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Endogenous asymmetrical dimethylarginine and hypertension associated with puromycin nephrosis in the rat

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1 The present experiments were designed to investigate the role of asymmetrical N^G,N^G-dimethyl-L-arginine (ADMA) in causing hypertension associated with the focal and segmental glomerulosclerosis (FSGS) produced by a single bolus of puromycin aminonucleoside (PAN) and successive injection of protamine for 7 days in rats which had undergone unilateral nephrectomy.

2 After the unilateral nephrectomy, and administering PAN and protamine, histological examinations of the kidney revealed a typical FSGS, that is, evident abnormalities including segmental mesangial proliferation, obliteration of glomerular capillary lumens and adhesions between the glomerulus and Bowman's capsule could be observed. Changes in the glomerular epithelial cells consisted of the swelling with bleb formation.

3 In the FSGS rats, urine volume and urinary protein were significantly ($P<0.05$ and $P<0.005$) increased throughout 4-week experimental period, while the creatinine clearance was significantly ($P<0.005$) and transiently decreased, and recovered 4 weeks later. These changes were associated with the sustained elevation of the systolic blood pressure.

4 ADMA levels in aortic endothelial cells, plasma and urine were significantly ($P<0.05$ and $P<0.005$) increased in the FSGS rats, but the level in the kidney remained unchanged.

5 The basal level and net production of cyclic GMP in the aortic vessel wall with endothelium when stimulated by norepinephrine and acetylcholine were significantly ($P<0.05$ and $P<0.01$) attenuated in the FSGS rats.

6 There were significant and positive correlations between systolic blood pressure (y) and ADMA levels (x) in endothelial cells ($y=4.43x+122.2$, $r=0.979$, $P<0.0001$), plasma ($y=0.10x+71.9$, $r=0.921$, $P<0.001$) and urine ($y=0.48x+126.9$, $r=0.699$, $P<0.005$), but not significant in the kidney ($y=0.06x+102.7$, $r=0.252$, NS).

7 These findings suggest that ADMA as an endogenous inhibitor of NO synthesis may play an important role for the pathogenesis in the hypertension associated with the experimental FSGS in the rat.

Keywords: ADMA; endogenous NOS inhibitor; FSGS; hypertension; puromycin

Introduction

Experimental evidence had demonstrated that the L-arginine-NO pathway plays an important role for the maintenance of peripheral vascular tone. In humans, the infusion of N^G-monomethyl-L-arginine (L-NMMA) into the brachial artery significantly reduced forearm blood flow (Vallance *et al*., 1989). Recently, it has been reported that ADMA plays an important role for the pathogenesis of salt-sensitive hypertension (Matsuoka *et al*., 1997). In addition, Vallance *et al*. (1992) have reported that accumulation of endogenous ADMA might contribute to the hypertension associated with chronic renal failure in human. However, details causing hypertension associated with renal impairment remain still obscure.

Focal and segmental glomerulosclerosis (FSGS) is believed to represent a pathologic pattern of a number of clinical renal disorders (Baldwin, 1982; Brenner *et al*., 1982). Laboratory models also exist with glomerular abnormalities that are very similar to the human pathologic process. The administration of puromycin aminonucleoside (PAN) in unilaterally nephrectomized rats provides a well-characterized model of FSGS and interstitial fibrosis that results in the development of massive proteinuria and renal failure (Glasser *et al*., 1977; Velosa *et al*., 1977; Grond *et al*., 1984; Ebihara *et al*., 1993; Nakamura *et al*., 1993). On the basis of these findings, the present experiments were designed to investigate the role of ADMA in the pathogenesis of hypertension associated with the experimental FSGS in rats.

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Methods

Preparation of the FSGS model

Male Sprague-Dawley rats, 10 weeks of age, were used. These rats were purchased at 6 weeks of age and housed in a temperature ($23\pm1^{\circ}$C)- and humidity (50 ± 20%)-controlled room and were fed regular chow (CLEA Rodent Diet: CE-2, Japan CLEA, Tokyo, Japan) throughout the experimental periods. Systolic blood pressure and body weight were determined twice a week and once a week, respectively, from 2 weeks before to 4 weeks after starting the experiments. Animals were randomly divided into three groups of A, B and C, each consisting of 15 rats. Under ether anaesthesia and semisterile conditions, animals of groups B and C were subjected to the right unilateral

