Effects of Sodium Depletion on Inactive and Active Renin from Dog Kidney and Plasma

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SUMMARY The relationship of active renin and inactive renin (trypsin-activated angiotensin-I-forming enzyme) to sodium depletion was examined in renal and peripheral plasma and at the subcellular level in the kidneys of dogs. Subcellular fractionation was carried out by discontinuous sucrose density (1.5 and 1.6 M) centrifugation. Sodium depletion selectively caused a six- to sevenfold increase in the renal content of inactive and active renins in the original homogenate, while the subcellular distribution patterns of these enzymes were little changed. Of the total granule fractions of 1.5 M sucrose (F1), 1.6 M sucrose (F2), and sediment (F3), approximately 80% of inactive renin was recovered in F1, which was rich in microsomes, while about 50% of active renin was in F2. The ratio of inactive to active renin was 0.02 in F1 and 0.003 to 0.004 in F2. Sodium depletion also caused a 20-fold increase in active renin and a twofold increase in inactive renin in peripheral plasma. The renal venous-arterial concentration difference of inactive renin was statistically significant in low-sodium dogs, although it was not significant in controls. The ratio of inactive to active renin was 0.2 to 0.4 in plasma from low-sodium dogs, while it was 1.5 to 3 in plasma from control dogs. These results suggest that plasma inactive renin originates, at least in part, in the kidney.

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Key Words • active renin • inactive renin • renin granules • sodium depletion • trypsin

It is now widely accepted that human plasma contains an inactive form of renin, which can be activated with trypsin. However, little is known of the physiological mechanisms controlling the activation of inactive renin. Recently, inactive renin was detected in human kidney extracts and also in the renin granules. Plasma inactive renin is thought to originate, at least to some extent, in the kidney, although an extrarenal origin has also been suggested. In laboratory experiments, inactive renin was detected in dog and rat plasma and dog and hog kidneys.

It is well known that plasma active renin usually reflects the active renin content of the kidney. In particular, sodium intake is a major determinant in controlling the active renin in plasma and kidney. However, only limited information exists on the responses of inactive renin to sodium depletion in both the plasma and kidney. As renin is known to be synthesized in a precursor form in the microsomal fraction, the inactive renin content of the kidney should also be examined at subcellular levels. Therefore, we have evaluated the effect of sodium restriction on renin concentration of renal and peripheral plasma and on subcellular distribution of kidney renin.

Material and Methods

Experimental Protocol

Sixteen male mongrel dogs weighing 18 to 20 kg were kept in metabolic-balance cages and given tap water ad libitum. For over 2 weeks before the study, the animals were fed a standard laboratory dog chow (Japan Clea Company, Osaka, Japan) containing 57.5 mEq sodium and 88 mEq potassium per daily intake. Each daily urine collection was analyzed for sodium and potassium contents. At the beginning of the study, these animals were separated into low-sodium and normal-sodium (control) groups of eight dogs each. In the

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low-sodium group, sodium depletion was accomplished by feeding a low-sodium diet containing 5 mEq sodium and 76 mEq potassium per daily intake (Japan Clea Company) for 10 days and by administering 40 mg furosemide intravenously for the first 5 days of the low-sodium diet. The control group was given standard laboratory dog chow for 10 days.

Blood Sampling and Preparation of Subcellular Fractionation of Kidney

Blood samples were collected into tubes containing EDTA, immediately cooled, and centrifuged at 4°C. The plasma was promptly frozen and stored at −70°C. On the last day (Day 0) before and the 11th day (Day 11) after separation into two groups, blood samples were obtained from the jugular vein of the conscious dogs. On Day 11, the dogs were anesthetized with sodium pentobarbital (30 mg/kg), and a flank incision was made to expose the left kidney. A cannula was inserted into the renal vein through the left splanic vein. Cannulas were also inserted into the jugular vein and femoral artery. After blood samples from these vessels had been collected, the kidneys were excised and immediately washed by gentle infusion of 50 ml of isotonc saline solution through the renal artery. The kidney cortex was sliced and gently homogenized with 0.45 M sucrose (1:7, wt/vol), as previously reported. After centrifugation of the homogenate at 500 g for 10 minutes, 30 ml of the supernatant (original homogenate) was placed on a discontinuous sucrose-density-gradient solution (two 15 ml layers each of 1.5 and 1.6 M sucrose). This gradient was selected because dog renin granules are mainly recovered in the interphase of 1.5 and 1.6 M sucrose. After centrifugation at 60,000 g for 90 minutes, the gradient had two visible bands and some sediment. The gradient was separated into three fractions, F0 (volume 25.2 ± 0.3 ml), F1 (volume 12.6 ± 0.2 ml), and F2 (volume 18.9 ± 0.2 ml) from the top of tube, by using a ISCO Model 185 fractionator (Instrumentation Specialties Company, Nebraska). The sediment was resuspended in 10 ml of 0.45 M sucrose and was designated F3.

