Effect of Sodium Intake on Urinary Renin Excretion in Rats

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The present study was carried out to investigate the effect of sodium intake on urinary renin excretion in rats. Renin activity was measured before and after acidification, by radioimmunoassay of angiotensin I generated by incubation with partially purified homologous renin substrate. The molecular weight of renin was determined by gel filtration. Low sodium intake for 4 weeks resulted in a 16-fold increase in urinary renin excretion and led to a 15-fold increase in plasma renin activity. A 2.5-fold increase in renal renin content was simultaneously observed. In contrast, high sodium intake for 4 weeks decreased urinary renin excretion, plasma renin activity and renal renin content to about 20%, 25% and 35% of the control level, respectively. No significant change in renin activity after acidification was observed in all the urine samples. The molecular weight of renin in the urine from rats on low or high sodium intake was 40000, the value being identical with that from control rats. These results indicate that urinary renin excretion is well correlated with plasma renin activity, and that only active renin with a molecular weight of 40000 is excreted in the urine of rats, even under conditions of altered sodium intake.

Keywords — rat urine; sodium intake; urinary renin excretion; plasma renin activity; renal renin content; acidification; renin molecular weight

Introduction

Renin (EC 3.4.23.15) is an aspartic protease which catalyzes the formation of angiotensin I (AI) from angiotensinogen and plays a role in the regulation of blood pressure and body fluid homeostasis. Renin has been found in the urine of humans and experimental animals.¹⁻⁷⁾ Bailie *et al.*,⁶⁾ in stop flow studies in dogs, suggested that renin filtered through the glomerular capillaries was metabolized and/or reabsorbed at the proximal tubule, and that renin was added to the tubular fluid at the distal tubule. We have recently purified renin from rat urine and demonstrated that this renin has biochemical and immunological properties compatible with those of renal renin.⁸⁾ These findings indicate that the determination of urinary renin excretion might provide useful information for the biosynthesis and secretion of renal renin. To clarify the significance of assessment of urinary renin excretion, it is essential to estimate quantitatively the excretion rate of renin in the urine and compare it with the plasma renin level and renal renin content. The present study was designed, therefore, to evaluate the urinary excretion of renin, under conditions of altered

sodium intake which is known to negatively affect plasma renin activity and renal renin content.⁹⁻¹²⁾

Materials and Methods

Animal Experiments and Urine Collection — Male Wistar rats weighing about 150 g were used. For 1 week before the study, the rats were fed a standard laboratory rat chow and provided tap water *ad libitum*. The rats were divided into control and two experimental groups (low sodium group and high sodium group). The control group received a standard diet (172 meq sodium/kg diet) and tap water; the low sodium group received a low sodium diet (3.45 meq sodium/kg diet) and distilled water; and the high sodium group received a high sodium diet (1850 meq sodium/kg diet) and 1% NaCl solution. These diets were obtained from Oriental Yeast Co., Tokyo, and consisted of the same components as described in our previous paper.¹⁰⁾ All rats were placed in individual stainless-steel metabolic cages and maintained on standard, low or high sodium intake. After the experimental period of 4 weeks, 24 h urine samples were collected in flasks containing

toluene to prevent bacterial growth. After the exclusion of toluene, the urine was centrifuged at 3000 rpm at 4 °C for 10 min to remove solid debris and stored at -20 °C to prevent possible cryoactivation.^{13,14)}

Processing of Blood Samples and Preparation of Renal Cortical Extract — After urine collection, the peritoneal cavity of the rat was opened under pentobarbital anesthesia (40 mg/kg, i.p.). The renal artery and vein were ligated, and both kidneys were removed and immediately cooled in ice-cold physiological saline. An arterial blood sample was withdrawn through the aorta with a syringe wetted with heparin and centrifuged at 3000 rpm at 4 °C for 15 min. The kidney cortex was removed from the medulla, sectioned into thin slices and transferred into 50 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose (10%, w/v). Homogenization was performed in a Potter-Elvehjem homogenizer at 1800 rpm at 4 °C for 3 min. The homogenate was centrifuged at $105000 \times g$ at 4 °C for 60 min, and the supernatant was used as the renal cortical extract. The obtained plasma and renal cortical extract were stored at -20 °C until analyzed.

