Different Mechanisms for Testosterone-Induced Relaxation of Aorta Between Normotensive and Spontaneously Hypertensive Rats

Hideo Honda, Tamao Unemoto, Hiroshi Kogo

Abstract—The tension in isolated ring preparations of the thoracic aortae from Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) was measured isometrically to study the differences in testosterone-induced relaxation between WKY and SHR aortic rings. Testosterone (9 to 300 μmol/L) induced a concentration-dependent relaxation in both WKY and SHR aortic rings, and the relaxation induced by testosterone was greater in SHR than WKY. The relaxation induced by testosterone was significantly reduced by denudation of endothelium in SHR but not WKY. Indomethacin, an inhibitor of cyclooxygenase, and N⁶-nitro-L-arginine, an inhibitor of nitric oxide (NO) synthase, showed little influence on the relaxation induced by testosterone in both WKY and SHR aortic rings. Glibenclamide, a selective inhibitor of ATP-sensitive potassium channels, significantly reduced the relaxation induced by testosterone in both WKY and SHR aortic rings, although the extent of reduction was greater in WKY than SHR. On the other hand, 4-aminopyridine, a selective inhibitor of voltage-dependent potassium channels, and tetraethylammonium, an inhibitor of calcium-activated potassium channels, significantly reduced the relaxation induced by testosterone in SHR but not WKY. These results suggest that the mechanisms of testosterone-induced vasorelaxation in both WKY and SHR involve, in part, ATP-sensitive potassium channels in the thoracic aortae and that in SHR aortic rings, testosterone may release endothelium-derived substances that may cause hyperpolarization of the cells by a mechanism that involves potassium channels. Moreover, the data show differences between WKY and SHR in the function of ATP-sensitive, voltage-dependent, and calcium-activated potassium channels. (Hypertension. 1999;34:1232-1236.)

Key Words: testosterone □ endothelium □ rats, inbred SHR □ rats, inbred WKY □ aorta □ potassium channels

Hypertension and coronary heart disease occur more frequently in men than in premenopausal women.¹ A decrease in LDLs and an increase in HDLs are considered to be one of the mechanisms by which estrogen reduces the risk factors of coronary heart disease.² Studies have reported that hypertensive men and premenopausal women have lower levels of plasma androgen but higher levels of plasma estrogen than controls.³,⁴ Males become more hypertensive than females in genetic and nongenetic rat models of hypertension, and this sexual dimorphism is reduced by gonadectomy.⁵,⁶ Also, reports have indicated that androgen may contribute to the development of hypertension in spontaneous hypertensive rats (SHR) through sustained enhancement of tyrosine hydroxylase activity, which leads to increased norepinephrine (NE) levels in blood vessels.⁷,⁸ On the other hand, several reports consider the direct relaxing effects of estrogen on the vasculature in vitro,⁹–¹³ but few consider the direct relaxing effects of testosterone on the vasculature,¹⁴,¹⁵ and only normotensive animals have been used in these studies. Whether these sex steroids affect pharmacological or physiological actions on the vasculature is unknown. However, the purpose of the present study is to compare the effects of testosterone on vascular reactivity in thoracic aorta isolated from Wistar-Kyoto rats (WKY) and SHR and to discover the difference between hypertension and normotension in the reactivity of the aortae.

Methods

Animals and Tissues

All procedures were performed in accordance with institutional guidelines 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 euthanatized by exsanguination. The thoracic aorta was isolated and placed in modified Krebs-Henseleit solution (pH 7.4) of the following composition (in mmol/L): NaCl 118.0, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25.0, and glucose 11 at 37°C gassed with 95% O₂/5% CO₂. The aortic tissue was cleaned by removing connective tissue. The thoracic aorta was cut into rings ~4 mm long. Contraction and relaxation were measured by suspend-
Effects of Potassium Channel Blockers on Testosterone-Induced Relaxation

To determine the possible effects of ATP-sensitive, voltage-dependent, or calcium-activated potassium channels on testosterone-induced relaxation, glibenclamide, a selective inhibitor of ATP-sensitive potassium channels (Glu, L-NA), N \textsuperscript{G}-nitro-l-arginine (L-NA), an inhibitor of NO synthase, or teatethylyphamonium (TEA), an inhibitor of calcium-activated potassium channels, was added to the solution 5 minutes before treatment with NE.

Effects of \textsuperscript{N}G-nitro-l-arginine and Indomethacin on Testosterone-Induced Relaxation

\textsuperscript{N}G-nitro-l-arginine (L-NA), an inhibitor of NO synthase, or indomethacin, an inhibitor of cyclooxygenase, was added to the solution 5 minutes before treatment with NE to observe the effects on testosterone-induced relaxation.

Drugs and Chemicals

Testosterone and glibenclamide (Sigma Chemical Co) were dissolved in ethanol (final concentration of ethanol in bath ≤0.5%, with no influence on NE-induced contraction). NE hydrochloride, ACh chloride, SNP, 4-aminoypyridine, TEA, and L-NA (Sigma) were dissolved in distilled water. Indomethacin was dissolved in 4% (wt/vol) NaHCO\textsubscript{3}. Other chemicals were of analytical grade and obtained from Wako Pure Chemical Co Ltd.
without endothelium, respectively. A significant difference ($P < 0.05$) existed between WKY aortic rings with and without endothelium.

