Original Article

Decreased Ornithine Decarboxylase Activity in the Kidneys of Dahl Salt-Sensitive Rats

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To assess the roles of polyamines (putrescine, spermidine, and spermine) and ornithine decarboxylase (ODC), the rate-limiting enzyme of polyamine synthesis, in the development of salt-sensitive hypertension, we evaluated activity and expression of ODC, urinary polyamine excretion, and antizyme (endogenous ODC inhibitor protein) expression in Dahl salt-sensitive (SS) and salt-resistant (SR) rats after they were fed on a low (0.3%) or high (4%) salt diet for 4 weeks. We also examined the effects of spermidine and difluoromethyl-ornithine (DFMO: a specific inhibitor of ODC) on the systolic blood pressure and ODC protein expression in SS rats fed a high salt diet. Renal ODC activity and urinary polyamine excretion in SS rats were lower than those in SR rats after 4 weeks treatment with a low or high salt diet. The renal ODC protein expression of SS rats was paradoxically increased as compared to the SR group. A high salt diet did not alter ODC activity but increased ODC protein only in SS rats. ODC mRNA and antizyme protein expressions were not significantly different among the four groups. Spermidine supplementation attenuated and DFMO exaggerated hypertension in SS rats fed a high salt diet. Spermidine down-regulated and DFMO up-regulated renal ODC protein in SS rats on a high salt diet. ODC activity was decreased but protein was paradoxically increased in kidneys of SS rats. ODC protein was suggested to increase in compensation for the inhibition of its activity. Impaired ODC activity and polyamine production in the kidney may exaggerate salt-sensitive hypertension in SS rats. (*Hypertens Res* 2002; 25: 787–795)

Key Words: ornithine decarboxylase, polyamine, Dahl rat, kidney, salt-sensitive hypertension

Introduction

Polyamines (putrescine, spermidine, and spermine) are naturally occurring aliphatic amines that exist as normal cell components of almost all-living species (1, 2) and exert a number of cellular activities, including cell proliferation and differentiation (3). Ornithine decarboxylase (ODC) is the initial and the rate-limiting enzyme involved in the polyamine

biosynthetic pathway. ODC receives negative feedback control from its products, polyamines (4). Polyamine levels control the production of antizyme, a protein inhibitor of ODC. Translation of antizyme mRNA necessitates frameshifting, which in turn is dependent on polyamines (5, 6). The antizyme specifically binds to ODC, forms an inactive complex, sequesters ODC into the 26S proteasome, and promotes its degradation (6, 7). In almost all cells, ODC is constitutively expressed in small amounts, and its expression is

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This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labor and Welfare, Japan (No. 2-635-812).

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Received March 4, 2002; Accepted in revised form June 17, 2002.

dramatically induced by cell growth signals (1–4). The physiological roles of ODC and polyamines in renal function remain unknown. In the kidney, ODC is rather highly expressed in proximal tubules, and its expression is also strongly up-regulated in response to diabetes induction (8), uninephrectomy (9), and injection of hormones such as testosterone (10), growth hormone, and hydrocortisone (1). In streptozotocin diabetic rats, renal ODC and polyamines have been implicated in kidney hypertrophy (8) and increased proximal reabsorption (11), although the findings of Tovar *et al.* (12) contradict these relationships.

In some experimental models of hypertension, the roles of vascular ODC activity in development of hypertension and vascular remodeling have been studied by using a specific ODC inhibitor, difluoromethylornithine (DFMO). DFMO treatment attenuated blood pressure elevation in spontaneously hypertensive rats (SHR) (13), deoxycorticosterone acetate-salt hypertensive rats (14), and rats receiving continuous angiotensin II infusion (15). Moreover, Kai *et al.* reported a correlation between left ventricular hypertrophy and cardiac polyamine concentration in Tsukuba hypertensive mice (16). All of these previous studies were concerned with vascular and cardiac ODC activities. To our knowledge, however, the relationship between renal ODC and the development of hypertension has not yet been examined. Therefore, in the present study, we examined renal ODC activity, ODC expression, and antizyme expression in Dahl salt-sensitive and salt-resistant rats on a low or high salt diet. We here report on the decreased ODC activity and paradoxical increase in ODC protein in the kidneys of Dahl salt-sensitive rats. The reason for this discrepancy and the role of renal ODC in the development of salt-sensitive hypertension are also discussed.

