Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 472 (2003) 119–126
www.elsevier.com/locate/ejphar

Differences in the mechanisms for relaxation of aorta induced by 17β-estradiol or progesterone between normotensive and hypertensive rats

Tamao Unemoto, Hideo Honda*, Hiroshi Kogo

Department of Pharmacology, Tokyo University of Pharmacy and Life Science, 1432-1, Horinouchi, Hachioji, Tokyo 193-0392, Japan

Received 23 January 2003; received in revised form 16 April 2003; accepted 15 May 2003

Abstract

The tension in isolated ring preparations of the thoracic aorta from Wistar–Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) was measured isometrically to study if there are any differences in the mechanisms of 17β-estradiol- or progesterone-induced relaxation between WKY and SHR aortic rings. 17β-Estradiol and progesterone caused dose-dependent vascular relaxation of the thoracic aorta precontracted with norepinephrine in both WKY and SHR, and the relaxation induced by 17β-estradiol was greater in SHR than WKY. However, no difference was observed in progesterone-induced relaxation between SHR and WKY. With the exception of tetraethylammonium, an inhibitor of Ca^{2+}-activated K^+ channels, glibenclamide, a selective inhibitor of ATP-sensitive K^+ channels, or 4-aminopyridine, a selective inhibitor of voltage-dependent K^+ channels, significantly reduced 17β-estradiol-induced relaxation only in SHR, but not in WKY. Both 17β-estradiol and progesterone inhibited Ca^{2+}-induced vasocontraction of the thoracic aorta in K^+ depolarization medium in WKY and SHR. These results suggest that the mechanisms of 17β-estradiol-induced relaxation in SHR aorta are at least partially mediated via ATP-sensitive and voltage-sensitive K^+ channels in addition to the inhibition of Ca^{2+} channels, although those of progesterone-induced relaxation in both WKY and SHR are mainly concerned with the inhibition of Ca^{2+} channels rather than the operation of K^+ channels. Moreover, a difference in 17β-estradiol-induced relaxation between WKY and SHR aorta suggests a possibility that vascular response in SHR is modified by hypertension.

Keywords: 17β-Estradiol; Progesterone; Endothelium; Inbred SHR, rat; Aorta; K^+ channel

1. Introduction

The incidence of hypertension and cardiovascular disease is lower in women than men if the age is similar (Wild and Bartholemew, 1988; Adams et al., 1995). It is reported that estrogen replacement therapy reduces the risk of coronary and cerebrovascular diseases in postmenopausal women (Sullivan et al., 1988; Stampfer et al., 1991; Godsland et al., 1987). An increase in plasma high density lipoprotein (HDL) cholesterol and a decrease in low density lipoprotein (LDL) cholesterol may be one of the mechanisms by which estrogen reduces the risk of these diseases. However, analysis of lipid changes in postmenopausal women taking estrogen shows that they do not fully account for the cardiovascular protective effects (Adams et al., 1990; Clarkson et al., 1990). Indeed, several studies have shown that estrogen causes vasorelaxation in a range of species including humans (Belfort et al., 1996; Jiang et al., 1991; Chan et al., 2001). Another important mechanism may be the modulation of arterial tone. In contrast to estrogen, little is known about the effects of progesterone, which is commonly used in hormone replacement therapy and is supposed to oppose some vascular actions of estrogen (Yamaguchi et al., 2001; Gerhard and Ganz, 1995).

Clinical studies have demonstrated that hypertensive men and premenopausal women have lower levels of plasma androgen but higher levels of plasma estrogen than normotensive men (Khaw and Barret-Connor, 1988; Hughes et al., 1989). There is no report on the relationship between hypertension and the levels of plasma progesterone. In genetic and nongenetic rat models of hypertension, males become more hypertensive than females and this sexual difference is abolished by gonadectomy (Cambotti et al., 1984; Share et al., 1988). Recently, we have indicated that testosterone-induced vasorelaxation in aortic rings is greater

* Corresponding author. Tel.: +81-426-76-4529; fax: +81-426-76-4529.
E-mail address: hhonda@ps.toyaku.ac.jp (H. Honda).

0014-2999/03/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01858-2

in spontaneously hypertensive rats (SHR) than Wistar–Kyoto rats (WKY), and have suggested that the mechanisms of testosterone-induced vasorelaxation in both WKY and SHR involve, in part, ATP-sensitive K^+ channels in the thoracic aorta (Honda et al., 1999). Moreover, we have shown differences between WKY and SHR in the function of ATP-sensitive, voltage-dependent, and Ca^{2+}-activated K^+ channels (Honda et al., 1999). That is, we found that there was a difference in the vascular response caused by testosterone between WKY and SHR. To our knowledge, no one has considered the direct relaxing effects of female hormones on the vasculature in hypertensive animals. So the purpose of this study was to determine and compare the vasorelaxing actions of 17β-estradiol and progesterone on vascular reactivity in the thoracic aorta isolated from WKY and SHR and to contribute to the vascular pathophysiology of hypertension.

