Kor J Physiol (1996) 30(2), 269~278

Endothelium-dependent Contraction of Aorta in One-kidney, One-clip Goldblatt Hypertensive Rat

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=ABSTRACT=

The mechanism of impaired endothelium-dependent relaxation in the aorta of one-kidney, one clip Goldblatt hypertensive (1K,1C-GBH) rats was investigated. 8 week-old Wistar-Kyoto (WKY) rats were made hypertensive by left renal artery stenosis with contralateral nephrectomy.

Endothelium-dependent relaxation was significantly reduced in 1K,1C-GBH rats as compared with WKY rats. However, the relaxation by sodium nitroprusside in 1K,1C-GBH rats was not reduced as compared with WKY rats. The impairment of endothelium-dependent relaxation in 1K,1C-GBH rats was partially restored by the pretreatment of indomethacin or SQ29548.

When the nitric oxide production was inhibited by L-nitroarginine methyl ester, acetylcholine (ACh) induced a endothelium-dependent contraction that was greater in 1K,1C-GBH rats than in WKY rats. Endothelium-dependent contraction by ACh was completely abolished by indomethacin or SQ 29548. However, imidazole, tranylcypromine and superoxide dismutase did not affect the endothelium-dependent contraction in 1K,1C-GBH rats.

These results suggest that impaired endothelium-dependent relaxation in the 1K,1C-GBH rats might be due to the simultaneous release of EDCF, and that prostaglandin H_2 may be involved as a mediator of endothelium-dependent contraction.

Key Words: Vascular smooth muscle, Hypertension, Endothelium-dependent contraction

INTRODUCTION

Endothelial cells modulate underlying vascular smooth muscle tone by releasing endothelium-derived relaxing factor (EDRF) and endothelium-derived contracting factor (EDCF) (Furchgott, 1983; Gryglewski et al, 1988; Furchgott & Vanhoutte, 1989; Vanhoutte, 1989). An imbalanced production of relaxing and contracting factors may initiate as well as sustain the abnormal vasoconstriction of hypertension (Rees et al, 1989; Lüscher, 1990).

Endothelium-dependent relaxation was impaired in genetic hypertensive animals such as spontaneously hypertensive rat (SHR) or Dahl rat as well as in acquired hypertensive animals by coarctation of the aorta, constriction of the renal artery, or administration of mineralocorticoid with salts (Lüscher et al, 1987, 1988; Lockette et al, 1986; Van de Voorde & Leusen, 1986; Mayhan et al, 1987; Miller et al, 1987). Correction of the hypertension by removing the causes of hypertension restores endothelium-dependent relaxation (Lüscher et al, 1987; Lockette et al, 1986).

The mechanism of impaired endothelium-dependent relaxation in the hypertensive rats is still

unknown. An alteration in the balance between the release of EDRF and EDCF may be the underlying mechanism for the impaired endothelium-dependent relaxation. Endothelium-dependent contraction by acetylcholine has been demonstrated in the aorta of SHR (Lüscher & Vanhoutte, 1986). And several substances have been suggested as a mediator of endothelium-dependent contraction. The cyclooxygenase products - prostaglandin H_2 (PGH_2), thromboxane A_2 (TXA_2) - have been shown to be EDCF in the systemic circulation (Bennett et al, 1993). Also, it was reported that superoxide anions could be mediator of the endothelium-dependent contraction in the mesenteric artery of SHR (Jamenson et al, 1993).

However, it is still unknown about the mechanism of impaired endothelium-dependent relaxation and a mediator of endothelium- dependent contraction in the acquired hypertensive rats such as renovascular hypertensive rats and Goldblatt hypertensive rats. Therefore, the present study was aimed to investigate the mechanism of impaired endothelium-dependent response in acquired hypertensive rats by Goldblatt procedure.

