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Differential Function of EDRF in Systemic Arterial and Pulmonary Arterial System of Renal Hypertensive Rats

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ABSTRACT

To investigate the endothelium dependent vascular reactivity of the systemic arterial and the pulmonary arterial system in acute renal hypertensive rats of 2-kidney, 1-ligation type (RHRs), acetylcholine (ACh)-induced vasodilation and depressor effects were evaluated in isolated arteries and in vivo, respectively, in the presence and absence of functional endothelium. ACh (10^{-5} M) relaxed the intact thoracic aortas from RHRs and normotensive rats (NRs), but the effect was significantly smaller for those from RHRs (34 and 86%, respectively, $p<0.01$). ACh-induced vasodilation was completely abolished after removal of endothelial cell or pretreatment with EDRF inhibitors, L-NAME and MB, indicative of its dependence on intact endothelial or EDRF function. ACh also induced vasorelaxation of the intact pulmonary arteries from RHRs and NRs; however, unlike the effects on the thorcic aorta, no significant difference in amplitude was noted between two groups. ACh (0.1~10 μg/kg, i.v.) reduced mean systemic arterial pressure in anesthetized RHRs and in NRs to the similar magnitude (% change: 39 and 46% at 10 μg/kg, respectively) and these hypotensive effects were significantly decreased after pretreatment with L-NAME (30 mg/kg, i.v.). Deprssor effects of ACh on mean pulmonary arterial pressure were similar in RHRs and NRs with and without pretreatment of L-NAME. However, in both NRs and RHRs, the depressor effects of ACh on mean pulmonary arterial pressure were significantly reduced compared with those for mean systemic arterial pressure, and the increment of mean pulmonary arterial pressure noted after L-NAME (0.1~100 mg/kg, i.v.) was significantly smaller than that for mean systemic arterial pressure. These results indicate that in RHRs the endothelial cell function was impaired, at least in part, in systemic arterial system, but not in pulmonary arterial system, and both ACh-evoked and basal release of EDRF was less in the pulmonary arterial system than in systemic arterial system of both NRs and RHRs.

Key Words: Renal hypertensive rat, Systemic arterial system, Pulmonary arterial system, EDRF
Abbreviations: RHRs; renal hypertensive rats, NRs; normotensive rats, ACh; acetylcholine, L-NAME; N-nitro-L-arginine methyl ester, EDRF; endothelium-derived relaxing factor

INTRODUCTION

It is known that endothelial cells lining blood vessels release substances, which profoundly affect vascular tone and platelet function. They include a potent vasoconstrictor peptide endothelin and inhibitory substances such as endothelium-derived relaxing factor (EDRF or nitric oxide), prostacyclin (PGI2) and endothelium-derived hyperpolarizing factor (EDHF). Under certain

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conditions endothelial cells can also release angiotensin II, thromboxane A_2 and cyclooxygenase-dependent contracting factors (Lüscher, 1989). Alterations in vascular structure and reactivity has been reported in hypertension (Gabbiani *et al.*, 1979). It was reported that during hypertensive process the aorta undergoes many histological abnormalities including swelling of endothelial cell, thickening of endothelial cell, increase in intimal permeability, and expansion of subendothelial space, the findings that vary among types of hypertension (Gabbiani *et al.*, 1979; Haudenschild *et al.*, 1980). These morphological changes in vascular structure, especially changes in the vascular endothelium in the hypertensive state are thought to indicate their relevance to pathophysiology of hypertension, as based on physiological evidence that endothelial cells appear to play a key role in modulation of vascular tone (Rees *et al.*, 1989). It has been shown that the function of EDRF appears to dominate in normal arteries, whereas in diseased arteries, the release and action of EDRF is impaired and that of endothelium-dependent contracting factors is increased (Lüscher, 1989). These findings appear to concur with the studies which have shown an impaired EDRF function in experimental models of hypertension (Van de Voorde *et al.*, 1984; 1986), atheroma (Chappel *et al.*, 1987) and diabetes (Oyama *et al.*, 1986). More recently, it has been reported that patients with essential hypertension had an endothelium-dependent abnormality of vascular relaxation (Panza *et al.*, 1990).

Although extensive studies have been conducted in spontaneously hypertensive rats, an animal model for human essential hypertension, to elucidate the role of vascular endothelium and EDRF in human hypertension, little studies were conducted in renal hypertensive rats, a model for human hypertension of renal origin. The mechanisms responsible for the induction and maintenance of hypertension in this renal hypertensive rat model are not clear. However, various physiological factors such as the renin-angiotensin system, extracellular volume distribution, autonamic nervous system, lack of vasodepressor substance and production of a renal vasopressor factor other than renin and interaction among these multiple factors have been proposed as possible underlying mechanisms (Cangiano *et al.*, 1979). Moreover, the recent introduction of new biotechnologies to cardiovascular research has demonstrated that the renin-angiotensin system known to play a principal role in renal hypertension operates as both a circulating (endocrine) and a tissue (autocrine/paracrine) system (Ferrario, 1990). Thus, it appears that the pathophysiology of renal hypertension could be more complicated than ever thought, and the components involved vary among animal models of renal hypertension.