Materials and Methods

DFMO was purchased from Ilex Oncology, Inc. (San Antonio, USA). Spermidine was from Sigma (St. Louis, USA). All other chemicals were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). L-[1-¹⁴C] ornithine (59 mCi/mmol) was from Moravsek Biochemicals Inc. (Brea, USA). Anti-ODC monoclonal antibody was a product of Neo Markers (Union, USA). Rat ODC cDNA was purchased from ATCC (Manassas, USA) as a gift. A polyclonal antibody raised against highly purified antizyme from Yasuko Murakami of the Jikei University School of Medicine (17).

Animals and Experimental Protocols

All studies were done in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Kobe University Graduate School of Medicine. All rats were exposed to a 12-h light/12-h dark cycle throughout the experiment, under controlled temperature (22 ± 2°C) and humidity (55 ± 5 %).

Study 1: Twenty male Dahl/Rapp salt-sensitive (SS) and 20 salt-resistant (SR) rats (Seac Yoshitomi, Fukuoka, Japan) were given standard chow and free access to water. At 4 weeks of age, they were divided into 2 groups of 10 animals each, respectively. SS and SR rats were maintained on a low salt (0.3% NaCl) (SS-L, SR-L) or high salt (4% NaCl) (SS-H, SR-H) diet and distilled water for 4 weeks.

Study 2: Four week-old SS rats ($n = 20$) were divided into a control (high salt diet only) group and spermidine supplementation group, each containing 10 rats. The control group was maintained on a high salt (4% NaCl) diet (SS-H), and the spermidine group was fed a high salt diet containing 0.05% spermidine (SS-H-Sp) for 5 weeks. Both groups were given distilled water as drinking water.

Study 3: Four-week-old SS rats ($n = 20$) were divided into a control (high salt diet only) group and a DFMO-treated group. Both groups were maintained on a high salt (4% NaCl) diet for 5 weeks. The control group was given distilled water (SS-H), and the DFMO-treated group was given water containing 1% DFMO (SS-H-DFMO).

Systolic blood pressure (systolic BP) and body weight were measured every week. Systolic BP was measured by the tail-cuff method using an automatic blood pressure measurement system (MK-1100; Muromachi Kikai Co., Ltd., Tokyo, Japan). At the end of 4 weeks (age 8 weeks) in Study 1, and at the end of 5 weeks (age 9 weeks) in Studies 2 and 3, 24-h urine samples were collected for measurement of polyamine excretion.

The rats were sacrificed at the end of the study, and the left kidney was removed under pentobarbital anesthesia, rapidly cleaned, weighed, and immediately frozen in liquid nitrogen for later experiments.

In Study 1, three rats in the SR-H group and SS-H group were transcardially perfused under pentobarbital anesthesia (50 mg/kg-body wt) with cold phosphate-buffered saline (PBS; 50 mmol/l, pH 7.4) followed by cold 4% paraformaldehyde and 10% sucrose overnight at 4°C for immunohistological examination.

Assay of ODC activity

ODC activity was assayed by the release of ¹⁴CO₂ from L-[1-¹⁴C]ornithine as previously described with slight modification (18). Briefly, the tissue was homogenized in a Polytron homogenizer three times for 10 s in a buffer containing 2.5 mmol/l dithiothreitol, 0.1 mmol/l pyridoxal 5-phosphate, 0.1 mol/l tris (hydroxymethyl) aminomethane (Tris), and 0.1 mmol/l EDTA, pH 7.2. The homogenate was centrifuged for 30 min at 30,000 × *g* at 4°C. Then 90 μl of the supernatant was added to a glass tube containing 4.625 kBq of L-[1-¹⁴C]ornithine (10 μl) and 80 nmol of L-ornithine (5 μl). The test tube was sealed with a rubber stopper equipped with a disposable syringe needle, on which a paper disk (8 mm in diameter) containing 50 μl Soluen-350 (Packard Instruments, Downers Grove, USA) was impaled, and was incubated at 37°C for 1

h. The CO₂ released by the addition of 1 ml of 2 mol/l citrate through the rubber stopper was trapped by the Soluen-350 absorbed on the paper disk, and was then transferred to a vial containing 5 ml toluene scintillation fluid (Omuifluor; Dai-ichi Chemicals, Tokyo, Japan). Radioactivity was measured using a liquid scintillation counter (Beckman LS-5801; Beckman, Fullerton, USA).

