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Interaction of Nitric Oxide and Renin Angiotensin System in Pulmonary Arterial Circulation of RHR

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We investigated the interaction between nitric oxide and the renin angiotensin system in regulating isolated pulmonary arterial tension and pulmonary arterial pressure (PAP) in renal hypertensive rats (RHR) made by complete ligation of left renal artery. Losartan induced a depressor response that was smaller in RHR than in normotensive rats (NR) (3.3 and 7.0 mmHg, respectively, at 3.0 mg/kg, $p<0.05$), and the response was significantly reduced by N^G-nitro-L-arginine methyl ester (L-NAME). Angiotensin II elevated the PAP (7.6 and 10.8 mmHg at 0.1 μg/kg; 20.3 and 23.6 mmHg at 1.0 μg/kg, respectively) and contracted the isolated pulmonary artery (pD_2: 8.79 and 8.71, respectively) from both NR and RHR with similar magnitude, and these effects were significantly enhanced by L-NAME in NR, but not in RHR. Acetylcholine lowered the PAP slightly less effectively in RHR than in NR (3.8 and 6.0 mmHg at 10 μg/kg, respectively) and relaxed the pulmonary artery precontracted with norepinephrine in both rats with similar magnitude (E_{max}: 60.8 and 63.6%, respectively), and the effect being completely abolished after pretreatment with L-NAME or removal of endothelial cells. These results suggest that nitric oxide interacts with renin angiotensin system to control the pulmonary vascular tension and pulmonary arterial circulation of RHR.

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

INTRODUCTION

Nitric oxide (NO) appears to be the endogenous prototype of the nitrovasodilator drugs, which act by stimulating soluble guanylate cyclase to increase intracellular levels of cyclic GMP in vascular smooth muscle and result in vasodilation (Moncada *et al.*, 1991; Ignarro, 1989; Henderson, 1989). NO is synthesized from the amino acid L-arginine by an enzyme, the NO synthase (Prince and Gunce, 1993), under the basal conditions and during stimulation by various vasoactive hormones such as acetylcholine, histamine, serotonin, adenosine diphosphate, thrombin and etc. (Knowles and Moncada, 1992; Henderson, 1989). Increasing evidence has suggested that interactions among NO and vasoconstrictor and vasodilator hormones have a severe influence on the regulation of vascular functions (Guan *et al.*, 1996; Ruilope *et al.*, 1994). A recent published report has suggested that angiotensin (Ang) II or its degradation products can stimulate NO release in rabbit brain arterioles (Haberl *et al.*, 1991). This finding is in line with other reports that the inhibition of NO synthesis led to an increase in basal tone and marked augmentation of Ang II-induced afferent arteriolar (Ito *et al.*, 1991) and renal arterial constriction (Zhang *et al.*, 1995). It has also been documented that L-arginine infusion significantly reduced serum angiotensin converting enzyme activity and lowers plasma Ang II significantly in humans (Higashi *et al.*, 1995). 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 (Lee *et al.*, 1993a) and afferent arteriolar constriction (Ito *et al.*, 1991). It has been also reported that NO stimulated renin secretion from isolated rat kidney (Gardes *et al.*, 1994; Scholz and Kurtz, 1993), and this findings has been further supported by Schricker *et al.* (1995), who suggested that renin mRNA levels are tonically increased by NO and that the action of NO is counteracted by Ang II, although contradictory findings suggest that NO inhibist the renin system (Vidal *et al.*, 1988). 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

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normal, as in renal hypertension and Ang II-induced hypertension, particularly, Ang II and NO may become an increasingly important factors in the regulation of the pulmonary arterial system. There are little information, however, regarding interaction of NO and the renin angiotensin system in pulmonary arterial circulation of renal hypertension. Accordingly, the present work was undertaken to elucidate the interaction between NO and the renin angiotensin system in pulmonary arterial circulation of renal hypertensive rats, a model of chronic increase of Ang II levels.