Renal hypertensive rats of 2-kidney, 1-ligation type (RHRs) are one of animal models for human hypertension of renal origin, which is frequently used for the elucidation of the pathogenetic mechanism of the renal hypertension and for the development of non-peptidic angiotensin II receptor antagonists, antihypertensive drugs of new generation (Timmersmans *et al.*, 1993; 1991; Wada *et al.*, 1992; Wong *et al.*, 1991) because of its excellent degree of reproducibility and reliability (Fernandez *et al.*, 1976). It has been shown that in this model peripheral plasma renin activity levels correlate well with elevation in systemic arterial pressure, thus indicating the usefulness of plasma renin activity as an index of acute hypertension of renal origin (Fernandez *et al.*, 1976; Fekete *et al.*, 1971).

It has been reported that endothelium-dependent acetylcholine (ACh)-induced relaxation is decreased in large conducting vessels such as thoracic aorta from RHRs (Van de Voorde *et al.*, 1984). However, little is known as to whether endothelium-dependnet ACh-induced relaxation is altered in other types of vessels such as small resistance vessel as well as pulmonary artery from RHRs and what implications it has in the regulation of blood pressure in the systemic and pulmonary arterial system of RHRs.

To study whether EDRF-dependent vascular reactivity is altered in the systemic and pulmonary arterial system of acute renal hypertensive rats, and the altered function, if any, contributes to developement and maintenance of hypertension in RHRs, we characterized RHRs of 2-kidney, 1-ligation type by measuring plasma renin activity and blood pressure change with time after ligation and examined ACh-induced relaxation and depressor effects using isolated arteries and anesthetized open-chest RHRs in the acute

phase of hypertension.

MATERIALS AND METHODS

Materials and solutions

Acetylcholine chloride, (−) norepinephrine hydrochloride, methylene blue, N-nitro-L-arginine methyl ester, urethane, α-chloralose were purchased from Sigma Chemical Company (USA), and other drugs and reagents used to prepare Krebs Ringer bicarbonate solution were purchased from Junsei Chemical Co. (Japan). Acetylcholine chloride with high hygroscopicity was made as 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 in propylene glycol with heating. All the other drugs were dissolved just before use. All the solutions were prepared in distilled and deionized water for in vitro experiment and in isotonic saline (0.9 w/v% NaCl soluton) for intravenous injection. The composition of Kreb's Ringer bicarbonate buffer (below Kreb's 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. High potassium solution (20 mM KCl) had a composition similar to Kreb's buffer except that NaCl was replaced with equimolar KCl.

Animals

Male Sprague-Dawly rats weighing 350~450 g were used in this study. They were perchased from the Department of Experimental Animal, KRICT (Korea) and kept in a storage room under the conditions of constant temperature, relative humidity 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 (RHRs)

RHRs of 2-kidney, 1-ligation type were prepared as follows. Rats were anesthetized with ether and a small incision (1 cm in length) was made on the left side of abdomen. 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 metalic clips. To delineate and confirm the development of high blood pressure and plasma renin activity over days of ligation, animals that underwent the operation were divided into groups of days 0 (before operation), 6, 7, 8 and 28 postoperatively for the measurement of systolic blood pressure and plasma renin activity. Indirect systolic blood pressure was measured using the tail cuff method with Multichannel 8000 (TSE, Germany) from the conscious rat. An average of three consecutive measurements was taken from each rat.

For the measurement of plasma renin acitivity, 1.5 milliliter of blood was withdrawn by heart puncture from each animal under light ether anesthesia into a prechilled syringe containing sufficient EDTA solution (final concentration: 1 mg/ml). Collected samples were maintained in an ice bath and the plasma separated by centrifugation at 4℃ for 15 minutes (1200xg). The clear plasma was stored frozen at −80℃ until the day of assay. After thawing of the frozen plasma samples in an ice bath, plasma renin activity was determined by radioimmunoassay (Haber *et al.*, 1969) using angiotensin I [^{125}I] assay kit (Du Pont-New England Nuclear), and the activity was expressed as ng/ml/hr of angiotensin I generated. As a measure of the hypertrophy of the kidney, the wet kidney weight to body weight ratio (KW/BW×100) was also determined after sacrifice. As it was shown that animals from groups of day 6, 7 and 8 revealed good correlation between systolic blood pressure and plasma renin activity, 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.

Preparation of isolated vascular rings

On the day of the experiment, normotensive and renal hypertensive rats were killed by a blow on the head and exsanguination. Thoracic aorta and main pulmonary artery were isolated and cleaned of adhering fat and connective tissue. Each artery was cut into two rings 2~3 mm wide, with extreme care taken to preserve endothelium

intact. In one of two rings, the endothelial layer was destroyed by gently rubbing the luminal surface with a cotton swab moistened with Kreb's solution. Arterial rings with the endothelium intact or denuded was suspended between wire hooks in an organ bath containing 20 ml of Kreb's buffer bubbled with mixture gas (95% O_2, 5% CO_2) and maintained at 37℃. The thoracic aortic rings and the pulmonary arterial rings were allowed to equilibrate for 60 minutes under the resting tension of 2 g and 1 g, respectively. Isometric contractile activity was measured with a force displacement transducer (Grass FT03) and displayed on a chart recorder (Multicorder MC 6625, Hugo Sachs Electronik, Germany). Removal of the endothelium was confirmed pharmacologically by the absence of endothelium-dependnet relaxation to ACh (10 μM) in tissues precontracted with norepinphrine (10^{-7} M).

