Exaggerated Vascular Response Due to Endothelial Dysfunction and Role of the Renin-Angiotensin System at Early Stage of Renal Hypertension in Rats

Jin Hoshino, Tetsuo Sakamaki, Tetsuya Nakamura, Masato Kobayashi, Misa Kato, Hironosuke Sakamoto, Toshiaki Kurashina, Atsuko Yagi, Kunio Sato, Zenpei Ono

We investigated whether endothelial dysfunction might contribute to the exaggerated vasoconstriction that was induced by the administration of norepinephrine at the early stage of one-kidney, one-clip renal hypertension (1K1C) in rats. We also studied the role of the renin-angiotensin system in this phenomenon. Male Wistar rats were killed 48 hours after the induction of renal artery stenosis or sham operation, and ring preparations of the thoracic aorta were obtained. The isometric contraction and relaxation of aortic strips produced by norepinephrine and acetylcholine, respectively, were recorded with a force-displacement transducer. The aorta of 1K1C rats showed a significantly (P<0.05) exaggerated contractile response to norepinephrine as compared with that of control rats. Rubbing the endothelium and treatment with methylene blue or N\textsuperscript{\textbf{\textit{O}}}monomethyl L-arginine acetate augmented the contractile responses to norepinephrine to a greater extent in control rats than in 1K1C rats; therefore, the responses of the groups did not differ significantly. In the second experiment, rats received 0.05% captopril, 0.02% enalapril, or 0.02% nicardipine in the drinking water for 1 day before and for 48 hours after the induction of renal artery stenosis or sham operation. The increased contractile responses of the aorta to norepinephrine in 1K1C rats were normalized to the level of the control rats by treatment with either captopril or enalapril but not with nicardipine. These results suggest that the endothelial dysfunction may contribute to the exaggerated norepinephrine-induced vasoconstriction observed in the 1K1C rats and that angiotensin I-converting enzyme inhibitors can restore the endothelial function. (Circ Res. 1994;74:130-138.)

**Key Words**  • endothelium  • nitric oxide  • one-kidney, one-clip renal hypertension  • captopril  • enalapril  • nicardipine

Although an increased vascular reactivity to vasoconstrictor substances has been observed both in clinical hypertension\textsuperscript{1,2} and in animal models of hypertension,\textsuperscript{3-9} the mechanisms are not completely understood. Folkow et al\textsuperscript{10} suggest that this hyperresponsiveness in hypertension is due to vascular wall hypertrophy or medial hypertrophy that results in an increased vessel wall to lumen ratio. Findings of other investigators\textsuperscript{4,5,11} indicate that such mechanisms are not the only explanation. For instance, Prewitt et al\textsuperscript{11} reported that no vascular wall hypertrophy was observed in resistance vessels of one-kidney, one-clip renal hypertensive (1K1C) rats, despite a structural reduction in the size of the lumen. Others\textsuperscript{4,5} have reported that an exaggerated pressor response and increased sensitivity (change in threshold) to norepinephrine occur in rabbits with renal artery stenosis at an early stage, even before the onset of hypertension. These studies\textsuperscript{4,5,11} indicate that the exaggerated pressor response may be due to some alteration in the responsiveness of the vessels without hypertrophy of the vascular wall and suggest that the responsiveness of the vascular smooth muscle cells itself may be enhanced. However, confirmatory evidence is lacking.

The response of isolated blood vessels to a vasoactive agonist is reportedly modulated by endothelial cells.\textsuperscript{12-16} Endothelium-derived relaxing factor (EDRF), first described by Furchgott and Zawadzki,\textsuperscript{17} has been characterized pharmacologically. The properties of EDRF and nitric oxide (NO) are identical.\textsuperscript{18} The guanidino nitrogen group of L-arginine is the physiological endogenous precursor of the NO molecule.\textsuperscript{19} This biosynthetic process, the endothelium-dependent relaxation of vascular rings, and the vasodilatation induced by acetylcholine are all inhibited by the L-arginine analogue N\textsuperscript{\textbf{\textit{O}}}monomethyl L-arginine acetate (LNMMA).\textsuperscript{20,21} NO reportedly activates cytoplasmic guanylate cyclase, elevates tissue levels of cGMP, produces a marked relaxation of vascular smooth muscle,\textsuperscript{22-24} and modulates the vasoconstrictor action of such vasoactive substance\textsuperscript{25,26} as norepinephrine. Recent in vitro studies on isolated conduit arteries, mainly the aorta, have demonstrated that endothelium-dependent responses are reduced in several models of hypertension\textsuperscript{14,16,29} and that an angiotensin I-converting enzyme (ACE) inhibitor could prevent or reverse endothelial dysfunction in hypertensive animals.\textsuperscript{27,28}