Renin Assay — Renin activity in the urine, plasma and renal cortical extract was measured by radioimmunoassay of AI¹⁵⁾ generated after incubation with partially purified homologous renin substrate, which was prepared from the plasma of nephrectomized rats according to the procedure described previously.^{10,16)} The incubation medium consisted of: 1) samples containing renin, 200 μ l; 2) renin substrate (400 ng as AI equivalent), 50 μ l; 3) 0.1 M sodium phosphate buffer (pH 7.0) containing 15 mM disodium ethylenediaminetetraacetic acid and 0.1% (w/v) neomycin, 730 μ l; 4) 20 mM phenylmethylsulfonyl fluoride, 10 μ l; 5) 17 mM 8-hydroxyquinoline sulfate, 10 μ l. The mixture was incubated at 37 °C for 0.5–3 h and then placed in a boiling water bath to terminate the renin reaction. Renin activity was expressed as the amount of AI generated per ml of the sample per hour of incubation.

Acidification — Aliquots of renin-containing samples were dialyzed against 50 mM glycine-HCl buffer (pH 3.0) containing 0.1 M

NaCl at 4 °C for 24 h, and then neutralized by dialysis against 50 mM sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl at 4 °C for 24 h. As a control, a sample was dialyzed separately against 50 mM sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl at 4 °C for 48 h.

Determination of Molecular Weight of Renin — The molecular weight of renin was estimated by gel filtration. A portion (1.5 ml) of the sample was applied to a Sephadex G-100 column (1.6 $\times$ 95 cm, Pharmacia) previously equilibrated with 40 mM sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl and 0.02% (w/v) NaN₃ as an antimicrobial agent. The column was washed with the same buffer at a flow rate of 8 ml/h, and 1 ml fractions were collected at 4 °C. Void volume of the column was estimated by using blue dextran. Bovine serum albumin (M.W. 67000), ovalbumin (M.W. 43000), α -chymotrypsinogen A (M.W. 25000) and ribonuclease A (M.W. 13700) were used as molecular weight standards.

Determination of Electrolyte Concentration — Sodium and potassium concentrations in the plasma and urine were measured by flame photometry.

Statistical Analysis — The Student's *t*-test was used to determine whether differences between the experimental and control groups were significant. Differences were not considered significant if $p > 0.05$.

Results

After the experimental period of 4 weeks, no significant difference could be detected in average body weight between the control group and experimental groups, although the experimental groups (low sodium group; 282 ± 11.9 g, high sodium group; 307 ± 5.78 g) weighed less than the control group (319 ± 7.82 g). Urinary sodium excretion was significantly decreased by low sodium intake and increased by high sodium intake. Urine volume was significantly increased by either low or high sodium intake. However, there were no significant changes in plasma sodium or potassium level and urinary potassium excretion between the control group and the two experimental groups (Table I).

TABLE I. Effect of Sodium Intake on Urine Volume, Plasma Concentration and Urinary Excretion of Electrolytes

	Control	Low sodium	High sodium
Plasma sodium (meq/l)	138.1 ± 1.03	137.3 ± 1.69	138.8 ± 0.88
Plasma potassium (meq/l)	3.90 ± 0.27	4.28 ± 0.24	3.91 ± 0.28
Urine volume (ml/d/100 g B.W.)	5.12 ± 0.54	8.75 ± 1.08 ^{a)}	37.72 ± 2.22 ^{b)}
Urinary sodium excretion (meq/d/100 g B.W.)	0.511 ± 0.037	0.012 ± 0.0004 ^{b)}	12.2 ± 0.37 ^{b)}
Urinary potassium excretion (meq/d/100 g B.W.)	0.683 ± 0.019	0.729 ± 0.036	0.750 ± 0.024

Each value represents the mean ± S. E. of nine animals. Values are significantly different from the control value; ^{a)} $p < 0.01$, ^{b)} $p < 0.001$.

