Diminution of angiotensin II-induced contraction of the abdominal aorta isolated from Watanabe heritable hyperlipidemic rabbits

Katsunari TASAKI¹, Ichiro WAKABAYASHI¹, Tetsuro SHISHIDO², Satoshi TAKASAKI², Yasuchika TAKEISHI², Isao KUBOTA³, Tsunekata ITO⁴, Yumi KATANO⁴ and Hitonobu TOMOIKE⁵

¹Department of Hygiene & Preventive Medicine,
²First Department of Internal Medicine,
³Laboratory Animal Center,
⁴Division of Clinical Nursing and Pathophysiology,
Yamagata University School of Medicine, Yamagata 990-9585, Japan
⁵National Cardiovascular Center, Suita, Osaka 565-8565, Japan

Abstract

The purpose of this study was to investigate the changes in vasocontractile responses in atherosclerosis, using abdominal aortic strips isolated from Watanabe heritable hyperlipidemic (WHHL) rabbits and Japanese White (control) rabbits. The aortic strips from WHHL rabbits showed a significantly lower contractile response to angiotensin II than that in strips from control rabbits. The contractile responses to phenylephrine and 5-hydroxytryptamine were not different in WHHL and control groups. The contractile response to angiotensin II was higher in endothelium-denuded aortic strips than in endothelium-intact strips, but to a greater extent in the control group than in the WHHL group. The contractile response to angiotensin II in the absence of the endothelium was also lower in the WHHL group than in the control group. Pretreatment with N⁶-nitro-L-arginine significantly increased the contractile response to angiotensin II in the endothelium-intact aortic strips in both the WHHL and control groups, while pretreatment with diclofenac did not affects the aortic contractile response to angiotensin II. The contractile responses to angiotensin II in the presence of N⁶-nitro-L-arginine and diclofenac were lower in the WHHL group than in the control group. The contractile response to angiotensin II in the presence of PD123319 was also lower in the WHHL group than in the control group. Endothelium-dependent relaxation by acetylcholine occurred to the same extent in the WHHL and control groups. These results suggest that the WHHL rabbit abdominal aorta displays attenuated angiotensin II-induced contraction, mainly due to an abnormality in the angiotensin II-specific contractile pathway of the medial smooth muscle.

Key words: hyperlipidemia, atherosclerosis, vasoconstriction, angiotensin II, endothelium

Correspondence to: Ichiro Wakabayashi, Department of Hygiene & Preventive Medicine, Yamagata University School of Medicine, Iida-Nishi 2-2-2, Yamagata 990-9585, Japan
Phone: +81-23-628-5252 Fax: +81-23-628-5255 e-mail: wakabaya@med.id.yamagata-u.ac.jp
Introduction

Both dyslipidemia and hypertension are major risk factors during the progress of atherosclerosis. As a positive correlation between blood pressure and serum cholesterol level is known from epidemiological studies (Kannel, 1988), it is of interest to investigate how dyslipidemia affects arterial tone. Abnormalities in vasocontractile responses have been reported in animals with atherosclerosis induced by hypercholesterolemia. 5-Hydroxytryptamine-induced contraction is known to be increased in arteries of Watanabe heritable hyperlipidemic (WHHL) rabbits (Yokoyama et al., 1983) as well as in those of rabbits with diet-induced hyperlipidemia (Henry and Yokoyama, 1980; Merkel et al., 1990; Chin et al., 1990). However, the reported change in angiotensin II contraction in atherosclerotic vessels is not consistent (Merkel et al., 1990; Dam et al., 1997; Yang et al., 1998). The rabbit thoracic aorta has often been used for the study of vasocontractility in experimental atherosclerosis. On the other hand, little is known about the vascular response of the abdominal aorta isolated from WHHL rabbits. We have recently reported that angiotensin II-induced contraction was attenuated in the thoracic aorta isolated from WHHL rabbits (Shishido et al., 2004). However, the detailed mechanism of this attenuation of angiotensin II contraction still remains to be determined. Angiotensin II is a major vasoactive substance involved in the pathophysiology of hypertension and atherosclerosis. In addition to having a potent vasoconstrictive action, angiotensin II induces the release of vasodilatory substances such as nitric oxide (NO) and prostacyclin, mainly from the vascular endothelium (Vane and Botting, 1993). These suppress the contractile component of the vascular response to angiotensin II. Thus, the angiotensin II-induced vasocontractile response is increased in the absence of the endothelium (Yilmaz et al., 1987; Zhang et al., 1994). In the present study, we investigated whether angiotensin II-induced contraction was altered in the abdominal aorta of WHHL rabbits and was modulated by vasodilatory substances released from the vessels.