The time (in minutes) to reach the half-maximal and maximal relaxation induced by testosterone (75 $\mu$mol/L) was not different between SHR and WKY aortic rings, but a significant difference was seen in the maximal relaxation induced by testosterone (75 $\mu$mol/L) between SHR and WKY aortic rings (Table 2).

**Effects of Glibenclamide, 4-Aminopyridine, and TEA on Testosterone-Induced Relaxation**

To examine the involvement of potassium channels in the relaxant action of testosterone, the effects of pretreatment with glibenclamide, 4-aminopyridine, and TEA were investigated. Application of glibenclamide (3 $\mu$mol/L) significantly reduced testosterone-induced relaxation in both WKY and SHR aortic rings, and the reduction was greater in WKY than SHR (Figure 3A). 4-Aminopyridine (1 mmol/L) significantly reduced testosterone-induced relaxation only in SHR aortic rings and had no influence on the relaxation in WKY aortic rings (Figure 3B). TEA (1 mmol/L) significantly reduced testosterone-induced relaxation in SHR aortic rings and somewhat reduced the relaxation in WKY aortic rings (Figure 3C).

**Effects of L-NA and Indomethacin on Testosterone-Induced Relaxation**

Preincubation with L-NA (100 $\mu$mol/L) or the prostaglandin synthase inhibitor indomethacin (10 $\mu$mol/L) did not significantly reduce testosterone-induced relaxation in both SHR and WKY aortic rings (Table 3).
voltage-dependent calcium channels in the vascular smooth muscle have been attributed to the inhibition of calcium influx in the vascular smooth muscle cell. In contrast to estrogen, the rapid vasorelaxing effects of testosterone have not been attributed to the inhibition of calcium influx in the vascular smooth muscle. The concentrations of testosterone (38 μmol/L) that induced the relaxation of SHR and WKY thoracic aorta are almost the same as those in the previous reports in rabbit coronary arteries and Sprague-Dawley rat aorta, and these concentrations are 1000 times higher than those found in normal men (35 nmol/L) and SHR (17±3 nmol/L from 9 rats in our laboratory). It is well known that a disparity exists between plasma levels of testosterone and the levels that induce in vitro vasorelaxation.

L-NA, which has been reported to inhibit endothelial NO synthase, failed to affect the relaxation induced by testosterone in SHR and WKY aortic rings. Further, indomethacin, which inhibits the synthesis of prostaglandin, had little influence on the relaxation induced by testosterone in SHR and WKY aortic rings (Figure 4). These results indicate that the release of vasodilator NO and prostanoids is not involved in testosterone-induced relaxation in SHR and WKY aortic rings. Our present results are in accordance with recent results in rabbit aorta and coronary artery that indicate that vasodilator NO and prostanoids are not candidate contributors to testosterone-induced relaxation. However, Costarella et al indicated that L-NA methyl ester, an inhibitor of NO synthase, suppressed the inhibitory effect of testosterone on phentolamine-induced contraction, which suggests that the vasorelaxing effect of testosterone in the endothelium-intact aorta from Sprague-Dawley rat can be attributed to the release of NO. These discrepancies may be due to differences in experimental conditions, species, or strains.

Glibenclamide, an inhibitor of ATP-sensitive potassium channels, significantly reduced testosterone-induced relaxation in both SHR and WKY aortic rings, and the reduction was greater in WKY than SHR aortic rings. Notably, the dependence of arterial smooth muscle on open of ATP-sensitive potassium channels is greater in WKY than SHR in testosterone-induced relaxation. We suggest that spontaneous hypertension causes dysfunction of ATP-sensitive potassium channels in arterial smooth muscle. These results appear to show that ATP-sensitive potassium channels play an important role in the pathophysiology of hypertension in SHR. 4-Aminopyridine, an inhibitor of voltage-dependent potassium channels, significantly reduced testosterone-induced relaxation in SHR but not WKY aortic rings. TEA, which blocks large-conductance calcium-activated potassium channels when used at appropriate concentrations, significantly reduced testosterone-induced relaxation in SHR aortic rings. However, TEA could not significantly reduce testosterone-induced relaxation in WKY aortic rings. These results also suggest that the mechanism of testosterone-induced relaxation mainly involves ATP-sensitive potassium channels in vascular smooth muscle in WKY. In SHR, testosterone may release, in part, endothelium-derived substances that may cause hyperpolarization of the underlying smooth muscle cells by a mechanism that involves both voltage-dependent and calcium-activated potassium channels. The relaxation induced by testosterone was greater in aortic rings from SHR than WKY, which suggests that both voltage-dependent and
calcium-activated potassium channels may take part in the ability of testosterone to induce relaxation in aortic rings from SHR. Furthermore, because of the dysfunction of ATP-sensitive potassium channels in vascular smooth muscle, both voltage-dependent and calcium-activated potassium channels may be modified and contribute to the suppression of the development of a severe hypertension in SHR.

In conclusion, we have demonstrated that testosterone induces both endothelium-dependent and -independent relaxation in SHR aortic rings but only endothelium-independent relaxation in WKY aortic rings. Testosterone-induced relaxation is greater in SHR than WKY. The mechanism may involve vascular smooth muscle potassium channels in both SHR and WKY. Furthermore, testosterone-induced relaxation in SHR appears to be mediated by the release of endothelium-derived substances that may open both voltage-dependent and calcium-activated potassium channels in the vascular smooth muscle.

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References

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