Our objective was to determine the role of depressed synthesis and/or release of EDRF on the exaggerated vasoconstrictive response to norepinephrine at the early stage of hypertension before the development of vascular wall hypertrophy or an increased vascular wall to lumen ratio. The present study was designed to clarify the role of the renin-angiotensin system in endothelial
dysfunction, using ACE inhibitors in 1K1C rats, because circulating angiotensin II is suggested to produce vascular and/or endothelial injury.29,30 Two different ACE inhibitors, captopril and enalapril, were administered to evaluate the possibility that the sulfhydryl moiety of captopril would protect NO against the superoxide-mediated breakdown. In addition, nicardipine as calcium antagonist was administered to determine the effect of a non-ACE inhibitor on the endothelial dysfunction in 1K1C rats.

Materials and Methods

Male Wistar rats that were 15 to 16 weeks old (Imai, Saitama, Japan) were used. They were individually caged, fed rat chow and water ad libitum, and maintained on a 12-hour light-dark cycle until the experiments were performed. Systolic blood pressure was measured by tail-cuff methods (model UR5000, Ueda Co, Tokyo, Japan). Rats were anesthetized with ethyl ether. After an incision was made in each flank, a silver clip 0.45 mm in diameter was put on the right renal artery, and the left kidney was removed. Control rats were similarly treated, except that no clip was applied (sham-operated rats). In the preliminary study in seven rats, we observed that systolic blood pressure rose from 130±8 to 225±45 mm Hg 4 weeks after the placement of a clip of this size. A prophylactic antibiotic (carumomon, 30 mg/kg IM) was injected after the surgery.

Forty-eight hours after the induction of renal artery stenosis or the sham operation, the rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg) and then exsanguinated. The thorax was opened, and the descending thoracic aorta was immediately excised. The aorta was carefully removed to avoid damaging the endothelium. After removing the loose connective tissue, one or two cylindrical segments 3 mm long were cut from the aorta. The number of aortic rings obtained from each rat depended on the protocol (described below). Two stainless-steel wires (outer diameter, 0.1 mm) were inserted through the lumen of the aortic ring. One wire was anchored for stationary support, and the other was connected to a force-displacement transducer (model UR-50GR Minebea Co, Ltd, Nagano, Japan). The preparation was bathed in 10 mL Krebs’ bicarbonate solution aerated with a mixture of 95% O2–5% CO2 and maintained at 37°C. The composition of the Krebs’ bicarbonate solution was (mmol/L) NaCl, 120; KCl, 5.2; CaCl2·H2O, 2.4; MgSO4·7H2O, 1.2; NaHCO3, 25; Na2-EDTA, 0.03; and dextrose, 11 (pH 7.4). Agonists were applied to the bath in volumes of 0.1 mL using a calibrated pipette. The agonists were further diluted with the bath solution to achieve the final concentrations indicated in each protocol. The bath solution was continuously bubbled to obtain rapid mixing of the drug. To obtain a dose-response curve, the drug was added cumulatively from low to high concentrations. In a preliminary study, we tested addition of the drug from high to low concentrations, washing out the bath at each dosage, and obtained results similar to those seen with the cumulative application.

Rings were suspended under 2 g of tension. The force of isometric contraction was measured using a force-displacement transducer. Preparations were allowed to equilibrate for 90 minutes. In all cases, 100 μL of norepinephrine (10−5 mol/L) was initially applied to the bath to preconstrict the aortic strip. The concentration of norepinephrine in the final bath was 10−7 mol/L, as described above. After the contraction had reached a plateau at ~2 minutes, the aorta was relaxed using 10−7 mol/L acetylcholine to confirm that the endothelium was intact. The bath solution was then washed out with Krebs’ bicarbonate solution and allowed to equilibrate for 30 minutes.

