Antagonism of ANG II type 1 receptors protects the endothelium during the early stages of renal hypertension in rats

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Hoshino, J, in, Tetsuya Nakamura, Toshiaki Kurashina, Yuichiro Saito, Hiroyuki Sumino, Zenpei Ono, Hironosuke Sakamoto, Keiko Kowase, and Ryozo Nagai. Antagonism of ANG II type 1 receptors protects the endothelium during the early stages of renal hypertension in rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1950–R1957, 1998.—The degree of involvement of the renin-angiotensin system in endothelial dysfunction was investigated by using a one-kidney, one-clip (1K,1C) model of renal hypertension. Male Wistar rats received 0.02% enalapril, 0.02% losartan, or tap water for 1 day before and for 48 h after the induction of renal artery stenosis or sham operation. The aorta of 1K,1C rats showed increased contraction and decreased relaxation responses produced by norepinephrine and acetylcholine, respectively, vs. control responses. Exposure to 10⁻⁵ mol/l N⁶-monomethyl-L-arginine acetate augmented the contractile responses to norepinephrine to a greater extent in control rats than in the 1K,1C rats. The increased contraction and decreased relaxation responses to these agonists in the 1K,1C rats were normalized by enalapril or losartan. The addition of HOE-140 to the bath did not alter these normalized responses. Results suggest that angiotensin II causes endothelial dysfunction and reduces nitric oxide levels in 1K,1C rats. Such endothelial dysfunction enhanced the norepinephrine-induced contraction during the early-stage hypertension in 1K,1C rats.

Acetylcholine; sodium nitroprusside; bradykinin

An increased reactivity to pressor agents is seen in patients with hypertension (2, 6) as well as in experimental models of hypertension (9, 11, 25). The mechanism underlying this phenomenon has not been elucidated clearly, but Folkow et al. (3) have proposed that an increased reactivity to pressor agents results from a thickening of the vascular wall or medial hypertrophy. However, other researchers contend that the enhanced pressor response is not fully explained by such a mechanism (9, 20). For instance, Prewitt et al. (20) failed to find any thickening of the resistance vessels in rats with one-kidney, one-clip (1K,1C) renal hypertension. It has also been reported that an enhanced pressor response to norepinephrine can be detected in rabbits with renal artery stenosis at an early stage, even before the onset of hypertension (9). These findings suggest that the pressor response may be enhanced by changes in vascular reactivity even in the absence of thickening of the vascular wall.

Recent studies show that the vascular response to vasoactive agents is regulated by endothelial cells (12, 16, 26). Endothelium-derived relaxing factor was first described by Furchgott and Zawadzki (4) in 1980, and was subsequently identified as nitric oxide (NO) (19). NO is synthesized from L-arginine and O₂ by NO synthase (19) and acts on such adjacent target cells as smooth muscle cells to induce relaxation of smooth muscle (22). The intravenous administration of N⁶-monomethyl-L-arginine acetate (L-NMMA), an analog of L-arginine, has been shown to selectively inhibit NO synthase and to increase the blood pressure (23). Studies using aortic preparations have shown that endothelium-dependent responses are reduced in various models of hypertension (12, 17, 26). In a previous study, we demonstrated that impairment of NO synthesis or a reduction in NO release could explain the enhanced vasoconstrictor response to norepinephrine before the occurrence of vascular wall thickening during the very early stages of 1K,1C renal hypertension in rats (8). Rubbing the endothelium augmented the contractile responses to norepinephrine to a greater extent in control rats than in the 1K,1C rats; therefore, the response of the groups did not differ significantly (8). We also demonstrated that angiotensin-converting enzyme (ACE) inhibitors can prevent or reverse endothelial dysfunction in this model (8). Other investigators have also reported that ACE inhibitors improve endothelial function (1, 15).

ACE inhibitors reduce the formation of angiotensin II and prevent the degradation of bradykinin, an endothelium-dependent vasorelaxant that stimulates NO release. In the present study, we investigated the involvement of the renin-angiotensin system and the kinin-kallikrein system in endothelial dysfunction during the early stage of hypertension in the 1K,1C rat model to determine whether angiotensin II interferes with the action of NO. For this purpose, we evaluated the ability of an angiotensin II type 1A receptor antagonist (losartan) and a bradykinin B₂ receptor antagonist (HOE-140) to restore the endothelial function in this model.

