Laboratory Studies

Urinary Excretion of Renin and Its Biochemical Properties in Dogs

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SUMMARY The amount and biochemical properties of renin excreted by anesthetized dogs were investigated to elucidate the significance of urinary excretion in the metabolism of renin. Mean arterial blood pressure was 127 ± 4 mm Hg, renal blood flow was 170 ± 8 ml/min, glomerular filtration rate, 38.6 ± 2.3 ml/min, and urine flow rate, 0.37 ± 0.09 ml/min (n = 11). Urinary renin concentration (URC) was 9.2 ± 2.1 ng angiotensin I (ANG I)/ml-hr (n = 11), as determined by radioimmunoassay for ANG I generated by incubation with semipurified homologous renin substrate. The ANG I-producing activity was inhibited by more than 90% by a specific antibody to dog kidney renin. The renin secretion rate from the left kidney into the renal vein was 76.4 ± 13.3 ng ANG I/ml-hr per min (n = 11), and the simultaneous urinary excretion rate of renin was 2.3 ± 0.4 ng ANG 1/ml-hr per min (n = 11). Molecular weight of the urinary renin was 40,000 daltons. The pH dependent curves of the angiotensin-forming capacity of renin showed an optimum between pH 5.5 to 6.0, and the estimated Michaelis constant was 0.42 /J,M. These biochemical parameters were similar to the findings in the case of renin in the plasma and the kidney. Moreover, neither acid nor trypsin treatment altered the renin activity in the urine. Thus, the active form of renin with a molecular weight 40,000 was excreted into the urine in dogs. Urinary excretion of renin was a small percentage of the renin secretion rate, thereby indicating the minor role of urinary excretion in the metabolism of renin. (Hypertension 6: 837-842, 1984)

KEY WORDS • antirenin antibody • renin secretion rate • high performance liquid chromatography

RENIN (EC 3.4.23.15) is excreted into the urine in humans and experimental animals in both normal and pathological conditions." Since the molecular weight of circulating renin is about 40,000 daltons, it should be partially filtered through the glomerular capillaries. A micropuncture study confirmed that renin concentration in the ultrafiltrate in Bowman's capsule was about 20% of the arterial plasma concentration. Using autoradiographic techniques, we found that intravenously injected 125I-labeled renin accumulated mainly in the renal cortex in mice, particularly in the proximal tubules. Bailie et al., in stop-flow studies in dogs, suggested that filtered renin was reabsorbed in the proximal nephron segment. Such evidence led to the conclusion that renin was filtered through the glomerular capillaries, then reabsorbed at the proximal tubule as is the case of other small proteins, and excreted into the urine in small amounts as filtered renin. For an accurate assessment of renin metabolism, it is essential to estimate quantitatively the amount of renin excreted in the urine and compare it with the amount of renin secreted from the kidney and with the renin concentrations in the arterial and renal venous plasma.

Biochemical properties of renin in the urine have not been elucidated, although there are reports related to renin in the kidney and plasma. We report herein the physiological and biochemical characteristics of urinary renin in dogs.

Methods

Animal Preparation

Mongrel dogs of both sexes weighing 11 to 17 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and ventilated artificially with a Harvard respirator. The right brachial vein was cannulated for continuous infusion of creatinine and of additional anesthetics. A priming dose of creatinine (100 mg/kg) was given intravenously, followed by a maintenance dose (50 mg/kg/hr). Saline solution was infused via a venous catheter at a rate of 0.5 to 2.0 ml/min throughout the experiment. The right brachial artery was cannulated for arterial blood sampling. A retroperitoneal flank incision was made to expose the left kidney. A
Abbreviations

ANG angiotensin
BP blood pressure
EDTA ethylenediaminetetraacetic acid
GFR glomerular filtration rate
HPLC high performance liquid chromatography
MES morpholinoethanesulphonate
PMSF phenylmethylsulphonylfluoride
PRC plasma renin concentration
RBF renal blood flow
RIA radioimmunoassay
RSR renin secretion rate
UER urinary excretion rate of renin
UF urine flow rate
URC urinary renin concentration

A catheter was placed in the left renal vein through the spermatic or ovarian vein, for the collection of renal venous blood. Renal blood flow (RBF) was measured with an electromagnetic flowmeter (Nihon Kohden, MFV-1200, Tokyo, Japan), and systemic blood pressure (BP) was monitored by a pressure transducer (Model P23ID, Nihon Kohden) connected to a catheter placed in the abdominal aorta, via the right femoral artery. A polyethylene catheter was inserted into the left ureter for urine collections.

Experimental Protocols

Group 1

In 11 dogs, after 60 to 90 minutes of equilibration, urine samples were collected for 30 minutes and used for the assay of urinary renin concentration (URC). At the midpoint of the urine collection, arterial and renal venous blood samples were obtained for determination of plasma renin concentration (PRC) and creatinine.