Experimental protocol for in vitro studies

To study whether the endothelial cell function and its two components EDRF and EDHF are altered in the thoracic aorta and pulmonary artery from RHRs, the in vitro experiments were conducted as follows. Matched pairs of arterial rings with endothelium intact and removed were precontracted with norepinephrine (10^{-7} M: submaximal concentration). After plateau was reached, dose-relaxant response curves to ACh (10^{-10}~10^{-5} M) were obtained and in some tissue after EDRF inhibitors, methylene blue and L-NAME (10^{-5} M), were added to the bath 10 and 15 minutes, respectively, prior to exposure to ACh.

To examine relative contribution of EDRF and EDHF to endothelium-dependent ACh-induced relaxation, cumulative dose-relaxant response curves to ACh (10^{-10}~10^{-5} M) were obtained after the normal Kreb's buffer was replaced by high potassium solution ($[K^+]$=20 mM), which blocked ACh-induced, EDHF-dependent hyperpolarization, due to a shift in potassium equilibrium potential, with or without the addition of L-NAME (10^{-5} M) to the bath. Data were expressed as percentage of contractile response to NE (10^{-7} M).

In vivo studies

Both normotensive and renal hypertensive rats 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 (Harvard apparatus, UK; stroke volume, 1 ml/100g, 60 cycles/min). Systemic arterial pressure (SAP) was measured and continuously monitored via a catheter (heparinized, 20 IU/ml) inserted in the left carotid artery, which was connected to Grass P23XL pressure transducer and a Gould 2000 physiograph. Pulmonary arterial pressure (PAP) was recorded via the catheter inserted through the surface of right ventricle in pulmonary artery after thoracotomy. Heart rate was derived from the systemic arterial pressure pulse by 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 consistent control values for all the parameters were possible to obtain, the experiment was started.

To study the role of EDRF in the regulation of basal systemic and pulmonary arterial pressure in RHRs, NO synthase inhibitor L-NAME (1.0~100 mg/kg) was succesively administered at 5 minute interval, and in some animals, a single bolus injection (30 mg/kg, i.v.) was given to delineate the time course of L-NAME effects. In separate experiments, ACh-induced (0.1~10 μg/kg, i.v.) hypotensive response was measured with and without pretreatment with L-NAME (30 mg/kg, i.v.). Drugs were administered via a catheter inserted into the left femoral vein in volumes of 1 ml/kg. Data were expressed as percentage change of blood pressure and heart rate from baseline values.

Statistical analysis

Data were expressed as mean±S.E.M. The difference between groups was evaluated by Student's t-test for paired or unpaired data as appropriate, with $p<0.05$ being considered statistically significant.

RESULTS

Rat model for acute renal hypertension

In eighty to ninety percentage of animals, systolic blood pressure started increasing on day

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 154.99±1.83 mmHg to 190~125 mmHg), and thereafter droped a little bit, yet still significantly greater than control values ($p<0.01$). Plasma renin activity showed a significant changes ($p<0.01$) from control level of 7.31±0.63 ng/ml/hr A I to 19~22 ng/ml/hr A I on days 6~8 after the renal arterial ligation, but on day 28 returned to control level, despite the consistence of high blood pressure, indicating good correlation between development of hypertension and PRA, only in the acute phase of renal hypertension in this model.

The ratio of the left kidney weight to body weight (KW/BW×100) was significantly greater on day 7, but smaller on day 28 of ligation than on day 0 (before ligation). About 10~20% of rats that underwent the surgery for renal arterial ligation did not develop hypertension with an apparent morphological evidence of complete renal infarction in kidney on the side subject to surgery and no change in PRA. Table 1 lists results on development of blood pressure and PRA over time after the ligation of left renal artery.

In vitro studies

As shown in Fig. 1, ACh (10^{-10}~10^{-5} M) dose-dependently relaxed the intact aorta from both NRs and RHRs precontracted with NE (10^{-7} M), but with a significant difference in efficacy (E_{max}: 85.94±2.96% and 34.31±6.45% at 10^{-5} M, respectively, $p<0.01$), the effect being almost completely abolished after removal of endothelial cells pretreatment with two EDRF inhibitors of different mode of action, L-NAME (a specific inhibitor of EDRF formation from L-arginine, Rees *et al.*, 1989, 1990) and methylene blue (a guanylate cyclase inhibitor, Yamakuchi *et al.*, 1988). ACh also induced a dose-dependent relaxation of the intact pulmonary arteries from NRs and RHRs; however, the significant difference in efficacy observed for tho-

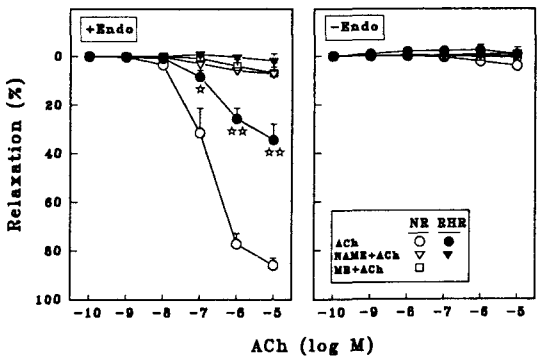

Fig. 1. Relaxant effects of ACh on the isolated thoracic aorta from normotensive (NRs) and renal hypertensive rats (RHRs) in the presense (+Endo) and absense (−Endo) of functional endothelium. Muscle strips were pretreated with L-NAME and MB (10^{-5} M) for 15 and 10 minutes, respectively. Data are expressed as percentage decrease from NE (10^{-7} M)-induced contraction. Each point represents the mean±S.E.M. of 5-8 experiments.
* $p<0.05$, *** $p<0.01$ compared with NR ACh group with intact endothelium.

