Interaction of Nitric Oxide and the Renin Angiotensin System in Renal Hypertensive Rats

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ABSTRACT—We investigated the interaction between nitric oxide and the renin angiotensin system in regulating isolated aortic tension and mean arterial pressure in renal hypertensive rats (RHR). Acetylcholine (ACh) relaxed aorta precontracted with norepinephrine from RHR significantly less than that from normotensive rats (NR) (E_{max}: 34.3% and 86.0%, respectively, $P<0.01$). The ACh-induced relaxation was significantly enhanced by losartan ($P<0.05$) and completely abolished by removal of endothelium or N^G-nitro-L-arginine methyl ester (L-NAME). ACh lowered the mean arterial pressure slightly less effectively in RHR than in NR (6.8 and 13.0 mmHg, respectively, at 0.1 μg/kg), whereas the depressor effect was reduced by L-NAME (−15.5 and 10.3 mmHg, respectively, at 0.1 μg/kg), but rather enhanced by further treatment with losartan (9.9 ($P<0.05$) and 17.3 mmHg, respectively, at 0.1 μg/kg). Angiotensin II induced similar contractile and pressor responses in both RHR and NR, and these effects were significantly enhanced by L-NAME, except for the pressor effect in RHR. L-NAME induced a similar pressor response in RHR and NR (15.9 and 15.2 mmHg, respectively, at 0.1 mg/kg), the effect being decreased by pretreatment with losartan. Losartan induced a depressor response that was smaller in RHR than in NR (34.0 and 48.8 mmHg, respectively, at 0.3 mg/kg), and the response was significantly reduced by L-NAME. These results suggest that nitric oxide interacts with the renin angiotensin system to control the vascular tension and systemic arterial circulation in RHR.

Keywords: Nitric oxide, Renin angiotensin system, Renal hypertensive rat, Losartan, Endothelium

Nitric oxide (NO) appears to be the endogenous prototype of the nitrovasodilator drugs that act by stimulating soluble guanylate cyclase to increase intracellular levels of cyclic GMP in vascular smooth muscle to result in vasodilation (1-3). NO is synthesized from the amino acid L-arginine by an enzyme, NO synthase (4), under the basal condition and during stimulation by various vasoactive substances such as acetylcholine (ACh), histamine, serotonin, adenosine diphosphate and thrombin (2, 5). Increasing evidence has suggested that interaction between NO and vasoconstrictor/vasodilator substances have a severe influence on the regulation of vascular functions (6, 7). A recently published report has suggested that angiotensin II (Ang II) or its degradation products can stimulate NO release in rabbit brain arterioles (8). This finding is in line with other reports that the inhibition of NO synthesis led to an increase in basal tone and a marked augmentation of Ang II-induced afferent arteriolar (9) and renal arterial constriction (10). It has also been documented that infusion of L-arginine significantly reduced serum angiotensin converting enzyme activity and lowers plasma Ang II level in humans (11). Moreover, NO may also mediate the tachyphylactic response seen with administered Ang II because acute NO blockade greatly increased the duration of Ang II-induced aortic (12) and afferent arteriolar constriction (9). It has been also reported that NO stimulated renin secretion from isolated rat kidney (13, 14), and this finding has been further supported by Schricker et al. (15), who suggested that renin mRNA levels are tonically increased by NO and that the action of NO is counteracted by Ang II (15), although contradictory findings suggest that NO inhibits the renin system (16). All of these observations suggest that NO interacts with the renin angiotensin system, with NO serving as a counterregulatory influence on the vascular actions of Ang II. When Ang II levels increase above normal, as in renal hypertension and Ang II-induced hypertension, Ang II and NO may become increasingly important factors in the regulation of the systemic arterial system. Accordingly, the present work was

undertaken to elucidate the interaction between NO and the renin angiotensin system in renal hypertensive rats, a model of chronic increase in Ang II levels.

MATERIALS AND METHODS

Materials and solutions

Acetylcholine chloride, (−)norepinephrine hydrochloride, N^G-nitro-L-arginine methyl ester (L-NAME), urethane, α-chloralose and Ang II acetate were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and other drugs and reagents used to prepare Krebs bicarbonate solution were purchased from Junsei Chemical Co. (Tokyo). Losartan was synthesized at the Korea Research Institute of Chemical Technology (KRICT, Taejon, Korea). Acetylcholine chloride with high hygroscopicity was made as a stock solution of 10 mM and 1 mg/ml in water, and the stock solutions were divided into a large number of aliquots and stored at −20℃, each aliquot being used for each experiment with serial dilution. Urethane was dissolved in saline and α-chloralose dissolved in propylene glycol by heating. Other drugs were dissolved just before use. All the solutions were prepared in distilled and deionized water for the in vitro experiments and in isotonic saline (0.9 w/v% NaCl solution) for intravenous injection. The composition of Krebs bicarbonate buffer (Krebs buffer) was as follows: 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4 and 11.0 mM glucose.