2. Materials and methods

2.1. Animals and tissues

All procedures were performed in accordance with the institutional guideline for animal research at Tokyo University of Pharmacy and Life Science. Ten-week-old male WKY/NCrj and SHR/NCrj, which were supplied by Charles River Japan, Yokohama, Japan, were anesthetized with ether and euthanized by exsanguination. The thoracic aorta was isolated and placed in modified Krebs–Henseleit solution

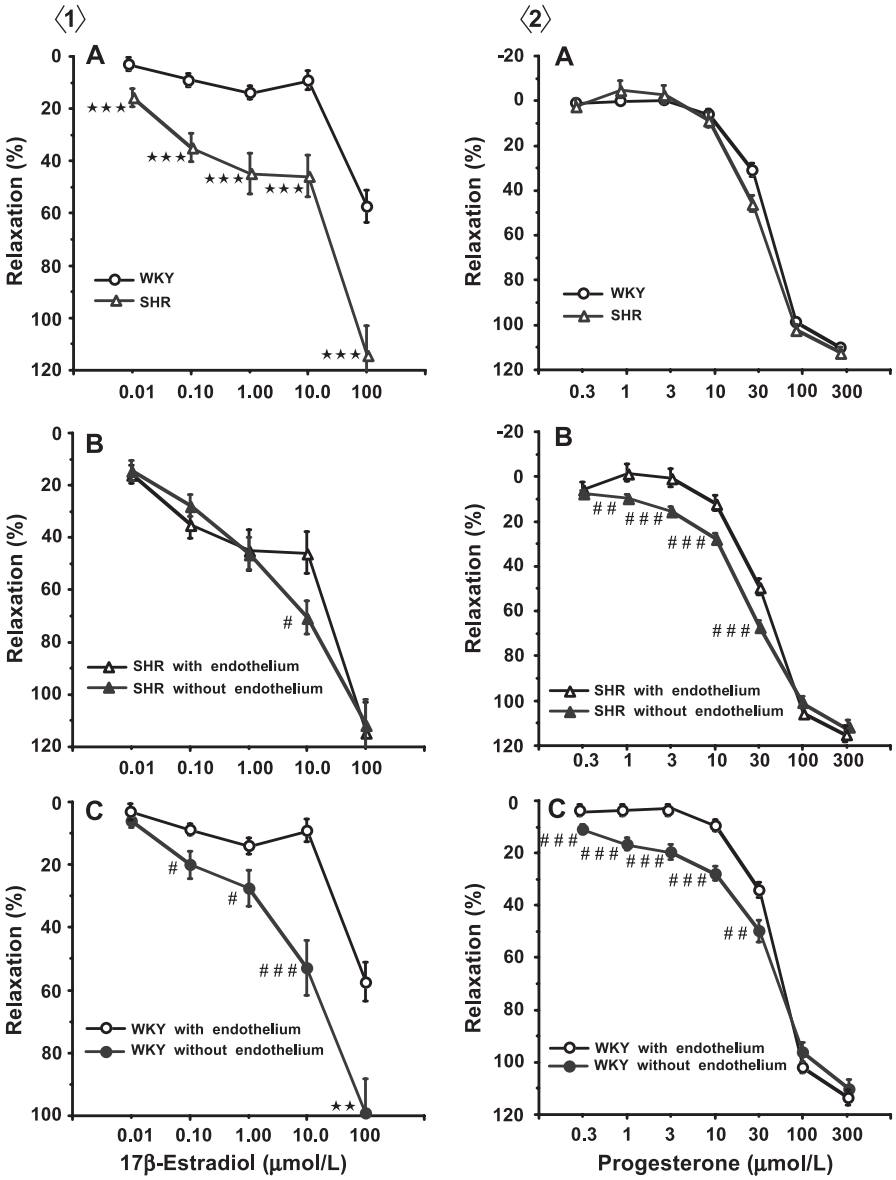

Fig. 1. Cumulative relaxation to 17β-estradiol ⟨1⟩ or progesterone ⟨2⟩ in aortic rings precontracted with norepinephrine in WKY and SHR, together (A) and separately (B and C). Each value is mean ± S.E. from 8 to 20 experiments. ★★★$P<0.001$ from WKY; #$P<0.05$, ##$P<0.01$, ###$P<0.001$ with endothelium.

Table 1
Time to reach the half ($T_{1/2}$) and maximal (T_{max}) relaxation induced by 17β-estradiol or progesterone

Rats	Time (min)		Maximal relaxation (%)
	$T_{1/2}$	T_{max}	
17β-Estradiol			
SHR	10.4 ± 1.2	34.7 ± 3.5	90.4 ± 14.9[a]
WKY	10.3 ± 1.1	34.4 ± 3.3	53.6 ± 2.3
Progesterone			
SHR	9.1 ± 0.5	27.2 ± 1.0	96.9 ± 1.5
WKY	8.9 ± 0.5	26.6 ± 1.1	93.1 ± 2.4

Each value represents the mean ± S.E. from 11 or 12 experiments. $T_{1/2}$ indicates half-maximal, and T_{max} maximal relaxation. Both 17β-estradiol and progesterone were used at 100 μm.
[a] $P < 0.01$ from WKY.