Table 1. Plasma renin activity levels (PRA), systolic arterial pressure (SAP), heart rate (HR) and ratio of kidney weight to body weight (KW/BW×100) before and 6, 7, 8 and 28 days after ligation of left renal artery. Data are expressed as mean±S.E.M.

	Days after renal artery ligation				
	0	6	7	8	28
PRA (ng/ml/hr)	7.31±0.63	22.00±2.82**	20.16±3.42**	19.74±2.29**	6.41±0.88
SAP (mmHg)	154.29±1.83	191.50±4.25**	206.86±9.45**	214.78±13.72**	173.67±3.12**
HR (beats/min)	355.88±24.00	364.25±16.80	407.71±34.12	427.22±22.08	345.22±13.06
KW/BW×100	0.46±0.010		0.67±0.055**		0.045±0.004**
No. of animals	34	8	7	9	9

**$p<0.01$ compared with normal rats ("0" days).

racic aorta was not noted between pulmonary arteries from NRs and RHRs (E_{max}: 63.57±3.17% and 60.84±4.56% at 10^{-5} and 10^{-6} M, respectively) (Fig. 2). ACh-induced vasorelaxation of pulmonary arteries from both NRs and RHRs was completely abolished after removal of endothelial cells, as for thoracic aorta, but only partially inhibited after treatment with L-NAME unlike thoracic aorta, thus resulting in a significant difference in the remaining portion of ACh-induced vasorelaxation between two types of arteries (E_{max} for aorta and pulmonary arteries: NRs, 5.75±4.67 % and 28.01±5.99%; RHRs, 0.25±1.43% and 18.02 ±4.41% and 10^{-6} M, respectively, $p<0.05$). The remaining portion of ACh-induced vasorelaxation was almost completely abolished after further treatment with high potassium solution ($[K^+]=20$ mM), the experimental condition thought to preclude EDHF component from endothelium-dependent, ACh-induced vasorelaxation (Fig. 3). These results appear to indicate the involvement of both EDRF and EDHF in the mediation of ACh-induced vasorelaxation of arteries from NRs and RHRs. As for arteries from NRs, ACh-induced vasorelaxation was signficantly smaller ($p<0.01$) for the intact pulmonary artery than for the intact thoracic aorta, while for from RHRs, it is greater for the pulmonary artery than for thoracic aorta.

In vivo studies

The aim of the in vivo studies was to study the role of basal and ACh-evoked EDRF function in the regulation of systemic and pulmonary arterial pressure in RHRs and NRs. L-NAME (0.1～100 mg/kg, i.v.) dose-dependently increased systemic arterial pressure in NRs and RHRs to a similar magnitude (E_{max} for mean system arterial pressue: 77.0±6.45 and 75.75±6.32 mmHg, respectively) (Fig. 4), having a differential effect on the systolic and diastolic pressure such that the pulse pressure decreased gradually with dose in both groups of animals (Data not shown). L-NAME (30 mg/kg, i.v.), at a single bolus injection,

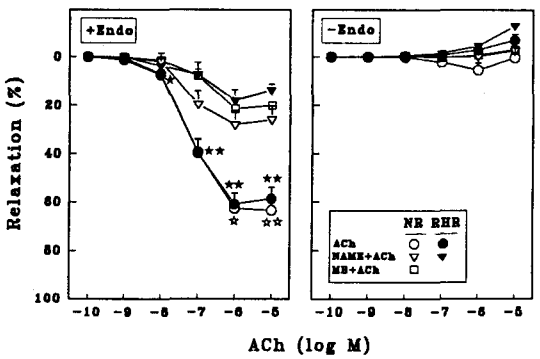

Fig. 2. Relaxant effects of ACh on the isolated pulmonary artery from normotensive (NRs) and renal hypertensive rats (RHRs) in the presense (+Endo) and absense (−Endo) of functional endothelium. Muscle strips were pretreated with L-NAME and MB (10^{-5} M) for 15 and 10 minutes, respectively. Data are expressed as percentage decrease from NE (10^{-7} M)-induced contraction. Each point represents the mean±S.E.M. of 5-8 experiments.
* $p<0.05$, ** $p<0.01$ compared with NR ACh group for intact thoracic aorta.
* $p<0.05$, ** $p<0.01$ compared with RHR ACh group for intact thoracic aorta.

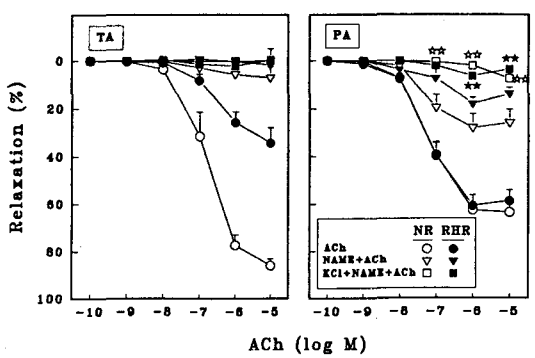

Fig. 3. Relaxant effects of ACh on the isolated thoracic aorta (TA) and pulmonary artery (PA) from normotensive (NRs) and renal hypertensive rats (RHRs) in 20 mM K^+ soluton. Muscle strips were pretreated with L-NAME (10^{-5} M) for 15 minutes. Data are expressed as percentage decrease from NE (10^{-7} M)-induced contraction. Each point represents the mean±S.E.M. of 4 experiments.
** $p<0.01$ compared with NR PA ACh group pretreated with NAME in normal Kreb's soln.
** $p<0.01$ compared with RHR PA ACh group pretreated with NAME in normal Kreb's soln.