Group 2

In six dogs, urine samples were taken before and after intravenous injection of dog kidney renin that had been semipurified according to the methods described by Haas et al. Each animal was given 1 ml of the dog kidney renin intravenously, the concentration of which was 18 ng ANG I/ml-hr. The renal hemodynamics remained unchanged following the intravenous injection. Urine was collected for three consecutive 10-minute periods after the injection, and at the midpoint of each period, arterial and renal venous blood samples were obtained.

Assay Procedures

Blood samples of 5 ml were collected into chilled tubes containing ethylenediaminetetraacetic acid (EDTA), centrifuged at 3000 rpm for 10 minutes at 4°C, and then preserved at -20°C. The URC and PRC were measured by radioimmunoassay (RIA) of the ANG I generated during 1 or 3 hours of incubation at 37°C, pH 7.4, with 0.05 M phosphate buffer in the presence of 10 mM EDTA and 2 mM phenylmethylsulphonylfluoride (PMSF) with semipurified dog plasma renin substrate, prepared by the method of Morimoto et al.13 Incubation was terminated by placing the tubes containing the mixture of renin and renin substrate into an ice bath. Renin substrate was added to the incubation mixture at a final concentration of about 250 ng ANG I equivalent/ml. The rate of generation of ANG I was linear in plasma and in dialyzed samples of urine incubated for as long as 3 hours; the generated ANG I levels were 0.5 to 5 ng/ml during the incubation. Since the levels of ANG I generated were much less than the substrate titers, such would be sufficient to compare the amounts of renin in plasma and urine.

Renin concentration was expressed as ANG I equivalent in ng/ml of urine and plasma. The renin secretion rate (RSR) was calculated as follows:

\[ RSR = \frac{\text{renal venous PRC} - \text{arterial PRC} \times RBF \times (1 - Hct)}{100} \]

Before incubation with renin substrate, urine was dialyzed for 24 hours against 0.05 M phosphate buffer, pH 7.4, containing 0.1 M NaCl at 4°C, to adjust the osmolarity and pH of the urine samples. The renin-substrate reaction may be influenced by the high osmolarity of urine, 750-1300 mOsm/kgH2O. The urinary excretion rate of renin (UER) was calculated by multiplying URC and urine flow rate (ml/min). The glomerular filtration rate (GFR) was estimated by creatinine clearance, and creatinine was determined by colorimetry.14

In four of 11 dogs, identification of urinary renin was made by using antibody15 raised against dog pure renin. The antibody GLN 816 was specific for dog kidney renin and inhibited renin activity at a titer of 1:50,000.15 Urine samples were dialyzed, as described above; 200 µl was incubated with 200 µl of antiserum (1:500) for 30 minutes at 37°C and then further incubated with renin substrate (final concentration: 250 ng ANG I equivalent/ml). As a control, urine was incubated with the vehicle of antiserum, 0.05 M phosphate buffer, and renin activity was determined by RIA of ANG I after the incubation with renin substrate.

Experiments with Excreted Urine from Dogs

Six mongrel dogs were kept individually in metabolic cages, and urine was collected in plastic bottles containing sodium azide. With the use of a Centriflo membrane cone (Model CF25, Amicon, Lexington, Massachusetts), urine was concentrated until the renin concentration was about 50 to 100 ng/ml hr. Concentrates were used for determination of the molecular weight of urinary renin, the alterations of activity after treatment with trypsin, and pH dependency. Molecular weight of urinary renin was estimated by high performance liquid chromatography (HPLC) with a TSK G3000SW column (Toyo Soda, Yamaguchi, Japan).16 Samples in amounts of 100 µl were applied to the column. Fractions of 0.5 ml were collected into ice-cold tubes for the assay of renin activity.

The pH dependency of renin activity was determined in a similar manner with the following buffers at 0.2 M; Gly-HCl for pH 3.0 to 5.0, morpholinoeth-
anesulphonate (MES) for pH 5.0 to 6.5, sodium phosphate for pH 6.5 to 7.5, and Tris-HCl for pH 7.5 to 8.5. The final pH values of each mixture of renin and renin substrate were determined before and after the incubation.

The Michaelis constant (K_m) for the reaction between renin and renin substrate was determined as follows. Substrate concentration was measured by extensive incubation with excess amounts of semipurified dog kidney renin. Samples of concentrated urine were applied to different concentrations of renin substrate, and the preparations were incubated for 1 hour at 37° C. Rates of ANG I generation were linear with time. The K_m was determined by Lineweaver-Burk plots.

To determine whether incubation of urinary renin with trypsin altered the activity of urinary renin, trypsin (Type III, Sigma Chemical Company, St. Louis, Missouri) was added at a final concentration of 25 to 500 Mg/ml, and the mixture was then incubated for 30 minutes at 25° C. The reaction of trypsin was terminated by soybean trypsin inhibitor (Sigma) at a concentration three times higher than that of trypsin. The activity of renin was determined in each mixture by the generation of ANG I.