nephrectomy *via* a midline incision of abdomen. Glasser *et al*., (1977) demonstrated that the unilateral nephrectomy augmented proteinuria and markedly accelerated the development of FSGS in rats given PAN. On the day of nephrectomy, animals received either the injection of 0.4 ml of 0.9% saline 100 g^{-1} body weight (group B) or puromycin aminonucleoside (5 mg in 0.9% saline 100 g^{-1} body weight) (PAN; Wako Pure Chemicals, Tokyo, Japan) (group C) *via* the caudal vein (Week 0) according to Diamond & Karnovski (1986) who demonstrated that one-third of the usual dosage of PAN, when injected as a single intravenous bolus, resulted in the glomerular injury consistent with FSGS. Coincidentally with the intravenous PAN injection, rats in group C received intravenous daily dose of protamine sulphate (4.5 mg 0.1 ml^{-1} 100 g^{-1} body weight) (Grade II from salmon, Sigma, St. Louis, MO, U.S.A.) for 7 days according to Saito *et al*., (1987) who have demonstrated that PAN-induced nephrosis is enhanced by the co-administration of protamine sulphate. Group A served as control without any operation and agent. Systolic blood pressure was determined in warmed conscious rats using the indirect tail cuff method (Programmable Sphygmomanometer PS-100, Riken Kaihatsu, Tokyo, Japan). One, 2 and 4 weeks after the nephrectomy, urine volume, urinary protein excretion and creatinine clearance were determined from an overnight collection of urine using metabolic cages. During the collection period, rats were given free access to water and food. Blood samples were withdrawn *via* the jugular vein under ether anaesthesia. At week 1, 2 and 4, five rats each were sacrificed under ether anaesthesia. The left kidney was removed. The upper half of the left kidney was fixed in a 10% neutral solution of formaldehyde for histological examinations. Remaining lower half was frozen for the determinations of ADMA, DNA and protein levels. This study complied with the Animal Welfare Regulations of Tokyo Medical and Dental University and the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society.

Determination of ADMA and SDMA

In order to determine the ADMA and symmetrical N^G,N'^G-dimethyl-L-arginine (SDMA) concentrations in endothelial cells and cyclic GMP production by the aortic vessel wall, additional 126 rats were divided into three groups corresponding to groups A (normal control without any treatment), B (uninephrectomized) and C which had undergone the unilateral nephrectomy and received PAN and protamine, each consisting of 42 rats. Twenty one rats from each group were sacrificed under ether anaesthesia at week 1 and 4. Thoracic aorta was rapidly excised and kept in the cold Krebs solution. After removal of the fat and connective tissue, endothelial cells from seven rats each were collected from the luminal surface according to the method described previously (Azuma *et al*., 1995) and pooled for processing the determination of ADMA and SDMA, which was performed by means of automated high-performance liquid chromatography (HPLC) according to the method described previously (Azuma *et al*., 1995; 1997; Hamasaki *et al*., 1997). Three determinations for each group were performed.

Determination of cyclic GMP

Ring preparations weighing approximately 8 mg with intact endothelium were cut off with razor blade from the thoracic aorta of each rat in groups A, B and C. The cyclic GMP level was determined according to the method described by Honma *et al*., (1977) and Rapoport & Murad (1983) with minor modifications. The preparations which had been processed without damaging luminal surface were preincubated in the modified Krebs solution for 5 min at 37°C, transferred into the fresh Krebs solution and followed by further 30 min-incubation until preparations were rapidly transferred into 10% trichloroacetic acid (TCA) with liquid nitrogen in order to stop the reaction. 10^{-5}M Norepinephrine and 10^{-5}M acetylcholine were added immediately and 15 min after transferring the preparations into the Krebs solution, respectively. All experiments were performed in the presence of 10^{-4}M 3-isobutyl-1-methylxanthine (IBMX) as a non-selective phosphodiesterase inhibitor. The net production of cyclic GMP was expressed as the difference between the production with 10^{-5}M norepinephrine plus 10^{-5}M acetylcholine and that with 10^{-5}M norepinephrine plus 10^{-5}M acetylcholine plus 10^{-4}M N^G-nitro-L-arginine as an inhibitor of nitric oxide synthase (Kobayashi & Hattori, 1990). The basal level was given as the value without any agonist and antagonist except for IBMX.

Determinations of DNA and protein

The microassay of DNA was performed according to the fluorometric method described by Kissane & Robins (1958). Protein concentration was measured with bovine serum albumin as a standard by the method of Lowry *et al*., (1951).

Histological examinations

The kidney specimens were embedded in paraffin after dehydration with ethanol. Thin sections were stained with hematoxylin eosin (HE) or periodic acid-Schiff (PAS) reagent for light microscopic analysis.

Statistical analysis

Results are given as mean $\pm$ s.e.m. The statistical analysis was carried out by a two-way analysis of variance (ANOVA). If the mean values were found to be different at $P<0.05$ by ANOVA, Student's *t*-test was also applied. Correlation was examined by Pearson's correlation coefficient (*r*) and Fischer's *z*-translation (analysis of the statistical significance). Regression equation was obtained by the least square method.