Western Blotting

The kidneys were homogenized in lysis buffer 10 mmol/l Tris-HCl (pH 7.4) containing 1 mmol/l EDTA (pH 8.0), 1 mmol/l phenylmethylsulfonyl fluoride, 1 mmol/l dithiothreitol, 1 µg/ml leupeptin, and 1 µg/ml pepstatin, and their protein concentrations were determined with a Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, USA). Total tissue homogenate (50 µg) was run on a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (Clear Blot Membrane-P; ATTO, Tokyo, Japan). After electrophoretic transfer, the membrane was blocked overnight at 4°C with blocking reagent (0.5% casein, 0.05% NaN₃/Tris-buffered saline with 0.5% Tween 20 (TBS-T)). After washing with TBS-T, the membrane was incubated for 1 h at room temperature with monoclonal antibody to ODC. After washing, the membrane was incubated with anti-mouse IgG conjugated with horseradish peroxidase for 1 h at room temperature. Detection of ODC proteins was achieved with an ECL plus detection kit (Amersham Biosciences, Buckinghamshire, UK). The densities of immunoreactive bands were analyzed using NIH image 1.55 software (National Institutes of Health; Bethesda, USA).

Immunohistochemistry

The transcardially perfused kidneys were frozen after fixation. Frozen sections (7-µm thick) were cut on a sliding microtome. After blocking in PBS containing 3% H₂O₂ for 5 min at room temperature, the sections were incubated with monoclonal antibody to ODC, which was diluted 1:1,000 in Tris-HCl buffer (50 mmol/l; pH 7.6) for 24 h at 4°C. Immunoreactivity was visualized by the avidin-biotin-peroxidase method (DAKO LSAB Kit; DAKO Corp., Carpinteria, USA). Some sections were counterstained with haematoxylin-eosin to show the distribution of the immunoreactive cells.

Northern Blotting

Total RNA was extracted from the kidneys using ISOGEN (a modified acid guanidium thiocyanate phenol/chloroform method; Nippon Gene, Tokyo, Japan). Total RNA (20 µg) was electrophoresed on formaldehyde containing 1.2% agarose gel and transferred to a nylon membrane (Hybond-N; Amersham Biosciences). The filters were hybridized to a random-primed, ³²P-labeled rat ODC cDNA. The membrane was

washed and exposed on an imaging plate, and the fragments were visualized using a BAS 2000 bio-imaging analyzer (FUJIX, Tokyo, Japan). The amount of detected mRNA was densitometrically quantified using software supplied with the FUJIX BAS 2000. The variability of the assay was controlled by hybridization with a ³²P-labeled rat GAPDH cDNA.

Urinary Polyamine Excretion

All rats were individually housed in metabolic cages. Urine samples were collected 24 h after treatment for measurement of polyamine. Urinary polyamine levels were measured using a previously described enzymatic assay method (19). Briefly, conjugated polyamines (putrescine, spermidine, and cadaverine) in urine were hydrolyzed by incubation with an acylpolyamine amidohydrolase at 30°C for 1 h. Then, free polyamines were separated by cation-exchange chromatography and incubated with a putrescine oxidase at 30°C for 30 min. The amount of hydrogen peroxide formed in this reaction was measured spectrophotometrically (at 514 nm).

Statistical Analysis

Data are presented as the mean ± SEM. Differences in mean values were analyzed by analysis of variance (ANOVA) followed by Student's *t*-test analyses using statistical software (Statview ver. 4; Abacus Concepts, Inc., Berkeley, USA). Values of *p* < 0.05 were considered to indicate statistical significance.

Results

Table 1 shows the blood pressure, heart rate, body weight, and kidney-to-body weight ratio of Dahl salt-resistant (SR) and salt-sensitive (SS) rats after 4 weeks of treatment with a low (0.3% NaCl) (SR-L, SS-L) or high (4% NaCl) (SR-H, SS-H) salt diet, respectively. Systolic blood pressure was elevated in the SS groups and was higher in rats fed on a high salt diet than in those on a low salt diet. The kidney-to-body weight ratio was significantly increased in the SS-H group as compared with the other groups, indicating that kidney hypertrophy occurred only in SS-H rats.