Experiment 1: Alteration of Vasostrictive Response to Norepinephrine in 1K1C Rats Compared With Control Rats

In these experiments, we tested whether norepinephrine-induced vasoconstriction might be altered in 1K1C rats compared with sham-operated rats. The effects of three different kinds of treatment, ie, endothelial removal, soluble guanylate cyclase inhibition, and NO synthesis inhibition, were studied to evaluate the roles of endothelium, EDRF, and endothelium-derived NO on norepinephrine-induced vasoconstriction, respectively. Two aortic strips were excised from each rat. One strip was used to compare the response to norepinephrine between 1K1C and control rats and also served for the control experiments of another strip, which was used for the treatment of endothelial removal, guanylate cyclase inhibition with methylene blue, or NO synthesis inhibition with LNMMA.

Experiment 1A: Role of endothelium on norepinephrine-induced vasoconstriction. Dose-related vasoconstriction to norepinephrine was studied in 1K1C and sham-operated rats with or without endothelium. Endothelium was removed in one of two aortic rings obtained from each rat by gently rubbing the intimal surface with a wooden stick.17 After the presence or absence of endothelium was confirmed by acetylcholine-induced vasodilation as described above, norepinephrine was added cumulatively to the bath to achieve the maximal contraction from 10−10 to 10−6 mol/L. Then, the dose-response curve of isometric contractile responses to norepinephrine was determined.

Experiment 1B: Effect of soluble guanylate cyclase inhibition with methylene blue on norepinephrine-induced vasoconstriction. cGMP is reportedly a second messenger of EDRF,22-24 and methylene blue blocks soluble guanylate cyclase activity.31 To evaluate the roles of cGMP and soluble guanylate cyclase, the effect of methylene blue on norepinephrine-induced vasoconstriction was examined. One of the two aortic preparations from each rat of either 1K1C or control rats was incubated in the Krebs’ bicarbonate solution with methylene blue (10−6 mol/L) for 30 minutes until equilibration. Another strip was incubated in the Krebs’ bicarbonate solution without methylene blue as a control condition. A dose-response curve of contractile responses to norepinephrine was determined as in experiment 1A.

Experiment 1C: Effect of NO synthesis inhibition with LNMMA on norepinephrine-induced vasoconstriction. The L-arginine analogue LNMMA can act as a NO synthase inhibitor.20,21 To evaluate the role of endothelium-derived NO on norepinephrine-induced vasoconstriction, the dose-related vasoconstriction to norepinephrine was determined in the presence and absence of LNMMA treatment. Aortic rings were incubated in Krebs’ bicarbonate solution with or without LNMMA (10−2 mol/L) for 30 minutes to equilibrate, and the dose-response curve of contractile responses to norepinephrine was determined as in experiments 1A and 1B.

Experiment 2: Role of the Renin-Angiotensin System in Endothelial Function and Norepinephrine-Induced Vasoconstriction in Control and 1K1C Rats

To determine the role of the renin-angiotensin system in endothelial dysfunction in 1K1C rats, an ACE inhibitor, captopril or enalapril, was administered orally to each group. We tested the effectiveness of ACE inhibition in a preliminary study. Three concentrations of captopril (0.01%, 0.05%, and 0.1%) or of enalapril (0.01%, 0.02%, and 0.05%) were given in the drinking water to normal Wistar rats (200 to 250 g body weight) for 3 days. The rats were anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg). The carotid artery and jugular vein were cannulated, and pressor responses to an intravenous infusion of angiotensin I (200 ng·kg−1·min−1) and to angiotensin II (200 ng·kg−1·min−1)
were observed. The increase in mean arterial pressure in response to intravenous angiotensin I was suppressed by the ACE inhibitors, being 33 ± 5, 26 ± 8, and 6 ± 3 mm Hg after captopril was given at doses of 0.01%, 0.05%, and 0.1%, respectively. The response was 29 ± 3, 20 ± 6, and 12 ± 4 mm Hg after enalapril was given at doses of 0.01%, 0.02%, and 0.05%, respectively. However, the increase in mean arterial pressure in response to angiotensin II was unaffected by the administration of ACE inhibitors, being 45 ± 6, 59 ± 1, and 39 ± 9 mm Hg after captopril was given at doses of 0.01%, 0.05%, and 0.1%, respectively. The response was 51 ± 4, 42 ± 7, and 43 ± 4 mm Hg after enalapril was given at doses of 0.01%, 0.02%, and 0.05%, respectively. In the present study, we selected doses of 0.05% for captopril and 0.02% for enalapril to be given in the drinking water to inhibit ACE activity.