Materials and Methods

All procedures were approved by the Animal Care and Use Committee of Gunma University School of Medicine and were
performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council (revised 1996). Male Wistar rats aged 15–16 wk (Imlai, Saitama, Japan) were anesthetized with ethyl ether. After an incision was made in each flank, a silver clip of 0.45 mm in diameter was placed on the right renal artery; the left kidney was removed. Control rats were similarly treated, except that no clip was applied (sham-operated rats). A prophylactic antibiotic (carumonam, 30 mg/kg) was injected after the operation. Systolic blood pressure was measured by using the tail-cuff method (model UR5000; Ueda, Tokyo, Japan). In preliminary studies conducted in 8 rats, the plasma levels of angiotensin II were significantly higher in 1K,1C (1,245 ± 72 pg/ml) than in control (462 ± 83 pg/ml) rats 48 h after the induction of renal artery stenosis or the sham operation. We also observed that systolic blood pressure rose from 130 ± 7 mmHg to 223 ± 43 mmHg 4 wk after the placement of a clip of this size in six rats.

Forty-eight hours after the induction of renal artery stenosis or the sham operation, the rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). After the thoracic cavity was carefully opened, one or two cylindrical segments 3 mm long were cut from the tail. The extra peritoneal injection of pentobarbital sodium (50 mg/kg) resulted in anesthetization of the rats. The aorta was cut and divided into six rings of isometric contraction was measured by using a force-displacement transducer (model UR-50GR; Minebea, Nagano, Japan). Each drug was progressively added from a low concentration to the bath in a volume of 0.1 ml to achieve the final concentration indicated in the protocol. In all cases, 100 µl of 10⁻⁵ mol/l norepinephrine was initially applied to the bath to preconstrict the aortic strip. The concentration of norepinephrine in the final bath was 10⁻⁷ mol/l. When the contraction had reached its maximum after ~2 min, the aorta was relaxed using 10⁻⁵, 10⁻⁶, 10⁻⁷, and 10⁻⁸ mol/l sodium nitroprusside were recorded.

The tissue preparation was bathed in 10 ml of Krebs bicarbonate solution aerated with a mixture of 95% O₂ and 5% CO₂ and maintained at 37°C. The composition of the Krebs bicarbonate solution was 120 mmol/l NaCl, 5.2 mmol/l KCl, 2.4 mmol/l CaCl₂, 1.2 mmol/l MgSO₄, 25 mmol/l NaHCO₃, 0.03 mmol/l Na₂-EDTA, and 11 mmol/l dextrose (pH 7.4). The arterial rings were suspended under a g of tension. The force of isometric contraction was measured by using a force-displacement transducer (model UR5000; Ueda, Tokyo, Japan). Each drug was progressively added from a low concentration to the bath in a volume of 0.1 ml to achieve the final concentration indicated in the protocol. In all cases, 100 µl of 10⁻⁵ mol/l norepinephrine was initially applied to the bath to preconstrict the aortic strip. The concentration of norepinephrine in the final bath was 10⁻⁷ mol/l. When the contraction had reached its maximum after ~2 min, 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 an additional 30 min. The arteries were not treated with indomethacin.

**Experiment 1: Alteration of Vasoconstrictor and Vasodilator Responses to Norepinephrine and Acetylcholine**

**Experiment 1A: Effect of NO synthesis inhibition with L-NMMA on norepinephrine-induced vasoconstriction.** The dose-related vasoconstrictor response to norepinephrine was evaluated in 1K,1C and sham-operated rats. To evaluate the influence of endothelium-derived NO on norepinephrine-induced vasoconstriction, one of the two aortic preparations from each 1K,1C or sham-operated rat was equilibrated in Krebs bicarbonate solution with 10⁻⁵ mol/l L-NMMA for 30 min. Another strip was incubated in Krebs bicarbonate solution without L-NMMA as a control. The rings were then preconstricted by adding 10⁻⁷ mol/l norepinephrine. After contraction had reached a plateau, the relaxation responses produced by 10⁻⁵, 10⁻⁶ mol/l acetylcholine were recorded.