MATERIALS AND METHODS

Materials and solutions

Acetylcholine chloride (ACh), (-)norepinephrine hydrochloride (NE), 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' Ringer bicarbonate solution were purchased from Junsei Chemical Co. (Tokyo, Japan). Losartan was synthesized at the Korea Research Institute of Chemical Technology (KRICT, Taejon, Korea). ACh 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 °C, each aliquot being used for each experiment with serial dilution. Urethane was dissolved in saline and α-chloralose dissolved in propylene glycol with heating. Other drugs were dissolved just before use. All the solutions were prepared in distilled and deionized water for the in vitro experiment and in isotonic saline (0.9 w/v % NaCl solution) for intravenous injection. The composition of Krebs' Ringer bicarbonate buffer was as follows (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $NaHCO_3$, 25; $MgSO_4$, 1.2; KH_2PO_4, 1.2; and glucose, 11.0.

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-h light, 12-h 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 (Lee and Shin, 1994; Lee *et al.*, 1993b). 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 traumatized 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 (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.* (1969). 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.

Experimental protocol for in vivo studies

Both normotensive rats (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; 1ml/100 g, 60 cycles/min; Harvard Apparatus, South Natick, MA, USA). Pulmonary arterial pressure (PAP) was measured and continuously monitored via a catheter (heparinized, 20 IU/ml) inserted through the surface of right ventricle in pulmonary artery after thoracotomy, which was connected to a Grass P23XL pressure transducer and a Gould 2000 physiograph. Rectal temperature was maintained at 36.5±0.5°C by the thermistor-controlled radiant heat. Forty minutes after surgery, when it was possible to obtain consistent control values for PAP were possible to obtain, the experiment was started. NR and RHR were divided into three groups at random. In first group of both NR and RHR, losartan (0.1~3 mg/kg, i.v.) was successively administered with and without pretreatment of L-NAME (30 mg/kg, i.v.) 15 minutes prior to administration of losartan. In 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 minutes prior to administration of Ang II. In third group, 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 minutes prior to administration of ACh, respectively. In some animals, a single bolus injection (30 and 3 mg/kg, respectively, i.v.) was given to delineate the time course of L-NAME and losartan effects. Drugs were administered via a catheter into the left femoral vein in volume of 1 ml/kg. Results are expressed as mmHg change of systolic PAP from the baseline values.

Experimental protocol for in vitro studies

On the day of the experiment, NR and RHR were killed by a blow on the head and exsanguination. The

pulmonary artery was isolated and cleaned of adhering fat and connective tissue. Each artery was cut into two rings 2 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 pulmonary arteries 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°C. The pulmonary arteries were allowed to equilibrate for 60 minutes under the resting tension of 1 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 decrease of endothelium-dependent relaxation to ACh (10^{-5} M) in tissues precontracted with norepinephrine (NE, 10^{-7} M).

The matched pairs of pulmonary arteries with endothelium intact and denuded were precontracted submaximally with 10^{-7} M of NE, washed out 3 times for 45 minutes, and rechallenged with NE to obtain a reproducible and stable response. After the NE response reached the plateau, Ang II (10^{-10}~3×10^{-8} M) were cumulatively added to the matched pairs of arterial rings with endothelium intact and denuded, whereas other tissues were pretreated with L-NAME (10^{-5} M) 15 minutes prior to exposure to Ang II. In separate experiments, ACh (10^{-9}~10^{-5} M) were cumulatively added to the tissue bath with and without pretreatment of L-NAME (10^{-5} M, 15 minutes), an NO synthase inhibitor (Rees *et al.*, 1990), or losartan (10^{-5} M, 30 minutes), an angiotensin AT_1 receptor antagonist (DeGraaf *et al.*, 1993; Timmermans *et al.*, 1993), alone or in combination. The results were expressed as percent decrease in tension from the NE contraction for ACh-induced relaxation and as percent of the reference contraction with high K^+ solution (NaCl was substituted with equimolar KCl) for Ang II-induced contraction.

Statistical analyses

All values are expressed as means±S.E.M. Data were analyzed by the unpaired Student's *t* test and one-way analysis of variance (ANOVA) followed by the Dunnett's test for multiple comparisons (Sigma Stat, Jandel Co., San Rafael, CA, USA). In all the comparisons, the difference was considered to be statistically significant at $P<0.05$.