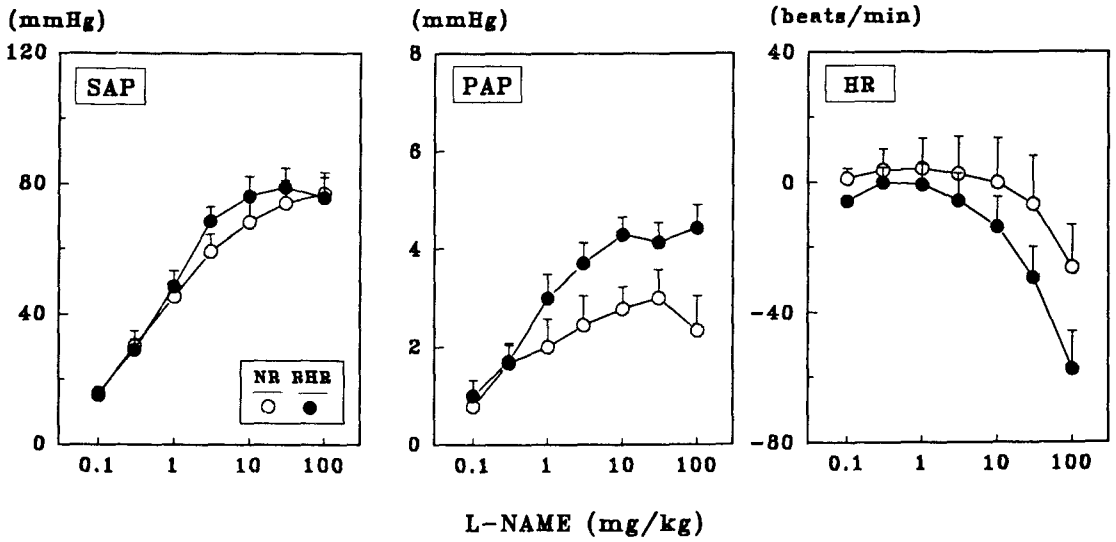

Fig. 4. Effects of L-NAME on mean systemic arterial (SAP), mean pulmonary arterial pressure (PAP) and heart rate (HR) in anesthetized open-chest normotensive (NRs) and renal hypertensive rats (RHRs). Each point represents the mean±S.E.M. of 5-9 experiments.

induced pressor response which reached a plateau within 3-5 minutes and lasted throughout the whole experiment (60~90 minutes) in both NRs and RHRs. The effects of L-NAME on pulmonary arterial pressure in both NRs and RHRs had a similar pattern to that for the effects on the systemic arterial pressure except that the increment of pulmonary arterial pressure was singificantly smaller than that for systemic arterial pressure (E_{max} for mean systemic and pulmonary arterial pressure: NRs, 77.0±6.45 vs. 3.00 ±0.58 mmHg [100.62±14.38 vs. 19.70±3.78% from basal value]; RHRs, 75.75±6.32 vs. 4.43±0.48 mmHg [±95.38±8.24% vs. 28.56±3.79% from basal value], respectively, $p<0.01$). The dose-dependent increase in arterial pressure induced by L-NAME was accompanied by a sustained bradycaria in NRs and RHRs.

ACh (0.1~10 ug/kg, i.v.) lowered the mean systemic arterial pressure in NRs and RHRs in a dose-dependent manner (E_{max}: 46.43 ± 1.31% and 38.63±3.01% at 10 μg/kg, respectively) with a slightly weaker effects at all doses tested in RHRs (Fig. 5). ACh-induced decrease in systolic sys-

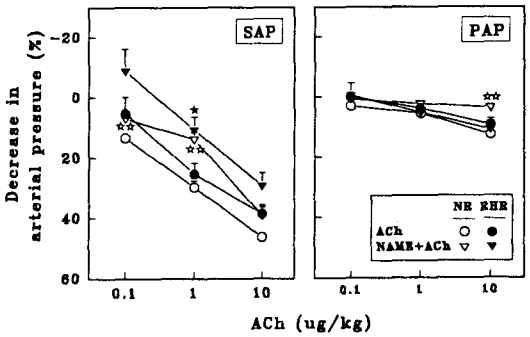

Fig. 5. Hypotensive effects of ACh on mean systemic arterial (SAP) and mean pulmonary arterial pressure (PAP) in anesthetized open-chest normotensive (NRs) and renal hypertensive rats (RHRs). Rats were pretreated with L-NAME (30 mg/kg, i.v.). Each point represents the mean ±S.E.M. of 4-8 experiments.

* $p<0.01$ compared with NR ACh group for SAP and PAP, respectively.