Animals

The experiments were performed on male Sprague-Dawley rats weighing 350−450 g. They were provided by the Department of Experimental Animals, KRICT and kept in a breeding room under the conditions of constant temperature and illumination (12-hr light, 12-hr dark cycle) until the day of experiment, with free access to food and tap water.

Preparation of renal hypertensive rats

The renal hypertensive rats (RHR) were prepared by complete ligation of the left renal artery as described previously (17, 18). Briefly, rats were anesthetized with ether, and the left renal artery was separated from the vein near the junction with the aorta, taking care not to traumatize the vein, and then a complete ligature of 4-0 sterile silk was placed on the renal artery. After ligation, the incision was closed by carefully suturing the muscle layer with 4-0 silk and then the skin with metallic clips. Plasma renin activity (PRA) was measured by radioimmunoassay (angiotensin I (Ang I) [^{125}I] assay kit; Dupont Co., Billerica, MA, USA) for Ang I generated by a modification of the technique of Haber et al. (19). Six to eight days after the renal artery ligation, a good correlation between systolic blood pressure and PRA was shown, and thus rats from these groups were considered a model for acute renal hypertension and used as hypertensive rats in this study when systolic blood pressure was more than 180 mmHg. In this model, 85% of the rats develop hypertension after renal artery ligation. However, rats with total renal infarction did not develop hypertension and no changes in PRA were observed. Rats were sham-operated for the control normotensive group.

Preparation of isolated vascular rings

On the day of the experiment, normotensive rats (NR) and RHR were killed by a blow on the head and exsanguination. The thoracic aorta was isolated and cleaned of adhering fat and connective tissue. Each artery was cut into two rings 2- to 3-mm-wide, with extreme care to preserve the endothelium intact. In one of two rings, the endothelial layer was destroyed by gently rubbing the luminal surface with a cotton swab moistened with Krebs solution. The aortic preparations with the endothelium intact or denuded were suspended between wire hooks in an organ bath containing 20 ml of Krebs buffer bubbled with a gas mixture (95% O_2, 5% CO_2) and maintained at 37℃. The aortic preparations were allowed to equilibrate for 60 min under the resting tension of 2 g. The isometric contractile activity was measured with a force displacement transducer (model FT03; Grass Ins., Quincy, MA, USA) and displayed on a chart recorder (Multicorder MC 6625; Hugo Sachs Electronic, March, Germany). The removal of the endothelium was confirmed pharmacologically by the absence of endothelium-dependent relaxation to ACh (10^{-5} M) in tissues precontracted with norepinephrine (NE) (10^{-7} M).

Experimental protocol for in vitro studies

The matched pairs of aortic preparations with endothelium intact and denuded were precontracted submaximally with 10^{-7} M of NE, washed out 3 times for 45 min, and rechallenged with NE to obtain a reproducible and stable response. After the NE response reached the plateau, ACh ($10^{-10}-10^{-5}$ M) was cumulatively added to the tissue bath with and without pretreatment of L-NAME (10^{-5} M, 15 min), an NO synthase inhibitor (20), or losartan (10^{-5} M, 30 min), an angiotensin AT_1-receptor antagonist (21, 22). In separate experiments, Ang II ($3\times10^{-10}-10^{-7}$ M) were cumulatively added to the bath holding the matched pairs of arterial rings with endothelium intact and denuded, whereas other tissues were pretreated with L-NAME (10^{-5} M) 15 min prior to exposure to Ang II. The results were expressed as percent decrease in tension from the NE contraction for ACh-in-

duced relaxation and as percent of the reference contractile response to high K^+ solution (NaCl was substituted with equimolar KCl) for Ang II-induced contraction.