Experiment 2A: Effect of ACE inhibition with captopril on the norepinephrine-induced vasoconstriction. Rats were divided into four groups: two groups of rats with renal artery stenosis and two sham-operated groups, with or without the oral administration of captopril. Two groups of rats were given 0.05% captopril in drinking water (150 mg·kg⁻¹·d⁻¹), and two groups received tap water. For each group, the protocol was begun 1 day before the induction of renal artery stenosis or the sham operation and continued for 48 hours after surgery. Renal artery stenosis and sham operation were performed as described in experiment 1. One aortic ring preparation was obtained from each rat, and the dose-response curve of the contractile responses to norepinephrine was determined as in experiment 1.

Experiment 2B: Effect of ACE inhibition with enalapril on the norepinephrine-induced vasoconstriction. Captopril has a sulfhydryl moiety in its structure, whereas enalapril does not. This moiety is reported to scavenge superoxide anion and protect EDRF breakdown. Therefore, we used enalapril in this experiment to inhibit ACE activity. The same protocol as in experiment 3A was performed using enalapril instead of captopril. Enalapril (100 mg·kg⁻¹·d⁻¹) was given in the drinking water at a concentration of 0.02%. One aortic ring preparation was obtained from each rat, and the dose-response curve of contractile responses to norepinephrine was determined as in experiment 1.

Experiment 3: Alteration of Endothelium-Dependent Relaxation Induced by Acetylcholine in 1K1C Rats Compared With Control Rats

Acetylcholine is an endothelium-dependent vasodilator. We determined whether there might be a difference in acetylcholine-induced vasodilation between 1K1C and control rats. The role of endothelium-derived NO in acetylcholine-induced vasodilation was also studied using LNMMa in both groups of rats. Two aortic strips were excised from each rat: one was used for the control study and the other was used after treatment with LNMMa. Aortic rings were incubated in Krebs' bicarbonate solution with or without LNMMa (10⁻⁵ mol/L) for 30 minutes to equilibrate and then preconstricted by norepinephrine (10⁻⁷ mol/L). After the contraction had reached a plateau, 100 μL acetylcholine was cumulatively applied to the bath to achieve final bath concentrations of 10⁻⁸ to 10⁻⁵ mol/L, and the relaxation responses were observed.

Experiment 4: Role of the Renin-Angiotensin System in Endothelial Function and Acetylcholine-Induced Vasodilation in Control and 1K1C Rats

We studied the effect of ACE inhibition with captopril on the vasodilation induced by acetylcholine. Captopril (0.05%) was administered in the drinking water for 48 hours after operation. One aortic strip was excised from each rat. Aortic rings were incubated in Krebs' bicarbonate solution for 30 minutes to equilibrate and then preconstricted by norepinephrine (10⁻⁷ mol/L). After the contraction had reached a plateau, 100 μL acetylcholine was cumulatively applied to the bath to achieve final bath concentrations of 10⁻⁸ to 10⁻⁵ mol/L, and the relaxation responses were observed.
putative maximal response was referred to as pD₂. The putative maximal vasoconstrictor response was considered to be the level before preconstriction by norepinephrine, and the response to each dose was expressed as the percentage of the putative maximal vasodilation. Results are presented as mean±SEM. Data were evaluated by ANOVA, followed by Duncan’s multiple-range test. The level of statistical significance was considered to be P<.05.

Results

Table 1 summarizes body weight, systolic blood pressure, and heart rate, measured by tail-cuff methods, in eight groups of rats. There were no significant differences in body weight among the groups on the day when the aortic ring preparations were obtained. The systolic blood pressure or heart rate did not differ among groups before the induction of renal artery stenosis or sham operation. Systolic blood pressure rose slightly (P<.05) in 1K1C rats 48 hours after renal artery stenosis as compared with sham-operated rats. Treatment with captopril or enalapril prevented the rise in systolic blood pressure in the 1K1C rats. The presence of renal artery stenosis or the administration of captopril or enalapril did not significantly affect the heart rate. Treatment with nicardipine prevented the rise in systolic blood pressure in the 1K1C rats. The administration of nicardipine increased the heart rate significantly (P<.05) in 1K1C rats.