(pH 7.4) of the following composition (in mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, and glucose 11 at 37 °C gassed with 95% O_2/5% CO_2. The aortic tissue was cleaned by removing connective tissue. The thoracic aorta was cut into rings approximately 4 mm long. Contraction and relaxation 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 San-ei Instruments). Isometric tension changes were recorded on a polygraph (LECT-HORIZ-8K NEC San-ei) as previously described (Tamura et al., 1997).

2.2. Relaxation of the aorta precontracted with norepinephrine

Each preparation was equilibrated in the 10 ml bathing 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 relaxation studies, the submaximal tone (80% of the maximal tone) was induced with norepinephrine (300 nM) and then 17β-estradiol or progesterone was added in a cumulative fashion, and the relaxing effects were compared between endothelium-intact and endothelium-denuded rings. The intact endothelium and the endothelial removal were confirmed with acetylcholine. Responses were expressed as relaxation percentage of norepinephrine-induced tone, and the relaxation in the absence of drugs was taken to be 0%. To assess the role of endothelium in the vascular response to 17β-estradiol or progesterone, some thoracic aortae were deendothelialized before being mounted by gentle rubbing of the luminal surface with stainless steel.

2.3. Effects of K^+ channel blockers on 17β-estradiol- or progesterone-induced relaxation

To determine the possible effects of ATP-sensitive, voltage-dependent or Ca^{2+}-activated K^+ channels on 17β-estradiol or progesterone-induced relaxation, glibenclamide, a selective inhibitor of ATP-sensitive K^+ channels, 4-aminopyridine, a selective inhibitor of voltage-dependent K^+ channels, or tetraethylammonium, an inhibitor of Ca^{2+}-activated K^+ channels, was added to the solution 5 min before treatment with norepinephrine.

2.4. Effects of N^G-nitro-L-arginine (L-NA), indomethacin or metyrapone on 17β-estradiol- or progesterone-induced relaxation

To observe the effects of 17β-estradiol- or progesterone-induced relaxation, N^G-nitro-L-arginine (L-NA), an inhibitor of nitric oxide (NO) synthase, or indomethacin, an inhibitor of cyclooxygenase, or metyrapone, an inhibitor of cytochrome P-450 monooxygenase, was added to the solution 5 min before treatment with norepinephrine.

2.5. Effects of receptor antagonists on 17β-estradiol- or progesterone-induced relaxation

The involvement of receptors in 17β-estradiol- or progesterone-induced vasorelaxation was examined by pretreatment with ICI 182,780, an estrogen receptor antagonist or mifepristone, a progesterone receptor antagonist. These receptor antagonists were added to the solution 20 min before treatment with norepinephrine.

2.6. Effects of 17β-estradiol or progesterone on Ca^{2+}-induced contraction

The aortic rings were incubated in Ca^{2+}-free solution for 10 min. Then, Ca^{2+} concentration-dependent contraction curves were preformed in K^+-depolarization medium (50 mmol KCl). Subsequently, the rings were incubated with

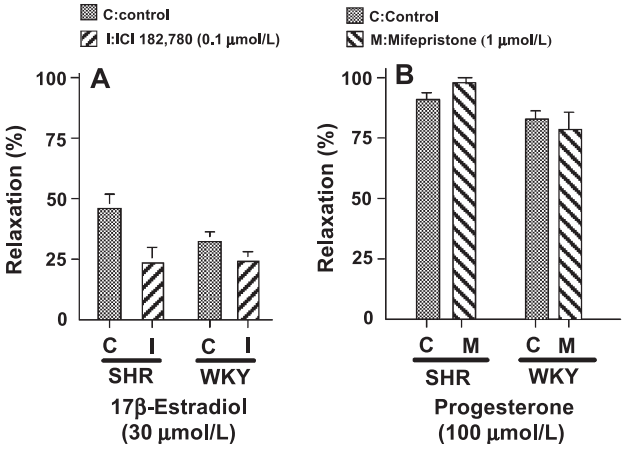

Fig. 2. Effects of (A) ICI 182,780 (0.1 μM), an estrogen receptor antagonist, and (B) mifepristone (1 μM), a progesterone receptor antagonist, on relaxation induced by 17β-estradiol (30 μM) or progesterone (100 μM), respectively, in aortic rings precontracted with norepinephrine in WKY and SHR. Each value is the mean ± S.E. from 5 to 14 experiments.

17β-estradiol or progesterone for 20 min and the Ca^{2+} concentration-dependent contraction curves were formed again.

2.7. Drugs and chemicals

17β-Estradiol, progesterone and glibenclamide (Sigma, St. Louis, MO, USA) were dissolved in ethanol (final concentration of ethanol in bath ≦0.5%, with no influence on norepinephrine-induced contraction.). 7-{9-[(4,4,5,5,5-pentafluoropentyl)sulphinyl]nonyl}-estra-1,3,5(10)-triene-3,17-diol (ICI 182,780) (Tocris, Ballwin, MO, USA) was dissolved in dimethylsulfoxide (final concentration of dimethylsulfoxide in bath ≦0.5%, with no influence on norepinephrine-induced contraction.). Mifepristone (ROUSSEL UCLAF), norepinephrine hydrochloride, 4-aminopyridine, tetraethylammonium, L-NA and metyrapone (Sigma) were dissolved in distilled water. Indomethacin (Sigma) was dissolved in 4% (wt/vol) $NaHCO_3$. The other chemicals were of analytical grade and obtained from Wako Pure (Osaka, Japan).

