**Original Article**

**Decreased Expression of Arginase II in the Kidneys of Dahl Salt-Sensitive Rats**

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Arginase catalyzes the hydrolysis of arginine to urea and ornithine. Urea is not only an important solute for concentrating urine but also inhibits Na-K-2Cl cotransport. To elucidate the roles of arginase in the development of salt-sensitive hypertension, we examined arginase activity and expression in the kidney and other organs of Dahl/Rapp salt-sensitive (SS) and salt-resistant (SR) rats before and after 4 weeks' administration of a 4% NaCl or control diet. At 4 weeks of age, arginase activity in the kidney was lower in SS rats than in SR rats. Kidney arginase activity was lower in SS rats than in SR rats at 8 weeks of age, and salt loading did not alter arginase activity. Arginase II (the dominant isoform in the kidney) mRNA and protein in the kidney of salt-loaded SS rats were also lower than those of salt-loaded SR rats. Arginase activities in the liver and cerebellum did not differ between SS and SR rats. To examine the effect of urea, the product of arginase reaction, on the development of hypertension, SS rats were given a 4% NaCl diet containing 5% kaolin or 5% urea. Six-week urea supplementation attenuated the development of hypertension in SS rats. These findings suggest that decreased arginase expression in the kidney may be at least partially responsible for the salt-sensitive hypertension in SS rats. (*Hypertens Res* 2002; 25: 411–418)

**Key Words:** arginase, Dahl rat, salt-sensitive hypertension, kidney, urea

**Introduction**

Arginase, which catalyzes L-arginine to urea and L-ornithine, is an important part of the urea cycle (for review, see Jenkinson et al. (1)). Arginase exists in two isoforms. Arginase I is a hepatic cytosolic form, and arginase II is an extrahepatic mitochondrial form (1, 2). The major site of urea synthesis is the liver, in which a complete urea cycle consists of ornithine carbamoyltranserase, argininosuccinate synthetase, argininosuccinate lyase, arginase I, and carbamoyl phosphate synthase (3, 4). Several organs in addition to the liver have an incomplete urea cycle. In the kidney, argininosuccinate synthetase and argininosuccinate lyase are present in the early proximal convoluted tubules, and arginase II exists in the cortical and to a greater extent in the outer medullary portions of the straight proximal tubules (5–9).

Urea, the major end product of the ammonia detoxification process in most mammals, plays several critical roles in kidney function. It is an important solute for the development of an osmotic gradient in the renal medulla (10). Urea is also reported to inhibit Na-K-2Cl cotransport in the medullary thick ascending limb cells (11). Increased Na-K-2Cl cotransport in the kidney of Dahl salt-sensitive rats has been demonstrated previously (12, 13). Furthermore, urea has been reported to lower blood pressure in several rat models of experimental hypertension (14, 15). However, the physiological roles of urea synthesized by kidney arginase are still unknown because the amount of urea produced in the kidney is much less than that filtrated from glomeruli (16).
Recently, increased arginase activity in the aorta of mineralocorticoid-salt hypertensive rats was reported (17). However, the relation between renal arginase activity and blood pressure has never been reported.

We postulated that arginase in the kidney may contribute to the sodium handling by regulating urea production in the kidney. The purposes of the present study were to examine arginase activity and expression, and the effect of urea supplementation on blood pressure and renal damage in Dahl salt-sensitive hypertensive rats.

**Materials and Methods**

**Materials**

All chemicals were purchased from Wako (Osaka, Japan) unless otherwise indicated. Male Dahl/Rapp salt-sensitive (SS) and salt-resistant (SR) rats (4 weeks old) were purchased from Seac Yoshitomi (Fukuoka, Japan). All animals were housed according to institutional guidelines, and studies were approved by the Institutional Animal Care and Use Committee of the Kobe University Graduate School of Medical Science.

