{max}) and affinity (K_d) were examined. No significant change was seen in the values of B_{max} and K_d between intact and ovariectomized rats. Further, administration of estradiol to ovariectomized rats did not affect these values (Table 2).

Table 2
Effects of ovariectomy and 17β-estradiol replacement on β-adrenoceptor binding characteristics in rat aortic membrane

Groups	B_{max} (fmol/mg protein)	K_d (pM)
Intact	281 ± 9.6	3.0 ± 0.13
Ovariectomy	333 ± 58.1	3.5 ± 0.54
Ovariectomy + 17β-Estradiol	341 ± 33.5	3.7 ± 0.47

17β-Estradiol (50 μg/kg/day) was administered subcutaneously for 3 days to ovariectomized rats. The aorta was collected 24 h after the final injection of vehicle or 17β-estradiol, or from rats in estrus. Crude membrane proteins (4 μg) from the aortas were incubated with 10 pg/ml [^{125}I]iodocyanopindolol in the presence of increasing concentrations (0–10 ng/ml) of unlabeled alprenolol. Data were obtained from the Scatchard analysis. Each value represents mean ± S.E.M. of three separate experiments involving 9 or 10 rats.

3.4. Effects of L-NOARG and SKF 525A on the suppressing action of 17β-estradiol on isoprenaline-induced relaxation

Pretreatment with indomethacin alone had no significant effect on the isoprenaline-induced relaxation (data not shown). Pretreatment with L-NOARG (100 μM) and indomethacin (10 μM) significantly inhibited the isoprenaline-induced relaxation in aortic rings from both vehicle- and estradiol-treated ovariectomized rats, and the suppression of relaxation induced by estradiol still remained (Fig. 2). Additional treatment with SKF 525A (10 and 30 μM) further inhibited the isoprenaline-induced relaxation (3 μM) in the presence of L-NOARG and indomethacin in vehicle-treated ovariectomized rats. However, in estradiol-treated ovariectomized rats, SKF 525A did not significantly inhibit the isoprenaline-induced relaxation. As a result, SKF 525A almost abolished the estradiol-induced suppression of the isoprenaline-induced relaxation (Fig. 3).

3.5. Effects of ovariectomy and 17β-estradiol replacement on CYP expression in the aorta

In order to clarify what kinds of CYP isoforms were significantly expressed in the aorta and to examine the effects of estradiol on the expression of these isoforms, we analyzed CYP mRNAs (Fig. 4A). CYP2C11, 2E1 and 3A1 mRNAs were detectable using 1 μg of poly (A) RNA isolated from the aorta, and the levels of CYP2C11 expression were the highest of the CYP mRNAs examined (data not shown). Ovariectomy significantly decreased CYP2C11 expression two weeks after the operation. Statistical analysis of the corrected densitometry readings showed that ovariectomy significantly decreased CYP2C11 mRNA levels in the aorta to about 30% compared with those in intact rats in estrus (intact: 1.00 ± 0.12 vs. ovariectomy: 0.30 ± 0.13, $P < 0.01$). However, estradiol treatment completely reversed the ovariectomy-induced decrease in CYP2C11 expression (ovariectomy + estradiol: 1.08 ± 0.20) (Fig. 4B). Fig. 4C shows a negative and a positive control, using samples from ovariectomized rats, with the RNA competitor added to the RT reaction. The expected size (300 bp) PCR product was obtained in lanes 2–4. In addition, the

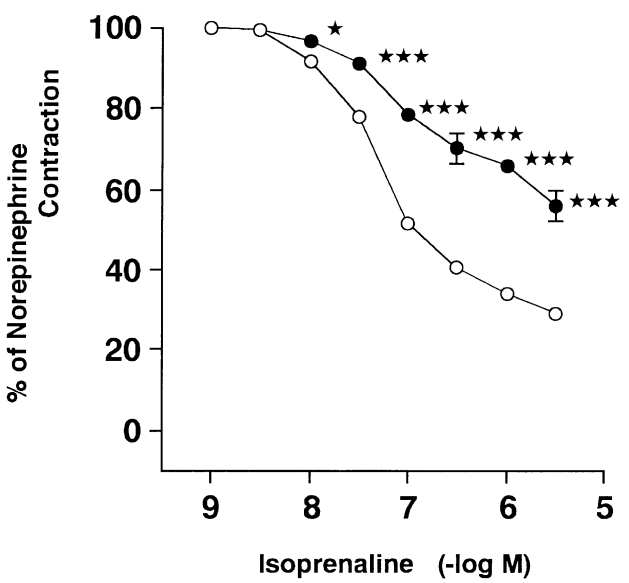

Fig. 2. Isoprenaline-induced relaxation of aortic rings in the presence of L-NOARG (100 μM) and indomethacin (10 μM) in ovariectomized rats (○) and 17β-estradiol-treated ovariectomized rats (●). Each value represents mean ± S.E.M. from 5 or 6 rats. $^{\star}P < 0.05$, $^{\star\star\star}P < 0.001$ from ovariectomized rats.