Experimental protocol for in vivo studies

Both NR and RHR were anesthetized with a combination of urethane (900 mg/kg, i.p.) and α-chloralose (90 mg/kg, i.p.). The rats breathed room air via a tracheotomy tube connected to a rodent ventilator (stroke volume, 1 ml/100 g, 60 cycles/min; Harvard Apparatus, South Natick, MA, USA). Arterial pressure was measured and continuously monitored via a catheter (heparinized, 20 IU/ml) inserted in the left carotid artery, which was connected to a Grass P23XL pressure transducer and a Gould 2000 physiograph (Gould Inc., Cleveland, OH, USA). Heart rate was derived from the arterial pressure pulse by the ECG/Biotacho amplifier module of the Gould 2000 physiograph. Rectal temperature was maintained at 36.5±0.5℃ by thermistor-controlled radiant heat. Forty minutes after surgery, when it was possible to obtain consistent control values for all the parameters, the experiment was started. NR and RHR were divided into four groups at random. In the first group of both NR and RHR, ACh (0.1−10 μg/kg, i.v.) was successively administered to rats pretreated with L-NAME (30 mg/kg, i.v.) and/or losartan (3 mg/kg, i.v.), alone or in combination, 15 and 10 min prior to administration of ACh, respectively. In some animals, a single bolus injection (30 mg/kg, i.v.) was given to delineate the time course of the L-NAME effects. In the second group, Ang II (0.01−3 μg/kg, i.v.) was successively administered to rats, with and without pretreatment of L-NAME (30 mg/kg, i.v.) 15 min prior to administration of Ang II. In the third group, L-NAME (0.1−100 mg/kg, i.v.) was successively administered with and without pretreatment of losartan (3 mg/kg, i.v.) 10 min prior to administration of L-NAME. In the fourth group, losartan (0.1−3 mg/kg, i.v.) was successively administered with and without pretreatment of L-NAME (30 mg/kg, i.v.) 15 min prior to administration of losartan. Drugs were administered via a catheter inserted into the left femoral vein in volumes of 1 ml/kg. Results were expressed as mmHg change of mean arterial pressure from the baseline values.

Statistical analyses

Data were expressed as means±S.E.M. The difference between groups was evaluated by the unpaired Student's *t*-test as appropriate, with $P<0.05$ being considered statistically significant.

RESULTS

Renal hypertensive rats

In 85% of the animals that underwent the ligation of left renal artery, systolic blood pressure started increasing on days 3 and 4, reached its maximum on days 6−8 after the ligation of the renal artery (with a significant change ($P<0.01$) from the control level of 154.29±1.83 to 190−215 mmHg), and thereafter dropped slightly, to a level that was still significantly greater than the control values ($P<0.01$) (Table 1). PRA showed a significant change ($P<0.01$) from the control level of 7.31±0.63 ng/ml/hr Ang I to 19−22 ng/ml/hr Ang I on days 6−8 after the renal arterial ligation, but on day 28, returned to the control level, despite the consistent high blood pressure, indicating there is a good correlation between development of hypertension and PRA only in the acute phase of renal hypertension in this model.

In vitro studies

The values of mean arterial pressure and PRA in RHR averaged 204.4±9.14 mmHg ($P<0.01$) and 20.6±2.8 ng/ml/hr Ang I ($P<0.01$), respectively, with a significant difference noted compared to those in NR (154.3±1.83 mmHg and 7.3±0.6 ng/ml/hr of Ang I, respectively). ACh (10^{-10}−10^{-5} M) caused a concentration-dependent relaxation of the intact aortic preparations from NR and RHR precontracted with NE (10^{-7} M). ACh-induced relaxation of the intact aortic preparation was significantly smaller in RHR (E_{max}: 34.3±6.5%, $P<0.01$) than in NR (E_{max}: 85.9±2.9%), and it was completely abolished

Table 1. Plasma renin activity levels (PRA), systolic blood pressure (SBP) and heart rate (HR) before and 6, 7, 8 and 28 days after ligation of the left renal artery

	Days after renal artery ligation				
	0	6	7	8	28
PRA (ng/ml/hr)	7.3±0.6	22.0±2.8**	20.2±3.4**	19.7±2.3**	6.4±0.9
SBP (mmHg)	154.3±1.8	191.5±4.3**	206.9±9.5**	214.8±13.7**	173.7±3.1**
HR (beats/min)	355.9±24.0	364.3±16.8	407.7±34.1	427.2±22.1	345.2±13.1
No. of animals	34	8	7	9	9

Values are each expressed as a mean±S.E.M. **$P<0.01$, compared with control normotensive rats ("0" days).