Materials and Methods

Animals

The study protocols regarding treatment of animals were in accordance with the Guidelines for Experiments Using Laboratory Animals in Yamagata University School of Medicine. Male WHHL rabbits and Japanese White (control) rabbits aged 3–4 months were used in the present study. Each rabbit was housed individually in a controlled environment with unlimited access to water and was fed standard rabbit chow (120 g/day, Labo R Grower, Nihon Nosen Kogyo, Ltd., Tokyo, Japan). The animals were anesthetized with an intravenous administration of 30 mg/kg sodium pentobarbital, and segments of the abdominal aorta were carefully removed and immediately immersed in ice-cold Krebs-Henseleit solution for isometric tension study.

Tissue preparation

Excess fat and connective tissue were carefully removed from the dissected abdominal aortae. The vessels were cut into 3 mm long rings which were then cut open. In some strips,
the endothelium was removed by gentle rubbing of the intimal surface with a moistened cotton swab.

**Tension measurement**

Each aortic strip was suspended in an organ bath containing 10 ml of Krebs-Henseleit solution. The composition of the solution was as follows (in mM): NaCl 118, KCl 4.7, NaHCO₃ 24.9, MgSO₄ 1.18, KH₂PO₄ 1.18, CaCl₂ 2.5, glucose 11.1, and ascorbic acid 0.057. The solution was saturated with a mixture of 95% O₂ and 5% CO₂ at 37°C (pH 7.4). The developed tension was recorded with an isometric force transducer (7T-15-240, Orientec, Tokyo, Japan). After an equilibration period of 1 hr with a resting tension of 1 g, each strip was contracted with 66.7 mM KCl repeatedly until a reproducible contraction was obtained. A solution containing a high concentration of K¹ solution was made by substituting NaCl with equimolar KCl. Contraction level was expressed as a percentage of the maximal contraction induced by the high potassium solution. Ascorbic acid (0.057 mM) did not affect angiotensin II-induced contraction of aortic strips isolated from WHHL and control rabbits. Relaxation level was expressed as a percentage of the pre-contractile tension induced by phenylephrine (100 nM). Removal of the endothelium was verified by the disappearance of relaxation induced by acetylcholine (1 μM) in strips precontracted with phenylephrine (100 nM).

**Measurement of blood lipids**

Blood was sampled from a marginal ear artery 18 hr after the last feed. Plasma was separated from the blood samples by centrifugation and stored at −80°C until measurement. Plasma triglyceride and total cholesterol concentrations were measured by enzymatic methods using commercial kits.

**Drugs**

The drugs used were as follows: angiotensin II, phenylephrine hydrochloride, 5-hydroxytryptamine creatinine sulfate, N⁶-nitro-L-arginine, diclofenac and PD123319 (Sigma Chemical, St. Louis, MO, U.S.A.). Angiotensin II, phenylephrine and 5-hydroxytryptamine were dissolved in distilled water to make stock solutions of 0.1 mM, 10 mM and 10 mM, respectively, and diluted with 0.9% NaCl before use. N⁶-nitro-L-arginine and diclofenac were dissolved in distilled water to make stock solutions of 10 mM and diluted with 0.9% NaCl before use. PD123319 was dissolved in dimethylsulfoxide to make a stock solution of 10 mM and diluted with 0.9% NaCl before use.

**Statistics**

Data was expressed as the mean ± S.E.M. Statistical analysis was done using repeated measures ANOVA (analysis of variance) followed by the Scheffé F-test. P<0.05 was considered to be statistically significant.
Table 1  Body weight and serum lipid levels of WHHL rabbits and control JW rabbits.