* $p<0.05$ compared with RHR ACh group for SAP.

temic arterial pressure at all doses tested was significantly smaller in RHRs than in NRs ($p<0.01$ or $p<0.05$) (Data not shown). The depressor effects of ACh were significantly reduced after pretreatment with L-NAME (30 mg/kg, i.v.) (NRs: $p<0.01$ at 0.1 and 1.0 μg/kg; RHRs: $p<0.05$ at 1.0 μg/kg). The depressor effect of ACh on pulmonary arterial pressure were similar in NRs and RHRs (E_{max}: 12.60±0.66 and 9.18±2.02% at 10 μg/kg, respectively). However, in both NRs and RHRs, depressor effects of ACh on mean pulmonary arterial pressure were significantly smaller than those for systemic arterial pressure ($p<0.01$) (Fig. 5).

DISCUSSION

Since Goldblatt first studied the important quantitative features of hypertension caused by renal artery constriction, many different types of animal model for renovascular hypertension have been developed, each type having different profile in the development of hypertension and plasma renin activity. In rat (Cangiano *et al.*, 1979; Fernandez *et al.*, 1977; Gross, 1971) and dog (Fekete *et al.*, 1971) of 2-kidney, 1-clip type, it has been shown that plasma renin activity is elevated in the acute phase of hypertension, but returns to the normal level despite the consistence of high blood pressure in chronic phase of hypertension (at 4 weeks of clipping or ligation). Findings by others and ours which confirmed their result appear to indicate the possible role of circulating renin angiotensin system in the development of hypertension in the acute phase, but not in the chronic phase of renal hypertension in this rat model.

To investigate the EDRF-dependent vascular ractivity in the rat model for the acute renal hypertension, with a special emphasis on differentiation of systemic and pulmonary arterial system, all the study was conducted in RHRs of 2-kidney, 1-ligation type on days 6~8 after ligation and vascular tissues from these animals. In the first part of the study, the physiological role of basally released EDRF in the regulation of systemic and pulmonary arterial pressure was investigated using L-NAME as a pharamacological tool for determining the physiological role of basally released EDRF in vivo (Rees *et al.*, 1990). In the present study with anesthetized renal hypertensive rats L-NAME dose-dependently increased both systemic arterial and pulmonary arterial pressure, the effect long-lasting at high dose (30 mg/kg, i.v.), as for systemic arterial pressure in studies with anesthetized wistar rats (Rees *et al.*, 1990; Whilttle *et al.*, 1989), and rabbits (Rees *et al.*, 1989). We demonstrated that the pressor effects of L-NAME on systemic and pulmonary arterial pressure were similar in RHRs and NRs, whereas the pressor effect on pulmonary arterial pressure was significantly smaller than that for systemic arterial pressure in both groups of animals. These observations suggest that the basal EDRF function which is thought to regulate and maintain arterial pressure in systemic and pulmonary circulation via modulation of vascular tone in resistance vessels is not altered in RHRs, and it is predominating in systemic arterial system in both NRs and RHRs. The dose-dependent bradycardia accompanying L-NAME-induced increase in arterial pressure may be mediated via the baroreceptor reflex (Yamazaki *et al.*, 1991) or through a neuronal L-arginine: NO pathway (Rees *et al.*, 1990).

In the second part of the study, the role of agonist-evoked EDRF in the regulation of systemic and pulmonary arterial system was investigated. In this study, it was shown that ACh induced a dose-dependent relaxation of isolated thoracic aorta and pulmonary artery, and lowered the mean systemic arterial pressure in vivo in a dose-dependent manner in both RHR and NRs with a slightly weaker effect in RHRs. Unlike its effects on systemic arterial pressure, the depressor effects of ACh on mean pulmonary arterial pressure were similar in RHRs and NRs. It is thought that ACh-induced hypotension is mediated primarily via its effects of EDRF function in resistant vessels rather than in conducting arteries although EDRF-dependent ACh-induced vasodilation was observed in these arteries in vitro, as suggested by Griffith *et al.* (1984), who reported that the activity of endogenous nitric oxide (NO) was greatest in large arterioles in which hydraulic resistance and shear stress were also highest. In both NRs and RHRs, EDRF-dependent ACh-induced depressor effect was significantly smaller

in pulmonary arterial system than in systemic arterial system, probably due to decrease in the ability of endothelium to release EDRF or an inability of smooth muscle to respond to EDRF in pulmonary arterial system. Difference in the EDRF function along the vascular tree was also supported by findings in humans that on the arterial side of the circulation, but not the venous side, there was a continuous release of NO that maintained a dilator tone. Differential depressor effects of ACh on systemic and pulmonary arterial pressure were in line with in vitro studies for NRs, but this was not true for RHRs, due to a decrease in EDRF function in aorta from RHRs. Results from in vitro studies revealed that ACh-induced EDRF-mediated relaxation of thoracic aorta from RHRs were significantly smaller than that for thoracic aorta from NRs unlike the effects of ACh on pulmonary arteries from RHRs and NRs. These results concur with the decreased amplitude of endothelium-dependent relaxation in response to ACh in aortas from RHRs (Van de voorde *et al.*, 1984; 1986) and spontaneously hypertensive rats (Konishi *et al.*, 1983; Lüscher *et al.*, 1986) and deoxycorticosterone acetate-treated rats (Van de Voorde *et al.*, 1984). These findings in line with results from in vivo study that in RHRs EDRF-dependent ACh-induced relaxations may be impaired in the systemic arterial system with high pressure, but not in the pulmonary arterial system with relatively low pressure. This observation indicate that an impairement of endothelium-dependent relaxation in systemic arterial system of RHRs may be due to abnormal function of endothelium arising from high pressure, the mechanism involving a diminished release of EDRF or an impairment of coupling between the endothelium and the smooth muscle (Van be Voorder *et al.*, 1986).

