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Direct Vascular Actions of Quercetin in Aorta from Renal Hypertensive Rats

朝鮮大學校 大學院
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신성 고혈압 쥐에서 Quercetin의 혈관 이완 작용

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신성 고혈압 쥐에서 Quercetin의 혈관 이완 작용

류 권 호(柳 權 浩)
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플라보노이드 일종인 quercetin은 실험적 고혈압 동물에 장기간 투여시 혈압하강 효과와 혈관내피층의 기능이 개선됨이 알려져 있다. 이 연구는 two-kidney, one clip (2K1C) 신성고혈압 모델에서 quercetin의 혈관 반응에 미치는 영향이 정상혈압 동물과 차이가 있는지 구명하고자 항산화제인 ascorbic acid의 효과와 비교 검토하였다.

흰쥐의 일측 신동맥에 직경 0.2 mm 의 clip을 장치하고 10 주 동안 사육하여 2K1C 고혈압을 유발시켰다. 대조군은 개복한 후 바로 봉합하였다. 실험당일 흉부 대동맥 표본을 채취하여 수조에 현수하고 그 등장성 장력변화를 생리기록기에 기록하였다.

Acetylcholine에 의한 이완반응은 2K1C 고혈압군에서 대조군에 비해 약화되었으며 quercetin 및 ascorbic acid는 acetylcholine의 이완반응을 고혈압군에서 강화시켰으나 대조군은 영향이 없었다. Sodium nitroprusside에 의한 이완반응은 고혈압군과 대조군에서 유사하였으며 양군 모두 quercetin과 ascorbic acid에 의해 영향받지 않았다. Phenylephrine에 의한 수축반응은 2K1C 고혈압군에서 대조군에 비해 증가되었으며 quercetin 및 ascorbic acid는 phenylephrine의 수축반응을 고혈압군에서 억제시켰으나 대조군은 영향이 없었다. Phenylephrine의 수축반응은 Nw-nitro-L-arginine methyl ester (L-NAME)에 의해 대조군에서 항진되었으나 고혈압군은 차이가 없었다. Quercetin 및 ascorbic acid는 L-NAME 처치하에서는 양군 모두 phenylephrine의 수축반응에 영향을 미치지 않았다. 혈관반응에 미치는 quercetin의 효과는 ascorbic acid와 유사하였다.

이상의 실험결과는 2K1C 신성고혈압 상태에서 quercetin이 혈관에 직접 작용하여 변이된 내피층 의존 이완반응을 개선시키고 수축반응을 억제시킴을 시사한다.

I . INTRODUCTION

Hypertension, a common risk factor of cardiovascular disease, is characterized by endothelial dysfunction in large conduit and small resistance arteries[1]. Indeed, endothelium-dependent vasodilation is impaired in a number of experimental models, including two-kidney, one clip (2K1C) renal hypertension[2-4]. Biological activity of nitric oxide (NO), an effective endothelium-derived relaxing factor, is primarily associated with endothelial NO synthase activity or its interaction with superoxide anion, which is produced in the vascular wall by free radical generating enzymes[5]. Endothelium-derived NO may be scavenged by superoxide anion, and caused to reduce the bioavailability of NO and diminish the vasorelaxation[6]. It is now well established that an endothelial dysfunction in hypertension is in part linked to the exaggerated production of superoxide anions[7,8] and oxidative stress is reponsible for the impaired endothelial modulation in 2K1C hypertension[9,10]. Therefore, antioxidants may exert the beneficial effects on the vascular complications associated with hypertension.

Quercetin is commonly found flavonol-type flavonoid which is widely distributed in dietary vegetables, fruits and wine[11]. Previous studies have demonstrated that flavonoid quercetin has various physiological effects involving antioxidant, antihypertensive effects and an improvement of vascular reactivity[12,13]. The vascular beneficial effects induced by quercetin are attributed mainly to its antioxidant properties which might interact with endothelium-derived NO system[14]. Evidence has been accumulated by the results that flavonoids increases the biological activity of NO by interacting with superoxide anions[15], and an impairment of vascular endothelial function was protected by chronic oral treatment with quercetin in experimental hypertension[16]. Besides the chronic effects of flavonoids associated with endothelial dysfunction in hypertension, when the acute exposure to quercetin in vitro, it restores the endothelium-dependent relaxation and inhibits the contractile responses to agonist in genetically hypertensive rats[11,17]. For this point of view, although it has been

demonstrated that chronic treatment with quercetin exerts antihypertensive and antioxidant effects or improves the endothelial function in renovascular hypertension,[18] acute effects of quercetin on vascular function in renal hypertension are still unclear.

The present study was undertaken to examine the direct effects of quercetin on vascular reactivity in chronic 2K1C hypertensive rats. The effects of quercetin on the endothelium-dependent or -independent relaxation to acetylcholine or sodium nitroprusside and the contractile responses to α1-adrenergic receptor agonist phenylephrine were examined in isolated aortae from 2K1C hypertensive and sham-clipped control rats. The effects of antioxidant vitamin ascorbic acid (vitamin C) on the vascular reactivity were also examined.