METHODS

Animals

The experiments were performed on Wistar-Kyoto (WKY) rats and one-kidney one-clip Goldblatt hypertensive (1K,1C-GBH) rats. 7~8 week-old WKY rats were anesthetized with ether. After the abdomen was incised, a stainless steel wire (diameter : 0.3 mm) placed on the left renal artery. The wire and renal artery were ligated together with nylon and the wire removed gently. Then, contralateral kidney was removed. The rats were under close observation with weekly monitoring of systolic blood pressure. The systolic blood pressure was measured in conscious restrained rats by the tail-cuff plethysmographic method (PE-300, Narco-Biosystems, Houston, Texas) after a short prewarming at 40°C.

Measurement of isometric tension of isolated aortic rings

The rats were neck-dislocated and exsanguinated. The aorta was quickly isolated and adhering adventitia and remaining fat were removed in the preparation bath under a stereoscopic microscope. Bubbling with 100 % O_2, the aorta was left to recover for 2 hours at room temperature. The aorta was then carefully cut into a rings (3~4 mm wide).

The rings of aorta were mounted vertically between stainless steel hooks (L-shaped) in a thermostatically controlled organ bath containing 50 ml of the Tris-buffered Tyrode's solution of the following composition (mM): NaCl 158, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, Glucose 6, and Tris 5 (pH 7.4 at 35°C). Organ bath solution was maintained at 35°C and continuously bubbled with 100% O_2. The hook anchoring the upper end of the ring was connected to the lever of a force transducer (F-60, Narco-Bio system) and the rings were suspended under a tension of 2 g. Each preparation was allowed to equilibrate for at least one hour. The presence of the endothelium was confirmed by the acetylcholine (ACh) (10^{-6} M)-induced relaxation in norepinephrine-contracted aortic ring (Furchgott & Zawadzki, 1980). Isometric tensions were recorded on a strip chart recorder (Coles Parmer, U.S.A.).

Statistical analysis

The results are expressed as means and S.E.M. Student's t-tests were used for statistical analysis. P values of less than 0.05 were considered to be statistically significant.

Drugs

Acetylcholine, norepinephrine and L-nitroarginine methyl ester (L-NAME), indomethacin, imidazole, superoxide dismutase were purchased from Sigma Chemicals, 1S [1α, 2B (5Z), 3B, 4α] - 7 - [3 - [2 (phenylamino) carbonyl] hydrazinomethyl] - 7 - oxabicycle (2.2.1) hept- 2 -yl -5 - hepenoic acid (SQ29548) was

purchased from BIOMOL research Laboratory Inc. Indomethacin was dissolved in distilled water containing 50 mM Na_2CO_3. SQ29548 was dissolved in ethanol. The final ethanol concentration in the bath solution was 0.1% or less. Vehicle did not affect the acetylcholine-induced response or the resting tone. The other drugs was dissolved in distilled water.

RESULTS

Blood pressure

Systolic blood pressure (SBP) of 1K,1C-GBH rats was sharply increased for 4 weeks after operation and then reached a plateau after next 4 weeks. The systolic blood pressures of 12 (4 weeks after operation) and 16 week-old 1K,1C-GBH rats (8 weeks after operation) were 185.0±13.1, 195.1±10.3 mmHg, respectively, which were significantly greater than those of WKY rats (Fig. 1).

General characteristics of 1K,1C-GBH rats and WKY rats

Body weights of 1K,1C-GBH rats were lesser than those of WKY rats, whereas heart and one kidney weights of 1K,1C-GBH rats were significantly greater than those of WKY rats (Table 1). The ratio of heart weight and kidney weight of 1K,1C-GBH rats versus those of WKY rats were 1.55 and 1.53, respectively. To evaluate the change of thickness of tunica media in aorta after the development of hypertension, we measured thickness of tunica media in aorta by using microcaliper of light microscope. Average thickness of tunica media in 1K,1C-GBH rats was significantly greater than that in WKY rats as their thickness was 118.3 ±10.2 μm for WKY rats and 139.9±4.3 μm for 1K,1C-GBH rats, respectively.