Results

Body weight change

The mean body weight was determined to be 318.3 ± 3.4 g for group A ($n=15$), 329.7 ± 6.8 g for group B ($n=15$) and 331.1 ± 6.6 g for group C ($n=15$) at week 0. These values were not significantly different from each other. Similar time course of the body weight change was observed in group A (normal control without any treatment) and group B which underwent unilateral nephrectomy but did not receive PAN and protamine. One, 2 and 3 weeks after the unilateral nephrectomy, and the administration of PAN and protamine, the body weight gain was significantly ($P<0.05$ and $P<0.005$) reduced in group C *vs* the corresponding value in groups A and B. However, there was no significant difference among three groups at week 4.

Changes in urine volume, urinary protein and creatinine clearance

Urine volume (ml 24 h^{-1}), urinary protein (mg 24 h^{-1}) and creatinine clearance (ml min^{-1} kg^{-1} body weight) were compared among three groups of A, B and C. There were no significant differences in the urine volume and urinary protein excretion between groups A and B (Figure 1, I and II). Creatinine clearance in group B tended to be decreased at week 1 and 4 or significantly ($P<0.05$) decreased at week 2 *vs* corresponding value in group A (Figure 1, III). Urine volume and urinary protein in group C were, however, significantly ($P<0.05$ and $P<0.005$) increased as compared to those values in group A or partially in group B throughout the experimental periods (Figure 1, I and II). In addition, the creatinine clearance in group C was significantly ($P<0.005$) lower than that in group A at week 1 and 2, and recovered to the control level 4 weeks later (Figure 1, III). The value in group C at week 4 was significantly ($P<0.005$) higher than the corresponding value in group B.

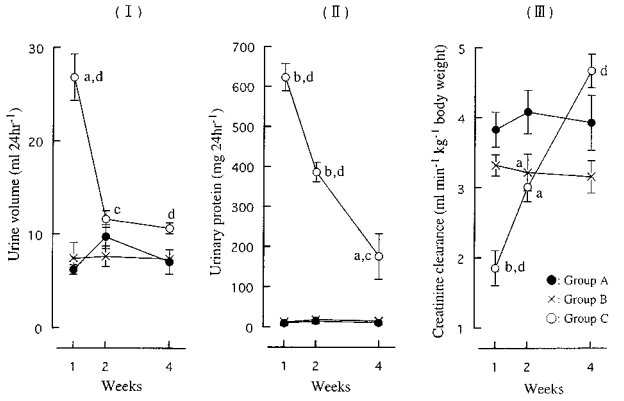

Figure 1 Changes in urine volume (I), urinary protein (II) and creatinine clearance (III) of groups A, B and C. Each point represents the mean value of five rats. [a]and [b]:Significant difference *vs* corresponding value in group A at $P<0.05$ and $P<0.005$, respectively. [c]and [d]:Significant difference *vs*. corresponding value in group B at $P<0.05$ and $P<0.005$, respectively. Vertical bars show s.e.m.

Histological findings

Figure 2 demonstrates histological changes 4 weeks after the unilateral nephrectomy, and the administration of PAN and protamine (group C). Evident abnormalities including segmental mesangial proliferation, obliteration of glomerular capillary lumens, and adhesions between the glomerulus and Bowman's capsule could be observed. Changes in the glomerular epithelial cells consisted of the swelling with bleb formation. Cellular crescent formation could be observed in the glomerulus. There were focal atrophy and a few proteinous cast formation in the uriniferous tubules, and diffuse but slight infiltration of small mononuclear cells in the interstitium. No abnormalities were observed in the kidneys of groups A and B.

Protein and DNA levels in the kidney

The protein and DNA levels (mg g^{-1} wet weight) in the kidney were compared among the three groups. Results are shown in Table 1. Both of the protein and DNA levels in group C and the protein level in group B were significantly increased as compared to the corresponding values in group A, suggesting the renal hypertrophy in group B, and the renal hypertrophy and increased mesangial proliferation in group C after the unilateral nephrectomy, and the administration of PAN and protamine.

Table 1 Protein and DNA levels in the kidney at week 4

Group	*Protein* (mg g^{-1} wet weight)	*DNA* (mg g^{-1} wet weight)
A	75.5 ± 0.7	2.26 ± 0.33
B	91.9 ± 0.8[a]	2.31 ± 0.18
C	98.7 ± 2.2[a]	3.55 ± 0.26[b]

Results are given as mean$\pm$s.e.m. of three determinations. [a] and [b]:Significant difference *vs* corresponding value in group A at $P<0.005$ and $P<0.05$, respectively.