2.8. Statistical analysis

Values were expressed or plotted as mean ± S.E., and the statistical analysis was performed with a Student's *t*-test or multiple Tukey test. Differences were considered significant at $P < 0.05$.

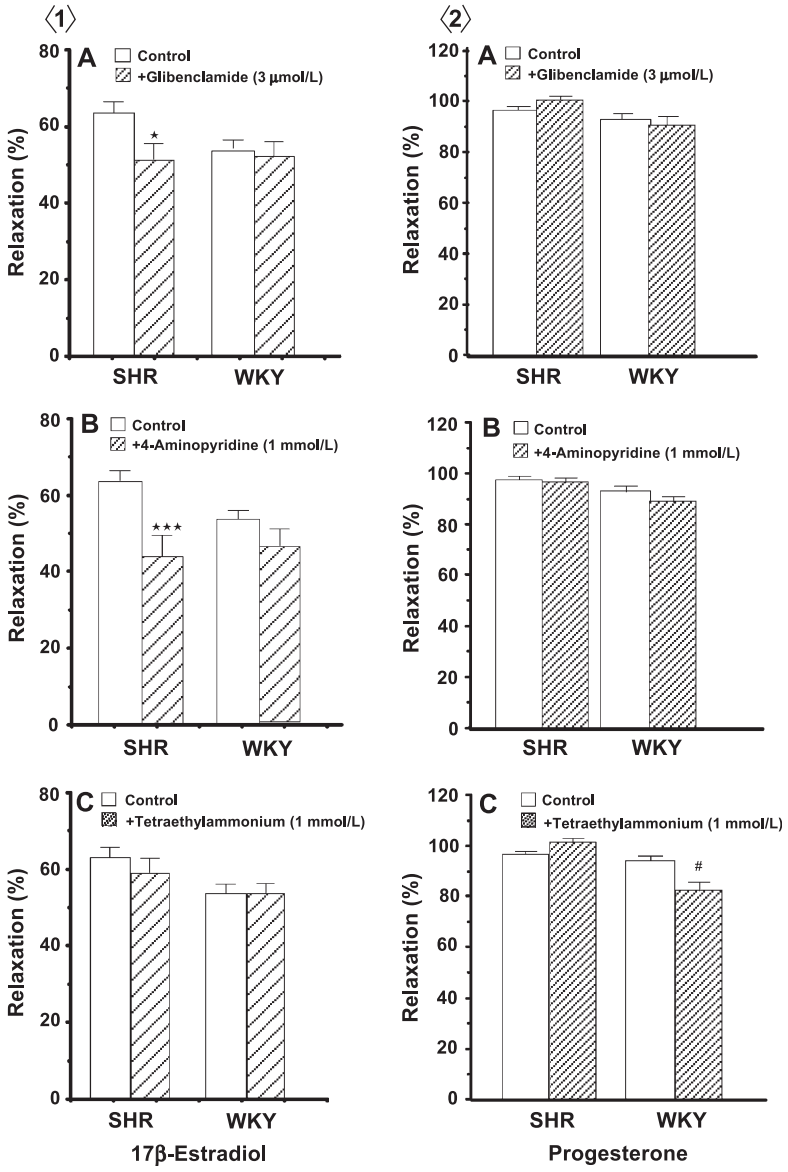

Fig. 3. Effects of K^+ channel blockers on relaxation induced by 17β-estradiol (30 μM) ⟨1⟩ or progesterone (100 μM) ⟨2⟩ in aortic rings precontracted with norepinephrine in WKY and SHR. Each value is the mean ± S.E. from 8 to 20 experiments. ★$P < 0.05$, ★★★$P < 0.001$ from SHR control; #$P < 0.05$ from WKY control.

3. Results

3.1. Relaxation induced by 17β-estradiol or progesterone

17β-Estradiol induced dose-dependent vascular relaxation of the thoracic aorta precontracted with norepinephrine in both WKY and SHR aortic rings, and the relaxation was greater in SHR than WKY (maximal relaxation percentage; WKY: 52.9 ± 5.7% vs. SHR: 116.0 ± 8.9%, $p < 0.01$, pD_2 values; WKY: 5.16 ± 0.27 vs. SHR: 6.37 ± 0.26, $p < 0.05$). (Fig. 1⟨1⟩A). Denudation of endothelium in SHR rings had a little influence on the relaxation caused by 17β-estradiol (Fig. 1⟨1⟩B). In contrast to SHR, removal of endothelium in WKY significantly enhanced the 17β-estradiol-induced relaxation, and significant differences were found at almost all the concentrations between the presence and the absence of endothelium (Fig. 1⟨1⟩C). progesterone also induced dose-dependent vascular relaxation of the thoracic aorta precontracted with norepinephrine in both WKY and SHR (maximal relaxation percentage; WKY: 113.3 ± 1.3% vs. SHR: 115.4 ± 2.0%, NS, pD_2 values; WKY: 5.36 ± 0.02 vs. SHR: 5.44 ± 0.11, NS). Unlike 17β-estradiol, there was no significant difference in progesterone-induced relaxation between WKY and SHR (Fig. 1⟨2⟩A). Denudation of endothelium significantly enhanced the progesterone-induced relaxation in both WKY and SHR (Fig. 1⟨2⟩B and C). The times (in min) to reach the half-maximal and maximal relaxation induced by 17β-estradiol or progesterone (each 100 μM) were not different between WKY and SHR, but a significant difference was seen in the maximal relaxation induced by 17β-estradiol only between WKY and SHR (Table 1).