TABLE II. Effect of Sodium Intake on Urinary Renin Excretion, Plasma Renin Activity and Renal Renin Content

	Control	Low sodium	High sodium
Urinary renin excretion (ng AI/h/d/100 g B.W.)	47.9 ± 6.36	774 ± 208 ^{a)}	9.58 ± 1.76 ^{b)}
Plasma renin activity (ng AI/ml/h)	6.33 ± 1.23	96.4 ± 16.3 ^{b)}	1.59 ± 0.464 ^{b)}
Renal renin content (μg AI/h/mg wet tissue)	0.539 ± 0.097	1.37 ± 0.179 ^{b)}	0.182 ± 0.022 ^{b)}

Each value represents the mean ± S.E. of nine animals. Values are significantly different from each control value; ^{a)} $p < 0.01$, ^{b)} $p < 0.001$.

TABLE III. Effect of Acidification on Renin Activity in the Urine, Plasma and Renal Cortex from Rats Maintained on Low and High Sodium Intakes

	Dialysis	Urine (ng AI/ml/h) ^{a)}	Plasma (ng AI/ml/h) ^{a)}	Renal cortex (μg AI/ml/h) ^{b)}
Control	pH 7.4/7.4	6.71 ± 1.27	6.57 ± 1.42	39.5 ± 8.60
	pH 3.0/7.4	7.50 ± 1.46	6.55 ± 1.33	40.8 ± 12.3
Low sodium	pH 7.4/7.4	74.9 ± 18.3	95.1 ± 17.2	89.2 ± 16.3
	pH 3.0/7.4	74.8 ± 21.7	99.7 ± 20.7	85.2 ± 14.1
High sodium	pH 7.4/7.4	0.27 ± 0.11	1.63 ± 0.68	10.2 ± 2.28
	pH 3.0/7.4	0.33 ± 0.17	2.26 ± 0.76	12.7 ± 2.72

Each value represents the mean ± S.E. of nine animals. ^{a)} Renin activity was expressed as ng of AI per ml of urine and plasma per hour. ^{b)} Renin activity was expressed as μg of AI per ml of extract of renal cortex per hour.

The effect of sodium intake on urinary renin excretion, plasma renin activity and renal renin content is shown in Table II. Low sodium intake for 4 weeks resulted in a 16-fold increase in urinary renin excretion and led to a 15-fold increase in plasma renin activity. A 2.5-fold increase in renal renin content was simultaneously ob-

served. In contrast, high sodium intake for 4 weeks decreased urinary renin excretion, plasma renin activity and renal renin content to about 20%, 25% and 35% of the control value, respectively.

We next examined whether an acid-activatable form of renin would be induced under

conditions of altered sodium intake. As shown in Table III, no significant change of renin activity after acidification was observed in the urine, plasma and renal cortex of rats maintained on standard, low or high sodium intake.

Furthermore, we determined the molecular weight of renin in the urine, plasma and renal cortex from the rats maintained on altered sodium intake. As shown in Fig. 1, when each urine sample from the three groups was applied to a Sephadex G-100 column, renin activity showed the same single peak with an apparent molecular weight of 40000. No other peaks of renin activity were observed on each chromatogram. These data indicate that renin with a molecular weight of 40000 is excreted into the urine, under conditions of altered sodium intake. The rats maintained on standard or low sodium intake had renin with a molecular weight of 40000 in the plasma and renal cortex, as reported elsewhere.¹⁷⁾ The same results were obtained in the rats maintained on high sodium intake.

Discussion

The present study showed that the amount of sodium intake negatively affects urinary renin excretion, as well as plasma renin activity and renal renin content. We also found that only active renin with a molecular weight of 40000 was excreted into the urine of rats, under conditions of altered sodium intake.