**Experiment 1B: Effect of NO synthesis inhibition with L-NMMA on acetylcholine-induced vasodilation.** The involvement of endothelium-derived NO in acetylcholine-induced vasodilation was also evaluated by administering L-NMMA to tissues from 1K,1C and control rats. One of the two aortic preparations from each 1K,1C or sham-operated rat was equilibrated in Krebs bicarbonate solution with 10⁻⁵ mol/l L-NMMA for 30 min. Another strip was incubated in Krebs bicarbonate solution without L-NMMA as a control. The rings were then preconstricted by adding 10⁻⁷ mol/l norepinephrine. After contraction had reached a plateau, the relaxation responses produced by 10⁻⁵, 10⁻⁶ mol/l acetylcholine were recorded.

**Experiment 1C: Confirmation of intact vasodilator response to sodium nitroprusside.** To verify that responsiveness of the vascular smooth muscle to NO is intact, the dose-related vasodilator response to sodium nitroprusside was evaluated in 1K,1C or sham-operated rats. One aortic preparation from each 1K,1C or sham-operated rat was equilibrated in Krebs bicarbonate solution for 30 min. The rings were then preconstricted by adding 10⁻⁷ mol/l norepinephrine. After contraction had reached a plateau, the relaxation responses produced by 10⁻⁵, 10⁻⁶ mol/l sodium nitroprusside were recorded.

**Experiment 2: Involvement of the Renin-Angiotensin System in Endothelial Function and Evaluation of Vasomotor Tone**

We tested the effectiveness of angiotensin-converting enzyme (ACE) inhibition with enalapril and angiotensin II antagonism with losartan in a preliminary study. For 3 days, three concentrations of enalapril (0.01, 0.02, and 0.05%) or of losartan (0.002, 0.01, and 0.02%) were added to the drinking water of normal Wistar rats (200–250 g, n = 6 in each group). The animals were anesthetized with pentobarbital sodium (50 mg/kg), and the carotid artery and jugular vein were cannulated. The increase in mean arterial pressure in response to intravenous angiotensin I, 200 ng·kg⁻¹·min⁻¹, was suppressed by the ACE inhibitor, being 28 ± 4, 19 ± 5, and 11 ± 4 mmHg at enalapril doses of 0.01, 0.02, and 0.05%, respectively. However, the increase in mean arterial pressure in response to angiotensin II, 200 ng·kg⁻¹·min⁻¹, was unaffected with each increase in dose of enalapril. From these results, we selected a dose of enalapril of 0.02% to inhibit ACE activity. The increase in mean arterial pressure in response to intravenous angiotensin II, 200 ng·kg⁻¹·min⁻¹, was suppressed by losartan, being 20 ± 9, 8 ± 3, and 3 ± 1 mmHg at doses of 0.002, 0.01, and 0.02%, respectively. We therefore selected a dose of losartan of 0.02%.

**Experiment 2A: Effect of ACE inhibition with enalapril on vasoconstriction induced by norepinephrine.** Rats were divided into four groups, two groups with renal artery stenosis and two sham-operated groups, with or without 0.02% enalapril in the drinking water (~100 mg·kg⁻¹·day⁻¹). In each group, the experiment was begun the day before the induction of renal artery stenosis or sham operation and continued for 48 h after surgery. One aortic ring preparation was obtained from each rat. The dose response of isometric contraction to norepinephrine in the presence of 10⁻¹⁰, 10⁻⁹, 10⁻⁸ mol/l norepinephrine was determined.

**Experiment 2B: Effect of ACE inhibition with enalapril on vasodilation induced by acetylcholine.** We evaluated the effect of ACE inhibition with enalapril on the vasodilation induced by acetylcholine. As in expt 2A, rats were divided into four groups. One aortic ring preparation from each rat was preconstricted by adding 10⁻⁷ mol/l norepinephrine to the bath. After the contraction had reached a plateau, 100 µl of acetylcholine was progressively applied to the bath as in expt 1C.

**Experiment 2C: Effect of angiotensin II type 1A receptor antagonism with losartan on vasoconstriction induced by norepinephrine.** We also studied the effect of the angiotensin II type 1A receptor antagonist losartan on the vasoconstriction induced by norepinephrine. The same protocol as used in expt 2A was followed, but with losartan instead of enalapril.
Losartan was administered in the drinking water at a concentration of 0.02% (~100 mg·kg⁻¹·day⁻¹).

Experiment 2D: Effect of angiotensin II type 1A receptor antagonism with losartan on vasoconstriction induced by acetycholine. The same protocol as in expt 2B was followed, with losartan used instead of enalapril. Losartan was given in the drinking water as in expt 2C. One aortic ring preparation was obtained from each rat, and the dose-response curve for the vasodilator responses to acetycholine was determined as in expt 1C.