Separation of Inactive Renin from Active Renin and Activation of Inactive Renin

The concentration of inactive renin was determined as previously described. Subcellular fractions were diluted with 1 volume of 0.05 M phosphate buffer that contained 0.1% Triton X-100 and 0.15 M NaCl (pH 7.2), and the preparation was centrifuged at 100,000 g for 30 minutes. The supernatant was used for measuring the amount of inactive renin. To remove the active renin, 5 ml of the supernatant or plasma was applied to a pepstatin-column. The breakthrough fraction was concentrated into 5 ml, and the aliquot was incubated with trypsin at 37°C for 0, 1, 5, 15, 30, and 60 minutes. Trypsin concentration was 1000 µg/ml in plasma, or 100 µg/ml in the case of subcellular fractions of the kidneys. After the reaction was stopped by the addition of soybean trypsin inhibitor, the renin activity of each sample was measured. The maximum value of renin activities obtained at each incubation time was defined to be the concentration of inactive renin. No activity was detected in 0 time incubation samples.

Measurement of Renin Activity

Tubes containing 50 µl portions of the sample, 20 µl of nephelometric dog renin substrate partially purified by the method of Morimoto et al. (maximum amount of angiotensin I (Al) that could be liberated = 100 ng), 10 µl of phenylmethyl-sulfonlyfluoride (250 mM), and 170 µl of phosphate buffer (0.1 M, pH 7.0) together with EDTA (15 mM), dimercaprol (4, 8 mM), and 8-hydroxyquinoline sulfate (2.4 mM) were incubated at 37°C for 1 hour. The reaction was stopped by immediate cooling (0°C). The quantity of Al generated was determined by radioimmunoassay (CEA-IRE-SOLIN, Italy), according to the method of Haber et al. Angiotensin I in a sample was measured before incubation, and this value was used as a baseline value.

Other Enzyme Activities and Protein

Succinate dehydrogenase (EC 1.3.99.1.) (a marker for mitochondria) was assayed according to the method of Slater and Bonner. N-acetyl-β-glucosaminidase (EC 3.2.1.30) (lysosomes) was assayed by the method of Leaback and Walker. Gamma-glutamyltranspeptidase (EC 2.3.2.2) (brush border membranes) was assayed with a commercial kit of γ-GTP C Test Wako (Wako Pure Chemicals, Osaka, Japan) in which γ-L-glutamyl-p-diethylaminoanilide and glycyglycine were used as a substrate. Glucose-6-phosphatase (EC 3.1.3.9) (microsomes) was assayed by the method of Morimoto et al. in which glucose-6-phosphate was used as a substrate, and the released inorganic phosphate was determined by a modification by Chen el. The method of Fiske and Subbarow. The protein content of the fraction was determined by the method of Lowry et al., with bovine serum albumin as the standard.

pH Profiles of Active Renin and Partially Purified Trypsin-Activated Renin

The F2 fraction obtained from low-sodium dogs was used for active renin. The specific activity was 10 ng/hr/µg protein. Plasma and homogenate of kidney cortex from low-sodium dogs were used for partial purification of plasma and kidney trypsin-activated renin. A portion (10 ml) of each sample was applied to the pepstatin column (2.5 × 30 cm), the breakthrough fraction was concentrated, and then was treated by trypsin as described above. The trypsin-treated sample was applied to the pepstatin column. The bound protein was eluted by 0.5 M Tris-acetate buffer (pH 7.5), and the buffer was substituted for the 50 mM phosphate buffer (pH 7.2) while the eluate was concentrated. The specific activities were 172 and 221 ng/hr/µg protein in plasma and kidney trypsin-activated renin, respectively.
Measurement of Electrolytes

Serum and urine Na\(^+\) and K\(^+\) concentrations were measured on a flame photometer (Hitachi 775, Hitachi Scientific Instruments, Tokyo, Japan).