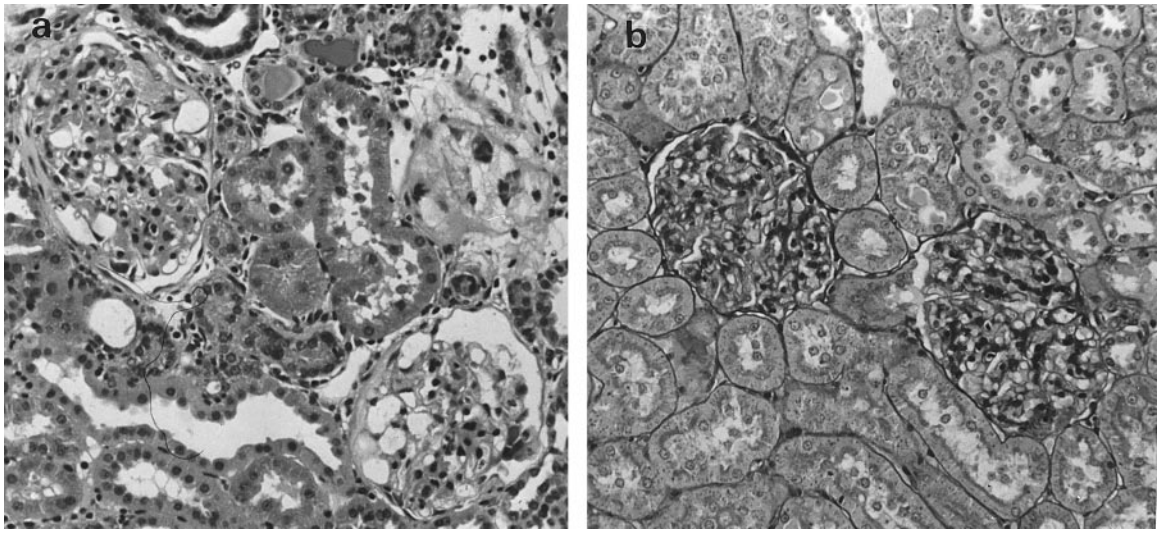

Figure 2 Representative histological findings in the kidney 4 weeks after unilateral nephrectomy, and administration of puromycin and protamine. (a) Evident abnormalities including segmental mesangial proliferation, obliteration of glomerular capillary lumens, and adhesions between the glomerulus and Bowman's capsule could be observed. PAS staining (original magnification $\times 50$). (b) Normal appearance.

Changes in systolic blood pressure

The systolic blood pressure was determined twice a week. The values in groups A and B were ranged between 124.9 ± 2.9 and 130.7 ± 1.3 mmHg and were not different from each other throughout the experimental periods. In group C which had undergone the unilateral nephrectomy and received PAN and protamine, significant ($P<0.005$ *vs* groups A and B) elevation of the systolic blood pressure was noted during the periods after the treatments. The blood pressure in group C was determined to be 130.6 ± 0.8 ($n=15$), 136.8 ± 0.8 ($n=15$), 147.0 ± 1.5 ($n=10$), 154.8 ± 3.3 ($n=5$) and 153.2 ± 4.5 ($n=5$) mmHg ($n=5$) at week 0, 1, 2, 3 and 4, respectively. These results are shown in Figure 3.

ADMA levels in endothelial cells, plasma, urine and kidney, SDMA level in endothelial cells, and ADMA clearance

The ADMA and symmetrical N^G, N'^G,-dimethyl-L-arginine (SDMA) concentrations in endothelial cells was calculated using the method described previously (Hamasaki *et al*., 1997) and compared between groups A, B and C (Table 2). In groups A and B, the ADMA concentration was ranged between 1.1×10^{-6} to 1.6×10^{-6}M at weeks 1 and 4, respectively. The ADMA concentration was significantly ($P<0.01$ and $P<0.005$) higher in group C than the corresponding values in groups A and B, and was determined as $(8.2 \pm 0.7) \times 10^{-6}$ M in group C at week 4. On the other hand, SDMA concentration in endothelial cells at weeks 1 and 4 remained unaltered in all groups (Table 2).

As shown in Figure 4, I–III, ADMA levels in plasma (pmoles ml^{-1}) and urine (nmoles $24\ h^{-1}$ urine) of group C were significantly higher *vs* corresponding values in groups A and B. The levels in groups A and B were not different from each other except for the plasma ADMA in group B at week 2, which was significantly different from corresponding value in group A. In addition, ADMA levels (pmoles mg^{-1} protein) in the kidney were not different from each other among three groups.