Experiment 3: Effect of Bradykinin B₂ Receptor Antagonism With HOE-140 on Restoration of Endothelial Function After ACE Inhibition

Preliminarily, we tested the effectiveness of the bradykinin B₂ receptor antagonism produced by HOE-140. Aortic rings from normal Wistar rats were equilibrated in Krebs bicarbonate solution with three concentrations of HOE-140 (10⁻⁹, 10⁻⁷, and 10⁻⁵ mol/L, final bath concentrations) or without HOE-140 for 30 min (n = 5 in each group). The rings were then preconstricted by adding 10⁻⁷ mol/L norepinephrine. The vasodilatory responses to 10⁻⁷, 10⁻⁶ mol/L bradykinin were abolished by 10⁻⁵ and 10⁻⁴ mol/L HOE-140. In the present study, we selected a dose of 10⁻⁵ mol/L for HOE-140.

Experiment 3A: Effect of bradykinin B₂ receptor antagonism on vasoconstriction induced by norepinephrine. Rats were divided into four groups: two groups with renal artery stenosis and two sham-operated groups, with or without 0.02% enalapril in the drinking water. One aortic ring preparation was obtained from each rat. Aortic rings from 1K,1C and control rats given enalapril were equilibrated in Krebs bicarbonate solution containing 10⁻⁵ mol/L HOE-140 for 30 min. Aortic rings from 1K,1C and control rats given tap water were incubated in Krebs bicarbonate solution without HOE-140. The dose-response curves for the contractile responses to norepinephrine were then determined for each aortic preparation as in expt 2A.

Experiment 3B: Effect of bradykinin B₂ receptor antagonism with HOE-140 on vasodilation induced by acetycholine. The same protocol as in expt 3A was performed in four groups of rats treated with or without enalapril. One aortic ring preparation was obtained from each rat. Aortic rings from 1K,1C and control rats given enalapril were equilibrated in Krebs bicarbonate solution containing 10⁻⁵ mol/L HOE-140 for 30 min as in expt 3A. Aortic rings from 1K,1C and control rats given tap water were incubated in Krebs bicarbonate solution without HOE-140. The dose-response curves for the vasodilator responses to acetycholine were then determined.

Drug Administration

Acetycholine (Sigma Chemical, St. Louis, MO) and L-NMMA (Calbiochem, La Jolla, CA) were dissolved in saline, frozen, and stored at −20°C for no more than 6 wk. Norepinephrine was a gift of Sankyo Pharmaceutical, Tokyo, Japan. These drugs were subsequently diluted with Krebs bicarbonate solution to the desired concentrations. Enalapril and losartan were dissolved daily in the drinking water. Enalapril and losartan were kindly donated by The University of Tokyo, Tokyo, Japan. HOE-140 was kindly donated by Hekist Pharmaceutical, Tokyo, Japan.

Data Analysis

In each experimental group, n refers to the number of animals from which the aortas were taken. Responses to norepinephrine or acetycholine were expressed according to the method of Konishi and Su (12) and our previous study (8). For vasoconstriction, the maximal response was taken to be the maximal force (mg) of the norepinephrine-induced vasoconstriction observed in the dose-response curve for each aortic preparation. The negative logarithm of the concentrations of norepinephrine that produced the half-maximal response was referred to as pD₂. For vasodilation, the negative logarithm of the concentrations of acetycholine that produced the half-maximal response to the drug was used and was referred to as pD₂. The putative maximal vasodilator response was taken to be the level that preceded the preconstriction induced by norepinephrine. The response to each dose was expressed as a percent of the putative maximal vasodilation. Data are expressed as means ± SE. Differences among data sets were evaluated by performing an ANOVA, followed by Duncan’s multiple-range test. A level of P < 0.05 was accepted as statistically significant.

RESULTS

Table 1 summarizes the body weights, systolic blood pressures, and heart rates of the six groups of rats studied. There was no significant difference in body weight among the groups on the day on which the aortic ring preparations were obtained. The systolic blood pressures and heart rates did not differ among the groups before the induction of renal artery stenosis or sham operation. However, the systolic blood pressures rose slightly (P < 0.05) in the 1K,1C rats 48 h after renal artery stenosis as compared with the sham-operated rats. Treatment with enalapril and losartan prevented the rise in systolic blood pressure in the 1K,1C rats. The presence of renal artery stenosis or the administration of enalapril or losartan did not significantly affect the heart rate.