The ODC activity and protein expression level in kidneys of SS rats were not significantly different from those of SR rats at 4 weeks of age (data not shown). The ODC activity in SS rats was lower than that in SR rats after 4 weeks of treatment with a low or high salt diet (8-week-old SR-L vs. SS-L: 297.1 ± 50.8 vs. 117.1 ± 11.4 cpm/mg protein/h, *p* < 0.01; 8-week-old SR-H vs. SS-H: 378.1 ± 18.8 vs. 158.1 ± 43.1 cpm/mg protein/h, *p* < 0.01) (Fig. 1). A high salt diet did not significantly alter the ODC activity either in SS or SR rats (Fig. 1).

To examine whether decreased ODC activity resulted in decreased polyamine production, urinary polyamine excretion was measured. Figure 2 shows the urinary polyamine excretion normalized to creatinine excretion after 4 weeks of treatment. The urinary polyamine levels in SS rats were low-

Table 1. Systolic Blood Pressure, Heart Rate, Body Weights, and Kidney and Body Weight Ratio of Salt-Resistant and Salt-Sensitive Rats Fed on 0.3% or 4% NaCl Diets

Treatment groups	Systolic blood pressure (mmHg)	Heart rate (beats/min)	Body weight (g)	KW/BW (mg/g)
SR-L	106 ± 10	431 ± 24	325 ± 9	5.9 ± 0.3
SR-H	109 ± 8	416 ± 31	316 ± 10	6.4 ± 0.4
SS-L	141 ± 6*	408 ± 19	311 ± 8	6.1 ± 1.4
SS-H	180 ± 8**	419 ± 16	308 ± 11	8.3 ± 0.3***

KW/BW indicates the ratio of kidney weight to body weight. SR indicates Dahl salt-resistant rats. SS indicates Dahl salt-sensitive rats. SR-L and SS-L rats had been fed on a 0.3% NaCl diet and SR-H and SS-H rats had been fed on a 4% NaCl diet for 4 weeks. Values are the mean ± SEM. * $p < 0.001$ compared with the SR-L group and ** $p < 0.0001$ compared with the SS-L group. *** $p < 0.001$ compared with the other groups. Each group consists of 10 rats.

Fig. 1. Kidney ODC activity of Dahl salt-resistant (SR) and salt-sensitive (SS) rats fed on a 0.3% NaCl diet (SR-L, SS-L) or 4% NaCl diet (SR-H, SS-H) for 4 weeks. Values are the mean ± SEM. * $p < 0.01$ compared to the SR-L group. ** $p < 0.01$ compared to the SR-H group. Each group consists of 7 rats.

Fig. 2. Urinary polyamine excretion (normalized to creatinine excretion) of Dahl salt-resistant (SR) and salt-sensitive (SS) rats on a 0.3% NaCl diet (SR-L, SS-L) or 4% NaCl diet (SR-H, SS-H) for 4 weeks. Values are the mean ± SEM. * $p < 0.05$ compared to the SR-L group. ** $p < 0.05$ compared to the SR-H group. Each group consists of 7 rats.

Fig. 3. Western blot analysis showing the protein expression of ODC in the kidneys of Dahl salt-resistant (SR) and salt-sensitive (SS) rats fed on 0.3% or 4% NaCl diets for 4 weeks. The results are expressed as a percentage of ODC protein in SR-L rats over the control, which was taken as 100%. Each group consists of 3 rats. Values are the mean ± SEM.

er than those in SR rats after a low or high salt diet (SR-L vs. SR-H vs. SS-L vs. SS-H: 603.1 ± 13.1 vs. 614.3 ± 16.7 vs. $481.9 \pm 24.2^*$ vs. $506.9 \pm 34.1^{**}$ μmol/g creatinine (Cr); * $p < 0.05$ vs. SR-L; ** $p < 0.05$ vs. SR-H). As in the case of ODC activity, a high salt diet did not alter the urinary polyamine excretion either in SS or SR rats.