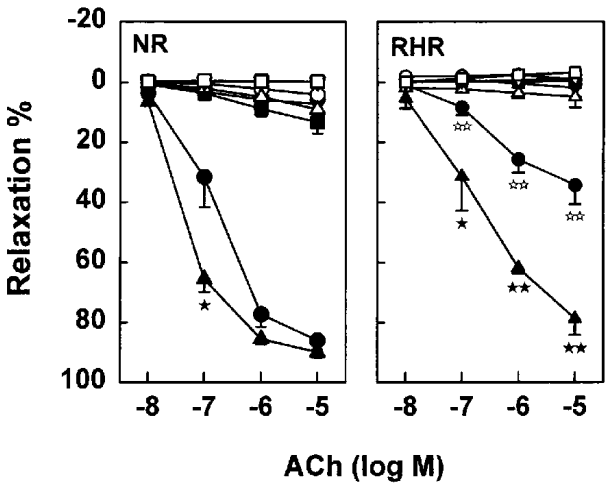

Fig. 1. The relaxant effects of acetylcholine (ACh) on the isolated aortic preparations from normotensive (NR) and renal hypertensive rats (RHR) in the presence (solid symbols) and absence (open symbols) of functional endothelium. The aortic preparations were pretreated with vehicle (circles), losartan (10^{-5} M, 30 min, triangles), N^G-nitro-L-arginine methyl ester (10^{-5} M, 15 min, reversed triangles) or a combination of both drugs (squares). Data are expressed as percentage decreases of norepinephrine (10^{-7} M)-induced contraction. Each point represents the mean±S.E.M. of 5–10 experiments. ☆☆P<0.01, compared with endothelium intact aorta from normotensive control rats. ★P<0.05, ★★P<0.01, compared with endothelium intact aorta from respective control rats.

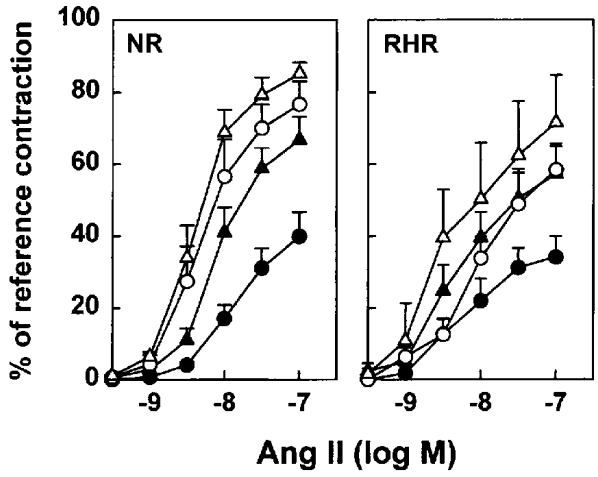

Fig. 2. The contractile effects of angiotensin (Ang) II on the isolated aortic preparations from normotensive (NR) and renal hypertensive rats (RHR) in the presence (solid symbols) and absence (open symbols) of functional endothelium. The aortic preparations were pretreated with either vehicle (circles) or N^G-nitro-L-arginine methyl ester (10^{-5} M, 15 min, triangles). Data are expressed as percentage of the reference contraction induced by high K^+ solution. Each point represents the mean±S.E.M. of 6–9 experiments.

after removal of endothelial cells or pretreatment with L-NAME (Fig. 1). ACh-induced relaxation was significantly increased by losartan (10^{-5} M) pretreatment of intact aortic preparations from NR ($P<0.05$ at ACh 10^{-7} M) and RHR ($P<0.05$ at 10^{-7} M, $P<0.01$ at 10^{-6} and 10^{-5} M). However, losartan had no significant effect on the ACh-induced relaxation following single pretreatment with L-NAME of either intact or rubbed aortic preparations from both NR and RHR. L-NAME and losartan had no effect on the basal tension of either intact or rubbed aortic preparations from NR and RHR. As shown in Fig. 2, Ang II (3×10^{-9}–10^{-7} M) produced a concentration-dependent contractile response in intact aortic preparations from both NR and RHR, with no significant difference between the groups (pD_2: 7.91±0.06 and 8.23±0.18; E_{max}: 40.0±6.6% and 34.2±5.8%, respectively). The removal of endothelial cells (pD_2: 8.25±0.09 and 8.02±0.09; E_{max}: 76.7±6.4%, $P<0.01$ and 60.7±7.1%, $P<0.05$, for aortas from NR and RHR, respectively) or pretreatment of L-NAME (pD_2: 8.12±0.15 and 8.35±0.23; E_{max}: 66.8±6.5%, $P<0.05$ and 61.4±8.6%, $P<0.05$, for aortas from NR and RHR, respectively) significantly shifted the concentration-response curve for Ang II to the left in a non-parallel manner with enhancement of the maximal response.