<table>
<thead>
<tr>
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<th>Body weight (kg)</th>
<th>Serum triglyceride (mg/dl)</th>
<th>Serum total cholesterol (mg/dl)</th>
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<tbody>
<tr>
<td>WHHL rabbits</td>
<td>2.30 ± 0.01*</td>
<td>2035.9 ± 445.4*</td>
<td>1131.5 ± 65.5*</td>
</tr>
<tr>
<td>Control rabbits</td>
<td>2.53 ± 0.01</td>
<td>65.0 ± 18.9</td>
<td>53.2 ± 3.6</td>
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</tbody>
</table>

Data are shown as the mean ± SE. Asterisks denote significant difference from values of the control rabbits (P<0.01). n=17-20.

Fig. 1. Representative macroscopic images of aortae isolated from Watanabe hereditary hyperlipidemic (WHHL) and Japanese White (control) rabbits. Note the marked plaques in the thoracic region of the WHHL aorta.

Results

Body weight and blood lipid levels

Table 1 provides a comparison of the body weight and blood lipid levels of the WHHL and control rabbits. Body weight was slightly but significantly lower in the WHHL rabbits than in the control rabbits. Serum triglyceride and total cholesterol levels were markedly higher in the WHHL rabbits than in the control group.

Macroscopic observation of the aortae

Figure 1 compares representative macroscopic images of a descending aorta isolated from both a WHHL and a control rabbit. In the control rabbit aorta, no atherosclerotic plaques were observed. In the WHHL rabbit aorta, the thoracic portion of the descending aorta displayed large plaques, while plaques were not apparent in the abdominal portion of the aorta.
Angiotensin II-induced contraction in hyperlipidemic rabbit aorta

![Graph showing contraction vs. phenylephrine concentration](image)

**Fig. 2.** Phenylephrine-induced contraction of abdominal aortic strips prepared from Watanabe heritable hyperlipidemic (WHHL) and control rabbits. E+, endothelium-intact; E−, endothelium-denuded. n=6-10.

![Graph showing contraction vs. 5-hydroxytryptamine concentration](image)

**Fig. 3.** 5-Hydroxytryptamine-induced contraction of abdominal aortic strips prepared from Watanabe heritable hyperlipidemic (WHHL) and control rabbits. E+, endothelium-intact; E−, endothelium-denuded. n=5-7.

**Contractile responses to KCl, phenylephrine and 5-hydroxytryptamine**

The maximum level of KCl-induced contraction of aortic strips was not significantly different between WHHL rabbits and control rabbits [8.39 ± 0.42 mN/mg tissue (WHHL) v.s. 8.53 ± 0.35 mN/mg tissue (control)]. Thus, we used this as the standard level of contraction for each strip. Both phenylephrine- and 5-hydroxytryptamine-induced contractile responses of aortic strips occurred to the same extent in both the WHHL and control groups (Figs. 2 and 3).

**Angiotensin II-induced contractile responses in the presence or absence of endothelium**

Figure 4 displays the concentration-force relationships of angiotensin II contracture of the aorta isolated from both WHHL and control rabbits in the presence or absence of endothelium. In each case, the angiotensin II-induced contraction was higher in strips without endothelium than in those with the endothelium. However, the increment in the angiotensin II-induced contraction which occurred after removal of the endothelium was less in the WHHL aorta than in the control aorta. Regardless of the presence of the endothelium, angiotensin II-induced contraction in the WHHL group was significantly lower than in the control group.
Fig. 4. Angiotensin II-induced contraction of abdominal aortic strips prepared from Watanabe heritable hyperlipidemic (WHHL) and control rabbits. E+, endothelium-intact; E−, endothelium-denuded. *, significantly different (P<0.05) from the values of strips isolated from the control rabbits; †, significantly different (P<0.05) from the values of strips with endothelium. n=5–12.