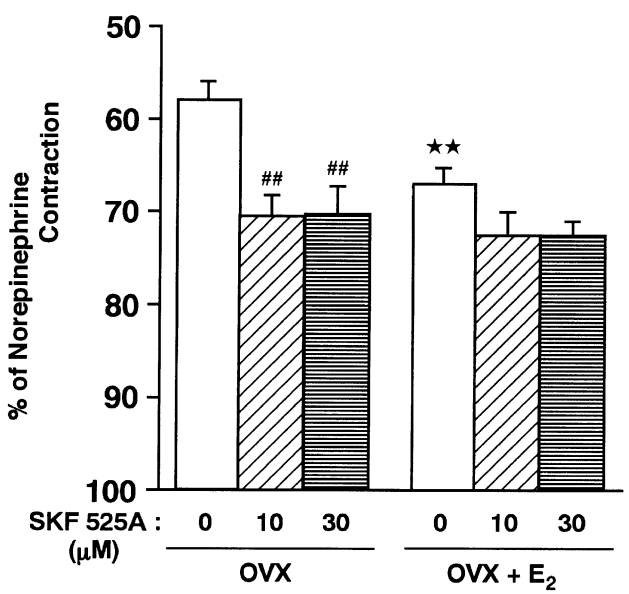

Fig. 3. Effects of SKF 525A on the relaxation induced by isoprenaline (3 μM) in the presence of L-NOARG (100 μM) and indomethacin (10 μM) in ovariectomized rats (OVX) and 17β-estradiol-treated ovariectomized rats (OVX + E_2). Each value represents mean ± S.E.M. from 3–10 rats. $^{\star\star}P < 0.01$ from OVX. $^{\#\#}P < 0.01$ from the absence of SKF 525A.