Experiment 1: Alteration of Vasoconstrictive Response to Norepinephrine in 1K1C Rats Compared With Control Rats

The isometric contraction of the aortic ring in response to norepinephrine was exaggerated in 1K1C rats as compared with control rats (Figs 1 through 4). Both the pD₂ and maximal constriction were significantly (P<.05) higher in 1K1C rats than in control rats (Tables 2, 3, and 5). Removal of the endothelium produced a leftward shift in the norepinephrine-induced vasoconstriction and increased the pD₂ and the maximal response in the control rats (Fig 1; Table 2, experiment 1A). Removal of the endothelium abolished the difference in norepinephrine-induced vasoconstriction between control and 1K1C rats. After the treatment with methylene blue and LNMMA, pD₂ and the maximal response increased significantly (P<.05) in both groups but were more pronounced in the control versus the 1K1C rats, and the difference in the vasoconstrictor response to norepinephrine between the two groups was diminished (Fig 2; Table 2, experiments 1B and 1C).

Experiment 2: Role of the Renin-Angiotensin System in Endothelial Function and Norepinephrine-Induced Vasoconstriction in Control and 1K1C Rats

Treatment with captopril (Fig 3) and enalapril (Fig 4) produced a rightward shift of norepinephrine-induced vasoconstriction in 1K1C rats but did not alter the response of control rats. Both pD₂ and the maximal response decreased significantly (P<.05) with captopril.

![Dose-response curves show the effect of endothelium removal (rubbed) on norepinephrine-induced vasoconstriction in one-kidney, one-clip (1K1C) and sham-operated (control) rats. Intact indicates intact endothelium.](http://circres.ahajournals.org/)}
TABLE 2. Norepinephrine-Induced Vasoconstriction

<table>
<thead>
<tr>
<th>Experiment</th>
<th>pD2</th>
<th>Max Const, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A: Effect of endothelial removal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=12)</td>
<td>7.66±0.09</td>
<td>1141±25</td>
</tr>
<tr>
<td>1K1C (n=12)</td>
<td>8.30±0.05*</td>
<td>1205±27*</td>
</tr>
<tr>
<td>Control+rubbed (n=12)</td>
<td>8.34±0.03*</td>
<td>1272±33*</td>
</tr>
<tr>
<td>1K1C+rubbed (n=12)</td>
<td>8.42±0.04*</td>
<td>1292±24*</td>
</tr>
<tr>
<td>1B: Effect of MB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=7)</td>
<td>7.16±0.21</td>
<td>999±112</td>
</tr>
<tr>
<td>1K1C (n=7)</td>
<td>7.77±0.19*</td>
<td>1275±132*</td>
</tr>
<tr>
<td>Control+MB (n=7)</td>
<td>8.14±0.15‡</td>
<td>1590±103‡</td>
</tr>
<tr>
<td>1K1C+MB (n=7)</td>
<td>8.38±0.07‡</td>
<td>1571±142‡</td>
</tr>
<tr>
<td>1C: Effect of LNMMA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=7)</td>
<td>7.55±0.10</td>
<td>1037±83</td>
</tr>
<tr>
<td>1K1C (n=7)</td>
<td>8.17±0.14†</td>
<td>1190±99</td>
</tr>
<tr>
<td>Control+LNMMA (n=7)</td>
<td>8.38±0.11‡</td>
<td>1532±92‡</td>
</tr>
<tr>
<td>1K1C+LNMMA (n=7)</td>
<td>8.49±0.13†</td>
<td>1542±53‡</td>
</tr>
</tbody>
</table>

$pD_2$ indicates the negative logarithm of half-maximal constriction; Max Const, maximal constriction of aortic strips from one-kidney, one-clip (1K1C) and sham-operated (control) rats; n, number of rats; rubbed, endothelium removal; MB, methylene blue; and LNMMA, N\(^\text{0}\)-monomethyl L-arginine acetate. Values are mean±SEM.

*P<.05 and †P<.01 vs control; ‡P<.05 vs 1K1C.

and enalapril in 1K1C rats (Table 3, experiments 2A and 2B). There was no significant difference in $pD_2$ or the maximal response between 1K1C and control rats after treatment with captopril or enalapril.