Fig. 5. Angiotensin II-induced contraction of abdominal aortic strips (endothelium-intact) prepared from Watanabe heritable hyperlipidemic (WHHL) and control rabbits. LNA: The aortic strips were pretreated with Nω-nitro-L-arginine (LNA, 100 μM) for 20 min before stimulation with angiotensin II. *, significantly different (P<0.05) from the values of strips isolated from the control rabbits; †, significantly different (P<0.05) from the values of strips incubated with Nω-nitro-L-arginine. n=5–12.

Angiotensin II-induced contraction in the presence of nitro-L-arginine

In both the WHHL and control groups, pretreatment with nitro-L-arginine significantly enhanced the contractile response to angiotensin II in aortic strips with intact endothelium (Fig. 5). Angiotensin II-induced contraction in the presence of nitro-L-arginine was significantly lower in the WHHL group than in the control group.

Angiotensin II-induced contraction in the presence of diclofenac

In both the WHHL and control groups, the contractile response to angiotensin II in the aorta with the intact endothelium was not significantly affected by diclofenac (Fig. 6). The angiotensin II-induced contraction in the presence of diclofenac was significantly lower in the WHHL group than in the control group.
Angiotensin II-induced contraction in hyperlipidemic rabbit aorta

Angiotensin II-induced contraction in the presence of PD123319

In both the WHHL and control groups, PD123319 only slightly but not significantly increased the angiotensin II-induced contraction of aortic strips with an intact endothelium (Fig. 7). The angiotensin II-induced contraction in the presence of PD123319 was significantly lower in the WHHL group than in the control group.

Acetylcholine-induced relaxing response

Acetylcholine-induced relaxation of aortic strips occurred to the same extent in both the WHHL and control groups (Fig. 8).
Discussion

There have been conflicting results reported about the contractile responses of atherosclerotic arteries to angiotensin II. Dam et al. (1997) reported that contractile responses to angiotensin II and methoxamine were decreased in the thoracic aorta but not in the iliac artery of rabbits fed with a cholesterol diet (0.3%) for 12 weeks. On the contrary, Yang et al. (1998) found that angiotensin II contraction, as well as its type 1 receptor expression, was increased in the thoracic aorta isolated from rabbits fed with a diet of cholesterol (1%) and coconut oil (4%) for 10 weeks. Moreover, Merkel et al. (1990) observed no change in angiotensin II-induced contraction of the abdominal aorta isolated from rabbits fed with a cholesterol-free, casein-rich diet for 10 weeks. These discrepant results might be due to the stage and severity of the atherosclerosis in the vessels used for the experiments. On the other hand, little is known about changes in angiotensin II contraction in arteries of WHHL rabbits. Our recent study has shown that the thoracic aorta isolated from WHHL rabbits displayed a decreased contractile response to angiotensin II, while its type 1 (AT1) receptor expression was increased in the aorta of young WHHL rabbits at 3–4 months of age (Shishido et al., 2004). The present study is the first to demonstrate that angiotensin II-induced contraction was also specifically attenuated in the abdominal aorta of WHHL rabbits, while their 5-hydroxytryptamine-induced contraction was not significantly different from the aorta of control rabbits. However, both the abdominal aorta and the thoracic aorta of diet-induced atherosclerotic rabbits showed augmented contractile responses to 5-hydroxytryptamine (Henry and Yokoyama, 1980; Merkel et al., 1990; Chin et al., 1990). The WHHL rabbit aorta has also been reported to show increased contractility in response to 5-hydroxytryptamine (Yokoyama et al., 1983). The discrepant results of the 5-hydroxytryptamine contraction in the atherosclerotic aorta in these previous studies and those of the present study may also be due to the stage and severity of atherosclerosis, because atherosclerotic plaques were not macroscopically observed in the descending aortae used in the present study. Moreover, the aorta from 1-month-old animals was reportedly more sensitive to 5-hydroxytryptamine than that from 6-month-old
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animals (Wines et al., 1989), suggesting that age could also affect the contractile response to 5-hydroxytryptamine.