**Animals and Experimental Protocols**

**Series 1**

SS and SR rats were given standard rat chow and had free access to distilled water. At 4 weeks of age their body weight was measured, and their systolic blood pressure (systolic BP) was also evaluated by the tail-cuff method using an automatic blood pressure measurement system (MK-1100; Muromachi Kikai Co., Ltd., Tokyo, Japan). Ten SS and 10 SR rats were anesthetized with ether and decapitated, and their organs (kidneys, livers and cerebella) were frozen with liquid nitrogen and stored at -80ºC until assayed. The remaining SS and SR rats were maintained on a low salt (0.24% NaCl) diet until 4 weeks of age. Ten SS and 10 SR rats were given water containing 0.05% hydralazine (approx. 32 P-labeled rat GAPDH cDNA). Messenger RNA was densitometrically quantified using software supplied with the BAS 2000. The variability of the assay was controlled by hybridization with a 32P-labeled rat GAPDH cDNA.

**Series 2**

SS rats were maintained on a high salt (4% NaCl) diet containing 5% urea (SSH-U group; n = 10) or 5% kaolin (SSH-K group; n = 10) and given free access to distilled water for 6 weeks. We added 5% kaolin, in the form of potter’s clay, to the diet of the control group to adjust the amount of food intake. Systolic BP, body weight, urine volume, and blood and urinary urea nitrogen concentration were measured before and after treatment. The kidneys were removed and fixed in 20% formalin solution, and the histology was examined by periodic acid-Schiff (PAS) and Masson-trichrome staining.

**Measurement of Arginase Activity**

Arginase activity was measured according to Corraliza et al. (18). Briefly, the samples were homogenized using Polytron (Kinematica AG, Luzern, Switzerland) in a solution of 50 mmol/l Tris-HCl (pH 7.4) containing 1 mmol/l EDTA, 1 mmol/l dithiothreitol, 1 µmol/l (p-aminophenyl) methanesulfonfonyl fluoride, 1 µmol/l pepstatin A and 2 µmol/l leupeptin at 4 ºC. A 50 µl aliquot was mixed with 50 µl of 10 mmol/l MnCl	extsubscript{2} and 50 mmol/l Tris-HCl (pH 7.5) and activated for 10 min at 55 ºC. A 25 µl aliquot of 0.5 mol/l arginine (pH 9.7) was added to 25 µl of the activated lysate and incubated at 37 ºC for 60 min; the reaction was stopped by the addition of 400 µl of an acid mixture containing H:SO	extsubscript{4}, H:PO	extsubscript{4}, and H:O (1:3:7). Urea formation was colorimetrically quantified at 540 nm after the addition of 25 µl 9% 1-phenyl-1,2-propanedion-2-oxime and heating at 100 ºC for 45 min. A calibration curve was prepared using increasing amounts of urea between 1.5 and 30 µg/ml, and arginase activity was expressed as the amount of urea produced per mg protein (µmol urea/mg protein).

**Northern Blotting**

Total RNA was extracted from the kidneys using ISOGEN (the modified acid guanidium thiocyanate phenol/chloroform method; Nippon Gene, Tokyo, Japan). Total RNA (20 µg) was electrophoresed on a formaldehyde-containing 1.2% agarose gel and transferred to nylon membrane (Hybond-N; Amersham Pharmacia Biotech, Buckinghamshire, UK). The filters were hybridized to a random-primed, 32P-labeled rat arginase II cDNA (2) as a probe. The membrane was washed and exposed on an imaging plate, and the fragments were visualized in a BAS 2000 Bio-imaging analyzer (FUJIFILM, Tokyo, Japan). Messenger RNA was densitometrically quantified using software supplied with the BAS 2000. The variability of the assay was controlled by hybridization with a 32P-labeled rat GAPDH cDNA.