3.2. Effects of receptor antagonists on 17β-estradiol- or progesterone-induced relaxation

In order to clarify if ovarian hormone-induced vasorelaxation is mediated through its receptor, pretreatment with receptor antagonists were performed. Addition of ICI 182,780 (0.1 μM), a 17β-estradiol receptor antagonist, did not inhibit 17β-estradiol-induced relaxation (Fig. 2A), and the addition of mifepristone (1 μM), a progesterone receptor antagonist, also did not inhibit progesterone-induced relaxation (Fig. 2B).

3.3. Effects of glibenclamide, 4-aminopyridine, or tetraethylammonium on 17β-estradiol- or progesterone-induced relaxation

To examine the involvement of K^+ channels in the relaxant actions of 17β-estradiol and progesterone, the effects of pretreatment with glibenclamide, 4-aminopyridine or tetraethylammonium were investigated. Glibenclamide (3 μM), and 4-aminopyridine (1 mM) significantly reduced 17β-estradiol-induced relaxation only in SHR and had no influence on the relaxation in WKY (Fig. 3⟨1⟩A and B). On the other hand, tetraethylammonium (1 mM) had no influence on 17β-estradiol-induced relaxation in both WKY and SHR (Fig. 3⟨1⟩C). In contrast to 17β-estradiol-induced relaxation, the three inhibitors had no effect on the relaxation induced by progesterone in SHR (Fig. 3⟨2⟩A and B). And the relaxation by progesterone in WKY was slightly but significantly inhibited by tetraethylammonium only (Fig. 3⟨2⟩C).

3.4. Effects of L-NA, metyrapone or indomethacin on 17β-estradiol- or progesterone-induced relaxation

Preincubation with metyrapone (1 mM) or indomethacin (10 μM) did not affect 17β-estradiol-induced relaxation in both WKY and SHR (Fig. 4A). Preincubation with L-NA (100 μM), significantly enhanced 17β-estradiol-induced vasorelaxation in WKY only and had no influence on the relaxation in SHR (Fig. 4A).

Progesterone-induced relaxation in both WKY and SHR was not influenced by preincubation with L-NA (100 μM), metyrapone (1 mM) or indomethacin (10 μM) (Fig. 4B).

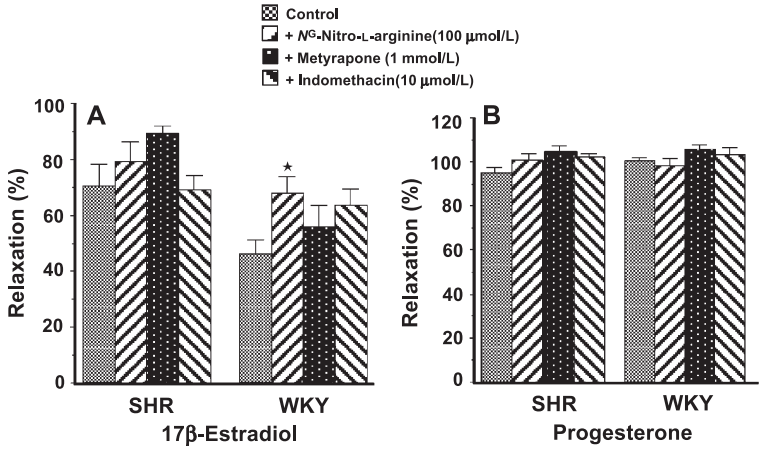

Fig. 4. Effects of N^G-nitro-L-arginine, metyrapone or indomethacin on relaxation induced by 17β-estradiol (30 μM) or progesterone (100 μM) of aortic rings precontracted with norepinephrine in WKY and SHR. Each value is the mean ± S.E. from 9 to 12 experiments. ★$P < 0.05$ from WKY control.

Table 2
Values of pD_2' for 17β-estradiol or progesterone and values of pA_2 for verapamil against $CaCl_2$-induced contraction in aortic rings depolarized by KCl

Drugs	WKY	SHR
pD_2' values		
17β-Estradiol	5.27 ± 0.63	5.40 ± 0.69
Progesterone	5.13 ± 0.60	5.03 ± 0.52
pA_2 values		
Verapamil	8.99 ± 0.73	8.61 ± 0.51

3.5. Effects of 17β-estradiol or progesterone on Ca^{2+}-induced contraction

The Ca^{2+} concentration-dependent contraction curves in K^+ depolarization medium (K^+ = 50 mM) were shifted to the right and downward after incubation with 17β-estradiol or progesterone in both WKY and SHR. For comparison, verapamil, a Ca^{2+} channel blocker, produced a marked antagonism, resulting in a rightward shift of the concentration–response curve to $CaCl_2$. The values of pD_2' for 17β-estradiol and progesterone, and the value of pA_2 for verapamil are shown in Table 2. No significant difference was seen in the values for 17β-estradiol, progesterone or verapamil between WKY and SHR.