Experiment 1: Alteration of Vasoconstrictor and Vasodilator Responses to Norepinephrine and Acetycholine

The isometric contraction of the aortic ring in response to norepinephrine was exaggerated in 1K,1C rats. The presence of renal artery stenosis or the administration of enalapril or losartan did not significantly affect the heart rate.

Table 1. Body weight, systolic blood pressure, and heart rate in one-kidney, one-clip rats and sham-operated rats before and after 48 h after renal artery stenosis or sham operation

<table>
<thead>
<tr>
<th>Body Wt, g</th>
<th>Systolic BP, mmHg</th>
<th>Heart Rate, Beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 h</td>
<td>Start</td>
<td>48 h</td>
</tr>
<tr>
<td>Sham (n = 5)</td>
<td>214 ± 2</td>
<td>126 ± 2</td>
</tr>
<tr>
<td>1K,1C (n = 5)</td>
<td>212 ± 2</td>
<td>127 ± 2</td>
</tr>
<tr>
<td>Sham + enalapril (n = 26)</td>
<td>209 ± 4</td>
<td>126 ± 3</td>
</tr>
<tr>
<td>1K,1C + enalapril (n = 26)</td>
<td>206 ± 3</td>
<td>128 ± 3</td>
</tr>
<tr>
<td>Sham + Losartan (n = 13)</td>
<td>208 ± 4</td>
<td>127 ± 3</td>
</tr>
<tr>
<td>1K,1C + Losartan (n = 13)</td>
<td>207 ± 4</td>
<td>126 ± 7</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. n = no. of animals. 1K,1C, one-kidney, one-clip rats; Sham, sham-operated rats; BP, blood pressure; Start, time at which renal artery stenosis or sham operation was performed; 48 h, 48 h after renal artery stenosis. *P < 0.05 vs. Start; †P < 0.05 vs. 1K,1C.
compared with control rats (Figs. 1A and 3). The pD$_2$ was significantly higher in the 1K,1C rats than in control rats (P < 0.05) (Table 2; expt 1A and Table 3). After treatment with L-NMMA, the pD$_2$ and maximal response increased significantly in both groups (P < 0.05). However, these increases were more pronounced in the control rats, and the difference in the vasoconstrictor response to norepinephrine between the two groups was diminished (Fig. 1A and Table 2, expt 1A).

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 reduced in 1K,1C rats compared to control rats (P < 0.05, Table 2; expt 1A). The pD$_2$ and maximal relaxation increased significantly in both groups (P < 0.05). However, these increases were more pronounced in the control rats, and the difference in the vasoconstrictor response to norepinephrine between the two groups was diminished (Fig. 1A and Table 2, expt 1A).

Data are expressed as means ± SE of the negative logarithms of half-maximal constriction or relaxation (pD$_2$) and the maximal constriction (mg) or relaxation (%) of aortic strips from 1K,1C and Sham rats. L-NMMA, N$^G$-monomethyl-L-arginine acetate. *P < 0.05, †P < 0.01 vs. Sham; ‡P < 0.05 vs. 1K,1C.

Table 2. Norepinephrine-induced vasoconstriction and acetylcholine- or sodium nitroprusside-induced vasodilation

<table>
<thead>
<tr>
<th>Experiment</th>
<th>pD$_2$ (Mg)</th>
<th>Maximal Relaxation (%)</th>
</tr>
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<tbody>
<tr>
<td>1A: Norepinephrine-induced vasoconstriction&lt;br&gt;Ef 1Ff of L-NMMA (n = 6)</td>
<td></td>
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</tr>
<tr>
<td>Sham</td>
<td>7.54 ± 0.15</td>
<td>1,054 ± 102&lt;br&gt;1K,1C</td>
</tr>
<tr>
<td>1B: Acetylcholine-induced vasodilation&lt;br&gt;Ef 1Ff of L-NMMA (n = 5)</td>
<td></td>
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</tbody>
</table>
| Sham | 7.91 ± 0.84 | 98 ± 5<br>1K,1C | 6.74 ± 0.83* | 85 ± 6*
| Sham + L-NMMA | -5.0± 0<sup>†</sup> | 6 ± 0.4‡<br>1K,1C + L-NMMA | -5.0± 0<sup>†</sup> | 5 ± 2‡ |
| 1C: Sodium nitroprusside-induced vasodilation<br>(n = 6) | | |
| Sham | 7.91 ± 0.23 | 100 ± 1<br>1K,1C | 7.86 ± 0.26 | 100 ± 1<br>