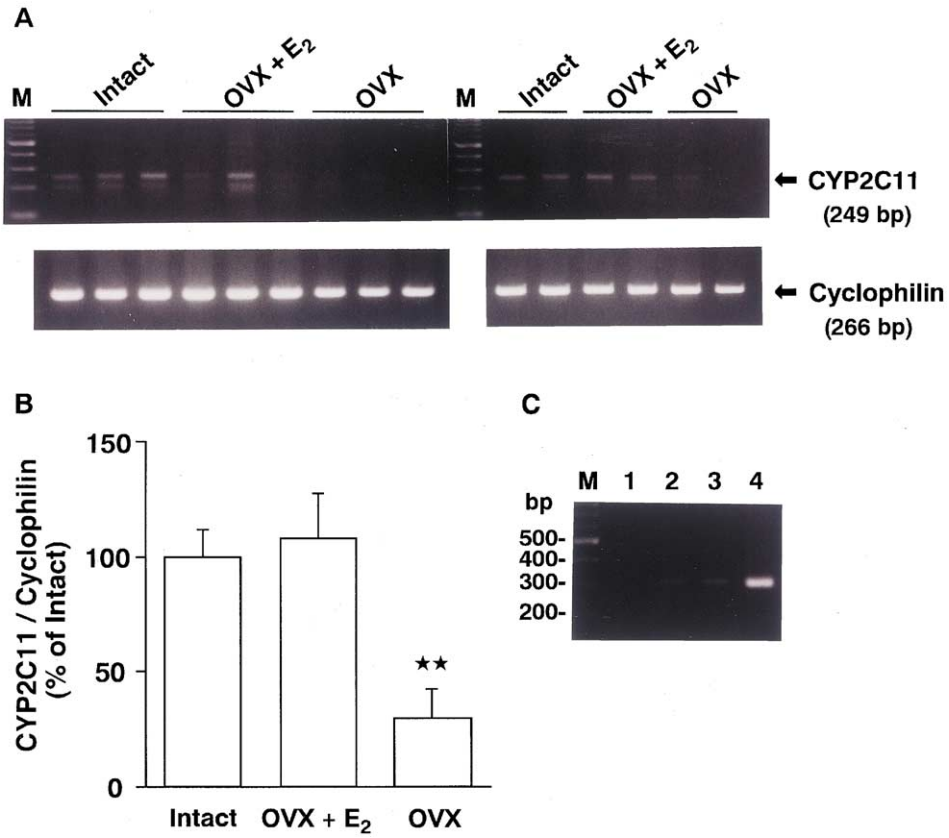

Fig. 4. Effects of ovariectomy and 17β-estradiol replacement on the expression of CYP2C11 in rat aorta detected by RT-PCR analysis. (A) 17β-Estradiol (50 μg/kg/day) was administered subcutaneously for 3 days to ovariectomized rats. The aorta was collected 24 h after the final injection of vehicle (OVX) or 17β-estradiol (OVX + E_2), or from rats in estrus (Intact). Each lane shows the data obtained from two animals. (B) The quantification data are expressed as a percentage of intact rats and means ± S.E.M. from 10 rats. $^{**}P < 0.01$ from intact. (C) RT-PCR reaction was performed without the competitor, using RNA samples from ovariectomized rats as a negative control (lane 1). The RNA competitor (lanes 2, 3: 1.6×10^6 copies, lane 4: 16×10^6 copies) was added to the samples and reverse-transcribed and amplified as a positive control.

results of CYP2E1 also showed the same tendency as that observed for CYP2C11 expression (data not shown).

4. Discussion

We have previously reported that administration of estradiol to female rats for 3 days significantly suppresses isoprenaline-induced relaxation in rat aortic rings (Honda et al., 1998b). In the present study, we used ovariectomized rats to clarify the actions of estradiol. The present results showed that estradiol treatment significantly suppressed the isoprenaline-induced relaxation of aortic rings isolated from ovariectomized rats. Acetylcholine-induced endothelium-dependent relaxation is promoted in some vessels when the levels of circulating estrogen are high during the late stage of pregnancy or after estrogen treatment (Kim et al., 1994; Honda et al., 1996, 1998a; Rahimian et al., 1997). In this study, however, estradiol treatment suppressed isoprenaline-induced relaxation of aortic rings. These results suggest that estradiol may control vascular tone through at least two types of receptors of the autonomic nerve system, that is, β-adrenoceptors and muscarinic receptors, and that it may be one of the vasoprotective actions of estrogen. The pA_2 value of ICI-118,551, a selective β_2-adrenoceptor antagonist (Bilski et al., 1983), was higher than that of atenolol, a selective β_1-adrenoceptor antagonist (Satake et al., 1996), indicating that the isoprenaline-induced relaxation is mediated mainly through β_2-adrenoceptors in rat aorta. There were no significant changes in the pA_2 value of atenolol and ICI-118,551 and in β-adrenoceptor capacity and affinity between vehicle- and estradiol-treated ovariectomized rats. Based on these results, we conclude that estradiol suppresses isoprenaline-induced relaxation without causing any alteration in β-adrenoceptor binding characteristics.

The suppressing action of estradiol on isoprenaline-induced relaxation remained even in the presence of L-NOARG and indomethacin. That is, the L-NOARG and indomethacin-resistant relaxation induced by isoprenaline in rat aorta was suppressed by estradiol treatment. The resistant part of the isoprenaline-induced relaxation may be