RESULTS

Renal hypertensive rats

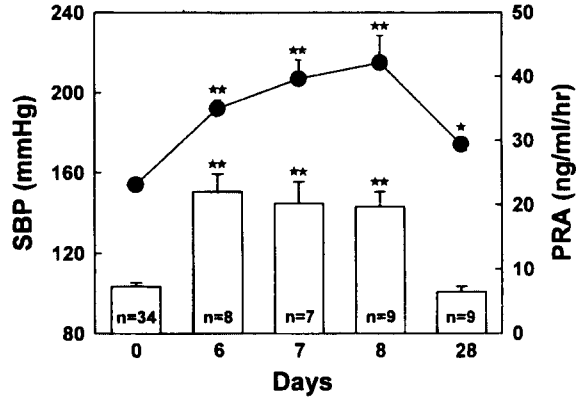

Fig. 1. Systolic blood pressure (SBP, solid circles) and plasma renin activity levels (PRA, bar graph) before (non-operative) and 6, 7, 8 and 28 days after ligation of left renal artery. Each point represents the mean±S.E.M. *P<0.05, **P<0.01 compared with normotensive control rats (0 days).

In eighty five percentage 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 154.3±1.8 to 190~215 mmHg), and thereafter dropped a little bit, to a level, yet still significantly greater than the control values ($P<0.05$) (Fig. 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 vivo studies

As shown in Fig. 2, losartan (0.1~3 mg/kg, i.v.) caused a dose-dependent decrease in PAP in both NR and RHR. The losartan-induced depressor response was reduced in RHR (3.5±0.7 and 3.3±0.3 mmHg at 1.0 and 3.0 mg/kg, respectively, $P<0.05$ vs. NR at 3.0 mg/kg) compared to those in NR (6.2±1.0 and 7.0±0.9 mmHg at 1.0 and 3.0 mg/kg, respectively). Pretreatment with L-NAME (30 mg/kg, i.v.) decreased the losartan-induced depressor response in both NR (3.0±0.8 and 3.8±1.0 mmHg at 1.0 and 3.0 mg/kg, respectively, $P<0.05$ at 1.0 and 3.0 mg/kg) and RHR (0.8±0.5 and 2.0±0.7 mmHg at 1.0 and 3.0 mg/kg, respectively, $P<0.05$ at 0.1, 0.3 and 1.0 mg/kg). Ang II (0.01~3 μg/kg, i.v.) caused a dose-dependent pressor response in both NR and RHR to a similar magnitude (7.6±0.8 and 10.8±2.1 mmHg at 0.1 μg/kg; 20.3±1.5 and 23.6±4.2 mmHg at 1.0 μg/kg, respectively) (Fig. 3). The Ang II-induced pressor response was significantly

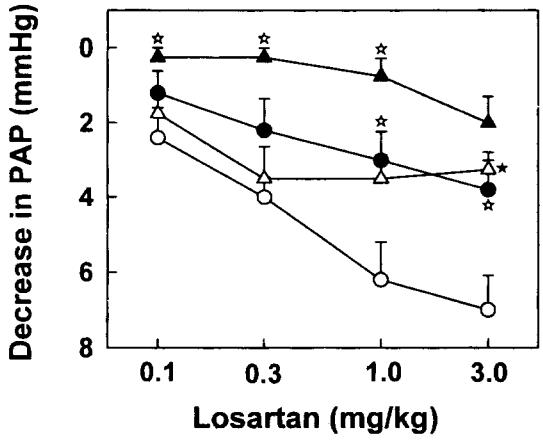

Fig. 2. The effects of losartan on pulmonary arterial pressure (PAP) in anesthetized normotensive (circles) and renal hypertensive rats (triangles). Rats were pretreated with either vehicle (open symbols) or N^G-nitro-L-arginine methyl ester (30 mg/kg, i.v., solid symbols). Each point represents the mean±S.E.M. of 4-5 experiments. *P<0.05 compared with normotensive control rats. ☆P<0.05 compared with respective control rats.