Ⅱ. METHODS

1. Induction of 2K1C renal hypertension

Male Sprague-Dawley rats(Samtaco Inc., Osan Korea), weighing 160 to 180 g, were anesthetized with intraperitoneal injection of sodium thiopental (40 mg/kg). Under antiseptic conditions, the left posterior side of the animal was shaved and sterilized with 70 % ethanol. An incision was made on the left flank to provide access to the left renal artery which was separated from the renal vein and cleaned of the connective tissue. A silver clip with an internal diameter of 0.2 mm was applied on the exposed renal artery. The clip was then turned so that the slit opening faces the abdomen, resulting in partial occlusion of renal perfusion. The contralateral kidney was not disturbed. The muscles and skin were sutured immediately and the rats were allowed to recover from anaesthesia. Control rats received a sham treatment: they were underwent the same surgical procedure as in 2K1C rats except for the clip placement. All rats were maintained on standard chow with free access to drinking water. They were used at 10 weeks after clipping, since the endothelial dysfunction is associated with a duration of hypertension[19]. Hypertensive rats were selected by measuring systolic blood pressure in a conscious state using the tail cuff method, and were considered to be hypertensive when systolic pressure was more than 160 mmHg.

2. Tissue preparation

At the time of experimentation, the descending thoracic aorta between the aortic arch and diaphragm was excised through a ventral incision and placed in cold, standard physiological salt solution (PSS) of the following composition (in mM): NaCl 118.3, KCl 4.7, NaHCO3 25, MgCl2 1.2, KH2PO4 1.2, CaCl2 2.5 and glucose 11.1. Vessels were cleaned of adherent fat and connective tissues, thereafter sectioned into cylindrical rings (2~3 mm in width) under a dissecting microscope. Care was taken not to stretch the artery or dislodge the vascular

endothelium.

The aortic rings were mounted between two triangle shaped stainless steel holders in the vessel lumen in organ chambers containing 15 mL of PSS maintained at 37±0.05 ℃, aerated with a mixture of 95 % oxygen and 5 % carbon dioxide to maintain a pH 7.4±0.01 throughout the experiment. One of the holders was fixed at the bottom of the chambers and the other was connected to a force displacement transducer (Grass FTO3) to measure isometric tension development (Fig. 1). Before initiating specific experimental protocols, aortic rings were stretched to the point of their optimal length-tension relationship 2 g, determined in similar preliminary experiments using repeated exposure to 60 mM KCl solution (obtained by equimolar replacement of NaCl by KCl in the physiological solution), and allowed to equilibrate during the period of at least 90 min. During this period of stabilization the bath solution was replaced every 15 min. After an equilibration period, aortic rings were stimulated with 60 mM KCl to test their functional viability. All experimental procedures were performed in the presence of indomethacin (10^{-5} M).

ISOLATED TISSUE BATH

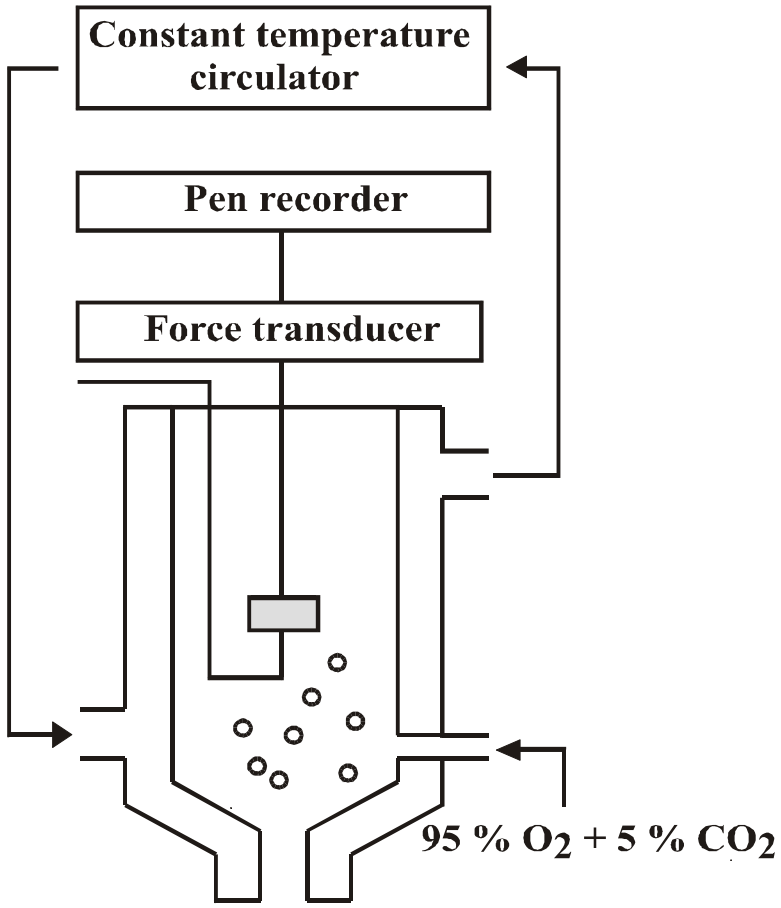

Fig. 1. A schematic representation of the recording system for isometric contraction with 15 mL tissue bath.

3. Protocols

Endothelium dependent and -independent relaxation to acetylcholine and sodium nitroprusside (SNP) were constructed in aortic rings from 2K1C and sham-operated rats under submaximally precontracted with phenylephrine (2×10^{-7} M in sham and 3×10^{-8} M in 2K1C), which were obtained in preliminary experiments. The responses of the aortic rings to cumulative addition of acetylcholine (from 10^{-9} to 10^{-5} M) and SNP (from 10^{-10} to $10^{-6.5}$ M) were examined in parallel rings, which had been incubated with vehicle, quercetin (10^{-5} M) or ascorbic acid (10^{-5} M) 20 min prior to the addition of phenylephrine.

In another set of experiments, aortic rings from 2K1C and sham rats were pretreated with vehicle, quercetin (10^{-5} M) or ascorbic acid (10^{-5} M) for 20 min and the contractile responses to cumulative addition of phenylephrine (from 10^{-9} to 10^{-5} M) were recorded. The experiments were repeated in the presence and absence of the NO synthase inhibitor Nw-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M).