Endothelium-dependent relaxation

The aortic rings with intact endothelium were contracted with 10^{-6} M norepinephrine (NE). After the contraction reached a plateau, ACh was added in a cumulative fashion (10^{-8}M~10^{-5}M). ACh induced a concentration-dependent relaxation in the aortic rings from both groups. However, the relaxant

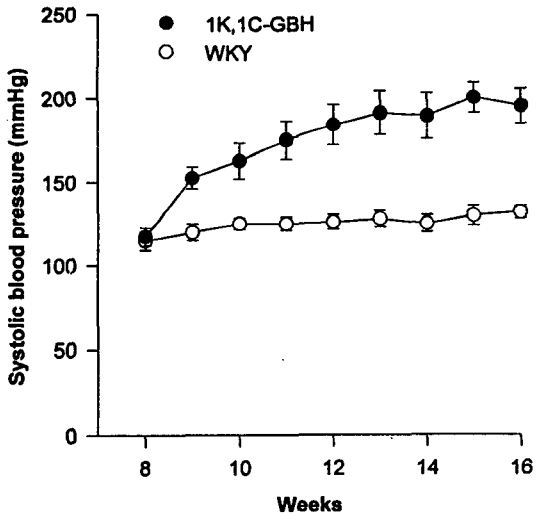

Fig. 1. Change of systolic blood pressure of Wistar-Kyoto rats (WKY) and one-kidney, one-clip Goldblatt hypertensive (1K, 1C-GBH) rats. Data points are means from 30 preparations and vertical bars are S.E.M..
** Systolic blood pressure was measured with Tail-Cuff method.*

Table 1. General characteristics of 16 week-old WKY rats and Goldblatt hypertensive rats (n=10)

Groups	Body weight (g)	Heart weight (g/kg of BW)	One kidney weight (g/kg of BW)	Aortic wall thicknesss (μm)
WKY	292.0±5.8	3.16±0.13	3.51±0.23	118.3±10.2
1K,1C-GBH	251.7±15.4	4.90±0.54	5.40±0.42	139.9±4.3

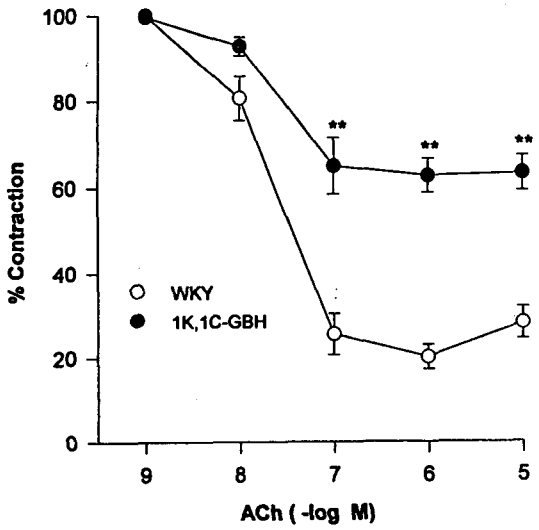

*Fig. 2. Endothelium-dependent relaxation by acetylcholine (ACh) in the aorta of Wistar-Kyoto (WKY) rats and one-kidney, one-clip Goldblatt hypertensive (1K,1C-GBH) rats. Contractions are expressed as percentage of the contraction induced by 10^{-6} M NE. Data points are means from 6 preparations and vertical bars are S.E.M.. **P<0.01 vs WKY.*