4. Discussion

The present results have indicated that ovarian hormones such as 17β-estradiol and progesterone produce relaxation in the precontracted thoracic aorta isolated from WKY and SHR, and that 17β-estradiol-induced relaxation was significantly greater in SHR than WKY aortic rings (Fig. 1⟨1⟩A) but no significant differences were observed in progesterone-induced relaxation between WKY and SHR (Fig. 1⟨2⟩A). The dose–response curves to 17β-estradiol in both WKY and SHR are not sigmoid, so there may be two different mechanisms. Denudation of endothelium in SHR exerted almost no influence on the 17β-estradiol-induced relaxation, although denudation of endothelium in WKY rings was significantly enhanced relaxation (Fig. 1⟨1⟩B and C). These results suggest that the mechanisms of endothelium-dependent inhibition to 17β-estradiol-induced relaxation in WKY aortic rings were impaired in SHR aortic rings. In contrast to 17β-estradiol-induced relaxation, removal of endothelium significantly enhanced progesterone-induced relaxation in both WKY and SHR (Fig. 1⟨2⟩B and C). These results show that endothelium-dependent inhibition to progesterone-induced relaxation was maintained in both WKY and SHR. Both 17β-estradiol- and progesterone-induced vasorelaxation appeared within minutes of each hormone application in both WKY and SHR, and there were no significant differences in the rate of relaxation between WKY and SHR (Table 1). These relaxations were not mediated through receptors of steroid hormone (Fig. 2). These facts suggest that each hormone-induced vasorelaxations were not mediated through the genomic mechanism, and indicate the existence of nongenomic mechanism of 17β-estradiol- and progesterone-induced relaxation in WKY and SHR. The concentrations of 17β-estradiol (0.1 μM) and progesterone (3 μM) that induced relaxation in both rats were almost the same as those in the previous reports in rat saphenous (Kakucs et al., 1998) and rabbit coronary (Jiang et al., 1991) arteries. These concentrations are approximately 100–1000 times higher than those found in premenopausal females (17β-estradiol: 74 pM, progesterone: 32 nM) (Wilson, 1996). It is well known that a disparity exists between the plasma levels of hormones and the levels which induce in vitro vasorelaxation (Yue et al., 1995). It is not known that whether the vascular reactivity of hormones in vitro connect with physiological and pharmacological responses. The main aim of this study is to contribute to the vascular pathophysiological characterization in normotensive and hypertensive aorta.

Glibenclamide, an inhibitor of ATP-sensitive K^+ channels (Standen et al., 1989), 4-aminopyridine, an inhibitor of voltage-sensitive K^+ channels (Ogata and Tatebayashi, 1993) or tetraethylammonium, which blocks large-conductance Ca^{2+}-activated K^+ channels when used at appropriate concentrations (Holland et al., 1996), fails to affect the relaxation induced by 17β-estradiol in WKY (Fig. 3⟨1⟩A, B and C) and the relaxation of WKY was smaller than that of SHR (Fig. 1⟨1⟩A). However, in SHR, both glibenclamide and 4-aminopyridine significantly reduced 17β-estradiol-induced relaxation (Fig. 3⟨1⟩A and B), which suggests that ATP-sensitive and voltage-sensitive K^+ channels partially take part in 17β-estradiol-induced vasorelaxation in SHR only. The function of K^+ channels may be modified in order to prevent the development of a severe hypertension in SHR. Indeed, we have already indicated that testosterone-induced relaxation is greater in aortic rings from SHR than those from WKY, and suggested that it may be due to a modification in the function of K^+ channels caused by hypertension (Honda et al., 1999). In contrast to 17β-estradiol-induced vasorelaxation, these three inhibitors of K^+ channels had no influence on progesterone-induced relaxation of both WKY and SHR aortic rings (Fig. 3⟨2⟩A, B and C) except for tetraethylammonium in WKY. Jiang et al. (1992) have also reported that glibenclamide does not affect progesterone-induced relaxation in rabbit coronary artery. It is of interest that the dependence of arterial smooth muscle on the opening of K^+ channels was greatest in 17β-estradiol-induced relaxation in SHR aortic rings and that progesterone-induced relaxation in SHR was through other mechanisms.

L-NA, which has been reported to inhibit eNOS (Ishii et al., 1990), significantly enhanced 17β-estradiol-induced relaxation in WKY but not in SHR. This is consistent with a previous report that L-NA causes a potentiation of the relaxation response to 17β-estradiol in male Sprague–Daw-

ley rats (Ie et al., 1997). This enhancement mechanism by L-NA in normotensive male rats was not been clarified. It is considered that vasorelaxing and vasocontracting substances which are suppressed and enhanced by NO, respectively, may be released from endothelial and/or smooth muscle cells. It has been reported that decreased levels of NO increase vasorelaxation mediated by endothelium-derived hyperpolarizing factor (EDHF) in porcine coronary arteries (Bauersachs et al., 1996). Furthermore, induction of inducible NOS by bacterial lipopolysaccharides suppressed EDHF-mediated relaxation in porcine coronary arteries (Kristof et al., 1997). Considering these reports, the increased vasorelaxation in WKY aorta may be related to EDHF, which is enhanced by a NOS inhibitor. It is also of interest that the aortic rings in SHR did not have the enhancement mechanisms of relaxation by L-NA. On the other hand, indomethacin, an inhibitor of cyclooxygenase (Ferreira et al., 1971), or metyrapone, an inhibitor of cytochrome P-450 monooxygenase (Satake et al., 1997), had little influence on 17β-estradiol-induced relaxation in both WKY and SHR. Progesterone-induced relaxation was not affected by L-NA, indomethacin or metyrapone in both WKY and SHR. These results indicate that the release of vasodilator prostanoids and P-450 arachidonic metabolites may not be involved in 17β-estradiol- and progesterone-induced relaxation in both WKY and SHR.