Table 3. Norepinephrine-induced vasoconstriction in the presence of enalapril or losartan

<table>
<thead>
<tr>
<th>Experiment</th>
<th>pD$_2$ (Mg)</th>
<th>Maximal Constriction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2A: Effect of enalapril (n = 7)</td>
<td></td>
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</tr>
<tr>
<td>Sham</td>
<td>7.57 ± 0.11</td>
<td>1,166 ± 107&lt;br&gt;1K,1C</td>
</tr>
<tr>
<td>2C: Effect of losartan (n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>7.65 ± 0.10</td>
<td>1,103 ± 102&lt;br&gt;1K,1C</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE of the negative logarithms of half-maximal constriction and the maximal constriction of aortic strips from 1K,1C and Sham rats. *P < 0.05, †P < 0.01 vs. Sham; ‡P < 0.05 vs. 1K,1C.

Table 4. Norepinephrine-induced vasoconstriction in the presence of enalapril with HOE-140 (n = 6)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>pD$_2$ (Mg)</th>
<th>Maximal Constriction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3A: Effect of enalapril with HOE-140 (n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>7.50 ± 0.11</td>
<td>1,131 ± 96&lt;br&gt;1K,1C</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE of the negative logarithms of half-maximal constriction and the maximal constriction of aortic strips from 1K,1C and Sham rats. *P < 0.05, †P < 0.05 vs. Sham; ‡P < 0.05, §P < 0.01 vs. 1K,1C.

Fig. 1. Vasoconstrictor response to norepinephrine and vasodilator response to acetylcholine or sodium nitroprusside. A: effect of N$^G$-monomethyl-L-arginine acetate (L-NMMA, 10$^{-5}$ mol/l) on norepinephrine-induced vasoconstriction in 1-kidney, 1-clip (1K,1C) and sham-operated (Sham) rats. B: effect of 10$^{-5}$ mol/l L-NMMA on acetylcholine-induced vasodilation in 1K,1C and Sham rats. C: vasodilator response to sodium nitroprusside in 1K,1C and Sham rats.
with control rats ($P < 0.05$) (Figs. 1B and 3). In addition, the $pD_2$ and maximal response values in 1K,1C rats were significantly smaller than the control values ($P < 0.05$) (Table 2, expt 1B, and Table 4). L-NMMA abolished the vasodilatory responses to acetylcholine in both groups (Fig. 1B and Table 2, expt 1B). No significant difference was observed in the response between the groups after treatment with L-NMMA (Fig. 1B and Table 2, expt 1B).

The cumulative addition of sodium nitroprusside produced concentration-dependent relaxations of aortic rings that were preconstricted with norepinephrine ($10^{-7} \text{ mol/l}$). No significant difference was observed in the response between 1K,1C and control rats (Fig. 1C and Table 2, expt 1C).

**Experiment 2: Involvement of the Renin-Angiotensin System in Endothelial Function and the Evaluation of Vasomotor Tone**

Treatment with enalapril (Fig. 2A) and losartan (Fig. 2B) produced a rightward shift of the norepinephrine-induced vasoconstriction in 1K,1C rats but did not alter the response in control rats. Both the $pD_2$ and the maximal response were decreased significantly following enalapril or losartan in 1K,1C ($P < 0.05$) (Table 3, expts 2A and 2C). There was no significant difference in either the $pD_2$ or the maximal response between 1K,1C and control after treatment with enalapril or losartan (Table 3, expts 2A and 2C).

Figure 3 and Table 4 show the effect of the oral administration of enalapril and losartan on acetylcholine-induced vasodilation in control and 1K,1C rats. The relaxation was augmented by enalapril (Fig. 3A) and losartan (Fig. 3B) in 1K,1C rats, with a significant shift of the dose-response curves toward the left ($P < 0.05$) (Table 4, expts 2B and 2D). In contrast to 1K,1C rats, neither enalapril nor losartan altered the dose-response curve in control rats (Table 4, expts 2B and 2D). There was no significant difference between the 1K,1C and control rats in the acetylcholine-induced vasodilation after treatment with enalapril or losartan.