ADMA clearance was compared among groups A, B and C. The values in groups A and B were ranged between $(1.94 \pm 0.59) \times 10^{-2}$ ($n=4$) to $(2.72 \pm 0.70) \times 10^{-2}$ ml min^{-1} kg^{-1} body weight at weeks 1, 2 and 4, and were not significantly different from each other. In contrast, a significant ($P<0.05$ and $P<0.005$) increase in the ADMA clearance was observed in group C. The values at weeks 1, 2 and 4, were determined to be $(5.41 \pm 0.99) \times 10^{-2}$ ($n=5$), $(16.29 \pm 2.89) \times 10^{-2}$ ($n=5$) and $(7.38 \pm 2.69) \times 10^{-2}$ ml min^{-1} kg^{-1} body weight ($n=4$), respectively.

Cyclic GMP production by the aortic vessel wall

The basal level and net production of cyclic GMP (pmole mg^{-1} protein) were compared among three groups. Not only basal level but also net production in groups A and B were not significantly different and remained unchanged at weeks 1 and 4. By dosing PAN and protamine in unilaterally nephrectomized rats (group C), the basal level and net production of the nucleotide were significantly ($P<0.05$ and $P<0.01$) attenuated in group C at weeks 1 and 4 *vs* corresponding values in groups A and/or B. These results are shown in Figure 5.

Relationship between systolic blood pressure and ADMA levels

Figure 6, I–IV shows the relationships between systolic blood pressure (y) and ADMA levels (x) in aortic endothelial cells,

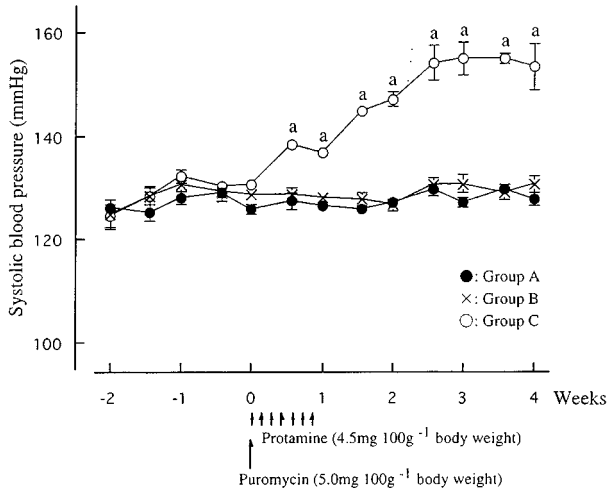

Figure 3 Changes in systolic blood pressure of groups A, B and C. Each point represents the mean value of 15 rats at ~ week 1, 10 rats at ~ week 2 and five rats at ~ week 4. [a]:Significant difference *vs* corresponding values in groups A and B at $P<0.005$.

Table 2 Changes in ADMA and SDMA concentrations in the aortic endothelial cells

	ADMA ($\times 10^{-6}$ M)		*SDMA* ($\times 10^{-7}$ M)	
Group	*Week 1*	*Week 4*	*Week 1*	*Week 4*
A	1.1 ± 0.1	1.5 ± 0.1	1.3 ± 0.4	1.7 ± 0.5
B	1.3 ± 0.1	1.6 ± 0.2	1.2 ± 0.4	1.4 ± 0.2
C	2.0 ± 0.1^{a}	$8.2 \pm 0.7^{b,c}$	1.5 ± 0.3	1.8 ± 0.4

Results are given as mean ± s.e.m. of three determinations. Each determination consists of seven rats (see text). [a] and [b]:Significant difference *vs* corresponding value in group A at $P<0.01$ and $P<0.005$, respectively. [c]Significant difference at $P<0.005$ *vs* corresponding value at week 1. ADMA: Asymmetrical N^G, N^G-dimethyl-L-arginine, SDMA: Symmetrical N^G, N'^G-dimethyl-L-arginine.

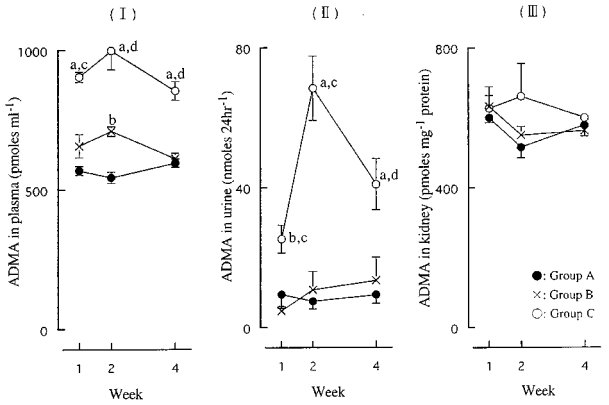

Figure 4 Asymmetrical N^G,N^G-dimethyl-L-arginine (ADMA) levels in plasma (I), urine (II) and kidney (III) of groups A, B and C. Each point represents the mean value of five rats. [a]and [b]:Significant difference *vs* corresponding value in group A at $P<0.005$ and $P<0.05$, respectively. [c]and [d]:Significant difference *vs* corresponding value in group B at $P<0.005$ and $P<0.05$, respectively. Vertical bars show s.e.m.