To elucidate the mechanism of decreased ODC activity in the kidneys of SS rats, we examined the protein and mRNA expression of ODC. Unexpectedly, the ODC protein expression in the kidneys of SS rats who received a low salt diet for 4 weeks was higher than that in the SR group (Fig. 3). Furthermore, the high salt diet resulted in a further increase of ODC protein in the kidneys of SS rats (SR-L vs. SR-H vs. SS-L vs. SS-H: 1.0 ± 0.16 vs. 1.14 ± 0.06 vs. $1.55 \pm 0.03^*$ vs. $2.33 \pm 0.09^{**}$; * $p < 0.05$ vs. SRL; ** $p < 0.01$ vs. SRL). Immunohistochemical analysis of the kidney samples showed that ODC immunoreactivity was localized in the pars convoluta of the proximal tubules both in SS-H and SR-H rats (Fig. 4). Consistent with the data from Western blotting, the intensity of ODC immunoreactivity was higher in SS-H rats

Fig. 4. ODC expression in the kidney of Dahl salt-resistant (SS) (a, c, e) and salt-sensitive (SR) (b, d, f) rats administered a high salt diet for 4 weeks. SS rats maintained on a high salt diet showed increased ODC-immunoreactivity inside the pars convoluta of the proximal tubules lumen compared to the SR group on a similar diet. Scale bars : a, b = 800 μ m ; c-f = 100 μ m.

than SR-H rats. ODC mRNA expression was not significantly different among the four groups (Fig. 5).

Next, we carried out Western blot analysis of antizyme, a polyamine inducible protein inhibitor of ODC. The expression of antizyme was not significantly different among the four groups (SR-L vs. SR-H vs. SS-L vs. SS-H: 1.0 ± 0.07 vs. 0.99 ± 0.03 vs. 1.20 ± 0.04 vs. 1.05 ± 0.04) (Fig. 6).

To test whether decreased ODC activity and polyamine production affected the development of hypertension, we performed two additional experiments.

In Study 2, SS rats fed with a high salt diet were treated

with or without a spermidine (0.05% in diet). Systolic BP in spermidine-supplemented rats (SS-H-Sp) was significantly lower than that in rats without spermidine supplementation (SS-H) after 5 weeks of treatment (SS-H vs. SS-H-Sp: 186 ± 12 vs. 160 ± 11 mmHg; $p < 0.01$; Fig. 7A). No significant difference in body weight was observed between the two groups (Fig. 7B). The urinary polyamine excretion of the SS-H-Sp group tended to be higher than that of the SS-H group, but the difference did not reach the level of statistical significance (SS-H vs. SS-H-Sp: 436.4 ± 16.4 vs. 494.8 ± 30.8 μ mol/g Cr; $n = 5$; $p = 0.13$). The amount of kidney ODC

Fig. 5. Northern blot analysis showing the mRNA expression of ODC in the kidneys of Dahl salt-resistant (SR) and salt-sensitive (SS) rats fed on a 0.3% NaCl diet (SR-L, SS-L) or 4% NaCl diet (SR-H, SS-H) for 4 weeks. The results are expressed as a percentage of ODC mRNA in SR-L rats over the control, which was taken as 100%. Each group consists of 3 rats. Values are the mean $\pm$ SEM.

Fig. 6. Western blot analysis showing the protein expression of antizyme in kidneys from Dahl salt-resistant (SR) and salt-sensitive (SS) rats fed on 0.3% or 4% NaCl diets for 4 weeks. The results are expressed as a percentage of antizyme protein in SR-L rats over the control, which was taken as 100%. Each group consists of 3 rats. Values are the mean $\pm$ SEM.

Fig. 7. Comparison of systolic blood pressure (a) and body weight (b) responses to 0.05% spermidine supplement with SS-H rats. Each group consists of 10 rats. Protein expression and densitometric scanning of ODC from SS-H and SS-H whose diets were supplemented with 0.05% spermidine (SS-H-Sp) are shown on panel c. Each group consists of 3 rats. The open circles and open bar indicate SS-H. The solid circles and solid bar indicate SS-H-Sp. Values are the mean $\pm$ SEM. * $p < 0.05$ compared to the SS-H group.

protein was significantly lower in the SS-H-Sp group than in the SS-H group (SS-H vs. SS-H-Sp: 1.0 ± 0.02 vs. 0.43 ± 0.27 ; $p < 0.05$; Fig. 7C).