Statistics

Statistical analyses were performed with Student’s \(t\) test for paired and unpaired data. Differences of \(p < 0.05\) were considered statistically significant. The mean and standard error are indicated.

Results

Basal Values of Low-Sodium and Control Dogs

The mean change in body weight, plasma and urine electrolytes, and hematocrit were examined in low-sodium and control dogs. In low-sodium dogs, sodium depletion caused a body weight loss (19.2 ± 0.3 to 18.0 ± 0.2 kg), hyponatremia (146 ± 1 to 140 ± 1 mEq/liter), lack of urinary sodium excretion (46.1 ± 2.9 to 0 mEq/day) and hemoconcentration (43 ± 1 to 48 ± 1 vol%). These values were statistically significant (\(p < 0.05\), paired \(t\) test). Plasma potassium concentration (4.38 ± 0.11 to 4.31 ± 0.14 mEq/liter) and urine potassium excretion (73.1 ± 4.8 to 71.6 ± 4.1 mEq/day) were unchanged. In control dogs, there were no changes in any basal values (data not shown).

Fractionation of Cortical Homogenate of Kidney in Low Sodium and Control Dogs

Figure 1 shows specific enzyme activities and protein concentration in the original homogenate of the renal cortex of low-sodium and control groups. Sodium depletion caused a six- to sevenfold increase in specific activity of both active and inactive renin, while little change was observed in the specific activity of other enzymes or in protein concentration. Inactive renin comprised about 0.7% to 0.8% of the total renin activity (inactive renin + active renin) in the original homogenate in low-sodium and control dogs.

Figure 2 shows the subcellular distribution patterns of enzyme activities and protein contents in the renal subcellular fractions from low-sodium and control dogs. Enzyme activities and protein content are expressed as a percentage of the total recovered from all fractions (F0 + F1 + F2 + F3). Although the content of both active and inactive renin was markedly increased by sodium depletion, the distribution patterns of active and inactive renins were similar in low-sodium and control groups. Sodium depletion appeared to cause no significant changes in the distribution patterns of other enzyme activities or protein content. Of total granular fractions (F1 + F2 + F3), about 50% of active renin was recovered in F2 while about 80% of inactive renin was recovered in F1. Glucose-6-phosphate, \(\gamma\)-glutamyl transpeptidase, and succinate dehydrogenase showed the highest activity in F1, which contained more than 80% in total granular fractions. N-acetyl-\(\beta\)-glucosaminidase showed the highest activity in F3. Inactive renin comprised about 1% to 2% of the total renin in F1 and about 0.2 to 0.3% of the total renin in F2.

Plasma Inactive and Active Renin in Dogs on Control and Low-Sodium Diets

The concentration of inactive or active renin in blood samples from the jugular vein of conscious dogs on Day 0 was 8.31 ± 1.33 ng/ml/hr or 2.81 ± 0.50 ng/ml/hr in the control group and 8.18 ± 1.29 ng/ml/hr or 2.63 ± 0.47 ng/ml/hr in the low-sodium group. These values were not significantly different. Table 1 shows the mean concentrations of inactive and active renin in the plasma of conscious vs anesthetized dogs fed control or low-sodium diets. In the anesthetized control or low-sodium dogs, there was about an 80% increase in the concentration of active renin and no change in inactive renin in samples from the jugular vein. In control dogs, there were statistically significant differences in the renal venous-arterial concentrations of active renin, but the levels of inactive renin were not statistically significant. Sodium depletion resulted in a 16-fold increase of active renin and a two-
Fold increase in inactive renin in blood samples from the jugular vein of conscious dogs. Differences in the renal venous-arterial concentrations were evident with both active and inactive renin under conditions of sodium depletion. The ratio of inactive to active renin was about 0.2 to 0.4 in the plasma from low-sodium dogs and about 1.5 to 3 in the control dogs (Table 1).

