Role of BK Channels in Testosterone-Induced Relaxation of the Aorta in Spontaneously Hypertensive Rats

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The previous data indicated that the testosterone (Tes)-induced relaxation of thoracic aorta is greater in spontaneously hypertensive rats (SHR) than in normotensive rats (Wistar-Kyoto rats; WKY) and that there were differences between SHR and WKY in the functions of KATP, K Ca, and K Ca channels. The present study was carried out to ascertain the mechanisms of the Tes-induced relaxation. Indomethacin (30 μM) pretreatment suppressed the Tes-induced relaxation. Following noradrenalin (NA)-induced vasoconstriction, the relaxation induced by Tes was significantly attenuated by endothelium removal in SHR (not in WKY), but the dilatary effect of Tes following KCl-induced vasoconstriction was not attenuated by endothelium removal. After tetraethylammonium (K Ca channel inhibitor) or iberiotoxin (large conductance, Ca 2+ activated BK channel inhibitor) pretreatment, the Tes-induced relaxation was attenuated in SHR, but not in WKY. This attenuation in SHR was not observed after endothelium removal. The above results suggest that the relaxation induced by Tes following NA-induced vasoconstriction in SHR results from hyperpolarization due to BK channel opening.

Key words testosterone; spontaneously hypertensive rat (SHR); BK channel; endothelium; indomethacin; iberiotoxin

MATERIALS AND METHODS

Animals and Tissues All animal-handling protocols and surgical procedures were approved by the Animal Care Committee at Tokyo University of Pharmacy and Life Sciences in compliance with the institutional guidelines for experimental animal care. Ten-week-old male WKY/NCnj and SHR/NCnj (250—285 g) were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). The rats were etherized and euthanatized by exsanguination. The chest was opened to excise the thoracic aorta and the isolated aorta was placed in modified Krebs-Henseleit solution (pH 7.4) of the composition (in mmol/l) of 118.0 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 25.0 mM NaHCO3 and 11 mM glucose at 37°C gassed with 95% O2/5% CO2. The aortic tissue was cleaned by removing the connective tissue. The thoracic aorta was cut into rings about 4 mm long. The contraction and relaxation were measured by suspending the rings between two stainless steel hooks, one of which was attached to

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the end of a bathing tube and the other to a force transducer (45196A NEC San-ei Instruments). The isometric tension changes were recorded on a polygraph (LECTHORIZ-8K NEC San-ei) as previously described.12

Relaxation of the NA-Precontracted Aorta Induced by Tes Each ring was equilibrated in the 10 ml bathing solution for 60 min before experiment. Following the equilibration, the ring contraction was confirmed using KCl (50 mM). After confirming the optimal loading weight for vessels in a preliminary study, the resting tension was set at 0.7 g, which was the optimal preload for force development in these blood vessels, as previously described.2,6 For the relaxation studies, the submaximal tone (80% of the maximal tone) was induced with NA (300 nM) and then Tes (9—150 μM) was added in a cumulative fashion, and the relaxing effects were compared between endothelium-intact and endothelium-denuded rings. To assess the role of the endothelium in the vascular response to Tes, the thoracic aortae were denudationed before being mounted by gently rubbing the luminal surface with stainless steel. The intact endothelium and the endothelial removal were confirmed with acetylcholine (ACh). Responses were expressed as the relaxation percentage of NA-induced tone, and the relaxation in the absence of the drug was taken to be 0%.

Effects of Ind on Tes-Induced Relaxation To examine the role of prostaglandins on Tes-induced relaxation, Ind (10 or 30 μM), an inhibitor of cyclooxygenase, was added to Krebs–Henseleit solution 10 min before NA-induced vasoconstriction.

Tes-Induced Relaxation between NA- and KCl-Induced Vasoconstriction To confirm the involvement of K⁺ channels, the relaxation induced by Tes (75 μM) was compared by using the aortic rings precontracted with NA (300 nM) or KCl (50 mM) in SHR and WKY, followed by the examination of the effects of endothelium removal on the relaxation of KCl-induced vasoconstriction.

Effects of TEA and Iberiotoxin (Ib-Tx) on Tes-Induced Relaxation To observe the involvement of K channels on the Tes (75 μM)-induced relaxation, TEA (KCa channel inhibitor; 1 mM) or Ib-Tx (BK channel inhibitor; 20 nM) was added to the Krebs–Henseleit solution 10 min before the NA-induced vasoconstriction.