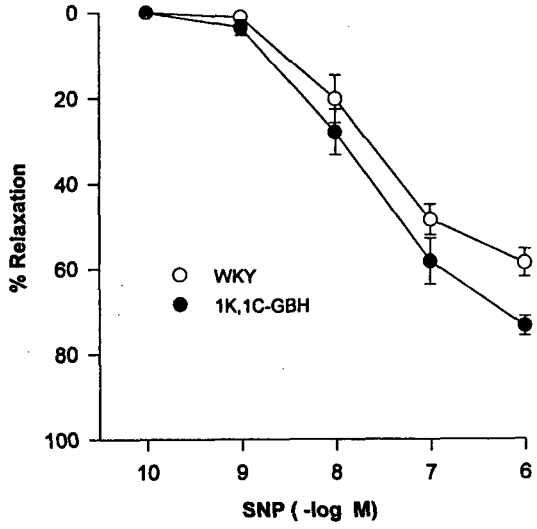

Fig. 3. Effect of sodium nitroprusside (SNP) on the 40 mM K^+-induced contraction in the aortic strips with rubbed endothelium of Wistar-Kyoto rats (WKY) and one-kidney, one-clip Goldblatt hypertensive (1K,1C-GBH) rats. Relaxations are expressed as percentage of the contraction induced by 40 mM K^+. Data points are means from 6 preparations and vertical bars are S.E.M..

responses to ACh in 1K,1C-GBH rats were lesser than those in WKY rats (Fig. 2).

To evaluate whether relaxant response of vascular smooth muscle in 1K,1C-GBH rats to nitric oxide was impaired or not, we compared the relaxant response to sodium nitroprusside (SNP) in the aortic rings from WKY and 1K,1C-GBH rats. The aortic rings were contracted with 40 mM K^+-Tyrode's solution and SNP was added in a cumulative fashion (10^{-9}~10^{-6}M). SNP caused a concentration-dependent relaxation in both groups. And there was no significant difference between WKY rats and 1K, 1C-GBH rats (Fig. 3).

The effect of indomethacin on the decreased endothelium-dependent relaxation in the aortic rings from 1K,1C-GBH rats was observed. The aortic rings were pretreated with indomethacin (2×10^{-6} M) for 15 minutes and contracted with 10^{-6} M NE. ACh was added in a cumulative fashion. Inhibition of cyclooxygenase by indomethacin caused a partial restoration of the endothelium- dependent relaxation in the 1K,1C-GBH rats. (Fig. 4).

Endothelium-dependent contraction

To compare endothelium-dependent contraction in the aortic rings from WKY rats and 1K,1C-GBH rats, the aortic rings with intact endothelium were pretreated with 10^{-4} M L-NAME, an inhibitor of nitric oxide synthase, and cumulative doses of ACh (10^{-7}~10^{-5}M) were added to the bath. When the EDRF production was inhibited by L-NAME, ACh caused a endothelium-dependent contraction in the aortic rings from both WKY and 1K,1C-GBH rats in a concentration-dependent manner (Fig 5). The endothelium-dependent contractions of 1K,1C-GBH

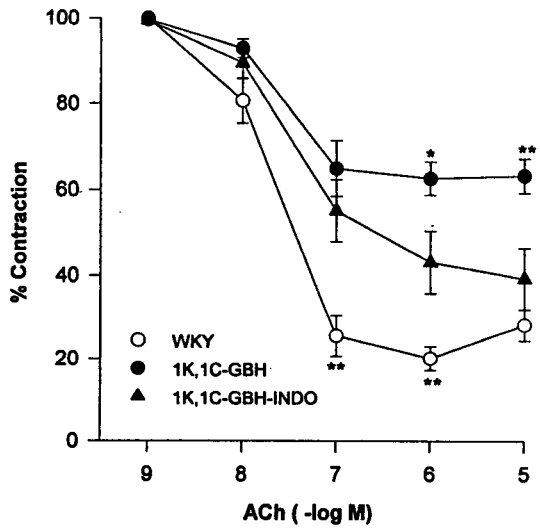

*Fig. 4. The effect of indomethacin (INDO) (2×10^{-6}M) on the endothelium-dependent relaxation by acetylcholine (ACh) in the aorta from one-kidney, one-clip Goldblatt hypertensive (1K,1C-GBH) rats. Contractions are expressed as percentage of the contraction induced by 10^{-6} M NE. Data points are means from 6 preparations and vertical bars are S.E.M.. *P<0.05, **P<0.01 vs 1K,1C-GBH-INDO.*