### Table 1. Inactive and Active Renin in the Plasma of Control and Low-Sodium Dogs on Day 11

<table>
<thead>
<tr>
<th>Dog</th>
<th>Plasma renin</th>
<th>Conscious dogs</th>
<th>Anesthetized dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active (A)</td>
<td>Jugular vein</td>
<td>Artery</td>
</tr>
<tr>
<td>Control</td>
<td>Inactive (I)</td>
<td>7.97 ± 1.36</td>
<td>8.31 ± 1.02</td>
</tr>
<tr>
<td></td>
<td>Active (A)</td>
<td>3.46 ± 0.45</td>
<td>6.11 ± 0.76*</td>
</tr>
<tr>
<td></td>
<td>I/A</td>
<td>2.66 ± 0.56</td>
<td>1.55 ± 0.29*</td>
</tr>
<tr>
<td>Low-sodium</td>
<td>Inactive (I)</td>
<td>16.68 ± 1.66</td>
<td>16.60 ± 1.66</td>
</tr>
<tr>
<td></td>
<td>Active (A)</td>
<td>56.59 ± 7.57</td>
<td>101.75 ± 13.31*</td>
</tr>
<tr>
<td></td>
<td>I/A</td>
<td>0.35 ± 0.08</td>
<td>0.20 ± 0.05*</td>
</tr>
</tbody>
</table>

Units are ng of angiotensin I/ml/hr. Values are means ± se of eight experiments.

* p < 0.01, compared to the value obtained from the jugular vein in conscious dogs (paired t test).

† p < 0.01, compared to the value obtained from artery (paired t test).

### pH Profiles of Active Renin and Partially Purified Trypsin-Activated Renin

As shown in Figure 3, the pH optima of partially purified trypsin-activated renins from plasma and kidney were identical to that of active renin. These enzymes reacted optimally with homologous renin substrate at around pH 7.0.
INACTIVE RENIN IN DOG KIDNEY AND PLASMA/Kawamura et al.

PLASMA TRYSIN-ACTIVATED RENIN
KIDNEY TRYSIN-ACTIVATED RENIN
ACTIVE RENIN

FIGURE 3. The pH profiles of active renin and partially purified trypsin-activated renins from plasma and kidney. Active renin and partially purified trypsin-activated renins were prepared as mentioned in the text. The incubation buffers used with renin substrate were 0.1 M citrate-HCl buffer (-x-) and 0.1 M phosphate buffer (-o-).

Discussion

In the present study, we found that responses of active and inactive renin to sodium depletion occurred concurrently in the plasma and kidney. In the subcellular distribution of kidney homogenate from low-sodium and control dogs, renin granules were mainly recovered in the fraction corresponding to 1.6 M sucrose (F2). Conversely, with regard to the granular fractions only (F1-3), inactive renin was mainly recovered in 1.5 M sucrose fraction (F1). This fraction was rich in microsomes containing rough-endoplasmic reticulum in which secretory protein is synthesized. As it is known that renin is synthesized in a precursor form, the inactive renin in F1 may be a precursor form of active renin. There are two explanations for low amounts of inactive renin in renin granules (F2). One is that inactive renin may be activated during transfer to storage granules. Another is that inactive renin may not be stored, but directly secreted. Fray and Park et al. suggest the existence of a renin secretion mechanism in which synthesized renin is not stored within the juxtaglomerular cells but, rather, is released quickly. Sodium depletion caused a marked increase in both active and inactive renin, while other marker enzymes and protein content were not changed, which indicates that both forms of renin are increased selectively by sodium depletion. As the distribution patterns of active and inactive renins were not changed, the increase in the renal content of both forms of renin may result from the stimulation of renin synthesis followed by an increase in the concentration of the stored renin.

Concomitant with the increase in the renal content of both active and inactive renin, sodium depletion also increased active and inactive renin in plasma (Figure 4). Although the renal venous and arterial concentrations of inactive renin were not significantly different in control dogs, the renal venous-arterial concentration difference of inactive renin was significantly altered in sodium-depleted dogs. In biochemical studies, trypsin-activated renins from plasma and kidney and active renin had an affinity for pepstatin, and these enzymes showed similar pH profiles (Figure 3). The activity of acid protease in the breakthrough fraction of the original homogenate on the pepstatin column was not increased by sodium depletion. The enzyme activities were not increased by trypsin treatment (Kawamura et

FIGURE 4. Ratio of inactive or active renin in low-sodium dogs to the corresponding value of each fraction and plasma in control dogs. The value of renin activities (fraction volume \times enzyme activity) in low-sodium dogs was divided by the corresponding value of each fraction in control (normal-sodium) dogs. The value of concentrations of active or inactive renin in low-sodium dogs was divided by the corresponding value in the original homogenate and plasma in control dogs. These data were obtained after the dogs had been anesthetized.
Acknowledgments

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