In this study, it was shown that ACh-induced vasorelaxation of thoracic aorta from RHRs and NRs was completely blocked after removal of endothelial cells or pretreatment with inhibitor of EDRF, whereas the effect of ACh on pulmonary artery was completely abolished after removal of the endothelial cells, but only partially inhibited after pretreatment with inhibitors of EDRF, with a substantial size of response remaining. These results indicate some factors other than EDRF may also be involved in ACh-induced vasorelaxation with a regional variation, exclusively in pulmonary artery in our study. In this regard Chen *et al.*, (188) reported that in addition to EDRF the endothelium of isolated rat pulmonary arteries release a nonprostanoid factor contributing to endothelium-dependent relaxation via its hyperpolarizing action on the vascular smooth muscle, thus named of endothelium dervied hyperpolarizing factor (EDHF). It is known that the effect of EDHF is not blocked by inhibitors of EDRF such as hemoglobin and methylene blue, is not mediated by cGMP (Chen *et al.*, 1988), and is mediated mainly via activation of membrane K^+ channels followed by K^+ efflux (Komori *et al.*, 1987), also possibly via the stimulation of Na^+/K^+ APTase (Chen *et al.*, 1988). Our finding that ACh-induced vasorelaxation of pulmonary artery was almost completely abolished after the combined application of L-NAME and high potassium solution (20 mM) although it was partially inhibited after separate treatment with any of them, appears to support the role of EDRF and EDHF in ACh-induced relaxation of pulmonary artery from NRs and RHRs.

In summary, our results indicate that in RHRs the endothelial cell function was impaired, at least in part, in systemic arterial system, but not in pulmonary arterial system, and both ACh-evoked and basal release of EDRF was less in the pulmonary arterial system than in systemic arterial system of both NRs and RHRs. Also, the relaxation induced by ACh on isolated thoracic aorta may be largely caused by EDRF, but on isoated pulmonary artery by both EDRF and EDHF.

REFERENCE

Cangiano J, Rodriguez-Sargent C and Martinez-Maldonado M: *Effects of antihypertensive treatment on systolic blood pressure and renin in experimental hypertension in rats. J Pharmacol Exp Ther 208(2): 310-313, 1979*

Chappel SP, Lewis MJ and Henderson AH: *Effect of lipid feeding on endothelium-dependnt relaxation in rabbit aortic preparations. Cardiovasc Res 21, 34-38, 1987*

Chen G, Suzuki H and Weston AH: *Acetylcholine release endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. Br J Pharmacol 95: 1165-1174,*

1988

Ferrario CM: *The renin-angiotensin system: Importance in physiology and pathology. J Cardiovasc Pharmacol 15 (Suppl. 3): S1-S5, 1990*

Fekete A, Forgacs I, Gaal K and Meszaros T: *Renin activity of renal venous blood in experimental hypertension induced by ligation of one renal artery in dogs. Acta medica Academiae Scientiarum Hungaricae 28(2): 181-196, 1971*

Fernandes M, Onesti G, Fiorentini R, Bellini G, Gould AB, Kim KE and Swartz C: *Role of adrenergic innervation in experimental renal hypertension. Life Sci 20: 623-626, 1977*

Fernandes M, Onesti G, Weder A, Dykyj R, Gould AB, Kim KE and Swartz C: *Experimental model of severe renal hypertension. J Lab Clin Med 87(4): 561-567, 1976*

Gabbiani G, Elmer G, Guelpa CH, Vallotton MB, Badonnel MC and Huttner I: *Morphologic and functional changes of the aortic intima during experimental hypertension. Am J Pathol 96: 399-422, 1979*

Griffith TM, Edwards DH, Lewis MJ, Newby AC and Henderson AH: *The nature of endothelium-derived vascular factor. Nature 308: 465-467, 1984*

Gross F: *The renin-angiotensin system and hypertension. Ann Int Med 75: 777-787, 1971*

Haber E, Koerner T, Page LB, Kliman B and Purnode A: *Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. J Clin Endocr 29: 1349-1355, 1969*

Haudenschild CC, Prescott MF and Chobanian AV: *Effects of hypertension and its reversal on aortic intimal lesions of the rat. Hypertension 2: 33-44, 1980*

Komori K and Suzuki H: *Heterogenous distribution of muscarinic receptors in the rabbit saphenous artery. Br J Pharmacol 92: 657-664, 1980*

Lüscher TF: *Endothelium-derived relaxing and contracting factors: potential role in coronary artery disease. Eur Heart J 10: 847-857, 1989*

Lüscher TF and Vanhoute PM: *Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. Hypertension 8: 344-348, 1986*

Oyama Y, Kawasaki H, Hattori Y and Kanno M: *Attenuation of endothelium-dependent relaxation in aorta from diabetic rats. Eur J Pharmacol 131: 75-78, 1986*

Panza JA, Quyyumi AA, Brush JE and Epstein SE: *Abnormal endothelium-dependent vasculature relaxation in patients with essential hypertension. New Engl J Med 323: 22-27, 1990*