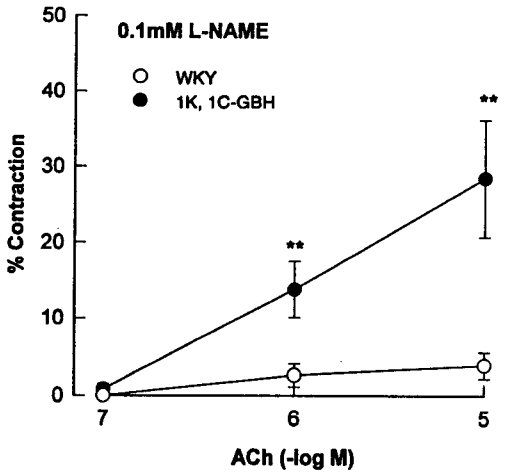

*Fig 5. Endothelium-dependent contractions by acetylcholine (ACh) after the pretreatment of L-NAME (10^{-4}M) in the aortic rings of Wistar-Kyoto (WKY) rats and one-kidney, one-clip Goldblatt hypertensive (1K,1C-GBH) rats. Contractions are expressed as percentage of the contraction induced by 10^{-6} M NE. Data points are means from 6 preparations and vertical bars are S.E.M.. **P<0.01 vs WKY.*

rats were significantly greater than those of WKY rats over the concentration range (10^{-6}~10^{-5}M).

To confirm that ACh-induced contraction was endothelium-dependent contraction, effect of ACh was observed in the aortic rings without endothelium. When the endothelium was removed, ACh did not induced any contraction in 1K-1C-GBH rats, indicating that the contractile response was an endothelium-dependent response (data not shown).

The effect of indomethacin, an inhibitor of cyclooxygenase, on the endothelium-dependent contraction by ACh was observed. The aortic rings from both groups were pretreated with indomethacin (2×10^{-6}M) and L-NAME (10^{-4}M) for 15 minutes and ACh was added to the bath. Endothelium-dependent contraction by ACh was completely abolished by the pretreatment of indomethacin in the aortic rings

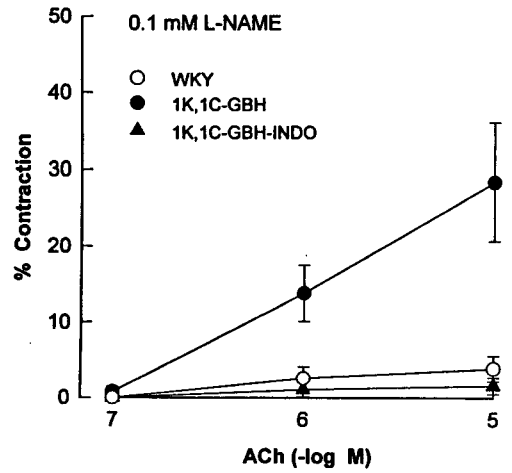

Fig 6. Effect of indomethacin (INDO) (2×10^{-6}M), an inhibitor of cyclooxygenase, on endothelium-dependent contraction by acetylcholine (ACh) after the pretreatment of L-NAME (10^{-4}M) in the aortic rings from one-kidney, one-clip Goldblatt hypertensive (1K,1C-GBH) rats. Contractions are expressed as percentage of the contraction induced by 10^{-6}M NE. Data points are means from 6 preparations and vertical bars are S.E.M..