Experiment 3: Alteration of Endothelium-Dependent Relaxation Induced by Acetylcholine in 1K1C Rats Compared With Control Rats

The cumulative addition of acetylcholine produced endothelium- and concentration-dependent relaxations of aortic rings that were precontracted with norepinephrine (10\(^{-7}\) mol/L). The relaxation induced by acetylcholine was significantly ($P<.05$) reduced in 1K1C rats compared with control rats. $pD_2$ and maximal response values in 1K1C rats were significantly ($P<.05$) smaller than the control values (Table 4). LNMMA abolished the vasodilator response to acetylcholine in both groups (Fig 5, Table 4). No significant difference was observed.
Table 3. Norepinephrine-Induced Vasocontriction Treated With Oral Administration of Captopril or Enalapril

<table>
<thead>
<tr>
<th>Experiment</th>
<th>pD2</th>
<th>Max Const, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>2A: Effect of captopril</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>7.66±0.10</td>
<td>1156±25</td>
</tr>
<tr>
<td>1K1C (n=10)</td>
<td>8.30±0.06*</td>
<td>1280±19</td>
</tr>
<tr>
<td>Control+captopril (n=10)</td>
<td>7.55±0.06†</td>
<td>1160±25$</td>
</tr>
<tr>
<td>1K1C+captopril (n=10)</td>
<td>7.63±0.03†</td>
<td>1100±29§</td>
</tr>
<tr>
<td>2B: Effect of enalapril</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>7.73±0.10</td>
<td>1075±120</td>
</tr>
<tr>
<td>1K1C (n=8)</td>
<td>8.28±0.14‡</td>
<td>1300±104</td>
</tr>
<tr>
<td>Control+enalapril (n=8)</td>
<td>7.85±0.09§</td>
<td>1025±62§</td>
</tr>
<tr>
<td>1K1C+enalapril (n=8)</td>
<td>7.83±0.06§</td>
<td>1037±89§</td>
</tr>
</tbody>
</table>

pD2 indicates the negative logarithm of half-maximal constriction; Max Const, maximal constriction of aortic strips from one-kidney, one-clip (1K1C) and sham-operated (control) rats; and n, number of rats. Values are mean±SEM.

*P<.01 vs control; †P<.01 vs 1K1C; ‡P<.05 vs control; and §P<.05 vs 1K1C.

in the response between the groups after the treatment with LNMMA (Fig 5).

Experiment 4: Role of the Renin-Angiotensin System in Endothelial Function and Acetylcholine-Induced Vasodilation in Control and 1K1C Rats

Fig 6 and Table 4 show the effect of oral administration of captopril on the acetylcholine-induced vasodilation in control and 1K1C rats. The relaxation was augmented by captopril in 1K1C rats, with a significant shift of the dose-response curves toward the left (Table 4). In contrast to 1K1C rats, captopril did not change the dose-response curve in control rats (Table 4). There was no significant difference in the acetylcholine-induced vasodilation between the 1K1C and control rats after treatment with captopril.

Experiment 5: Effect of the Calcium Antagonist on Norepinephrine-Induced Vasoconstriction in Control and 1K1C Rats

Fig 7 and Table 5 show the effect of oral administration of nicardipine on the norepinephrine-induced vasoconstriction in control and 1K1C rats. pD2 and the maximal response were significantly (P<.01) higher in 1K1C than in control rats (Table 5). Both pD2 and the
The major findings of the present study are enhanced vasoconstriction to norepinephrine and depressed endothelium-dependent relaxations to acetylcholine in aortic rings at the early stage in 1K1C rats as compared with control rats. After the removal of endothelium and treatment with methylene blue and L-NMMA, the difference in norepinephrine-induced vasoconstriction between 1K1C and control rats was diminished. L-NMMA abolished the vasodilator response to acetylcholine in both the control and 1K1C rats. Oral administration of either captopril or enalapril normalized the vasoconstrictor response to norepinephrine to control levels in 1K1C rats. Captopril also restored acetylcholine-induced vasodilation in 1K1C rats. However, oral administration of a non-ACE inhibitor, the calcium antagonist nicardipine, did not normalize the vasoconstrictor response to norepinephrine in 1K1C rats. These data suggest that the protective role of the endothelium against vasoconstrictive stimuli is less pronounced in the aorta of 1K1C than in normotensive animals and that the renin-angiotensin system may contribute to this alteration in endothelial function.