**Western Blotting**

The kidneys were homogenized in lysis buffer [1 mmol/l Tris-HCl (pH 7.4) containing 1 mmol/l EDTA (pH 8.0), 0.1 mg/ml phenylmethylsulfonyl fluoride, 1 mol/l dithiothreitol, 1 µg/ml leupeptin and 1 µg/ml pepstatin] and their protein concentrations were determined with the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, USA). Total tissue homogenate (50 µg) was separated on a 10% SDS-polyacrylamide gel under reduced conditions, transferred to a polyvinylidene difluoride membrane (Clear blot membrane-P; ATTO, Tokyo, Japan), and probed with anti-arginase II.
antibodies (9). Immunoreactive bands were visualized by horseradish peroxidase-conjugated anti-rabbit IgG using the ECL plus detection kit (Amersham Biosciences, Buckinghamshire, UK). The density of immunoreactive bands was analyzed using NIH image 1.55 software (National Institutes of Health, Bethesda, USA).

**Other Analytic Techniques**

Sodium and blood urea nitrogen (BUN) concentrations were measured by an autoanalyzer (Hitachi, Tokyo, Japan).

**Statistical Analysis**

Data are presented as the mean ± SE. Differences in mean values were analyzed by analysis of variance (ANOVA) followed by t-test analyses using StatView statistical software (ver. 4; Abacus Concepts, Inc., Berkeley, USA). A value of \( p < 0.05 \) was considered to indicate statistical significance.

**Results**

**Series 1**

Figure 1A shows the systolic BP values throughout the study. Systolic BP in the SSH group was significantly higher than that in the other groups at the end of the first week of salt loading. This significant difference continued until the end of the study. Treatment with hydralazine, a vasodilating agent, completely abolished the blood pressure elevation in the salt-loaded SS (blood pressure values after 4 weeks of treatment were as follows: SRL, 104 ± 3; SRH, 107 ± 2; SSL, 111 ± 3; SSH, 179 ± 3 \(* \); SSH-Hy, 109 ± 3 mmHg; \* \( p < 0.01 \) vs. SRH, \( \ddagger \) \( p < 0.01 \) vs. SSH; Fig.1A). No significant difference in body weight was observed among the five groups (Fig.1B).

Arginase activity in the kidney was lower in SS rats than in SR rats (SR vs. SS: 10.54 ± 0.36 vs. 8.26 ± 0.47 \( \mu \)mol urea/mg protein; \( p < 0.01 \)) at 4 weeks of age (Fig. 2A). On the other hand, arginase activity in the liver (Fig. 2C) and cerebellum (Fig. 2E) was not significantly different between SS rats and SR rats at 4 weeks of age. Arginase activity in the kidney at 8 weeks of age was lower than that at 4 weeks of age both in SS rats and SR rats, as previously reported in Wistar rats (Fig. 2B)(19). Kidney arginase activity of SS rats fed a low salt diet was lower than that of SR rats fed a low salt diet at 8 weeks of age (Fig. 2B). Four weeks of salt loading did not significantly alter arginase activity either in SR or SS rats. Hydralazine treatment had a tendency to increase arginase activity in salt-loaded SS rats, but the difference was not statistically significant. Arginase activity in the SSH-Hy group was not significantly different from that in either the SSH group or SRH group (SRL, 3.82 ± 0.16; SRH, 3.87 ± 0.50; SSL, 2.91 ± 0.31 \(* \); SSH, 2.72 ± 0.44 \( \ddagger \); SSH-Hy, 3.37 ± 0.53 \( \mu \)mol urea/mg protein; \* \( p < 0.05 \) vs. SRL, \( \ddagger \) \( p < 0.05 \) vs. SRH) (Fig. 2B). No significant difference was observed among the five groups in arginase activity in the liver (Fig. 2D) or cerebellum (Fig. 2F).