Several investigators¹⁸⁻²¹⁾ have reported that an acid-activatable form of renin is present in the plasma and kidney extract of humans and experimental animals. Although the physiological role of this acid-activatable form of renin is not completely understood, plasma levels of the acid-activatable renin were shown to differ under various pathological conditions.²⁰⁾ In normal rats, Morris and Johnston¹⁸⁾ observed the presence of an acid-activatable form of renin in the plasma and renin granules, whereas Sagnella *et al.*²²⁾ and Keijzer *et al.*²³⁾ failed to find the acid-activatable form. Although there has been no satisfactory demonstration for this discrepancy, Sagnella *et al.*²²⁾ have explained it by methodological differences such as the use of

Fig. 1. Gel Filtration Profiles of Renin in the Urine

Urine samples from the control (a), low sodium (b) and high sodium (c) groups were appropriately concentrated by using an Amicon YM-10 membrane. Each sample was applied to a Sephadex G-100 column (1.6 × 95 cm), as described in Materials and Methods. The elution positions of standard proteins are indicated by arrows; void volume (Vo), bovine serum albumin (BSA), ovalbumin (OVA), α -chymotrypsinogen A (CTA) and ribonuclease A (RNA).

homologous renin substrate. Using homologous renin substrate, we have also observed that no significant change in renin activity after acidification is detected in the plasma, renal cortical cytosol and renin granules from normal and sodium-restricted rats.¹⁷⁾ The present study supported this observation, and further showed that an acid-activatable form of renin was absent in the plasma and renal cortex, even under conditions of high sodium intake. In addition, only active renin with a molecular weight of 40000, the value being identical with that of renin in the plasma and renin granules,¹⁷⁾ was observed in the urine from rats maintained on standard, low

or high sodium intake. All these data taken together suggest that an active form of renin (M.W. 40000) stored in the kidney is secreted into the blood and urine without any changes in the molecular weight.

It is well known that alteration of sodium balance affects renal renin content.¹⁰⁻¹² Iwao and Michelakis¹² showed that the incorporation of tritium labeled leucine into the renin of renal cortical slices from mice fed a low sodium diet was higher than that from mice fed a high sodium diet. They suggested that changes of sodium balance affected the biosynthesis of renin which led to changes of renal renin content. Accordingly, our data which showed that renal renin content was altered by the amount of sodium intake would result from changes in the level of renin biosynthesis in the kidney. In humans and intact animals, changes in renin secretion from the kidney have traditionally been deduced by measuring plasma renin level. We observed that plasma renin activity was also altered by the amount of sodium intake. These findings suggested that sodium intake negatively affects both the biosynthesis and secretion of renal renin.

In the present study, urinary renin excretion was found to increase 16-fold of the control level by low sodium intake and to decrease to about 20% of the control level by high sodium intake. These values were similar to those obtained in plasma renin activity. This indicates that urinary renin excretion is well correlated with plasma renin activity which is considered to be an index of renin secretion from the kidney, under conditions of altered sodium intake.

However, the question remains as to whether the measurement of urinary renin excretion is useful as an index of renin secretion from the kidney, under various pathophysiological conditions. Kim *et al.*²⁴ have recently studied the fate of circulating renin by using highly purified ¹²⁵I-labeled rat renal renin, and showed that both the liver and the kidney are responsible for the clearance of circulating renin, with participation of the liver being predominant. In fact, it has been found that the reduced hepatic clearance contributes to increased plasma renin activity in canine experimental models for high-

output heart failure.²⁵ The elevated plasma renin activity has also been observed in patients with liver cirrhosis.²⁶ These findings imply that changes in plasma renin level also occur as a result of changes in the hepatic clearance of renin. On the other hand, it is likely that urinary excretion rate of renin is independent of the hepatic clearance of renin, on the basis of the findings that renal renin may be added to the tubular fluid at the distal nephron.⁶ Therefore, urinary renin excretion may reflect renin secretion from the kidney, even under conditions of altered hepatic clearance of renin.

Further studies, under conditions of altered hepatic clearance of renin, is required to clarify the significance of measurement of urinary renin excretion.

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