In vivo studies

ACh (0.1–10 μg/kg, i.v.) lowered the mean arterial pressure in NR and RHR in a dose-dependent manner (13.0±0.6 and 6.8±6.1 mmHg at 0.1 μg/kg; 29.0±2.0 and 24.8±6.2 mmHg at 1.0 μg/kg, respectively), with

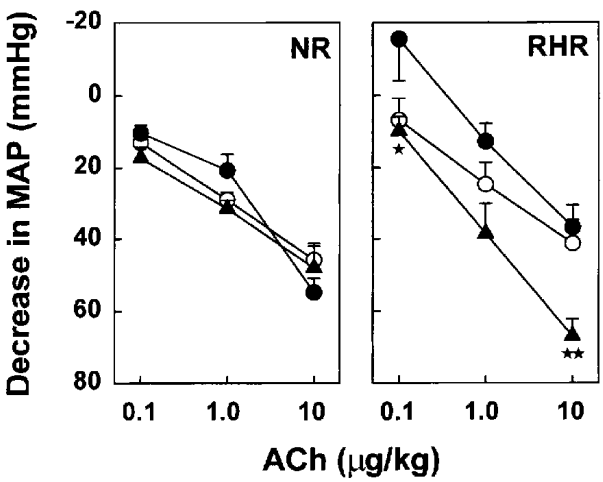

Fig. 3. The depressor effects of acetylcholine (ACh) on mean arterial pressure (MAP) in anesthetized normotensive (NR) and renal hypertensive rats (RHR). The rats were pretreated with vehicle (open circles), N^G-nitro-L-arginine methyl ester (L-NAME) (30 mg/kg, i.v., solid circles) or L-NAME in combination with losartan (3 mg/kg, i.v., solid triangles). Each point represents the mean± S.E.M. of 4–8 experiments. ★P<0.05, ★★P<0.01, compared with renal hypertensive rats pretreated with L-NAME.

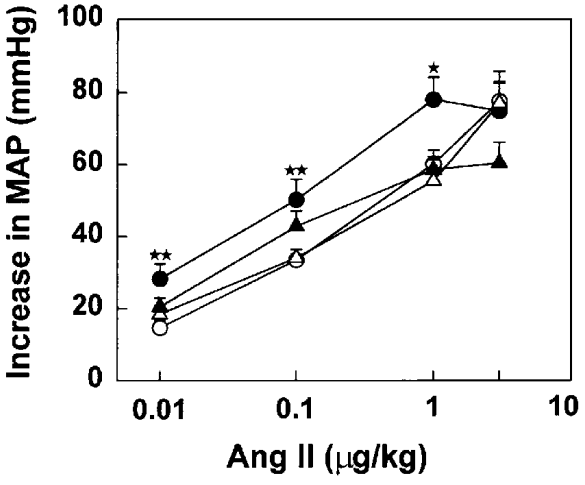

Fig. 4. The pressor effects of angiotensin (Ang) II on mean arterial pressure (MAP) in anesthetized normotensive (circles) and renal hypertensive rats (triangles). Rats were pretreated with either vehicle (open symbols) or N^G-nitro-L-arginine methyl ester (30 mg/kg, i.v., solid symbols). Each point represents the mean±S.E.M. of 5–6 experiments. ★P<0.05, ★★P<0.01, compared with normotensive control rats.

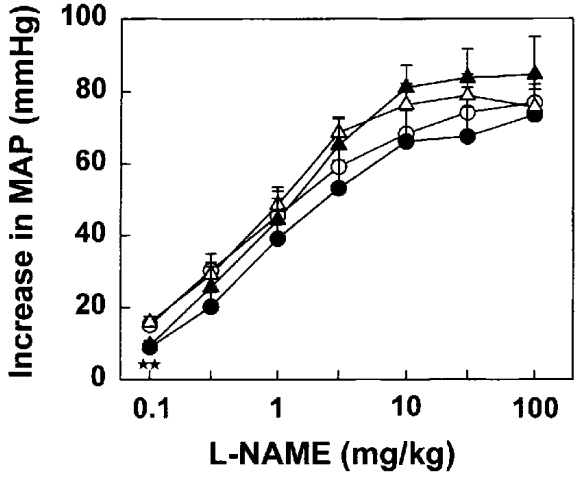

Fig. 5. Effects of N^G-nitro-L-arginine methyl ester (L-NAME) on mean arterial pressure (MAP) in anesthetized normotensive (circles) and renal hypertensive rats (triangles). Rats were pretreated with either vehicle (open symbols) or losartan (3 mg/kg, i.v., solid symbols). Each point represents the mean±S.E.M. of 4–9 experiments. ★★P<0.01, compared with normotensive control rats.