High-K^+ depolarization produces an increase in intracellular Ca^{2+} by activating voltage-dependent Ca^{2+} channels, which are inhibited by Ca^{2+} channel blockers such as verapamil and nifedipine (Honda and Sakai, 1987; Bolton, 1979; Yamagishi et al., 1992; Schachtele et al., 1989). Both 17β-estradiol and progesterone shifted Ca^{2+} concentration-dependent contraction curves to the right with the depression of maximal contraction in K^+ depolarization medium in both WKY and SHR. No significant differences in the values of pD_2' for ovarian hormones were seen between WKY and SHR (Table 2), suggesting that there is no difference in the antagonistic effect of 17β-estradiol or progesterone on voltage-dependent Ca^{2+} channels between WKY and SHR. These results suggest that the mechanisms of 17β-estradiol-induced relaxation in SHR aortae are mediated via not only the inhibition of Ca^{2+} channels but also the ATP-sensitive and voltage-sensitive K^+ channels, although those of progesterone-induced relaxation are mainly concerned with the inhibition of Ca^{2+} channels rather than the participation of K^+ channels.

Finally, a difference in 17β-estradiol-induced relaxation between WKY and SHR aortae suggests a possibility that vascular response in SHR aortae is modified by hypertension.

Acknowledgements

We wish to thank Prof. Seiichi Saida of Tokyo University of Pharmacy and Life Science for a critical proofreading of the manuscript.

References

Adams, M.R., Clarkson, J.R., Kaplan, J.R., Koritnik, D.R., 1990. Ovarian secretions and atherosclerosis. In: Naftoli, F., Gutmann, J.N., Decherney, A.H., Sarrel, P.M. (Eds.), Ovarian Secretion and Cardiovascular and Neurological Function. Raven Press, New York, NY, pp. 151–159.

Adams, M.R., Williams, J.K., Kaplan, J.R., 1995. Effects of androgens on coronary artery atherosclerosis and atherosclerosis-related impairment of vascular responsiveness. Arterioscler. Thromb. Vasc. Biol. 15, 562–570.

Bauersachs, J., Popp, R., Hecker, M., Sauer, E., Fleming, I., Busse, R., 1996. Nitric oxide attenuates the release of endothelium-derived hyperpolarizing factor. Circulation 94, 3341–3347.

Belfort, M.A., Saade, G.R., Suresh, M., Vedernlkov, Y.P., 1996. Effects of estradiol-17β and progesterone on isolated human omental artery from premenopausal nonpregnant women and from normotensive and preeclamptic pregnant women. Am. J. Obstet. Gynecol. 174, 246–253.

Bolton, T.B., 1979. Mechanisms of action of transmitters and other substances on smooth muscle. Physiol. Rev. 59, 606–718.

Cambotti, L.J., Cole, F.E., Gerall, A.A., Frohlich, E.D., Macphee, A.A., 1984. Neonatal gonadal hormones and blood pressure in the spontaneously hypertensive rat. Am. J. Physiol. 11, 58–264 (17β-estradiol).

Chan, H.Y., Yao, X., Tsang, S.Y., Chan, F.L., Lau, C.W., Huang, Y., 2001. Different role of endothelium/nitric oxide in 17β-estradiol- and progesterone-induced relaxation in rat arteries. Life Sci. 69, 1609–1617.

Clarkson, T.B., Shively, C.A., Morgan, T.M., Koritnik, D.R., Adams, M.R., Kaplan, J.R., 1990. Oral contraceptives and coronary artery atherosclerosis of cynomolgus monkeys. Obstet. Gynecol. 75, 217–222.

Ferreira, S.H., Moncada, S., Vane, J.R., 1971. Indomethacin and aspirin abolished prostaglandin release from spleen. Nature 231, 233–239.

Gerhard, M., Ganz, P., 1995. How do we explain the clinical benefits of estrogen? From bedside to bench. Circulation 92, 5–8.

Godsland, I.F., Wynn, V., Crook, D., Miller, N.E., 1987. Sex, lipoproteins, and atherosclerosis: prevailing assumptions and outstanding questions. Am. Heart J. 114, 1467–1503.

Holland, M., Langton, P.D., Standen, N.B., Boyle, J.P., 1996. Effects of BKCa channel activator, NS1619, on rat cerebral artery smooth muscle. Br. J. Pharmacol. 117, 119–129.