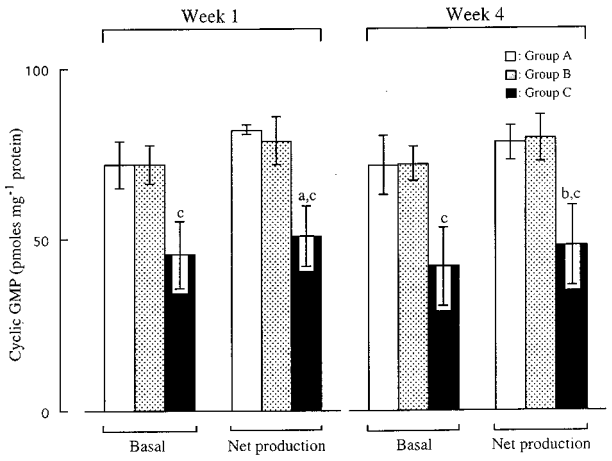

Figure 5 Basal and net production of cyclic GMP in the vessel wall with endothelium of groups A, B and C at week 1 and 4. Results are given as mean of four to five determinations. Vertical bars show s.e.m. [a]and [b]:Significant difference *vs* corresponding value at $P<0.01$ and $P<0.05$, respectively *vs* corresponding value in group A. [c]Significant difference at $P<0.05$ *vs* corresponding value in group B. All experiments were performed in the presence of 10^{-5} M 3-isobutyl-1-methylxanthine (IBMX) as a non-selective phosphodiesterase inhibitor. The net production of cyclic GMP was expressed as the difference between the production with 10^{-5} M norepinephrine plus 10^{-5} M acetylcholine and that with 10^{-5} M norepinephrine plus 10^{-5} M acetylcholine plus 10^{-4} M N^G-nitro-L-arginine as an inhibitor of nitric oxide synthase (Kobayashi & Hattori, 1990). The basal level was given as the value without any agonist and antagonist except for IBMX (see text).

plasma, urine and kidney at week 4. There were positive and significant correlations between systolic blood pressure and ADMA levels in endothelial cells ($y=4.43x+122.2$, $r=0.979$, $P<0.0001$), plasma ($y=0.10x+71.9$, $r=0.921$, $P<0.001$) and urine ($y=0.48x+126.9$, $r=0.699$, $P<0.005$). However, the relations were not significant in the kidney ($y=0.06x+102.7$, $r=0.252$, NS).

Discussion

Focal and segmental glomerulosclerosis (FSGS) is believed to represent a pathologic pattern of a number of clinical renal disorders (Baldwin, 1982; Brenner *et al*., 1982). In the present experiments, the administration of PAN and protamine in unilaterally nephrectomized rats provided typical FSGS that resulted in the development of massive proteinuria and renal failure. Similar results were reported by other investigators (Glasser *et al*., 1977; Velosa *et al*., 1977; Grond *et al*., 1984; Ebihara *et al*., 1993; Nakamura *et al*., 1993).

On the basis of these findings, the role of asymmetrical N^G, N^G-dimethyl-L-arginine (ADMA) in the pathogenesis of hypertension associated with the experimental FSGS was investigated. ADMA levels in aortic endothelial cells, plasma and urine were significantly increased in FSGS rats, but the level in the kidney remained unchanged. Experimental evidence has demonstrated that NO plays an important role in the maintenance of peripheral vascular tone. Selective inhibitors of NO synthase (NOS) such as N^G-monomethyl-L-arginine (L-NMMA) and ADMA can inhibit NO production (Palmer *et al*., 1988; Gross *et al*., 1990; Vallance *et al*., 1992). These inhibitors have equally potent vasoconstrictor and pressor actions (MacAllister & Vallance, 1994). In addition, the acute administration of L-NMMA produced an increase in the arterial blood pressure in guinea-pigs (Aisaka *et al*., 1989), rabbits (Rees *et al*., 1989), dogs (Chu *et al*., 1990) and rats (Whittle *et al*., 1989; Tolins *et al*., 1990; Hecker *et al*., 1990); these were mostly due to an increase in total peripheral resistance (Gardiner *et al*., 1990). Recently, Matsuoka *et al*., (1997) have provided evidence indicating that urinary ADMA excretion is significantly correlated with arterial pressure in Dahl salt-sensitive hypertensive rats and suggested the role of ADMA as an endogenous NOS inhibitor in the pathogenesis of salt-sensitive hypertension. In the present experiments, it was demonstrated that there were significant and positive correlations between systolic blood pressure and ADMA levels in endothelial cells, plasma and urine, although it may not be really legitimate to draw a correlation curve. These correlations may be important for understanding the pathogenesis of hypertension associated with FSGS.