4. Drugs and chemicals

Drugs used were acetylcholine, ascorbic acid, indomethacin, L-NAME, phenylephrine, quercetin and SNP. They were purchased from Sigma Chemical Co (St. Louis, MO, USA). All other chemicals were of reagent grade. Indomethacin and quercetin were dissolved in dimethylsulfoxide (DMSO) and the others were prepared in distilled water. Final bath concentrations of DMSO were less than 0.05 %, which did not alter the contraction or relaxation responses.

5. Statistical analysis

Values presented in the figures are expressed as the means and standard error of the means for the number of rats or tissues indicated in legends. The relaxant responses are given as the percent change in phenylephrine-induced contractile tension and the contractile responses are expressed as a percentage of the contraction developed by the 60 mM KCl. Statistical comparisons were

performed by Student's t-test or analysis of variance with repeated measurements and Fischer's *post-hoc* test. Differences were considered to be statistically significant when P value was less than 0.05.

Ⅲ. RESULTS

Ten weeks after surgery, the systolic blood pressure were 135±3 mmHg (n=43) and 192±4 mmHg (n=42) in sham-clipped control and 2K1C hypertensive rats, respectively ($P<0.05$, Fig. 2). The magnitude of KCl (60 mM)-induced isometric tension development was comparable between the two groups (1.45±0.12 g in control; 1.52±0.14 g in 2K1C).

Relaxant response to acetylcholine in aortic rings precontracted with phenylephrine was significantly attenuated in 2K1C rats as compared with sham rats (Fig. 3). Treatment with L-NAME (10^{-4} M) completely blocked the acetylcholine-induced relaxation in both 2K1C and sham groups (data not shown). In aortic rings from 2K1C rats, acetylcholine-induced relaxation was augmented by preincubation with quercetin or ascorbic acid. However, both chemicals did not affect the relaxant response to acetylcholine in sham rats (Fig. 4, Fig. 5). Relaxant response to SNP was not altered in 2K1C rats (Fig. 6). Quercetin or ascorbic acid did not affect the SNP-induced relaxation either in 2K1C or in sham rats (Fig. 7, Fig. 8).

Contractile response to phenylephrine was significantly enhanced in aortic rings from 2K1C rats as compared with sham rats (Fig. 9). Phenylephrine-induced contraction was inhibited by pretreatment with quercetin or ascorbic acid in 2K1C rats, while both compounds did not affect the contractile response to phenylephrine in sham rats (Fig. 10, Fig. 11). L-NAME markedly augmented the contractile response to phenylephrine in aortic rings from sham rats, while no significant differences were shown in 2K1C rats. In the presence of L-NAME, quercetin or ascorbic acid did not affect the phenylephrine-induced contraction either in 2K1C or in sham rats (Fig. 12, Fig. 13).

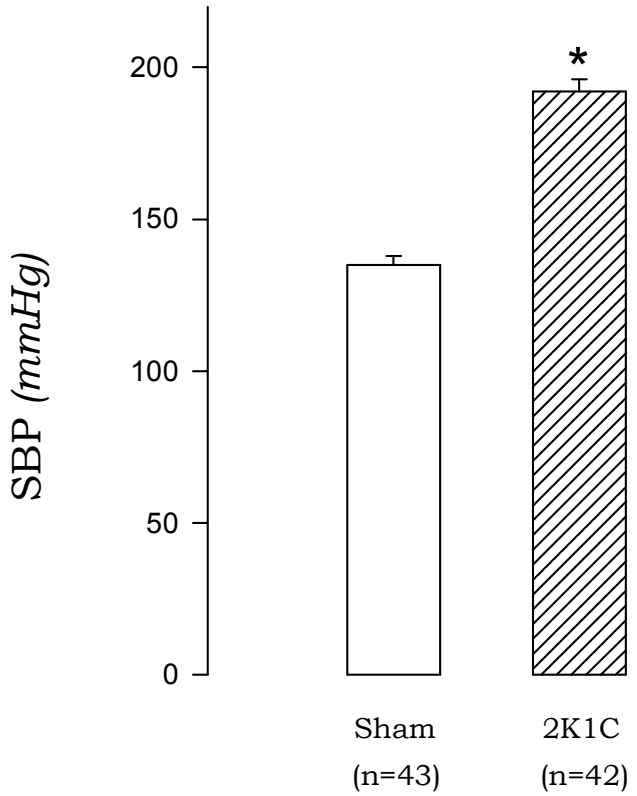

Fig. 2. Systolic blood pressure in 2K1C hypertensive and sham-clipped control rats. Points represent means±SE for number(n) of experiments in parentheses. * P <0.05, compared with the sham value.