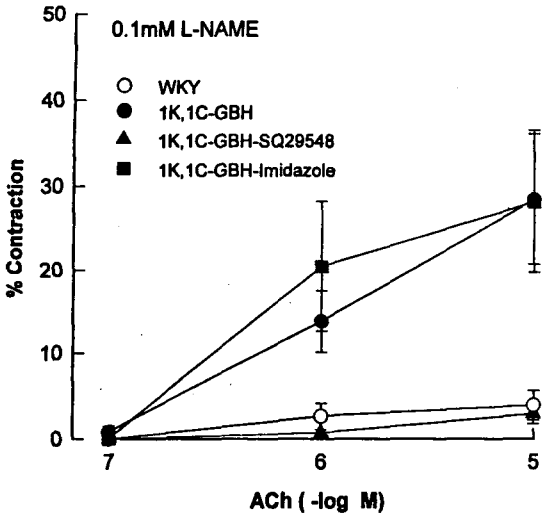

Fig. 7. Effect of SQ29548 (10^{-6}M), an inhibitor of prostaglandin H_2/thromboxane A_2 receptor, and imidazole (10^{-4}M), an inhibitor of thromboxane A_2 synthase, on endothelium-dependent contraction by acetylcholine (ACh) after the pretreatment of L-NAME (10^{-4} M) in the aortic rings from one-kidney, one-clip Goldblatt hypertensive (1K,1C-GBH) rats. Contractions are expressed as percentage of the contraction induced by 10^{-6}M NE. Data points are means from 6 preparations and vertical bars are S.E.M..

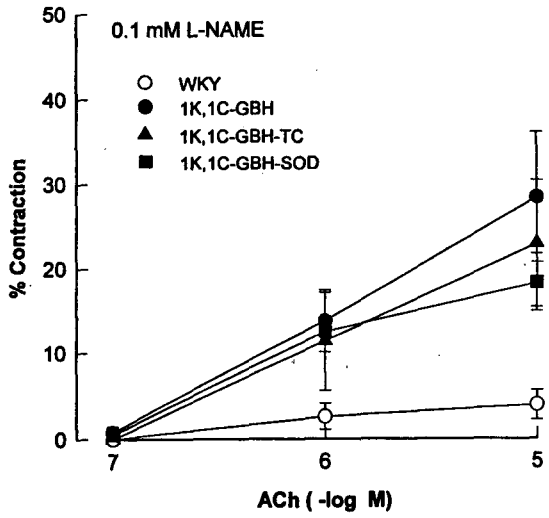

Fig. 8. Effect of tranylcypromine (10^{-4}M) (TC), an inhibitor of prostacyclin synthase, and superoxide dismutase (60 U/ml) (SOD), a scavenger of superoxide anion, on endothelium-dependent contraction by acetylcholine (ACh) after the pretreatment of L-NAME (10^{-4} M) in the aortic rings from one-kidney, one-clip Goldblatt hypertensive (1K,1C-GBH) rats. Contractions are expressed as percentage of the contraction induced by 10^{-6} M NE. Data points are means from 6 preparations and vertical bars are S.E.M.

from 1K,1C-GBH rats (Fig. 6).

To determine which substances of cyclooxygenase product are involved in the endothelium-dependent contraction, the aortic rings from 1K,1C-GBH rats were pretreated with L-NAME (10^{-4}M) and the effects of selective inhibitors of cyclooxygenase pathway on the endothelium-dependent contraction in 1K,1C-GBH rats were observed. When aortic rings were pretreated with SQ 29548(10^{-6}M), an inhibitor of TXA_2 / PGH_2 receptor, ACh did not induce any contraction at all (Fig. 7). However, when aortic rings were pretreated with imidazole (10^{-4}M), an inhibitor of TXA_2 synthesis, endothelium-dependent contraction was not affected (Fig. 7).

And then to evaluate the possibility whether superoxide and prostaglandin I_2 (PGI_2) can act as a mediator of endothelium-dependent contraction, aortic rings were pretreated with superoxide dismutase (60 U/ml) or tranylcypromine (10^{-4} M) in combination with L-NAME. Inhibition of prostacyclin synthesis with tranylcypromine, and scavenging of superoxide anion with superoxide dismutase did not affect the endothelium-dependent contraction in the 1K,1C-GBH rats (Fig. 8).