**Table 4. Acetylcholine-induced vasodilation in the presence of enalapril or losartan**

<table>
<thead>
<tr>
<th></th>
<th>Experiment 2B: Effect of enalapril (n = 7)</th>
<th>Experiment 2D: Effect of losartan (n = 6)</th>
<th>Experiment 3B: Effect of enalapril + HOE-140 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$pD_2$ Maximal Relaxation, %</td>
<td>$pD_2$ Maximal Relaxation, %</td>
<td>$pD_2$ Maximal Relaxation, %</td>
</tr>
<tr>
<td>Sham</td>
<td>$7.88 \pm 0.54$ $98 \pm 5$</td>
<td>$7.61 \pm 0.54$ $98 \pm 7$</td>
<td>$7.89 \pm 0.43$ $97 \pm 5$</td>
</tr>
<tr>
<td>1K,1C</td>
<td>$7.05 \pm 0.61^<em>$ $82 \pm 6^</em>$</td>
<td>$6.76 \pm 0.61^<em>$ $76 \pm 6^</em>$</td>
<td>$6.72 \pm 0.51^<em>$ $73 \pm 3^</em>$</td>
</tr>
<tr>
<td>Sham + enalapril</td>
<td>$7.78 \pm 0.49^<em>$ $99 \pm 3^</em>$</td>
<td>$7.42 \pm 0.48^<em>$ $96 \pm 5^</em>$</td>
<td>$7.72 \pm 0.51^<em>$ $97 \pm 3^</em>$</td>
</tr>
<tr>
<td>1K,1C + enalapril</td>
<td>$7.78 \pm 0.51^<em>$ $97 \pm 4^</em>$</td>
<td>$7.39 \pm 0.52^<em>$ $95 \pm 5^</em>$</td>
<td>$7.73 \pm 0.55^<em>$ $95 \pm 5^</em>$</td>
</tr>
<tr>
<td>Sham</td>
<td>$6.71 \pm 0.54$ $98 \pm 7$</td>
<td>$7.10 \pm 0.54$ $98 \pm 7$</td>
<td>$7.70 \pm 0.43$ $97 \pm 5$</td>
</tr>
<tr>
<td>1K,1C</td>
<td>$7.05 \pm 0.61^<em>$ $82 \pm 6^</em>$</td>
<td>$6.76 \pm 0.61^<em>$ $76 \pm 6^</em>$</td>
<td>$6.72 \pm 0.51^<em>$ $73 \pm 3^</em>$</td>
</tr>
<tr>
<td>Sham + losartan</td>
<td>$7.42 \pm 0.48^<em>$ $96 \pm 5^</em>$</td>
<td>$7.39 \pm 0.52^<em>$ $95 \pm 5^</em>$</td>
<td>$7.72 \pm 0.51^<em>$ $97 \pm 3^</em>$</td>
</tr>
<tr>
<td>1K,1C + losartan</td>
<td>$7.39 \pm 0.52^<em>$ $95 \pm 5^</em>$</td>
<td>$7.39 \pm 0.52^<em>$ $95 \pm 5^</em>$</td>
<td>$7.73 \pm 0.55^<em>$ $95 \pm 5^</em>$</td>
</tr>
</tbody>
</table>

Data are expressed as means $\pm$ SE of the negative logarithms of the half-maximal relaxation and the maximal relaxation of aortic strips from 1K,1C and Sham rats. $^*P < 0.05$, $^\dagger P < 0.01$ vs. Sham; $^\ddagger P < 0.05$ vs. 1K,1C.
Experiment 3: Effect of Bradykinin B2 Receptor Antagonism With HOE-140 on the Restoration of Endothelial Function After ACE Inhibition

Figure 2C shows the effect of HOE-140 on norepinephrine-induced vasoconstriction in control and 1K,1C rats treated with enalapril. The addition of HOE-140 to the bath did not alter the normalized contractile response of the aorta to norepinephrine in 1K,1C rats treated with enalapril. There was no significant difference in pD2 or in the maximal constriction between the control and the 1K,1C rats treated with enalapril plus HOE-140 (Table 3, expt 3A).

Figure 3C shows the effect of HOE-140 on the acetylcholine-induced vasodilation in control and 1K,1C rats treated with enalapril. The addition of HOE-140 to the bath did not alter the normalized relaxation responses of the aorta to acetylcholine in 1K,1C rats treated with enalapril. There was no significant difference in either the pD2 or the maximal dilatation between the control and the 1K,1C rats treated with enalapril plus HOE-140 (Table 4, expt 3B).