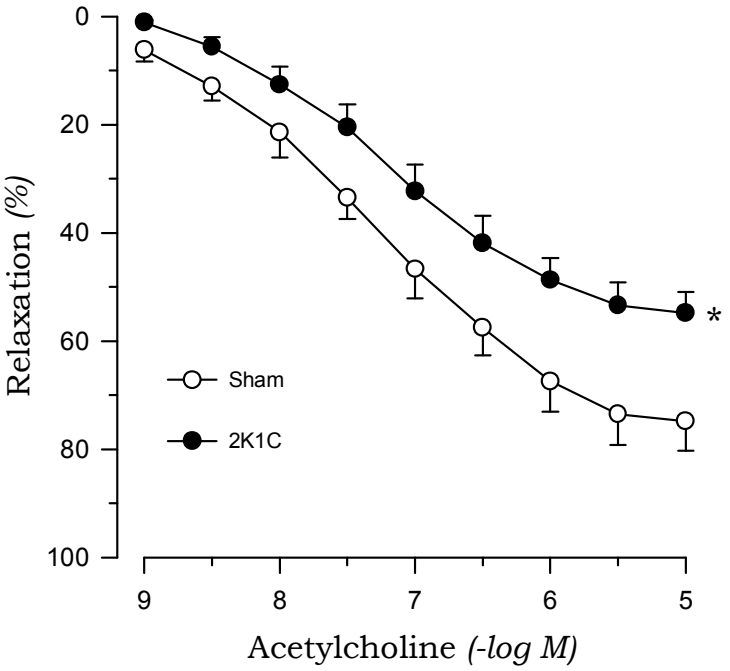

Fig. 3. Relaxant responses to acetylcholine in phenylephrine-precontracted aortic rings from 2K1C hypertensive and sham-clipped control rats. Points represent means±SE for six to eight experiments. * P 〈0.05, compared with sham values.

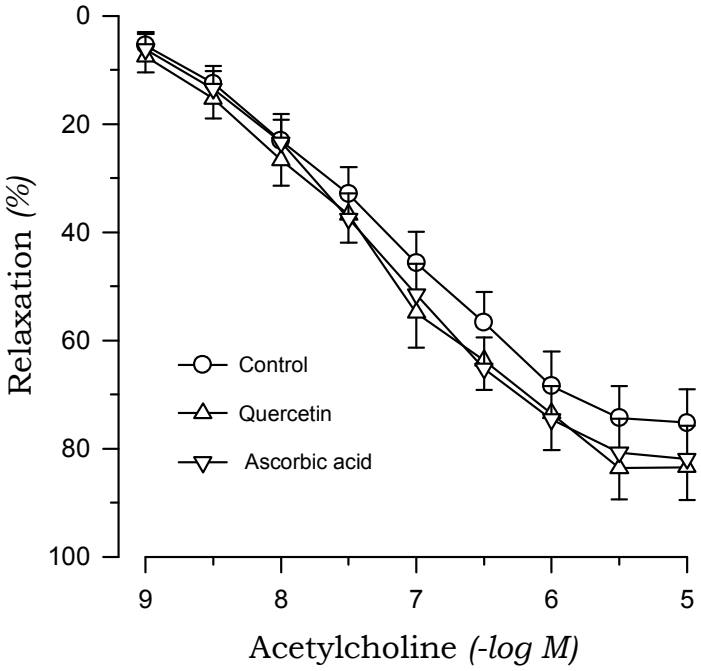

Fig. 4. Effects of quercetin or ascorbic acid on acetylcholine-induced relaxations in aortic rings from sham-clipped control rats. Points represent means±SE for six to eight experiments.

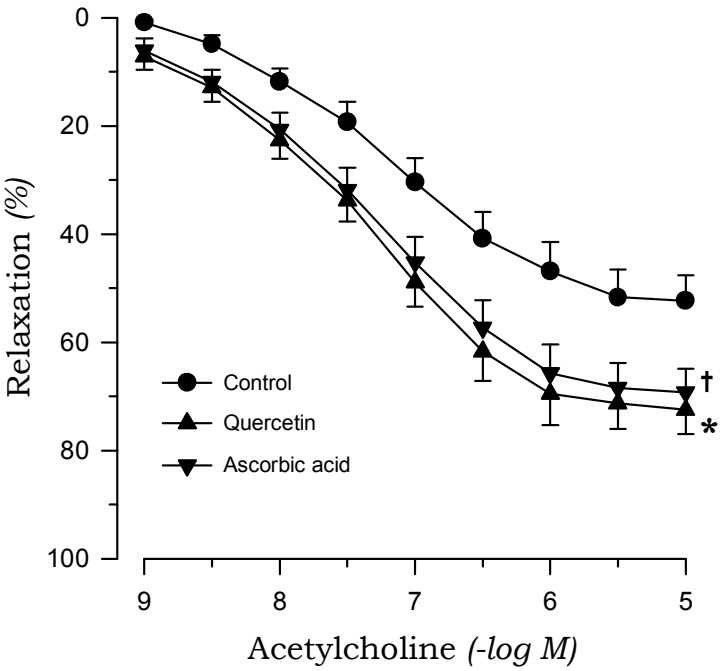

Fig. 5. Effects of quercetin or ascorbic acid on acetylcholine-induced relaxations in aortic rings from 2K1C hypertensive rats. Points represent means±SE for six to eight experiments. *, † P 〈0.05, compared with control values, respectively.

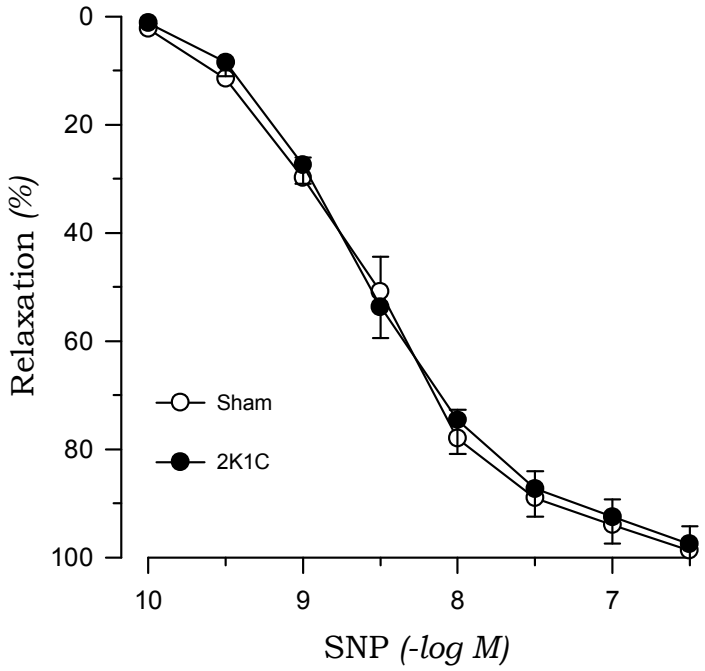

Fig. 6. Relaxant responses to sodium nitroprusside (SNP) in penylephrine-precontracted aortic rings from 2K1C hypertensive and sham-clipped control rats. Points represent means±SE for six to eight experiments.