All aspects of vasoconstriction seem to be enhanced in hypertension, particularly during the early stage of experimental hypertension.5,11 Exaggerated pressor responses have been reported both in hypertensive patients1,2 and in animal models of experimental hypertension.1,3,9,34 The arterioles of hypertensive animals show an increased sensitivity and reactivity to norepinephrine6,34 and other vasoactive agents,7 suggesting that exaggerated reactivity of resistance vessels leads to an enhanced pressor hyperresponsiveness. Our institute8,9 and others4,5 have reported that the pressor response to norepinephrine is enhanced in prehypertensive 1K1C rats and rabbits. These enhanced vascular contractile and pressor responses to vasoconstrictive agents could contribute to the initiation and maintenance of hypertension. The present study demonstrated that the isolated conduit artery also showed exaggerated contractile responses in 1K1C rats during the early stage of hypertension, when structural vascular changes should not yet occur. This early stage of experimental hypertension is especially important in the sense that structural alteration (vascular wall hypertrophy) and aorta regression (medial hypertrophy) have not been demonstrated in resistance vessels at either the early or chronic stage of hypertension in 1K1C rats.

An enhanced vasoconstrictor response could occur without medial hypertrophy if the sensitivity of vascular smooth muscle cells is increased or if other factors that modulate vasoconstriction, such as endothelial factors, were altered. We used an aortic ring preparation in the present study to examine the role of endothelium in this enhanced vascular reactivity because it is appropriate for the removal of endothelium5,9,17 and because a dysfunction of its endothelium has been established in this model of hypertension.15,16 Numerous studies12,13,15,36 have demonstrated that vascular endothelium is a source of vasoactive substances that mediate or modulate the vascular effects of various vasodilator and vasoconstrictor agents. EDRF is generated in the endothelium, is released to the vascular smooth muscle, and produces vasodilation.12,13,15,17,26 EDRF has recently been considered to be identical with NO and to be synthesized from L-arginine.10,20 Endothelium-derived NO has been demonstrated to be generated and/or released continuously, even without an exogenous endothelium-dependent vasodilator; the blockade of its synthesis using the L-arginine analogue L-NMMA induces vasoconstriction in isolated blood vessels.20,21 The basal release of endogenous NO could act as a functional antagonist to vasoconstrictors and may offer a protective role against hypertensive stimuli. In fact, the presence of the endothelium depresses the contraction evoked by norepinephrine.5,37 Conversely, a reduction of the release of endothelial NO could increase the vascular sensitivity to vasoconstrictive agents.

**TABLE 5. Norepinephrine-Induced Vasoconstriction Treated With Oral Administration of Nicardipine**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>pD2</th>
<th>Max Const, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>7.84±0.11</td>
<td>755±102</td>
</tr>
<tr>
<td>K1C (n=6)</td>
<td>8.37±0.06*</td>
<td>1278±51*</td>
</tr>
<tr>
<td>Control+nicardipine (n=6)</td>
<td>7.56±0.10</td>
<td>872±61†</td>
</tr>
<tr>
<td>K1C+nicardipine (n=6)</td>
<td>8.46±0.66*</td>
<td>1122±31*</td>
</tr>
</tbody>
</table>

pD2 indicates the negative logarithm of half-maximal constriction; Max Const, maximal constriction of aortic strips from one-kidney, one-clip (K1C) and sham-operated (control) rats; and n, number of rats. Values are mean±SEM.

*P<.01 vs control; †P<.01 vs K1C.
Interestingly, recent studies performed on isolated conduit arteries in vitro, primarily the aorta, have demonstrated that the release of EDRF stimulated by an endothelium-dependent vasodilator was reduced in several models of hypertension. In the present study, we also observed that acetylcholine-induced vasodilation, which was probably due to the reduced production of NO, was attenuated in 1K1C rats, since LNMMA treatment abolished the acetylcholine-induced vasodilation in both control and 1K1C rats. The present study demonstrated that norepinephrine-induced vasoconstriction, which was probably due to the reduced basal production of NO, was exaggerated in 1K1C rats, since the removal of the endothelium and the inhibition of soluble guanylate cyclase and NO synthase all enhanced the vasoconstriction more in control than in 1K1C rats. This evidence provides new insight into the mechanism of the enhanced vascular reactivity and pressor responsiveness to vasoconstrictive agents and could explain the role of reduction in EDRF in raising the vascular tone and blood pressure.