Effect of cyclooxygenase product on the endothelium-dependent relaxation

In order to evaluate the effect of PGH_2 on impaired endothelium-dependent relaxation in the aorta from 1K,1C-GBH rats, aortic rings were pretreated with SQ29548 and the endothelium-dependent re-

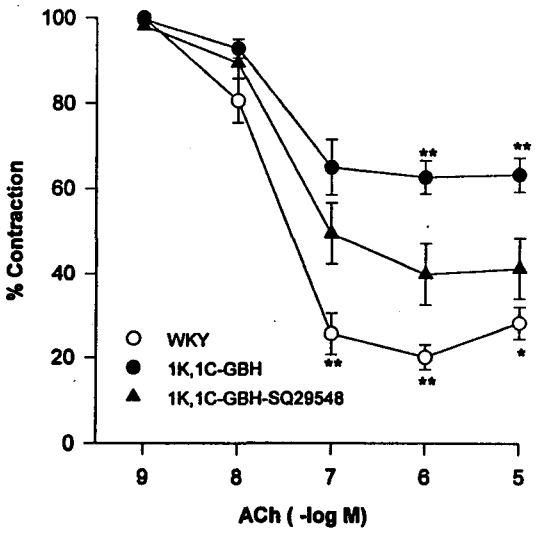

*Fig. 9. Effect of SQ29548 (10^{-6}M), an inhibitor of prostaglandin H_2/thromboxane A_2 receptor on the endothelium-dependent relaxation in the aortic rings from one-kidney, one-clip Goldblatt hypertensive (1K,1C-GBH) rats. Contractions are expressed as percentage of the contraction induced by 10^{-6}M NE. Data points are means from 6 preparations and vertical bars are S.E.M.. *P<0.05, **P<0.01 vs 1K,1C-GBH-SQ29548.*

laxation by ACh was observed. Aortic rings with intact endothelium were pretreated with SQ29548 and contracted by NE 10^{-6}M, and cumulative doses of ACh were added. The pretreatment of SQ29548 caused a partial restoration of the endothelium-dependent relaxation by ACh in the aortic rings of 1K,1C-GBH rats. However, the relaxant responses of SQ29548-pretreated aortic rings to ACh in 1K,1C-GBH rats were not restored completely when compared to those of WKY rats (Fig. 9).

DISCUSSION

An increase in peripheral resistance is a characteristic finding in established essential hypertension in humans and in spontaneously hypertensive rats. Chronic hypertension is associated with structural and functional changes in cardiovascular system. In our study, 1K,1C-GBH rats showed significant myocardial enlargement and thickened aortic wall, which is indicative of longstanding hypertension. It is well known that elevated arterial pressure is a major contributor to ventricular and arterial medial hypertrophy in hypertension (Folkow, 1978).

Endothelium-dependent relaxation in 1K,1C-GBH rats was lesser than that in WKY rats. The underlying causes of impaired endothelium-dependent relaxation in 1K,1C-GBH rats may be 1) decreased release of EDRF, 2) increased release of EDCF or 3) decreased response of smooth muscle to EDRF. The relaxant responses to the nitric oxide donor, sodium nitroprusside, which acts like a endothelium-derived relaxing factor and exerts its effect via formation of cGMP in smooth muscle (Rapoport et al, 1983) were not different between both groups. This result indicate that impaired endothelium-dependent relaxation in 1K,1C-GBH rats may be due to the functional alteration of the endothelium rather than vascular smooth muscle. It has been suggested that the impaired endothelium-dependent relaxation may be due to the increased release of contracting factor from endothelium (Bennett et al, 1993) or decreased formation of EDRF (Dohi et al, 1991) in renovascular hypertension. Luscher and Vanhoutte (1986) have observed the impaired endothelium-dependent relaxation to ACh in the SHR and suggested that the underlying cause of impaired endothelium-dependent relaxation was the simultaneous release of an EDCF. It is known that ACh induce contraction endothelium dependently in the aorta of spontaneously hypertensive rats (Luscher & Vanhoutte, 1986; Jeon et al, 1996). In the present study, ACh did not induced any contraction in the aorta of 1K,1C-GBH rats, when the endothelium was removed, indicating that ACh act on the endothelial cells and may induce the release of substances that mediate the contraction. Endothelium-dependent contraction by ACh was significantly enhanced and inhibition of cyclooxygenase pathway by indomethacin or SQ29548 caused partial restoration of