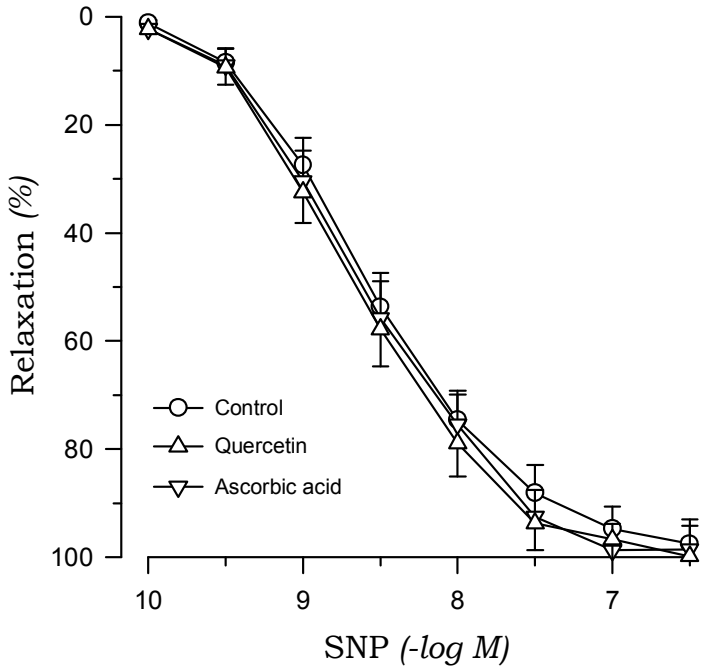

Fig. 7. Effects of quercetin or ascorbic acid on sodium nitroprusside (SNP)-induced relaxations in aortic rings from sham-clipped control rats. Points represent means±SE for six to eight experiments.

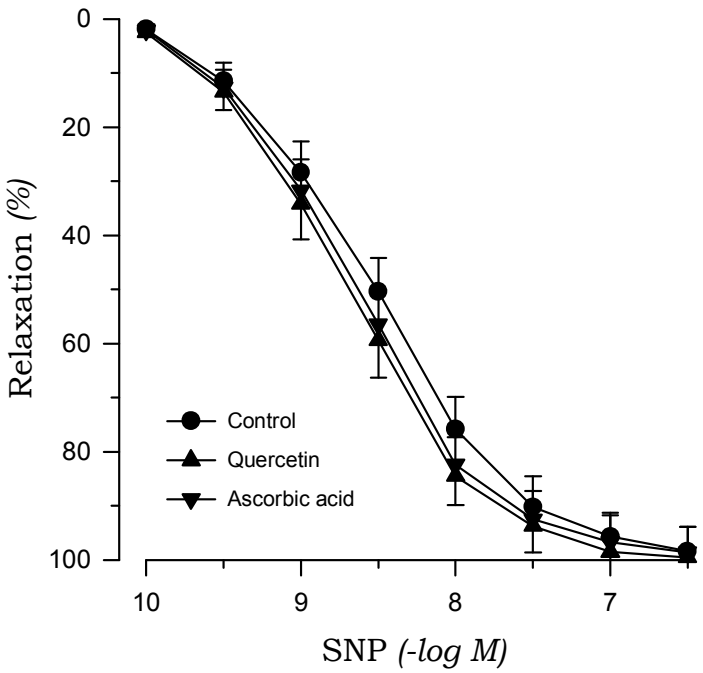

Fig. 8. Effects of quercetin or ascorbic acid on sodium nitroprusside (SNP)-induced relaxations in aortic rings from 2K1C hypertensive rats. Points represent means±SE for six to eight experiments.

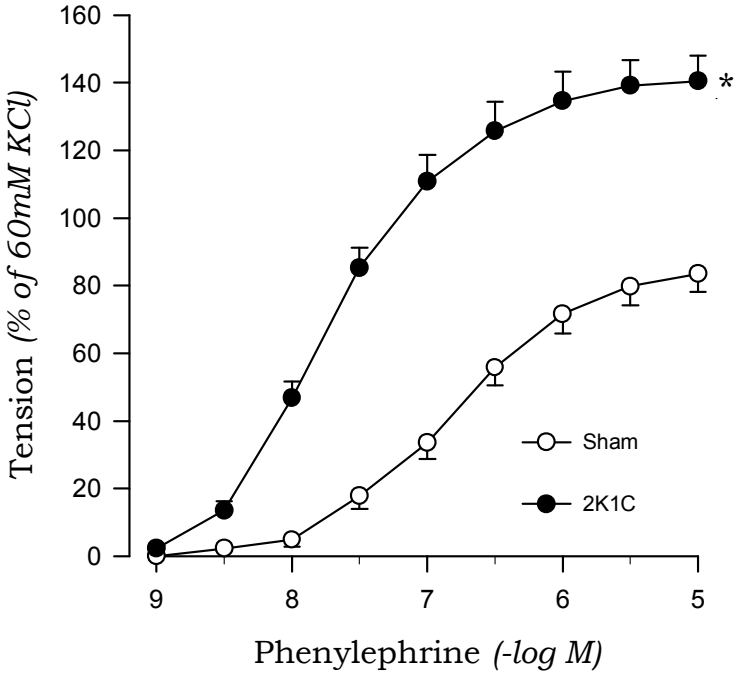

Fig. 9. Contractile responses to phenylephrine in aortic rings from 2K1C hypertensive and sham-clipped control rats. Points represent means±SE for six to eight experiments. * P <0.05, compared with sham values.