Drugs and Chemicals Testosterone (Wako Pure Chemical Co., Ltd., Tokyo, Japan) was dissolved in ethanol (the final concentration of ethanol was less than 0.5% in a bath, with no influence on the NA-induced contraction). Noradrenaline hydrochloride, acetylcholine chloride and TEA (Sigma) were dissolved in distilled water. Indomethacin was dissolved in 4% (wt/vol) NaHCO₃. Ib-Tx (Buthus tamulus; recombinant protein expressed in E. coli) was dissolved in Tris–HCl buffer (pH 7.5) containing 0.1% bovine serum albumin.

Statistical Analysis All data are expressed as means±S.E.M. The relaxation is expressed as percentage relaxation of contraction induced by agonists. The results were analyzed with Student’s t-test and Tukey–Kramer test. A probability value of less than 5% was considered significant.

RESULTS

Cumulative Relaxation by Tes with or without Endothelium in Thoracic Aorta Precontracted with NA in SHR and WKY Figure 1 shows a dose–response relationship for the relaxation induced by Tes in the thoracic aorta precontracted with NA in both WKY and SHR aortic rings. The Tes-induced relaxation was dose-dependent regardless of the presence or the absence of endothelium. But the relaxation in thoracic aorta with endothelium was clearly greater in SHR than that in WKY. Endothelium removal attenuated the Tes-induced relaxation in SHR, but not in WKY. On the basis of the results obtained here, the concentration of Tes was set at 75 μM in the subsequent experiments on Tes-induced relaxation.

Effects of Ind on Cumulative Relaxation by Tes in Thoracic Aorta Precontracted with NA in SHR To examine the involvement of prostaglandin in the relaxant action of Tes, the effects of pretreatment with Ind at 10 or 30 μM were investigated (Fig. 2). In SHR, the Tes-induced cumulative relaxation was significantly attenuated by pretreatment with Ind at 30 μM compared to control, though Ind at 10 μM only tended to inhibit the relaxation. In WKY, 10 or 30 μM Ind pretreatment had no effect (data not shown).

Differences in Tes-Induced Relaxation between NA- and KCl-Induced Vasoconstriction in SHR and WKY and the Effects of Endothelium Removal on the Relaxation Following NA- and KCl-Induced Vasoconstriction in SHR The Tes-induced relaxation was compared by...
using the aortic rings precontracted with NA or KCl, and the effects of endothelial denudation on the relaxation following NA- or KCl-induced vasoconstriction were examined (Fig. 3). In SHR, the Tes-induced relaxation following KCl-induced vasoconstriction was markedly attenuated compared to that following NA-induced vasoconstriction. On the other hand, there was no significant difference in WKY in the Tes-induced relaxation between NA- and KCl-induced vasoconstrictions. Moreover, there was no significant difference in the Tes-induced relaxation following the KCl-induced vasoconstriction between SHR and WKY. Furthermore, the Tes-induced relaxation of aortic rings precontracted with KCl in SHR was not affected by the presence or absence of endothelium.

Effects of TEA Pretreatment on Tes-Induced Relaxation Following NA-Induced Vasoconstriction in SHR and WKY and Effects of TEA on Relaxation of Tes in Endothelium-Removed SHR Whether K⁺ channels take part in the relaxation induced by Tes was examined. In SHR, the Tes-induced relaxation following KCl-induced vasoconstriction was markedly attenuated compared to that following NA-induced vasoconstriction. On the other hand, there was no significant difference in WKY in the Tes-induced relaxation between NA- and KCl-induced vasoconstrictions. Moreover, there was no significant difference in the Tes-induced relaxation following the KCl-induced vasoconstriction between SHR and WKY. Furthermore, the Tes-induced relaxation of aortic rings precontracted with KCl in SHR was not affected by the presence or absence of endothelium.

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**DISCUSSION**

The present study has indicated that a marked relaxation elicited by Tes following the NA-induced vasoconstriction in SHR aorta compared to that in WKY was considerably attenuated by endothelium removal (Fig. 1). We investigated the mechanisms of endothelium-mediated relaxation by focusing on the fact that endothelium removal attenuated the Tes-induced relaxation in SHR. Previously, the involvement of arachidonate metabolites in the Tes-induced relaxation has been investigated by using Ind (10 μM), and Ind showed a tendency to decrease the relaxation, though not significantly, in SHR. The present study was carried out by using 10 or 30 μM of Ind, and only in SHR, the Tes induced relaxation was significantly attenuated by pretreatment with 30 μM Ind (Fig. 2). These results suggest that the thoracic aorta relaxation in SHR might involve endothelium-derived PG (PGI₂, etc.). However, because the Tes relaxation was not significantly affected by 10 μM Ind, though the relaxation tended to be suppressed, other factors [nonspecific action(s)] may be involved.