slightly weaker effects in RHR at all doses tested (Fig. 3). The depressor effects of ACh on NR and RHR were decreased by pretreatment of 30 mg/kg L-NAME, with a conversion to a pressor effect in RHR at a dose of 0.1 μg/kg (10.3±2.0 and −15.5±11.5 mmHg at 0.1 μg/kg; 20.7±4.4 and 12.8±5.0 mmHg at 1.0 μg/kg, respectively), but rather enhanced by further pretreatment with 3 mg/kg losartan, especially in RHR (17.3±2.9 and 9.9±4.0 mmHg, P<0.05, at 0.1 μg/kg; 31.5±2.3 and 38.5±8.4 mmHg at 1.0 μg/kg; 48.0±6.1 and 67.0±4.8 mmHg, P<0.01, at 10 μg/kg, respectively). When administered alone, L-NAME or losartan had pressor and depressor effects in both NR and RHR, respectively, which lasted throughout the whole experiment (60 and 30 min, respectively) (data not shown). As shown in Fig. 4, Ang II (0.01–3 μg/kg, i.v.) caused a dose-dependent pressor response in both NR and RHR to a similar magnitude (14.6±2.0 and 18.4±2.3 mmHg at 0.01 μg/kg; 33.4±3.0 and 34.0±2.4 mmHg at 0.1 μg/kg, respectively). The Ang II-induced pressor response was enhanced by pretreatment with L-NAME (30 mg/kg, i.v.) in both NR and RHR (28.2±4.1, P<0.01 vs control, and 20.4±2.4 mmHg at 0.01 μg/kg; 50.2±5.6, P<0.01 vs control, and 42.8±4.3 mmHg at 0.1 μg/kg, respectively). L-NAME (0.1–100 mg/kg, i.v.) increased mean arterial pressure dose-dependently in both NR and RHR to a similar magnitude (15.2±2.3 and 15.9±0.8 mmHg at 0.1 mg/kg; 45.4±4.8 and 48.6 mmHg at 1.0 mg/kg, respectively) (Fig. 5). Pretreatment with losartan (3 mg/kg, i.v.) decreased the L-NAME-induced pressor response in both NR and RHR (9.0±2.4 and 9.5±1.1 mmHg, P<0.01 vs control, at 0.1 mg/kg; 39.2±7.7 and 44.3±8.1 mmHg at 1.0 mg/kg, respectively). Losartan (0.1–3 mg/kg, i.v.) caused a dose-dependent decrease in the mean arterial pressure in both NR and RHR (Fig. 6). The losartan-induced depressor response was decreased in RHR (21.3±3.2, 34.0±4.4 and 45.3±3.5 mmHg at 0.1, 0.3 and 3 mg/kg, respectively, P<0.05 vs NR at 3 mg/kg)

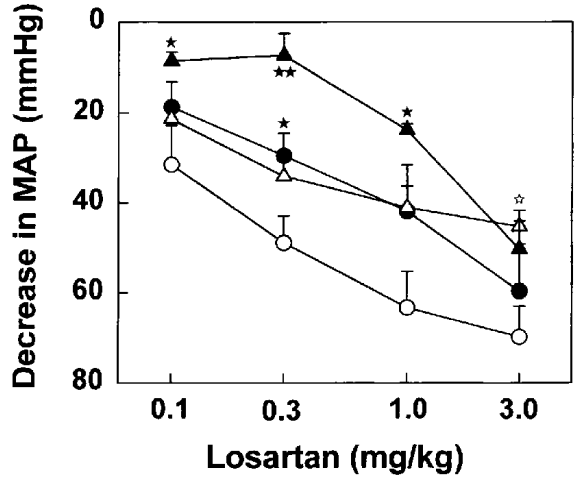

Fig. 6. The effects of losartan on mean arterial pressure (MAP) in anesthetized normotensive (circles) and renal hypertensive rats (triangles). Rats were pretreated with either vehicle (open symbols) or N^G-nitro-L-arginine methyl ester (30 mg/kg, i.v., solid symbols). Each point represents the mean±S.E.M. of 4–5 experiments. ☆P<0.05, compared with normotensive control rats. ★P<0.05, ★★P<0.01, compared with respective control rats.