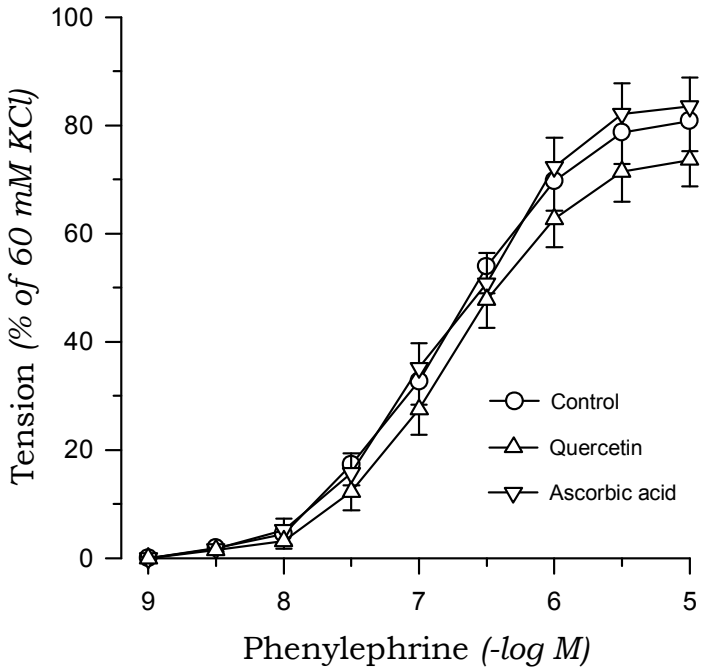

Fig. 10. Effects of quercetin or ascorbic acid on phenylephrine-induced contractions in aortic rings from sham-clipped control rats. Points represent means±SE for six to eight experiments.

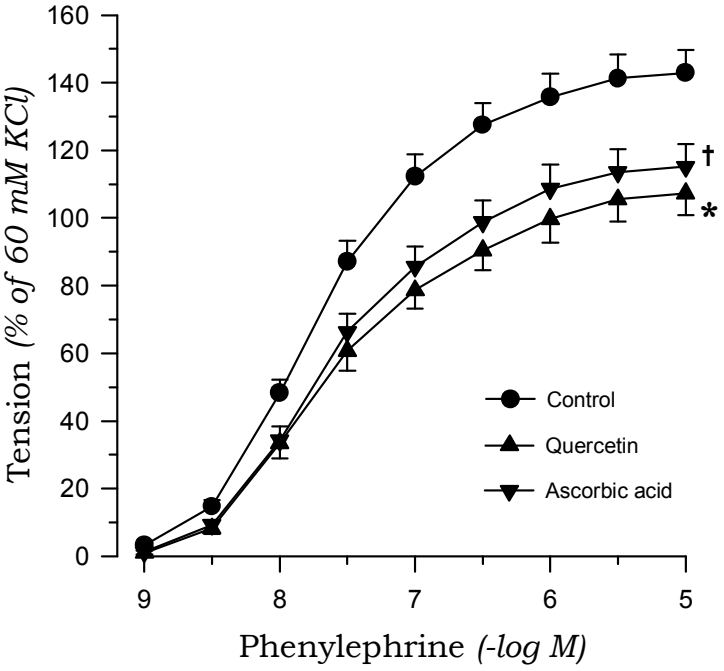

Fig. 11. Effects of quercetin or ascorbic acid on phenylephrine-induced contractions in aortic rings from 2K1C hypertensive rats. Points represent means±SE for six to eight experiments. *, † $P < 0.05$, compared with control values, respectively.

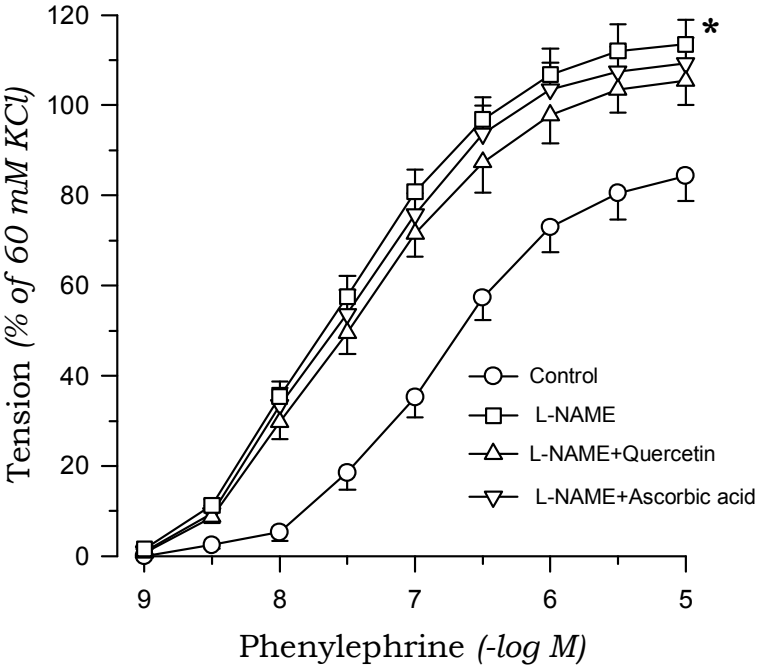

Fig. 12. Effects of Nw-nitro-L-arginine methyl ester(L-NAME) on phenylephrine-induced contractions in aortic rings from sham-clipped control rats. Effects of quercetin or ascorbic acid on phenylephrine-induced contractions in the presence of L-NAME are also shown. Points represent means±SE for six to eight experiments. * P 〈0.05, compared with control values.