due to the CYP pathway, because Satake et al. (1997) and our group (Honda et al., 1998b) have suggested that the CYP pathway is involved in the relaxation induced by isoprenaline in rat aorta. The metabolism of arachidonic acid by the CYP pathway results in the formation of numerous biologically active metabolites. To clarify the mechanisms of the suppression of the isoprenaline-induced relaxation by estradiol, we examined the relationship between the suppression of relaxation induced by estradiol and the CYP system in the aorta. First, we determined whether SKF 525A, a nonselective CYP inhibitor, affected the isoprenaline-induced relaxation and the suppression of relaxation induced by estradiol. High concentrations of CYP inhibitors may have another actions (Dong et al., 1997). Thus, we used 10 μM of SKF 525A in this study, because this concentration of SKF 525A is reported to have no influence on the open-state probability of potassium channels and NOS activity (Campbell et al., 1996). In the present study, this concentration of SKF 525A had no effect on either basal tension or the contraction elicited by norepinephrine. Furthermore, Dong et al. (1997) showed that SKF 525A did not affect exogenous NO-induced relaxation of endothelium-denuded preparations. Pretreatment with SKF 525A significantly inhibited the isoprenaline-induced relaxation in vehicle-treated ovariectomized rats in the presence of L-NOARG and indomethacin. Therefore, the suppression of relaxation induced by estradiol disappeared under this condition. Ovariectomy significantly decreased CYP2C11 expression, and estradiol treatment completely reversed the ovariectomy-induced decrease in CYP2C11 expression. It has been reported that members of the CYP2C gene family synthesize both epoxyeicosatrienoic acids (EETs) (Laethem et al., 1992; Imaoka and Funae, 1998; Fisslthaler et al., 1999) and 20-hydroxyeicosatetraenoic acid (20-HETE) (Laethem et al., 1992; Imaoka and Funae, 1998) from arachidonic acid, and in fact EETs and 20-HETE induce vasodilator (Carroll et al., 1992) and vasoconstrictor responses (Escalante et al., 1993), respectively. CYP2C11 purified from rat liver, kidney and lung metabolizes arachidonic acid into EETs and HETEs (Imaoka and Funae, 1998). Our data also indicated that the levels of CYP2C11 expression were the highest of the aortic CYP mRNAs we examined. Therefore, CYP2C11 may play an important role in vascular tone. The suppression of the isoprenaline-induced relaxation caused by estradiol in rat aorta may be related to an increase in CYP2C11 expression, resulting in an alteration in the balance of the amounts of EETs and 20-HETE. There is now much evidence that endothelium-dependent smooth muscle hyperpolarization is mediated by an endogenous factor referred to as endothelium-derived hyperpolarizing factor (EDHF). Bobadilla et al. (1997) suggested that pregnancy enhances the acetylcholine-stimulated release of EDHF from rat abdominal aorta, and that EDHF is released through a CYP-dependent pathway. Fisslthaler et al. (1999) suggested that CYP2C is an EDHF synthase in coronary arteries. Thus, that estradiol-induced CYP2C11 expression in the aorta may provide information about the association between the vascular protective actions of estrogen and EDHF-mediated responses.

It is known that estrogen affects endothelial NOS (eNOS) activity in guinea pig heart, kidney skeletal muscle and cerebellum (Weiner et al., 1994), and protein expression in rat renal medulla (Neugarten et al., 1997), and that endogenous and exogenous NO antagonize the vasodilation mediated by CYP-dependent arachidonic acid metabolites (Oyekan, 1995). We have already reported that short-term treatment with estradiol does not affect the eNOS protein levels in ovariectomized rat aorta (Tamura et al., 2000), suggesting that the suppressing effect of estradiol on isoprenaline-induced relaxation is dissociated from eNOS protein expression. So, it is suggested that eNOS-produced NO did not interfere with CYP2C11 activity in the estradiol-treated group. However, other possible collateral mechanisms should be considered. Estrogens are suggested to increase the α-adrenoceptor concentration (Larsson et al., 1984), which may result in a decrease in sensitivity to the relaxing response of β-adrenoceptor agonist.

We also investigated the effects of progesterone on the isoprenaline-induced relaxation. Progesterone treatment had no influence on the isoprenaline-induced relaxation. It has been reported that sex steroid hormones such as estradiol (Freay et al., 1997), progesterone (Perusquia et al., 1996) and testosterone (Honda et al., 1999) have rapid, direct relaxing effects in rat aorta in a nongenomic manner. The effects of estradiol have been suggested to contribute to cardiovascular protection. In this study, progesterone treatment for 3 days had no influence on the isoprenaline-induced relaxation. Furthermore, estradiol and/or progesterone treatment did not affect the norepinephrine-induced contraction. So, it is suggested that there are different mechanisms underlying the vascular responses induced by sex steroid hormones in vivo and in vitro. Progesterone inhibited the suppression of relaxation induced by estradiol. Interestingly, the vasomotor effects of estradiol were reduced when progesterone was combined with estradiol. The mechanism of this interaction is not clear, but it is known that estradiol and progesterone may also be antagonistic to each other in blood vessels. For example, it has been demonstrated that progesterone suppresses the enhancement of endothelium-dependent relaxation induced by estradiol in isolated dog coronary artery (Miller and Vanhoutte, 1991) and rat aorta (Rahimian et al., 1997).

In conclusion, our study indicates that 17β-estradiol treatment decreases the isoprenaline-induced relaxation in aortic rings isolated from ovariectomized rats, and that changes in the β-adrenoceptor-mediated vasorelaxation modulated by 17β-estradiol may be in part associated with an increase in CYP2C11 expression. These observations provide evidence that estrogen may control the cardiovascular system through the CYP pathway.

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