The ADMA concentration in endothelial cells was determined to be 8.2 μM 4 weeks after PAN and protamine, of which concentration may at least partially inhibit NO synthesis, since the median inhibitory concentration (IC_{50}) of ADMA for the cyclic GMP production was 18.5 μM, and the inhibition in a concentration of 8.2 μM was calculated to be 40% in the rabbit carotid artery strips with intact endothelium (Azuma: unpublished observation). In addition, the present experiments revealed that the net production of cyclic GMP in the aortic strips from FSGS rats was significantly ($P<0.05$) decreased (61.6% of the control), which would reflect the decreased NO biosynthesis and would be brought about by the accumulated ADMA in endothelial cells. It has been reported that native NO binds the heme group of soluble guanylate cyclase (Ignarro, 1990a), stimulating the production of cyclic GMP. Consequently, several groups of investigators have used the generation of cyclic GMP as an index of NO biosynthesis (Moncada *et al*., 1988; Ignarro, 1990b; Lüscher *et al*., 1990). Considering that endothelial cells are the major producers of NO in virtually all vascular territories, it would be logical to assume that the decreased NO production in endothelial cells resulting from the increased concentration of ADMA contributes to the increase in total peripheral resistance and to causing hypertension.

Mechanisms increasing ADMA levels in endothelial cells are not clarified in the present experiment, several possibilities are, however, considered. According to Bogle *et al*., (1995), transmembrane transport of the endogenous methylarginines might alter their concentrations in endothelial cells. Therefore, it seems possible to assume that the administration of PAN and protamine may result in an increase in the incorporation of ADMA by endothelial cells, which, in turn, increases the intracellular concentration of the methylarginine and inhibits the NO biosynthesis. This speculation may be supported by the findings that the ADMA concentration in endothelial cells was approximately 10 fold higher than that in the plasma 4 weeks after PAN and protamine (Figure 4, I and Table 2). However, further experiments will be necessary to confirm this hypothesis. Dimethylarginine dimethylaminohydrolase (DDAH) as an enzyme that metabolizes ADMA is localized in endothelial cells and blood vessels (MacAllister *et al*., 1996). The administration of PAN and protamine may inhibit the enzyme activity, resulting in a high level of ADMA in endothelial cells. This speculation may be supported in part by the findings that the endothelial concentration of symmetrical N^G, N'^G,-dimethyl-L-arginine (SDMA), which is not a substrate for DDAH (Ogawa *et al*., 1989), remained unchanged in all groups examined in the present experiments. In addition to the inhibition of DDAH, measurements of the increased biosynthesis of ADMA in endothelial

cells (Fickling *et al*., 1993) would be also helpful to reveal the mechanism increasing the inhibitor level. Details remain to be investigated.

Although the metabolic pathway of ADMA under the physiological conditions has been extensively investigated (Ogawa *et al*., 1987; 1989; Vallance *et al*., 1992; Fickling *et al*., 1993; Kimoto *et al*., 1993; MacAllister Vallance, 1994; MacAllister *et al*., 1994), we have little information regarding the altered metabolism in the pathological conditions (Yu *et al*., 1994; Azuma *et al*., 1995; Bode-Böger *et al*., 1996). ADMA is produced in several organs, released into blood and excreted from the kidney (Kakimoto & Akazawa, 1970; Ogawa *et al*., 1989; Kimoto *et al*., 1993). However, the plasma level and urinary excretion are mainly determined by the renal production and excretory capacity of ADMA (Ogawa *et al*., 1989; Vallance *et al*., 1992). Thus, it seems possible to assume that the increased plasma ADMA is due to the augmented renal functions to produce and that the increased urinary ADMA results from the increased renal excretion of ADMA in FSGS rats, in which ADMA clearance was significantly increased even when creatinine clearance was decreased. However, the detailed reason why the decreased creatinine clearance was accompanied by the increased ADMA clearance remains unclarified in the present experiments. Since the ADMA level in the FSGS rat kidney was not increased, the increased renal production of ADMA may not be reflected on the increase in tissue level of the inhibitor.