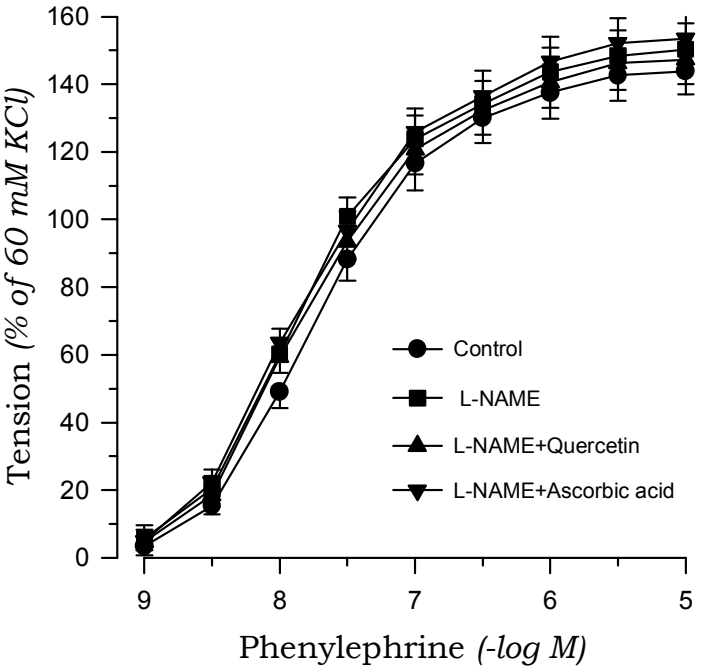

Fig. 13. Effects of Nw-nitro-L-arginine methyl ester(L-NAME) on phenylephrine-induced contractions in aortic rings from 2K1C hypertensive rats. Effects of quercetin or ascorbic acid on phenylephrine-induced contractions in the presence of L-NAME are also shown. Points represent means±SE for six to eight experiments.

Ⅳ. DISCUSSION

Dysfunction of the endothelial modulation is generally characterized by an alteration of the capacity of the vascular endothelium to dilate blood vessels and plays a critical role in the pathogenesis of hypertension[20]. Acetylcholine, which is known to cause vasodilatation via the release of endothelial factors such as NO from the endothelium, is widely used to determine the endothelial function in hypertension[20]. As has been shown recently in our previous report[21], endothelium-dependent relaxation to acetylcholine is markedly attenuated in 2K1C renal hypertensive rats as compared with that in sham-clipped control rats. Acetylcholine-induced vasodilatory effects were completely abolished in both 2K1C and sham rats by treatment with L-NAME, which is NO synthase inhibitor, suggesting that acetylcholine-induced vasorelaxation is mainly contribute to NO synthase-derived NO. As has been observed previously in genetically hypertensive rats[17], endothelium-dependent relaxation to acetylcholine was augmented by preincubation with quercetin or ascorbic acid in 2K1C rats, while both chemicals did not affect the relaxant response to acetylcholine in sham rats. The results indicate that endothelium-derived NO-mediated relaxation responses induced by acetylcholine are promoted by quercetin and ascorbic acid in 2K1C hypertensive aortae, and such mechanisms are absent in normotensive tissues. In association with these observations, previous studies[22,23] have proposed that blunted acetylcholine-induced vasorelaxation in hypertensive arteries is due to increased production of endothelium-derived factors such as vasoconstrictor prostanoids or superoxide anions. However, participation of vasoconstrictor prostanoids in acetylcholine-induced relaxation may be ruled out in 2K1C rats in this study, because all the experimental procedure were performed in the presence of indomethacin, cyclooxygenase inhibitor. Therefore, superoxide anions may be a possible candidate in impaired endothelium-dependent relaxation in hypertensive aortae. It may be postulated that the impaired relaxation to acetylcholine which is shown in 2K1C aortae is

attributed to harmful effects of superoxide anions or other free radicals on endothelium-derived NO. For the biological interaction NO with superoxide anions in vascular endothelium, it has been demonstrated that NO can be scavenged by superoxide anions to form peroxynitrite ($ONOO^-$)[24] and evoke to reduce the bioavailability of endothelium-derived NO caused by decreased NO synthesis or increased NO degradation. Taken together, as previously observed in genetic hypertension[17], an augmentation of acetylcholine-induced relaxation in 2K1C rats may be linked to superoxide anion scavenging properties by flavonoid quercetin or antioxidant vitamin.

The present study confirmed earlier observation[21], that endothelium-independent relaxations to SNP, which is an NO donor, were comparable in between aortic rings from 2K1C and sham rats. Quercetin or ascorbic acid did not affect the SNP-induced relaxation either in 2K1C or in sham rats, suggesting that NO-mediated vascular relaxation in hypertensive and normotensive rats are not altered by quercetin and ascorbic acid. It has been reported that flavonoids exert mainly their beneficial vascular effects through an accumulation on the surface between the endothelial and vascular smooth muscle cells and to improve NO bioavailability by either enhancing endothelium-derived NO production or inhibiting its destruction by free radicals[15]. Thus, it may be postulated that effects of quercetin or ascorbic acid are masked on which SNP-induced fully NO-mediated vasorelaxation, as has been shown previously in genetically hypertensive rats[17].