To elucidate the mechanism of decreased arginase activity
in the kidney of SS rats, we examined the mRNA and protein expression of arginase II because the kidney expresses this isoform almost exclusively (Fig. 3). Arginase II mRNA expression in the kidney was significantly lower in the SS group than in the SR group (SRL, 1.00 ± 0.02; SRH, 0.95 ± 0.02; SSL, 0.72 ± 0.02; SSH, 0.75 ± 0.01; **p < 0.01 vs. SRL, *p < 0.05 vs. SRH) (Fig. 3A). The amount of arginase II protein was also significantly lower in the SS group than in the SR group (SRL, 1.00 ± 0.02; SRH, 0.98 ± 0.02; SSL, 0.84 ± 0.05; SSH, 0.82 ± 0.03; *p < 0.05 vs. SRL, **p < 0.01 vs. SRH) (Fig. 3B).

### Series 2

To examine the effect of urea, the product of arginase reaction, on the development of hypertension, salt-loaded SS rats were given a high salt (4% NaCl) diet containing 5% kaolin (SSH-K) or 5% urea (SSH-U). After a 6-week treatment, plasma BUN (SSH-K vs. SSH-U: 21.4 ± 0.4 vs. 29.0 ± 1.7 mg/dl; p < 0.01) and urinary urea nitrogen excretion (SSH-K vs. SSH-U: 414 vs. 416 mg/dl; p < 0.01) were significantly lower in the SSH-K group compared to the SSH-U group.
were higher in SSH-U rats than in SSH-K rats. After 6 weeks, SBP in SSH-U was lower than in SSH-K (SSH-K vs. SSH-U; 151.0 ± 4.6 vs. 123.6 ± 2.2 mmHg; p < 0.01) (Fig. 4). Body weight was not significantly different between the two groups at the end of the experiment (data not shown). Histological observation of the kidney revealed glomerulosclerosis (Fig. 5A) and vascular lesions such as medial hypertrophy in the micro-vessel (Fig. 5C) in SSH-K rats. These findings of renal damage were attenuated in the kidneys of the urea-treated group (Fig. 5B, D).

**Discussion**

To study the roles of arginase in the pathogenesis of salt-sensitive hypertension, we examined the activity and expression of arginase in Dahl/Rapp SS and SR rats. We found that arginase II mRNA, arginase II protein, and arginase activity in the kidney were lower in SS rats than in SR rats before and after salt loading and development of hypertension. These findings suggest that decreased renal arginase may contribute to blood pressure elevation in SS rats. Because there was no difference in blood pressure between SS rats and SR rats at 4 weeks of age, despite the fact that renal agi-
nase activity was already lower in SS rats than in SR rats at this time point, arginase activity in the kidney could not have been the principal determinant of blood pressure. Arginase activity may be related to salt-sensitivity, since some physiological abnormalities (e.g., reduced natriuretic response to acute saline load) were observed before blood pressure elevation in SS rats (20). Interestingly, we previously observed that kidney arginase activity in spontaneously hypertensive rats, which are less salt-sensitive, was the same as that in normotensive control Wistar Kyoto rats (Iwata S and Tsujino T, unpublished data).

Arginase activity results in equimolar production of urea and ornithine. Microdissection studies by Levillain et al. revealed that urea production from arginine increased from the proximal convoluted tubules to the cortical and to a greater extent the outer medullary portions of the straight proximal tubules—that is, at the entrance to the loop of Henle—in all species (8). Immunohistochemical studies by Miyanaka et al. consistently showed that arginase II is localized in the outer stripes of the outer medulla, presumably in the proximal straight tubules, and in a subpopulation of the proximal tubules in the cortex (9). Because urea was reported to inhibit Na-K-2Cl cotransport, which is the target molecule of loop-diuretics such as furosemide, in the medullary thick ascending limb of the kidney (11), decreased local urea production might increase sodium resorption in SS rats. We also observed blood pressure reduction and amelioration of nephrosclerosis by urea supplementation in salt-loaded SS. Decreased arginase activity might exaggerate salt-sensitive hypertension and nephrosclerosis in SS rats.