In conclusion, our results suggest that ADMA may play a role for the pathogenesis of hypertension associated with

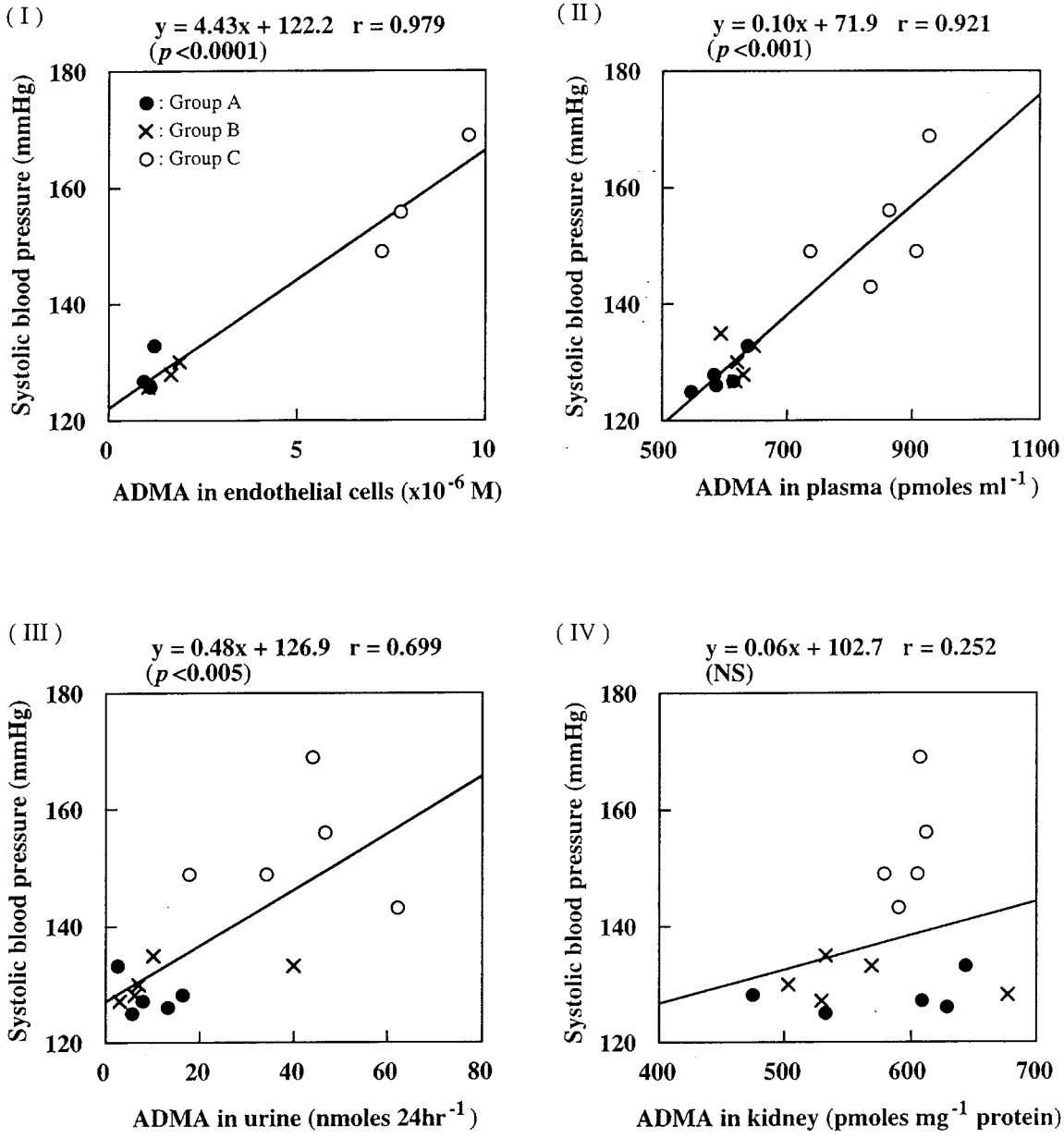

Figure 6 Relationships between systolic blood pressure and asymmetrical N^G, N^G-dimethyl-L-arginine (ADMA) levels in endothelial cells (I), plasma (II), urine (III) and kidney (IV) at week 4. Correlation was examined by Pearson's correlation coefficient (r) and Fischer's z-translation (analysis of the statistical significance). Regression equation was obtained by least square method. In (I), 21 rats from each group were sacrificed under ether anaesthesia at week 4. Endothelial cells of the thoracic aorta from seven rats each were collected from the luminal surface and pooled for processing the determination of ADMA. Three determinations for each group were performed. Systolic blood pressure of seven rats was averaged and demonstrated as three different points for each group in (I) (see text).

FSGS and that the increase in ADMA levels in endothelial cells, plasma and urine is not likely to be a secondary phenomenon in response to blood pressure elevation, since SHR had lower urinary ADMA excretion than WKY (Matsuoka *et al*., 1997). However, a further assessment of the hypertensinogenic significance of ADMA requires informations on the feasibility of chronically sustaining the elevation of blood pressure against the action of other compensatory mechanism.

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