Honda, H., Sakai, Y., 1987. The mode of action of ifenprodil tartrate in isolated canine cerebral and femoral arteries. Arch. Int. Pharmacodyn. 285, 211–225.

Honda, H., Unemoto, T., Kogo, H., 1999. Different mechanisms of testosterone-induced relaxation of aorta between normotensive and spontaneously hypertensive rats. Hypertension 34, 1232–1236.

Hughes, G.S., Mathu, R.S., Margolius, H.S., 1989. Sex steroid hormones are altered in essential hypertension. J. Hypertens. 7, 181–187.

Ie, Y., Fung, A., Forster, C., 1997. Role of gender and vascular endothelium in rat aorta response to 17β-estradiol. Can. J. Physiol. Pharm. 75, 1393–1397.

Ishii, K., Chang, B., Kerwin, J.F., Huang, Z.J., Murad, F., 1990. N^G-nitro-L-arginine: a potent inhibitor of endothelium-derived relaxing factor formation. Eur. J. Pharmacol. 176, 219–223.

Jiang, C.W., Sarrel, P.M., Lindsay, D.C., Poole-Wilson, P.A., Collins, P., 1991. Endothelium-independent relaxation of rabbit coronary artery by 17β-oestradiol in vitro. Br. J. Pharmacol. 104, 1033–1037.

Jiang, C.W., Sarrel, P.M., Lindsay, D.C., Poole-Wilson, P.A., Collins, P., 1992. Progesterone induces endothelium-independent relaxation of rabbit coronary artery in vitro. Eur. J. Pharmacol. 211, 163–167.

Kakucs, R., Varbiro, S., Szekacs, B., Nadasy, G.L., Acs, N., Monos, E., 1998. Direct relaxing effect of estradiol-17β and progesterone on rat saphenous artery. Microvasc. Res. 56, 139–143.

Khaw, K.T., Barret-Connor, E., 1988. Blood pressure and endogenous testosterone in men: an inverse relationship. J. Hypertens. 6, 329–332.

Kristof, A.S., Noorhosseini, H., Hussain, S.N., 1997. Attenuation of endothelium-dependent hyperpolarizing factor by bacterial lipopolysaccharides. Eur. J. Pharmacol. 328, 69–73.

Ogata, N., Tatebayashi, H., 1993. Differential inhibition of a transient K^+

current by chlorpromazine and 4-aminopyridine in neurons of the rat dorsal root ganglia. Br. J. Pharmacol. 109, 1239–1246.

Satake, N., Shibata, M., Shibata, S., 1997. Endothelium- and cytochrome P-450-dependent relaxation induced by isoproterenol in rat aortic rings. Eur. J. Pharmacol. 319, 37–41.

Schachtele, C., Wagner, B., Rudolph, C., 1989. Effect of Ca^{++} entry blockers on myosin light chain kinase and protein kinase C. Eur. J. Pharmacol. 163, 151–155.

Share, L., Crofton, J.T., Ouchi, Y., 1988. Vasopressin: sexual dimorphism in secretion, cardiovascular actions and hypertension. Am. J. Med. Sci. 295, 314–319.

Stampfer, M.J., Golditz, G.A., Willett, W.C., Manson, J.E., Rosner, B., Speizer, F.E., Hennekens, C.H., 1991. Postmenopausal estrogen therapy and cardiovascular disease. N. Engl. Med. 325, 756–762.

Standen, N.B., Quayle, J.M., Davies, N.W., Brayden, J.E., Huang, Y., Nelson, M.T., 1989. Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. Science 245, 177–180.

Sullivan, J.M., Vander-Zwaag, R., Lemp, G.F., Hughes, J.P., Maddock, V., Kroetz, F.W., Ramanathan, K.B., Mirvis, D.M., 1988. Postmenopausal estrogen use and coronary atherosclerosis. Ann. Intern. Med. 108, 358–363.

Tamura, K., Honda, H., Mimaki, Y., Sashida, Y., Kogo, H., 1997. Inhibitory effect of a new steroidal saponin, OSW-1, on ovarian functions in rats. Br. J. Pharmacol. 121, 1796–1802.

Wild, R.A., Bartholemew, M.J., 1988. The influence of body weight on lipoprotein lipids in patients with polycystic ovary syndrome. Am. J. Obstet. Gynecol. 159, 423–427.

Wilson, J.D., 1996. Hormones and hormone antagonists. In: Hardman, J.G., Goodman Gilman, A., Lee, E.L. (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th ed. McGraw-Hill, Health Professions Division, New York, NY, p. 1443.

Yamagishi, T., Yanagisawa, T., Taira, N., 1992. Ca^{++} influx induced by agonist U46619 is inhibited by hyperpolarization induced by the K^+ channel opener cromakalim in canine coronary artery. Jpn. J. Pharmacol. 59, 291–299.

Yamaguchi, K., Honda, H., Tamura, K., Kogo, H., 2001. Possible mechanisms for the suppressing action of 17β-estradiol on β-adrenoceptor-mediated vasorelaxation in rat aorta. Eur. J. Pharmacol. 427, 61–67.

Yue, R.Y., Chatterjee, K., Beale, C., Wilson, A.P., Collins, P., 1995. Testosterone relaxes rabbit coronary arteries and aorta. Circulation 91, 1154–1160.