Table 2. Basal mean arterial pressure after pretreatment with each drug in anesthetized normotensive (NR) and renal hypertensive rats (RHR)

	NR	RHR
Vehicle	94.1±6.4 (n=24)	96.9±7.1 (n=17)
L-NAME	132.8±5.9 (n=17)**	137.1±13.5 (n=13)**
L-NAME+Losartan	114.3±9.0 (n=4)	143.0±7.4 (n=8)**,†
Losartan	64.4±2.4 (n=5)*	74.5±5.0 (n=4)

Values are each expressed as a mean±S.E.M. Rats were pretreated with vehicle, N^G-nitro-L-arginine-methyl ester (L-NAME, 30 mg/kg, i.v.), losartan (3 mg/kg, i.v.) or a combination of both drugs. **$P<0.01$, compared with the respective vehicle-treated group (control). †$P<0.05$, compared with NR (L-NAME + losartan).

compared to those in NR (31.4±8.7, 48.8±6.0 and 69.8±6.9 mmHg at 0.1, 0.3 and 3 mg/kg, respectively). Pretreatment with L-NAME (30 mg/kg, i.v.) decreased the losartan-induced depressor response in both NR (18.6±5.6 and 29.4±5.0 mmHg, at 0.1 and 0.3 mg/kg, respectively, $P<0.05$ vs control at 0.3 mg/kg) and RHR (8.5±1.9 and 7.3±4.7 mmHg, $P<0.05$ and $P<0.01$ vs control at 0.1 and 0.3 mg/kg, respectively). We measured systolic blood pressure from each rat before starting the main experiments and included rats with a systolic blood pressure over 180 mmHg as the renal hypertensive rat model in these experiments. However, the mean arterial pressure was decreased by anesthesia with urethane-chloralose to the similar baseline values in renal hypertensive rats as well as in normotensive rats (Table 2). These phenomena could be due to the deep anesthesia caused by a sufficient dose of urethane-chloralose (900 and 90 mg/kg, i.p.).

DISCUSSION

The forms of hypertension associated with elevated circulating levels of Ang II may have unique vascular effects (23). RHR are one of the animal models that are thought to mimic human hypertension of renal origin and are frequently used to elucidate the pathophysiologic mechanism of renal hypertension. In this model, blood pressure elevation exhibits a biphasic profile, the drastic increase in blood pressure over 6 to 8 days after renal artery ligation (acute phase) followed by the maintenance period of moderately increased blood pressure (chronic phase). Hypertension in the acute phase is characteristically accompanied by the elevated PRA, suggesting a causative role of the renin angiotensin system in the induction of hypertension in the acute phase.

In the first part of the study, the effect of Ang II blockade on the ACh-evoked NO release and its role in the regulation of the systemic arterial system were investigated. In this study, it was shown that ACh-induced concentration-dependent relaxation of intact aortic preparations was significantly decreased in RHR ($P<0.01$) compared to that in NR (Fig. 1), and the findings concur with the previous observations (24). These findings are in line with the results from the in vivo study showing that ACh-induced depressor response in RHR was smaller than that in NR. Thus, results from both the in vitro and in vivo studies indicate that ACh-induced NO function could be impaired in the systemic arterial system of RHR. The pretreatment with losartan (10^{-5} M) significantly increased ACh-induced relaxation of the intact aortic preparation from both NR ($P<0.05$ at 10^{-7} M) and RHR ($P<0.05$ at 10^{-7} M, $P<0.01$ at 10^{-6} and 10^{-5} M), with a greater amplitude for tissues from RHR, but it had no effect when aortas were pretreated with L-NAME or denuded of endothelium. These findings were partially confirmed by the in vivo study in that the depressor effects of ACh were decreased after pretreatment with L-NAME (30 mg/kg), but rather enhanced by further pretreatment of losartan, especially in RHR (Fig. 3). Thus, our results indicate that in intact animals, especially RHR, ACh-induced endothelium-dependent depressor effects were enhanced by Ang II blockade even after pretreatment with L-NAME, unlike the findings from isolated intact aortas. These results are in line with other in vivo data that antihypertensive therapy with captopril (25), reserpine or hydralazine (26) could reverse the depressed NO-induced relaxation in hypertensive rats.