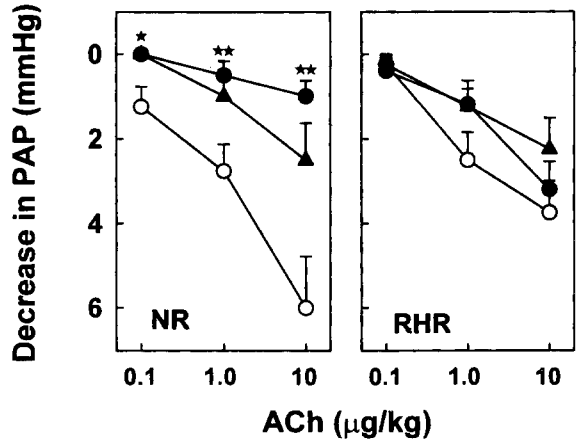

Fig. 4. The depressor effects of acetylcholine (ACh) on pulmonary arterial pressure (PAP) in anesthetized normotensive (NR) and renal hypertensive rats (RHR). The rats were pretreated with either vehicle (open circles), N^G-nitro-L-arginine methyl ester (L-NAME, 30 mg/kg, i.v., solid circles) or in combination 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.

enhanced by pretreatment with L-NAME (30 mg/kg, i.v.) in NR (15.0±3.8 and 35.0±2.7 mmHg at 0.1 and 1.0 μg/kg, respectively, P<0.05 and P<0.01 at 0.1 and 1.0 μg/kg, respectively), but not significantly enhanced in RHR (13.6±3.3 and 33.4±4.8 mmHg at 0.1 and 1.0 μg/kg. ACh (0.1~10 μg/kg, i.v.) lowered the PAP in NR and RHR in a dose-dependent manner (1.3±0.5 and 0.3±0.3 mmHg at 0.1 μg/kg; 2.8±0.6 and 2.5±0.7 mmHg at 1.0 μg/kg; 6.0±1.2 and 3.8±0.8 mmHg at 10 μg/kg, respectively), with slightly weaker effects in RHR at all doses tested (Fig. 4). The depressor effects of ACh on NR were significantly decreased by pretreatment of 30 mg/kg L-NAME (0.01±0.01, 0.5±0.3 and 1.0±0.4 mmHg at 0.1, 1.0 and 10 μg/kg, respectively, P<0.01), but not significantly decreased in RHR (0.4±0.4, 1.2±0.4 and 3.2±0.7 mmHg at 0.1, 1.0 and 10 μg/kg, respectively). These ACh-induced depressor responses were rather enhanced by further pretreatment with 3 mg/kg losartan in both NR and RHR (0.01±0.01 and 0.3±0.2 mmHg at 0.1 μg/kg; 1.0±0.4 and 1.3±0.6 mmHg at 1.0 μg/kg; 2.5±0.9 and 2.3±0.7 mmHg at 10 μg/kg, respectively), but not statistically significant. When administered alone, L-NAME or losartan had pressor or depressor effects in both NR and RHR, respectively, which lasted throughout the whole experiment (60 and 30 min, respectively, data not shown). The basal PAP after pretreatment with each drugs were presented in Table I.

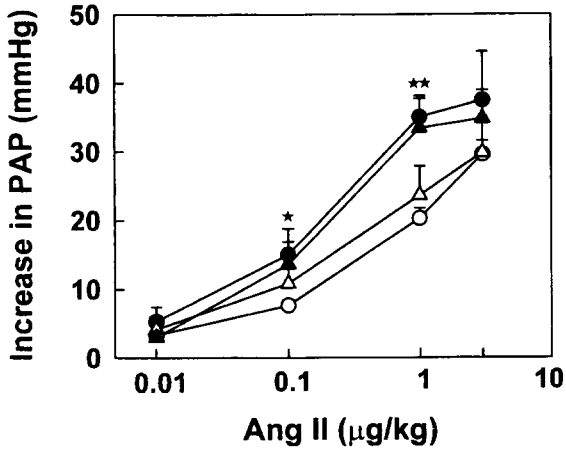

Fig. 3. The pressor effects of angiotensin (Ang) II on pulmonary arterial pressure (PAP) in anesthetized normotensive (circles) and renal hypertensive rats (triangles). Rats were pretreated with either vehicle (open symbols) or N^G-nitro-L-arginine methyl ester (30 mg/kg, i.v., solid symbols). Each point represents the mean±S.E.M. of 5-6 experiments. *P<0.05, **P<0.01 compared with normotensive control rats.