In the present study, we examined whether endogenous vasodilatory substances were involved in attenuation of the contractile response to angiotensin II in the WHHL rabbit aorta. The decrease in contractile force produced by angiotensin II in the WHHL aorta was also observed in the absence of the endothelium, indicating that the decrease in angiotensin II contraction was not due to vasoactive substances released from the endothelium. NO and prostacyclin are major vasodilatory substances that regulate vascular tone and that are produced in both the vascular intima and media (Moncada et al., 1991 Vane and Botting, 1993; Mitchell and Evans, 1998). Pretreatment of nitro-L-arginine, a NO synthase inhibitor, significantly augmented the contractile response to angiotensin II in the aorta with an intact endothelium in both WHHL and control rabbits. Thus, NO negatively regulates the contractile response to angiotensin II. However, angiotensin II-induced contraction in the presence of nitro-L-arginine was also lower in the WHHL group than in the control group, suggesting that NO is not involved in the diminution of angiotensin II contraction in the WHHL rabbit abdominal aorta. Moreover, acetylcholine-induced endothelium-dependent aortic relaxation, which is mainly due to NO release from the endothelium, was not significantly different between the WHHL and control groups. In contrast, several studies have shown a decrease in endothelium-dependent relaxation in the aorta of WHHL rabbits (Ragazzi et al., 1989; Kolodgie et al., 1990) as well as in the aorta of high-cholesterol-diet-induced atherosclerotic rabbits (Sreeharan et al., 1986; Jayakody et al., 1988). The lack of impairment of endothelium-dependent vasodilation in the WHHL rabbit abdominal aorta in the present study may also be due to a lesser degree of atherosclerosis. Moreover, our recent study demonstrated that attenuation of endothelium-dependent relaxation of the WHHL rabbit thoracic aorta, which displayed markedly large atherosclerotic plaques, was observed only in those animals with hypertriglyceridemia (Shishido et al., 2004), suggesting that hypertriglyceridemia aggravates hypercholesterolemia-induced functional impairment of endothelial cells. The contractile force of the aorta in response to angiotensin II was not affected by pretreatment with diclofenac, a cyclooxygenase inhibitor, while that in the presence of diclofenac was also lower in the WHHL group than in the control group. This finding agrees with the finding of a study by Forstermann et al. (1984) that prostaglandin release was enhanced by angiotensin II in both the rabbit coeliac artery and pulmonary artery but not in either the aorta or femoral artery. Therefore, cyclooxygenase products such as prostacyclin do not influence the vascular tone of the angiotensin II response and are not involved in attenuation of the angiotensin II contraction of the WHHL rabbit aorta. Angiotensin II contraction is mediated mainly via stimulation of AT1 receptors in vascular smooth muscle cells. In addition, angiotensin II has recently been reported to induce vasodilation directly via stimulation of its type 2 (AT2) receptor (Widdop et al., 2003). However, hypocontractility to angiotensin II of the WHHL rabbit aorta was also observed in the presence of PD123319, an AT2 receptor antagonist, indicating that AT2 receptor-mediated vasodilation is not involved in the hypocontractility of the WHHL rabbit aorta. The above findings suggest that the decrease in the angiotensin II contraction in the WHHL rabbit aorta is mainly due to an abnormality in smooth muscle contractility, and is not due to changes in the release of vasodilatory substances from the vessel wall. AT1 receptor
expression has rather been shown to be up-regulated in the thoracic aorta of WHHL rabbits (Shishido et al., 2004). Thus, further study including AT1 receptor coupling with downstream signals in vascular smooth muscle cells is needed to clarify the mechanism involved in the attenuated contractile response to angiotensin II in the WHHL aorta. Furthermore, it is also of interest to determine whether other angiotensin II-mediated biological actions in vascular smooth muscle, such as protein synthesis, mitogenesis and hypertrophy, are altered in arteries of WHHL rabbits.

In conclusion, the WHHL rabbit abdominal aorta displays attenuated angiotensin II-induced contraction, which is suggested to be mainly due to an abnormality in the AT1-receptor-mediated contractile pathway of medial smooth muscle.

References


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(Received December 24, 2004; Accepted February 10, 2005)