Rees DD, Palmer RMJ and Moncada S: *Role of endothelium-derived nitric oxide in the regulation of blood pressure. Proc Natl Acad Sci USA 86: 3375-3378, 1989*

Rees DD, Palmer RMJ, Schultz R, Hodson, HF and Moncada S: *Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br J Pharmacol 101: 746-752, 1990*

Timmermans PBMWM, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RT, Wexler RR, Saye JM and Smith RD: *Angiotensin II receptors and angiotensin II receptor antagonists. Pharmacological Reviews 45(2): 205-251, 1993*

Timmersmans PBMWM, Wong PC, Chiu AT and Herblin WF: *Nonpeptide angiotensin II receptor antagonists. Tips 12: 55-62, 1991*

Van de Voorde J and Leusen I: *Endothelium-dependent and independent relaxation effects on aorta preparations of renal hypertensive rats. Arch Int Physiol Biochim 92(4): 35-36, 1984*

Van de Voorde J and Leusen I: *Endothelium-dependent and independent relaxation of aortic rings from hypertensive rats. Am J Physiol 250: H711-H717, 1986*

Wada T, Inada Y, Shibouta Y, Ojima M, Kubo K, Kohara Y, Naka T and Nishikawa K: *Antihypertensive action of a nonpeptide angiotensin II (A II) antagonist, TCV-116, in various hypertensive rats. J Hypertens 10(Suppl 4): S114, 1992*

Whittle BJR, Lopez-Belmonte J and Rees DD: *Modulation of the vasodepressor actions of acetylcholine, bradykinin, substance P and endothelin in the rat by a specific inhibitor of nitric oxide formation. Br J Pharmacol 98: 646-652, 1989*

Wong PC, Hart SD, Chiu AT, Herblin WF, Carini DJ, Smith RD, Wexler RR and Timmersmans PBMWM: *Pharmacology of DuP 532, a selective and noncompetitive AT1 receptor antagonist. J Pharmacol Exp Ther 259(2): 861-870, 1991*

Yamazaki J, Fujita N and Nagao T: *NG-Monomethyl-L-arginine-induced pressor response at developmental and established stages in spontaneously hypertensive rats. J Pharamcol Exp Ther 259(1): 52-57, 1991*

=국문요약=

신성 고혈압쥐의 전신성 동맥계와 폐동맥계에 대한 EDRF 기능의 차이

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급성 신성 고혈압쥐 (2-kidney, 1-ligation type)의 전신성 동맥계와 폐 동맥계에 대한 내피의존적 혈관반응성을 규명하기 위하여, 적출 혈관 및 마취상태의 흰쥐에 대한 acetylcholine (ACh)의 혈관이완작용 및 혈압강하 작용을 측정하였다.

혈장 renin 활성(PRA)은 신동맥 결찰전 7.31±0.63 ng/ml/hr A I에 비해 결찰 6~8일후에는 19~22 ng/ml/hr A I으로 유의성있게 증가하였으며, 이는 수축기혈압의 상승과 (154±1.83 →190~215 mmHg) 일정한 상관성을 유지하였다. 신성 고혈압쥐 및 정상 혈압쥐의 흉곽 대동맥은 내피세포 존재시 ACh에 의해 용량의존적으로 이완되었으며, 이때 신성고혈압쥐에서의 반응은 정상 혈압쥐에 비해 유의성있게 감소하였다(각각 34% 및 86%, $p<0.01$). 또한 ACh은 신성 고혈압쥐 및 정상 혈압쥐의 폐동맥에 대해서도 내피세포 존재시에 이완반응을 초래하였다. 그러나, 흉곽 대동맥에서와는 달리 두 군간에 유의성있는 차이가 없었다. 이들 반응은 내피세포 제거후 또는 EDRF 억제제(L-NAME, MB, 10^{-5} M) 투여후 유의성있게 억제되었다. ACh(0.1~10 μg/kg, i.v.)은 신성 고혈압쥐 및 정상 혈압쥐에서 전신성 동맥압의 강하를 초래하였는데, 신성 고혈압쥐에서 다소 감소하였으나 유의성있는 차이는 없었으며(SAPm; 10 μg/kg에서 각각 39%, 46%), 이들 작용은 L-NAME(30 mg/kg, i.v.) 전처치후 유의성있게 억제되었다. ACh에 의한 폐동맥압 강하는 신성 고혈압쥐 및 정상 혈압쥐 사이에 서로 비슷하게 나타났다. 그러나, 신성 고혈압쥐 및 정상 혈압쥐에서 ACh에 의한 폐동맥압의 강하율은 전신성 동맥압의 강하율보다 유의성있게($p<0.01$) 작았으며, 또한 L-NAME (0.1~100 mg/kg, i.v.)에 의한 폐동맥압의 상승은 전신성 동맥압의 상승보다 유의성있게($p<0.01$) 작았다.

이상의 실험 결과들은 급성 신성 고혈압쥐의 전신성 동맥계에서는 내피세포 손상이 초래되지만, 폐동맥계에서는 초래되지 않는다는것을 제시해준다. 또 신성고혈압쥐 및 정상 혈압쥐에서 EDRF의 basal release 및 ACh 유발성 EDRF function은 전신성 동맥계에 비해 폐동맥계에서 적다는 것을 제시해준다.