In Study 3, SS rats fed a high salt diet were treated with or without a specific ODC inhibitor, DFMO (1% in drinking water). Systolic BP in DFMO-treated rats (SS-H-DFMO) was significantly higher than that in rats without DFMO treatment (SS-H) after 5 weeks of treatment (SS-H vs. SS-H-

DFMO: 170 ± 9 vs. 194 ± 12 mmHg; $p < 0.01$ vs. SS-H; Fig. 8A). No significant difference was observed in body weight between the two groups (Fig. 8B). The urinary polyamine excretion of the SS-H-DFMO group showed a significant decrease compared to that of the SS-H group (SS-H vs. SS-H + DFMO: 434.2 ± 18.1 vs. 352.5 ± 15.3 $\mu\text{mol/g Cr}$; $n = 5$; $p < 0.01$). The amount of kidney ODC protein was significantly higher in the SS-H-DFMO group than in the SS-H

Fig. 8. Comparison of systolic blood pressure (a) and body weight (b) responses to 1% DFMO treatment in SS-H rats. Each group consists of 10 rats. Protein expression and densitometric scanning of ODC from SS-H and SS-H treated with 1% DFMO (SS-H-DFMO) are shown on panel c. Each group consists of 3 rats. The open circles and open bar indicate SS-H. The solid circles and solid bar indicate SS-H-DFMO. Values are the mean $\pm$ SEM. * $p < 0.05$ compared to the SS-H group.

group (SS-H vs. SS-H-DFMO: 1.0 ± 0.08 vs. 1.48 ± 0.07 ; $p < 0.05$; Fig. 8C).

Discussion

To study the role of ODC and polyamines in the pathogenesis of salt-sensitive hypertension, we studied the kidney ODC activities and urinary polyamine excretion in Dahl rats, a representative model of salt-sensitive hypertension. Both ODC activity and urinary polyamine excretion were lower in SS rats than in SR rats. Moreover, in salt-loaded SS rats, blood pressure was lowered by supplementation with spermidine, one of the polyamines, and elevated by treatment with DFMO, a specific inhibitor of ODC. These results are the first to demonstrate that depletion of polyamine may at least partially contribute to the development of salt-induced hypertension. The magnitude of decrease in polyamine excretion was much lower than that of ODC activity, probably because the polyamines excreted in urine originated not only from the kidneys but also from other tissues.

ODC and polyamines have already been reported to play some roles in the pathogenesis of hypertension and vascular remodeling in several experimental models. ODC activation in blood vessels has been consistently shown to be involved in vascular remodeling in models such as spontaneously hypertensive rats (SHR) (13), deoxycorticosterone acetate-salt hypertensive rats (14), the angiotensin II infusion (15), and the coarctation hypertension (20). Contrary to our observations in SS rats, DFMO treatment has been reported to attenuate blood pressure elevation in SHR (13), deoxycorticosterone acetate-salt hypertensive rats (14), and the angiotensin II infusion model (15). We feel that this discrepancy may be due, at least in part, to the localization of ODC, which is responsible for the control of blood pressure. Activation of

vascular ODC may induce vascular hypertrophy and elevate blood pressure, but activation of renal ODC and increase in polyamines may lower blood pressure.

The mechanisms by which polyamines lower blood pressure in our model remain unclear. Our immunohistochemical studies showed that ODC was higher in the pars convoluta of the proximal tubules in SS rats than in those of SR rats. This finding was similar to that of Koibuchi *et al.* (10) that testosterone treatment increased ODC immunoreactivity in the pars convoluta of the proximal tubules. On the other hand, it is well known that sodium-glucose transporter 1 is localized to the proximal tubules (21). And polyamines have been shown to down-regulate sodium-glucose transporter in LLC-PK1, a cell line derived from porcine kidney (22). Polyamine depletion may up-regulate sodium-glucose transporter in the proximal tubules and thereby enhance sodium reabsorption.