It has been shown that vascular reactivity to contractile agonist is enhanced in disease states such as hypertension[25]. In accordance with our previous findings[26], the contractile response to phenylephrine was augmented in aortic rings from 2K1C hypertensive rats as compared with sham-clipped control rats. In the present study, either quercetin or ascorbic acid inhibit phenylephrine-induced contraction in aortic rings from 2K1C rats, while both compounds did not affect in sham rats. These results led us to hypothesize that whether a diminution of phenylephrine-induced contraction by quercetin and

ascorbic acid in 2K1C rats is responsible for improved biological activity of endothelium-derived NO by flavonoid or antioxidant vitamin. To investigate this issue, effects of quercetin or ascorbic acid on the contractile response to phenylephrine were examined in the presence of L-NAME. The contractile response to phenylephrine was markedly enhanced by L-NAME treatment in sham rats, while phenylephrine-induced contraction was comparable in 2K1C rats, suggesting that the role of endothelium-derived NO in isolated vascular segments to the vasoconstrictor is impaired in hypertensive rats. Similar results were also found in our previous study[26] that endothelium removal enhanced phenylephrine-induced contraction in 2K1C aortae, while it was without effect in sham tissue. These results imply that endothelium may modulate not only vasorelaxation but also vascular contraction induced by vasoconstrictor substances. It has been reported that the constrictors interact with the endothelium to cause the release of relaxing factors, which then have an inhibitory effect on vascular smooth muscle tone[3]. As expected, flavonoid quercetin or antioxidant ascorbic acid did not affect phenylephrine-induced contraction in the presence of L-NAME either in 2K1C or in sham rats. The results strongly suggest that a diminished effect by quercetin or ascorbic acid on the contractile response to phenylephrine in hypertensive rats is due to an altered biological activity of endothelium-derived NO. On the other hand, our results are inconsistent with the previous study by Ajay et al[17] that the magnitude of phenylephrine-induced contraction is similar in between spontaneous hypertensive and normotensive Wistar rats, and inhibitory effects by quercetin and ascorbic acid on the contractile response to phenylephrine in hypertensive rats are reversed in the presence of L-NAME. They also reported that the inhibitory effect of quercetin on phenylephrine-induced contraction in hypertensive aortae is more potent than ascorbic acid. These differences are complicate to explain, one possible discrepancy may be contributed to a different induction of experimental hypertension, animal species or experimental methods employed[25].

In the present study, the effects of flavonoid quercetin on the vascular function are similar with antioxidant vitamin ascorbic acid suggesting that an improved vascular reactivity by quercetin in 2K1C hypertensive rats is probably attributed to an antioxidant effect of flavonoid. To our knowledge, this is the first report showing that acute exposure to quercetin or ascorbic acid promote endothelium-dependent relaxation and inhibit the contractile responses in 2K1C rats. In addition, enhancement of acetylcholine-induced relaxation and diminishment of phenylephrine-induced contraction by quercetin or ascorbic acid in hypertensive rats are association with an alteration of biological activity of endothelium-derived NO. Previous studies[27,28] have demonstrated that superoxide anion levels are increased in renovascular hypertensive rats. Accumulating evidences suggest that maintenance of 2K1C hypertension is concerned with a systemically increase in oxidative stress[9,10] and we also reported recently that vascular oxidative stress may be involved in chronic 2K1C renal hypertension[29]. Therefore, as discussed above, it cannot be ruled out the possibility that an improved vascular reactivity by quercetin in 2K1C hypertensive rats may, at least in part, be linked to protective effects by flavonoid to vascular endothelium-derived NO through an biological interaction endothelial NO with superoxide anions, although the direct measurement of NO and superoxide anion levels which we were unable to perform in this study.

In summary, acute exposure to quercetin improves endothelium-dependent relaxation and inhibits α1-adrenoceptor mediated contractions, and the effects were similar with antioxidant vitamin ascorbic acid in aortic rings from 2K1C hypertensive rats. The effects of quercetin may, at least in part, be linked to its protective effects to vascular endothelial NO in renal hypertension.

V. SUMMARY

Chronic treatment with the dietary flavonoid quercetin is known to lower blood pressure and restores endothelial dysfunction in animal models of hypertension. The aim of the present study was to investigate the direct effects of quercetin on vascular response in chronic 2K1C renal hypertensive rats. The effects of antioxidant vitamin ascorbic acid on the vasoreactivity were also examined. The 2K1C renal hypertension was induced by clipping the left renal artery and age-matched rats receiving sham treatment served as controls. Thoracic aortae were mounted in tissue baths for measurement of isometric tension. Relaxant responses to acetylcholine were significantly attenuated in 2K1C rats than in sham rats. Quercetin or ascorbic acid augmented the acetylcholine-induced relaxation in 2K1C rats, while no significant differences were noted in sham rats. The relaxation response to sodium nitroprusside (SNP) was comparable in between 2K1C and sham rats and the SNP-induced relaxation was not altered by quercetin or ascorbic acid in both groups. Contractile response to phenylephrine was significantly enhanced in 2K1C rats than in sham rats. Phenylephrine-induced contraction was inhibited by pretreatment with quercetin or ascorbic acid in 2K1C rats, while both chemicals did not affect in sham rats. Nw-nitro-L-arginine methyl ester (L-NAME) markedly augmented the contractile response to phenylephrine in sham rats, while no significant differences were shown in 2K1C rats. Quercetin or ascorbic acid did not affect the phenylephrine-induced contraction in the presence of L-NAME either in 2K1C or in sham rats. These results suggest that acute exposure to quercetin improves endothelium-dependent relaxation and inhibits the contractile response with a similar effect of ascorbic acid in 2K1C hypertension. The results explain, in part, the vascular beneficial effects of quercetin in chronic renal hypertension.

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