We studied agonist-induced vasoconstriction and vasodilation of the aortic ring preparation 48 hours after the induction of renal artery stenosis or the sham operation. Systolic blood pressure was slightly higher in 1K1C than in control rats; however, endothelial dysfunction was already evident by acetylcholine-induced vasodilation at this early stage of renal hypertension. These observations suggest that neurohumoral factors, rather than hemodynamic factors, may contribute to the endothelial dysfunction in this model. The renin-angiotensin system has been implicated in hypertensive vascular injury. The oral administration of ACE inhibitors in the present study restored the impaired endothelial function. The result suggests that an elevated circulating level of angiotensin II and/or the enhanced renin-angiotensin system in the vascular tissue may produce endothelial damage. Clozel has reported that the ACE inhibitor cilazapril improves endothelial function in spontaneously hypertensive rats not by inhibiting the synthesis of vasoconstrictor prosstaglandin but by increasing either the release or the action of EDRF. Mombouli et al. have also reported that cilazapril increases production of EDRF in isolated canine arteries. Furthermore, Koivunen et al. have reported that acute intravenous infusion of captopril normalizes the exaggerated pressor response to norepinephrine in conscious 1K1C rabbits with renal artery stenosis of 3-day duration (renal prehypertensive rabbits). They have also observed that angiotensin II infusion increases the pressor response to norepinephrine in these captopril treated rabbits with renal artery stenosis and that circulating angiotensin II may play an important role in the enhanced pressor response to norepinephrine in this model. In the present study, oral treatment with nicardipine did not affect the dose-response curve of norepinephrine-induced vasoconstriction. This finding further supports the important role of the enhanced renin-angiotensin system in endothelial damage in 1K1C rats. Jayakody et al. have reported an absence of effect of nicardipine on endothelium-dependent relaxation in normal rabbit aorta. Superoxide anions reportedly inactivate EDRF by promoting its breakdown and shortening its half-life. Wei et al. have demonstrated that the oxygen radicals produced during acute hypertension abolish the dilation of cerebral arterioles in response to acetylcholine. They have also shown that the vasodilatory response to acetylcholine is restored by the topical application of enzymatic inhibitors of oxygen radicals. Therefore, oxygen radicals, which could be generated in greater number in hypertension, may interfere with the action of NO or may damage the endothelium. The sulfhydryl moiety of captopril can scavenge superoxide anions; this ability presents a possible mechanism for the restoration of endothelial function in renal hypertension. However, we observed that endothelial dysfunction was improved by the treatment not only with captopril, which has a sulfhydryl moiety, but also with enalapril, which does not. Our results indicate that ACE inhibitors improve endothelial dysfunction, irrespectively of a sulfhydryl moiety. These observations suggest that the renin-angiotensin system may be more important than the superoxide anion in producing endothelial dysfunction at this stage in the 1K1C rat.

ACE inhibitors could also block kininase II and increase the level of bradykinin, an endothelium-dependent vasodilator. The vasodilation induced by bradykinin is mediated via NO and prostacyclin. An intact endothelium is required for the action of bradykinin. It has been reported that bradykinin-induced vasodilation is depressed in 1K1C rats. Therefore, when endothelial function is normalized by the administration of an ACE inhibitor, the action of bradykinin in releasing endothelial vasodilators may also be restored, which could promote an antagonistic action of the endothelium against vasoconstrictive stimuli.

In conclusion, the aorta from 1K1C rats exhibited significantly exaggerated contractile responses to norepinephrine at the early stages of hypertension. Rubbing the endothelium and treatment with methylene blue or LNMMA augmented the contractile responses to norepinephrine to a greater extent in control than in 1K1C rats; therefore, the responses of the groups did not differ significantly. The relaxation response to acetylcholine was less in 1K1C than in control rats. The exaggerated contractile response of the aorta to norepinephrine in 1K1C rats was attenuated to the level found in control rats by treatment with captopril or enalapril. These results suggest that endothelial dysfunction may contribute to the exaggerated norepinephrine-induced vasoconstriction observed in the 1K1C rats and that angiotensin I-convertase enzyme inhibitors can restore the endothelial function. The actions of sulfhydryl moiety in captopril to scavenge superoxide anions do not appear to be responsible for the restoration of the endothelial function, because enalapril also normalized the exaggerated vasoconstriction in this model of experimental hypertension.

Acknowledgments

We are indebted to Prof Kazuhiko Murata for much helpful advice and discussion. The authors thank Toshie Ishihara, Akemi Yoguchi, Fusae Mizukawa, Chikako Kawamoto, Kyoko Yamashita, Shizuko Saiki, and Mari Kurosawa for their excellent technical assistance.

References


Exaggerated vascular response due to endothelial dysfunction and role of the renin-angiotensin system at early stage of renal hypertension in rats.

Circ Res. 1994;74:130-138
doi: 10.1161/01.RES.74.1.130
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/74/1/130

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/