However, it remains uncertain whether urea synthesized in the kidney plays a role in sodium excretion or blood pressure regulation, since the rate of such urea synthesis is much lower than that of urea flow from the glomerulus (e.g., 1 vs. 50

**Fig. 5.** Effect of urea supplementation on hypertensive nephropathy in Dahl salt-sensitive rats fed a high-salt diet (SSH). Photomicrographs show an increase in mesangial matrix in the glomeruli (A; PAS, x 200) and narrowing of the lumen of arterioles caused by thickening of the medial and intimal walls in the SSH supplemented with kaolin (C; Masson-trichrome, x 200). Compared with SSH supplemented with kaolin, these findings of nephrosclerosis were ameliorated in SSH supplemented with urea (B, PAS, x 200; D, Masson-trichrome, x 200). Scale bar, 50 μm. Arrow, glomeruli; arrowhead, arterioles.
increase in the kidneys of SS rats, and that arginine synthesis (29), 27 thase (nNOS) activity was reduced in Dahl salt-sensitive rats 25 24 impaired in Dahl salt-sensitive rats, and that arginine supple-

mented by L-arginine (17). However, this does not fully explain blood pressure elevation (28). In Dahl salt-sensitive rats, the rate of polyamine biosynthesis is increased, which may contribute to the development of hypertension (29). 

Arginase activity was decreased in SS rats. We also found that spermidine supplementation ameliorated salt-induced hypertension while an ODC inhibitor increased blood pressure in SS rats (Hayashi T et al., manuscript submitted). Therefore, decreased arginase activity may cause salt-sensitive hypertension by reducing polyamine production.

To examine whether blood pressure affected arginase regulation, we treated salt-loaded SS rats with hydralazine (SSH-Hy). Arginase activity in the SSH-Hy group had a tendency to increase, although not to a level that was significantly different from that of the SSH group. Changes in blood pressure or renal circulation may influence renal arginase activity. But hydralazine may also alter arginase activity by a mechanism other than decreasing blood pressure.

In any event, we must admit the possibility that the difference in renal arginase may not have functional significance. We could not perform conclusive experiments in this regard, because the currently available arginase inhibitors suppressed both arginase I (hepatic type) and arginase II (extrahepatic type). If the hepatic and kidney arginas have the same activity, then there will be simultaneous decreases in both locally produced urea and urea filtered from glomeruli. Arginase II knockout mice are also not available. Clearly, further studies will be needed to elucidate the pathophysiological role of renal arginase in salt-sensitive hypertension.

The effect of kidney arginase activity on the plasma arginine concentration is of interest because approximately 60% of net arginine synthesis in adult mammals occurs in the kidneys (4). Arginine is the only substrate of nitric oxide synthase (NOS). Chen et al. reported that NO production was impaired in Dahl salt-sensitive rats, and that arginine supplementation prevented hypertension in the same model (24, 25). We have also previously reported that type I NO synthase (nNOS) activity was reduced in Dahl salt-sensitive rats (26). NO plays a role in sodium excretion in the kidneys (27, 28). On the other hand, arginase II has been reported to down-regulate the NO production induced by lipopolysaccharide and interferon-γ in RAW 264.7 cells (29). We initially speculated that arginase activity might increase in the kidneys of SS rats, and that arginine synthesis might decrease. Contrary to our expectations, however, arginase activity was decreased in SS rats. We also found that plasma arginine concentration was not different between SS and SR rats (Iwata S and Tsujino T, unpublished data), which was consistent with previous studies (30, 31). A modest decrease in arginase activity appeared not to affect the arginine supply from the kidney to the general circulation.

In summary, we demonstrated decreased arginase activity, and decreased arginase II protein and mRNA expression in the kidneys of Dahl salt-sensitive rats. Moreover, urea supplementation attenuated blood pressure elevation and renal damage in Dahl salt-sensitive rats fed a high salt diet. Impaired arginase activity in the kidney may be partly responsible for blood pressure elevation in Dahl salt-sensitive rats, but more conclusive experiments will be needed to elucidate the pathophysiological role of renal arginase in salt-sensitive hypertension.

References


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