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

	NR	RHR
Vehicle	28.2±1.1 (n=24)	27.7±1.0 (n=17)
L-NAME	34.4±2.2 (n=17)*	30.4±1.6 (n=13)
L-NAME+Losartan	32.0±2.3 (n=4)	28.1±1.4 (n=8)
Losartan	27.8±0.5 (n=5)	26.7±1.5 (n=4)

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

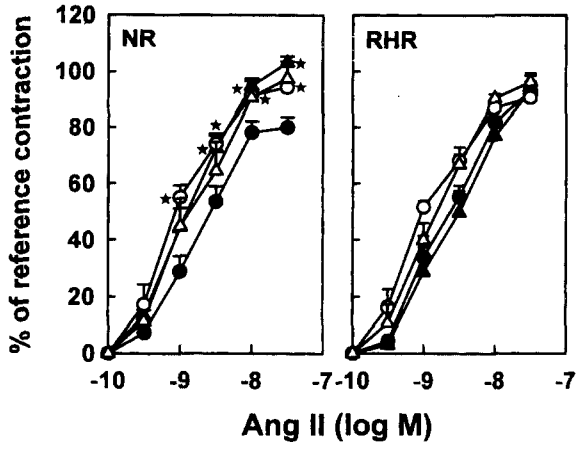

Fig. 5. The contractile effects of angiotensin (Ang) II on the isolated pulmonary arteries from normotensive (NR) and renal hypertensive rats (RHR) in the presence (solid symbols) and absence (open symbols) of functional endothelium. The pulmonary arteries were pretreated with either vehicle (circles) or N^G-nitro-L-arginine methyl ester (10^{-5} M, 15 minutes, 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. *P <0.05, compared with endothelium intact aorta from normotensive control rats.

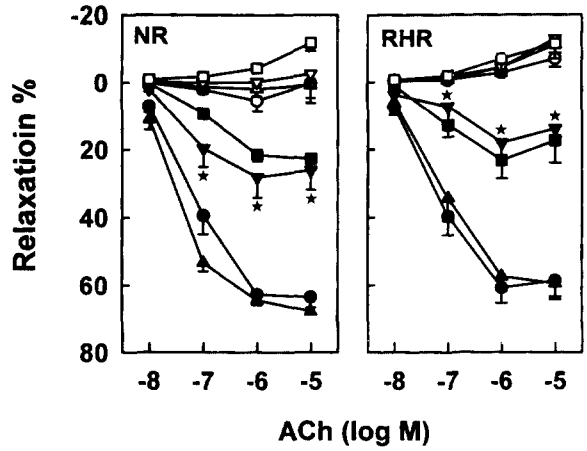

Fig. 6. The relaxant effects of acetylcholine (ACh) on the isolated pulmonary arteries from normotensive (NR) and renal hypertensive rats (RHR) in the presence (solid symbols) and absence (open symbols) of functional endothelium. The pulmonary arteries were pretreated with either vehicle (circles), losartan (10^{-5} M, 30 minutes, triangles), N^G-nitro-L-arginine methyl ester (10^{-5} M, 15 minutes, reversed triangles) or in 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<0.05, compared with endothelium intact aorta from respective control rats.