DISCUSSION

The present study showed an enhanced vasoconstrictor response to norepinephrine and a depressed endothelium-dependent relaxation response to acetylcholine in aortic rings studied during the early-stage hypertension in 1K,1C rats. After treatment with L-NMMA, the difference between the 1K,1C rats and the control rats in the norepinephrine-induced vasoconstriction was diminished. L-NMMA also abolished the vasodilator response to acetylcholine in the control and 1K,1C rats. The oral administration of enalapril or losartan normalized the vasoconstrictor and vasodilator responses to the control level in 1K,1C rats. These normalized responses to norepinephrine or acetylcholine by enalapril in 1K,1C rats were unaffected by coincubating the preparations with the bradykinin B2 receptor antagonist HOE-140. These data indicate that angiotensin II disturbs endothelial function in 1K,1C rats.

Exaggerated pressor responses have been reported in hypertensive patients (2, 6) as well as in animal models of hypertension (9, 11, 25). Our group (11) as well as others (9) have reported that the pressor response to norepinephrine is enhanced in prehypertensive 1K,1C rats and rabbits even before the onset of hypertension. These enhanced vascular contractile and pressor responses to vasoconstrictors may contribute to the initiation and maintenance of hypertension. This early stage of experimental hypertension is important, especially because such structural alterations as vascular wall hypertrophy, which could be one of the mechanisms leading to an enhanced pressor response (3), are not considered to occur, although an increase in the wall cross-sectional area (medial hypertrophy) of resistance vessels has not been demonstrated (20) in the early or the chronic stages of hypertension in 1K,1C rats.

We previously demonstrated that the aorta exhibits a significantly exaggerated contractile response to norepinephrine during early-stage hypertension in 1K,1C rats and that endothelial dysfunction contributes to this exaggerated response (8). In those experiments, the ACE inhibitors captopril and enalapril restored the endothelial function in 1K,1C rats. The renin-angiotensin system is activated during early-stage hypertension in 1K,1C rats, although its activity is not considered to
be enhanced during the chronic stage. The present study evaluated the agonist-induced vasoconstrictor and vasodilator responses of aortic ring preparations 48 h after the induction of renal artery stenosis or a sham operation. We evaluated the effects of an angiotensin II type 1A receptor antagonist on these responses in the present study. Systolic blood pressure was slightly higher in the 1K,1C rats than in the controls; however, endothelial dysfunction was already present, as documented by the acetylcholine-induced vasodilation observed 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. An important finding of the present study was that not only ACE inhibitors but also the angiotensin II type 1A receptor antagonist losartan restored endothelial function, indicating a close association between endothelial dysfunction and the renin-angiotensin system. The normalized responses to norepinephrine or acetylcholine by enalapril in 1K,1C rats were unaffected by coincubating the preparations with the bradykinin B2 receptor antagonist HOE-140.

ACE inhibitors reduce the formation of angiotensin II and prevent the degradation of bradykinin, an endothelium-dependent relaxant that works by stimulating NO release (5, 13). It has therefore been suggested that part of the antihypertensive action and thus the vascular protective capacity of the ACE inhibitors may be mediated by bradykinin and NO. If true, the improvement in endothelial function produced by the ACE inhibitor used in the present study could also be partly explained by an increase in the bradykinin level. However, intact endothelium is required for the release of NO by bradykinin, and the vasorelaxant response to bradykinin is reportedly reduced in 1K,1C rats (18). Because endothelial function was also improved by the angiotensin II type 1A receptor antagonist losartan, the inhibition of the renin-angiotensin system is of principal importance for the improvement of endothelial function. The normalized response to norepinephrine or acetylcholine produced by enalapril in 1K,1C rats was unaffected by coincubating the preparations with a bradykinin B2 receptor antagonist. This finding also suggests that angiotensin II is involved in endothelial dysfunction. Although receptors for angiotensin II include types 1A, 1B, and 2 (10), the response to losartan suggests that only the type 1A receptor is mainly involved in the endothelial cell damage and inhibition of NO production.

In conclusion, the present study demonstrated that an enhanced vascular response to norepinephrine was present during the early-stage hypertension in 1K,1C rats. Vasodilatory responses of aortic ring preparations 48 h after the induction of renal artery stenosis or a sham operation. The authors are grateful to Shizuko Saiki for excellent technical help.

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