Several studies have suggested that polyamines are important as antioxidants. Polyamines have been reported to inhibit lipid peroxide formation in rat liver microsomes (23) and protect DNA from attack of reactive oxygen species (24, 25). The organs that were exposed to oxidative stress induce ODC activity and increase the concentration of polyamines (26). Increased oxidative stress has been reported in human essential hypertension (27, 28). Enhanced oxygen free radical production was also reported in SS rats (29). Gonick *et al.* reported that 2,3-dimercaptosuccinic acid, a sulfhydryl-containing antioxidant, prevented nephrosclerosis and hypertension in SS rats (30). Taken together, these results suggest that polyamine depletion might induce oxidative stress and contribute to blood pressure elevation.

The mechanisms by which ODC activity and polyamine production are suppressed in SS rats remain unclear. Because the activity of arginase, which synthesizes ornithine from arginine, was decreased in the kidney of SS rats (31),

ornithine availability in the proximal tubules might also be decreased in this model.

In our study, there were two distinct dissociations between ODC activity and the amount of ODC protein in Dahl rats. One was a dissociation between SS rats and SR rats. In SS rats, ODC activity and polyamine excretion were decreased in spite of an increased expression of ODC protein, as compared to SR rats. The other dissociation was between the low-salt diet group and high-salt diet group in SS rats. The protein expression of ODC in the high-salt diet group was much higher than that in the low-salt diet group only in SS rats, even though there were no significant differences in ODC activity and polyamine excretion between the two groups. Different mechanisms might account for each of these cases, as described below.

ODC is known to receive negative feedback from its products, polyamines. Polyamines induce antizyme, which inactivates ODC and accelerates its degradation (6, 7). The decreased ODC activity, decreased polyamine excretion, and increased ODC protein seen in the present study suggest that ODC activity was primarily suppressed, and that the decrease in polyamine up-regulated ODC protein by negative feedback control in SS rats. This hypothesis was supported by our finding that spermidine supplementation decreased the amount of ODC protein and DFMO treatment increased it in SS rats fed a high salt diet.

A high salt diet also appeared to suppress ODC activity in SS rats, but decreased polyamine production was thought to increase ODC protein to maintain ODC activity and polyamine production at the same level as in SS rats fed a low-salt diet. It remains unknown why ODC protein in SS rats was not induced sufficiently to maintain ODC activity at the same level as in SR rats. At 4 weeks of age, and possibly earlier, ODC activity and protein were not different between these 2 strains. This finding suggested that the presence of mutations in coding lesions of the ODC gene was unlikely.

One possible mechanism that would account for the failure to maintain ODC activity in SS rats is that antizyme expression may not have been adequately suppressed. Because ODC activity and polyamine production were decreased in SS rats, we expected that antizyme levels were also decreased in this model. However, there was no significant difference in the expression of antizyme either between SS rats and SR rats or between groups fed a high salt diet and those fed a low salt diet. These findings meant that antizyme expression was not adequately suppressed in SS rats. Antizyme had been believed to inactivate ODC by degrading it. However, Murakami *et al.* recently reported that the 26S proteasome irreversibly inactivated ODC prior to its degradation (32). The "relatively increased" expression of antizyme and enhanced sequestration of ODC within the 26S proteasome without degradation may account for the decreased ODC activity in SS rats.

Moreover, agmatine, a product of arginine decarboxylation in mammalian cells, was reported to inhibit ODC activity

by antizyme-dependent and -independent pathways (33, 34). Arginine decarboxylase is expressed in the kidney cortex and outer medulla (35), and arginine metabolism is altered in SS rats (31, 36). Thus an alteration in agmatine metabolism might be responsible for the decreased ODC activity in this rat model.

In summary, we demonstrated that ODC activity was decreased, but that ODC protein was paradoxically increased, in the kidneys of SS rats, and especially in those fed a high salt diet. ODC protein was suggested to increase compensatorily in response to inhibition of its activity. And impairment of ODC activity and decreased polyamine production in the kidney was thought to exaggerate salt-sensitive hypertension in SS rats.

Acknowledgements

We would like to thank Prof. Yasuko Murakami, Jikei University School of Medicine for providing the antizyme antibodies. We also would like to thank Drs. Tomoya Yamashita, Masayuki Namiki, Yasuhiro Watari, and Hisashi Harada, Division of Cardiovascular and Respiratory Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine, for their immunohistological assistance (T. Y. and M. N.) and technical instructions (Y. W. and H. H.).

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