In the second part of the study, the role of NO in Ang II-stimulated vasoconstriction and pressor effects was investigated. Ang II-induced vasoconstriction was not significantly different between endothelium-intact aortas form NR and RHR (Fig. 2), and the removal of endothelial cells or pretreatment with L-NAME significantly shifted the concentration response curve for Ang II to the left in a non-parallel manner and enhanced the maximal response in both groups of tissues. These findings are in agreement with other reports that blockade of NO synthesis by N^G-nitro-L-arginine (L-NNA) augmented Ang II-induced vasoconstriction in isolated perfused afferent arterioles from the rabbit (9). On the other hand, the Ang II-induced pressor effect on mean arterial pressure was not significantly different between NR and RHR. However, it was significantly increased by pretreatment with L-NAME in NR, but not in RHR (Fig. 4). These results suggest that NO may play a major role in inhibiting Ang II-induced vasoconstriction in NR (10), but has an insignificant role in RHR. In RHR, the susceptibility of the Ang II-induced response in intact animals to L-NAME was less than that of isolated endothelium intact aorta, and this may be due to more markedly impaired

endothelial function along the smaller resistant vessels that plays a more important role in controlling blood pressure.

In the third part of the study, the physiological role of basally released NO in the regulation of mean arterial pressure was investigated with L-NAME. L-NAME did not constrict the aortic preparations from both NR and RHR, but when given to the animals, it had pressor effects on mean arterial pressure that were similar in NR and RHR (Fig. 5). These findings suggest that the basal NO function is not altered in RHR such that it may regulate and maintain systemic arterial pressure via modulation of vascular tone in resistance vessels. The pretreatment with losartan had a tendency to decrease the L-NAME-induced pressor response, although significantly only at low concentration, which was supported by a recent report that Ang II blockade attenuates the vasoconstriction elicited by L-NNA in rat juxtamedullary arterioles (27). Thus, the pharmacological suppression of Ang II activity may reduce the vasoconstriction elicited by L-NAME, presumably through a decrease in Ang II-stimulated NO levels.

In the last part of the study, the role of NO in the depressor effect of the angiotensin AT_1-receptor antagonist was measured. The losartan-induced depressor response was significantly smaller in RHR ($P < 0.05$) than in NR (Fig. 6), and it was significantly decreased after pretreatment with L-NAME in both NR ($P < 0.05$) and RHR ($P < 0.01$). These findings are in line with the recent results that the antihypertensive effect of losartan was attenuated by pretreatment with L-NAME (10 mg/kg) in aortic coarctated rats (6). One of the mechanisms for the decrease in depressor effect of losartan in both NR and RHR following treatment with L-NAME could be ascribed to a decrease in the pressor effect of Ang II due to an increase in basal mean arterial pressure by L-NAME. Other studies showed that as circulating levels of Ang II are increased, the role of NO in regulating the hemodynamics increases, balancing the amplified vasoconstrictor influence of Ang II (27), and that endothelial cells of the vascular bed in RHR are damaged due to persistence of high blood pressure with a malfunction of the NO pathway. The findings from studies by others suggested other possibilities to consider for our results such as mutual interaction between the NO pathway and renin angiotensin system and the alteration of this interaction depending on pathologic status. Accordingly, it may be thought that increased NO function could elevate Ang II level via stimulating renin secretion as reported in isolated rat kidney (13, 14), contributing to the maintenance of systemic arterial pressure, and thus the blockade of NO function by L-NAME obviates the Ang II increase and its contribution to blood pressure, thereby reducing losartan's depressor effect in both NR and RHR. The findings that the losartan-induced depressor effect is decreased in RHR ($P < 0.05$ at 3 mg/kg) compared to that in NR might be explained by a couple of possibilities. It may be possible that the vasodilator effect of losartan could be mediated in part by NO production, as suggested for the epicardial coronary circulation of dogs (28), and thus the ability of losartan to produce NO is reduced in RHR with partially impaired endothelial function. Most of the anesthetics including the combination of urethane and α-chloralose used in this study may increase PRA. Accordingly, another possibility may be that the combined anesthesia differentially increases PRA in NR and RHR. However, we could not rule out other possibilities as PRA was not measured from these animals under the anesthetic condition.

In conclusion, the present results show that despite the partial impairment of NO production, a significant level of NO function (both evoked and basal) is well-preserved in both RHR and in aortas isolated from these animals. The results from this study also raised the possibility that NO and Ang II may mutually interact in two different ways, counteracting the direct effect of the other at the level of smooth muscle and enhancing the function of the other probably via increasing the synthesis and release of the other, and this interaction plays an important role in regulating the vascular tone and systemic arterial circulation in RHR.

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