endothelium-dependent relaxation to ACh in the aortic rings of 1K,1C-GBH rats. These data suggest that endothelium dependent contracting factor play an important role in impaired endothelium-dependent relaxation in aortic rings of 1K,1C-GBH rats.

It was reported that the increase in PGH_2 production is closely related to the increase in blood pressure with aging or to the development of hypertension in SHR (Ito et al 1991; Iwama et al 1992, Kato et al 1990). The contractions in the rings from SHR induced by PGI_2 at high concentrations indicated the possibility that PGI_2 could be EDCF (Kato et al, 1990). And in the present study, the contractile response to ACh were completely abolished by indomethacin pretreatment. Therefore, we would like to define the nature of the putative cyclooxygenase-dependent EDCF released by ACh from the endothelium of 1K,1C-GBH rats using specific inhibitors of cyclooxygenase products. To identify EDCF, the possibility of TXA_2, PGH_2 and PGI_2 involvement was evaluated. The endothelium-dependent contraction was prevented by SQ29548, TXA_2/PGH_2 receptor antagonists, whereas imidazole, an inhibitors of thromboxane synthase (Miller & Vanhoutte, 1985; Salzman et al, 1980), are ineffective. Tranylcypromine, a prostacyclin synthase inhibitor (Johnson, 1980), had no effect on endothelium-dependent contraction. These findings suggest that PGH_2 may be the mediator of endothelium-dependent contraction by ACh in the aorta of 1K,1C-GBH rats and that PGI_2 is not increased in the tissue to the degree that induce vascular contractions. Superoxide anions is produced as a by-product of cyclooxygenase pathway and is known to be able to mediate endothelium-dependent contraction either by the breakdown of nitric oxide or direct effects on vascular smooth muscle (Kasusic & Vanhoutte, 1989; Rubanyi & Vanhoutte, 1986). However, superoxide dismutase did not affect endothelium-dependent contraction in aortic ring of 1K,1C-GBH rats. From the above data, it is suggested that PGH_2 may be an EDCF produced in response to ACh in the aorta from 1K,1C-GBH rats.

It is still unknown about mechanism of accentuated cyclooxygenase pathway in 1K,1C-GBH rats. Membrane fatty acid metabolism is accelerated in SHR arteries (Kawaguchi et al, 1986; Kawaguchi et al, 1987) and point mutations in the phospholipid-δ gene in SHRs may be causally related to the augmented phospholipase C activity (Yagisawa et al, 1991). Augmentation of phospholipase C activity, by increased intracellular calcium, could favor vasoconstriction. Similarly, ACh, by increasing cellular calcium (Gryglewski et al, 1986), may further increase arachidonate and phopholipid metabolism in the endothelial cell of 1K,1C-GBH rats.

In summary, endothelium-dependent relaxations by ACh are impaired in aorta of 1K,1C-GBH rats. ACh concomitantly releases an endothelium-derived substance that reverses the effects of EDRF in the 1K,1C-GBH rats. The production of this factor requires the activity of cyclooxygenase, as indomethacin normalizes endothelium-dependent relaxation in the 1K,1C-GBH rats. And the cyclooxygenase product which mediated endothelium-dependnent contraction may be PGH_2.

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