In vitro studies

As shown in Fig. 5, Ang II (10^{-10}~3×10^{-8} M) produced a concentration-dependent contractile response in intact pulmonary artery from both NR and RHR, with no significant difference between the groups (pD_2: 8.79 ±0.09 and 8.71±0.07; E_{max}: 80.0±3.7% and 93.1± 3.1%, respectively). The removal of endothelial cells (pD_2: 8.99±0.11 and 9.09±0.12; E_{max}: 94.5±2.1, P<0. 01, and 93.1±3.1% for pulmonary arteries from NR and RHR, respectively) or pretreatment of L-NAME (pD_2: 8.93±0.06 and 8.51±0.15; E_{max}: 103.2±2.2, P <0.01, and 95.4±6.7%) significantly shifted the concentration-response curve for Ang II to the left and enhanced the maximal response. ACh (10^{-9}~10^{-5} M) caused a concentration-dependent relaxation of the intact pulmonary artery from in NR and RHR precontracted with NE (10^{-7} M) (Fig. 6). ACh-induced relaxation of intact pulmonary artery in NR (E_{max}: 63.6±3.2 %) was similar to that in RHR (E_{max}: 60.8±4.6%) at all concentrations tested, the effect being significantly reduced after removal of endothelial cells or pretreatment with L-NAME. Losartan (10^{-5} M) did not alter the ACh-induced relaxation on intact pulmonary artery with and without pretreatment of L-NAME in both NR and RHR. L-NAME and losartan had no effect on the basal tension of either intact or rubbed pulmonary artery from NR and RHR.

DISCUSSION

The forms of hypertension associated with elevated circulating levels of Ang II may have unique vascular effects. RHR are one of the animal models that are thought to mimic human hypertension of renal origin. The RHR 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 in 6 to 8 days after renal artery ligation (acute phase) followed by the maintenance 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 role of NO in the depressor effect of the Ang II receptor antagonist was measured. The losartan-induced depressor response was significantly smaller in RHR (P<0.05) than that in NR (Fig. 2), and it was significantly decreased after pretreatment with L-NAME in both NR (P<0.05) and RHR (P<0.05). These findings are in line with the recent results that the antihypertensive effect of losartan was attenuated by the pretreatment of L-NAME (10 mg/kg) in aortic coarctated (Guan *et al.*, 1996) and renal artery ligated rats (Lee and Shin, 1997). 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 (Ohishi *et al.*, 1992), 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 (Scholz *et al.*, 1993 and Gardes *et al.*, 1994), contributing to the maintenance of PAP, 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 (Sudhir *et al.*, 1993), 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 the second part of the study, the role of NO in Ang II-stimulated vasoconstriction and pressor effects was investigated. The Ang II-induced pressor response on PAP 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. 3). Moreover, these results could be confirmed by the in vitro data which the removal of endothelial cells or pretreatment of L-NAME significantly shifted the concentration-response curve for Ang II on isolated endothelium-intact pulmonary artery from NR to the left, but not from RHR, although the Ang II-induced vasoconstriction was not significantly different between isolated pulmonary artery from NR and RHR (Fig. 5). These findings are in agreement with other reports that blockade of NO synthesis by NG-nitro-L-arginine (L-NNA) augmented Ang II-induced vasoconstriction in isolated perfused afferent arterioles from the rabbit (Ito *et al.*, 1991). These results suggest that NO may play a major role in inhibiting Ang II-induced vasoconstriction in NR (Zhang et al., 1995), but insignificant role in RHR.

In the third part of the study, the role of the agonist-evoked NO release in the regulation of the pulmonary arterial system was investigated. In this study, it was shown that the ACh-induced depressor response in RHR was smaller than that in NR, although statistically not significant (Fig. 4). The ACh-induced relaxation of isolated intact pulmonary artery was similar between on pulmonary artery from NR and RHR (Fig. 6). These results on pulmonary artery and PAP are not in agreement with those on the systemic artery and systemic arterial pressure because the endothelium-dependent response to ACh in aortas and systemic arterial pressure from RHR was significantly decreased (Lee and Shin, 1997; Van de Voorde and Leusen, 1986). This observation indicate that the endothelium-dependent relaxation in RHR might be impaired in the systemic arterial system due to high blood pressrue, however, less impaired in the pulmonary arterial system due to relatively low blood pressure. The depressor effects of ACh were significantly decreased after pretreatment with L-NAME (30 mg/kg) in NR, but not in RHR. This results were confirmed by the in vitro data which ACh-induced relaxation of intact pulmonary artery from NR was significantly increased by pretreatment with L-NAME or removal of endothelium, but not significantly increased in RHR.

In conclusion, the present results show that despite the partial impairment of NO production, a significant level of NO function is well-preserved in pulmonary arterial circulation of RHR. 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 pulmonary arterial circulation in RHR.

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