It has also been reported that there are differences in K⁺ (K<sub>ATP</sub>, K<sub>V</sub>, K<sub>Ca</sub>) channel functions between SHR and WKY; that is, in WKY, mainly K<sub>ATP</sub> channels act on the Tes relaxation, but, in SHR, three K⁺ channels associates, suggesting the involvement of K<sub>V</sub> and K<sub>Ca</sub> channels besides the K<sub>ATP</sub> channels.
as the compensatory mechanisms for endothelial dysfunction to suppress the development of severe hypertension in SHR. In the present study, in order to further confirm the involvement of K^+ channels in SHR, the relaxation induced by Tes following KCl vasoconstriction was compared with that in WKY (Fig. 3). In WKY, the Tes-induced relaxation following Na^- or KCl-induced vasoconstriction did not show any significant difference between the two vasoconstrictors. On the other hand, in SHR, the significant increment of the Tesla-induced relaxation following Na^-induced vasoconstriction did not appear in the case using KCl as vasoconstrictor, and in SHR, the relaxation induced by Tes after KCl contraction was of almost the same levels with or without the endothelium (Fig. 3). These findings suggest that, in SHR, Tes directly or indirectly acts on K^+ channels in smooth muscles to induce relaxation via endothelium-derived relaxing factors. The K_Ca channels of vascular smooth muscle cells can be divided into three groups; large-conductance Ca^{2+} activated K^+ channels (BK channels), intermediate-conductance Ca^{2+} activated K^+ channels (IK channels), and small-conductance Ca^{2+} activated K^+ channels (SK channels). In this study, we used TEA (a K_Ca channel inhibitor) and Ib-Tx (a BK channel inhibitor). In SHR, TEA pretreatment significantly decreased the Tes-induced relaxation, but had no effect on the relaxation in WKY (Fig. 4A). Moreover, TEA pretreatment did not affect the relaxation in endothelium-stripped SHR aortic rings (Fig. 4B). Therefore, it is possible that the Tes-induced relaxation of SHR thoracic aorta involves K_Ca channels, and that the K_Ca channels are endothelium-dependent. This mechanism does not appear to exist in WKY. Moreover, it is thought that, in SHR, K_Ca channels of vascular smooth muscles are compensatorily activated to suppress vascular tension. Apamin has been reported as an SK channel inhibitor. Prieto et al. have reported that, in penile resistance arteries, apamin had no effect on the relaxation elicited by sildenafil, though K_Ca channels were involved. Next, in order to evaluate BK channel involvement, Ib-Tx pretreatment was performed. Ib-Tx attenuated the relaxation induced by Tes in SHR, but not in WKY (Fig. 5A). The relaxation in SHR aortic rings was negated by removal of the endothelium (Fig. 5B), suggesting that there is an endothelium-dependent mechanism for BK channel activation, that the endothelium-mediated mechanism contributed to the difference in the Tes-induced vascular relaxation between SHR and WKY, and that the relaxation induced by Tes is associated with BK channel opening. Asano et al. indicated that BK channel activities in carotid arteries were greater in SHR than in WKY, and Ib-Tx caused a marked vasoconstriction in SHR, suggesting that there is a difference between SHR and WKY in the control mechanism of BK channels. While Ib-Tx attenuated the Tes-induced relaxation of porcine coronary arteries, it did not affect rabbit coronary arteries, suggesting differences in the responsiveness among species. It has also been reported that beraprost, a PG12 agonist, activates BK channels in guinea pig aorta, and induces the relaxation by functionally coupling with the PG12 receptor.

In conclusion, we have demonstrated that the marked relaxing response induced by Tes in thoracic aorta precontracted by NA in SHR was endothelium-dependent, and the mechanism may be associated with the hyperpolarization due to the endothelium-dependent BK channels induced by an endothelium-derived substance, like prostaglandin (PG12, etc.), in vascular smooth muscle cells. This relaxation mechanism was not recognized in WKY. The different responsiveness in the Tes-induced relaxation between SHR and WKY aorta suggests that the vascular response in SHR aorta may be modified by hypertension.

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