**Results**

**Urinary Excretion of Renin and Renal Hemodynamics**

The mean systemic BP of 11 anesthetized dogs was 127 ± 4 mm Hg, and the RBF was 170 ± 8 ml/min. The GFR and urine flow rate (UF) were 38.6 ± 2.3 and 0.37 ± 0.09 ml/min, respectively. These values were constant throughout the urine collection period. The URC was 9.2 ± 2.1 ng ANG I/ml-hr, while the protein concentration was 0.59 ± 0.17 mg/ml. Arterial PRC was 3.3 ± 0.6 ng ANG I/ml-hr. To investigate the relationship between URC and urinary protein concentration, URC and the UER of renin were plotted against the urinary protein concentration and excretion rate of protein. While there was no correlation between these values, the UER correlated well with the renal venous PRC and the RSR from the kidney (Figure 1).

To obtain direct evidence that the ANG I-producing activity in urine was caused by true renin, the enzyme activity was determined with or without pretreatment of anti-dog kidney renin antibody. Without antibody, the mean URC in four anesthetized dogs was 7.5 ± 4.5 ng ANG I/ml-hr. After incubation of the reaction mixtures containing urine and antibody solution (1:500), the URC of ANG I measured by RIA was 0.5 ± 0.4 ng ANG I/ml-hr. Thus, over 90% of the ANG I-producing activity in the urine was diminished by kidney renin antibody.

When semipurified kidney renin was injected intravenously to increase the PRC, the BP rose by 6 mm Hg without any change in RBF, GFR, and UF. Arterial PRC increased from 3.1 ± 0.9 to 8.3 ± 0.8 ng ANG I/ml-hr at 10 minutes after injection and then gradually decreased to the preinjection levels. There was a significant increase in UER from 2.1 ± 0.6 to 5.8 ± 1.7 ng ANG I/ml-hr per min (Figure 2). The RSR de-

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**Figure 1.** Correlation between urinary renin excretion rate against renal venous plasma renin concentration (PRC) (left panel) and renin secretion rate (RSR) following intravenous administration of 1 ml of semipurified dog kidney renin (18 fig angiotensin I [Al/mlhr]) in anesthetized dogs. Al = angiotensin I.

**Figure 2.** Arterial (•) and renal venous (○) plasma renin concentration (PRC), urinary excretion rate of renin (UER), and renin secretion rate (RSR) following intravenous administration of 1 ml of semipurified dog kidney renin (18 fig angiotensin I [Al/mlhr]) in anesthetized dogs. Data are shown as means ± SEM. The paired comparison t test was used for statistical analysis.
Biochemical Properties of Urinary Renin

Typical HPLC chromatograms of kidney, plasma, and urinary renin are shown in Figure 4. Activities of renin from three sources in dogs demonstrated a single peak with a retention time of 37 minutes, and the corresponding molecular weight was 40,000 daltons.

Figure 5 shows pH-dependent activity curves of renin from the kidney, plasma, and urine. The pH profiles of the renin activity showed optimum values between pH 5.5 and 6.0 and demonstrated the difference from nonspecific ANG I-forming enzymes in acidic conditions. The estimated $K_m$ of urinary renin was 0.42 $\mu$M and of kidney renin, 0.45 $\mu$M.

Incubation of concentrated urine with different concentrations of trypsin did not alter the rate of ANG I generation (Table 1). Even at 4°C, a 1-hour incubation with trypsin did not lead to activation of renin activity. When urine was titrated to pH 3.3 by 0.1 N HCl, kept

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<thead>
<tr>
<th>Trypsin concentration (mg/ml)</th>
<th>Renin activity (ng ANG I/ml • hr)</th>
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<tr>
<td>0</td>
<td>3.9±0.5</td>
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<tr>
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Data are means ± SEM.
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at 4°C for 1 hour, and the pH reverted to 7.4 by 0.1 N NaOH, there was neither a significant increase or decrease in the rate of ANG I formation. Acid and trypsin treatments did not alter the urinary renin activity, which indicated that an active form of renin was excreted into the urine of these dogs.

Discussion

After incubation of diluted (isotonic) dog urine with semipurified homologous renin substrate, ANG I was produced. Similar results have been reported with urine obtained from different species. It was not confirmed whether the ANG I production was due to renin. Renin substrates can be hydrolyzed and release decapptide ANG I not only by renin, but also by other proteases reportedly present in urine. Therefore, in the present experiments, we examined the ANG I production in urine biochemically, and we confirmed that this production was mostly due to renin, as deduced from the following evidence. First, the pH profile of the renin activity of urine showed a maximum yield of ANG I at pH 5.5 to 6.0, which is consistent with the optimum pH for both plasma and kidney renin. According to Smelby and Burnpus, nonspecific angiotensin-forming enzymes have a favorable acidic pH optima. Second, more than 90% of the ANG I-forming activity disappeared when the dog urine sample was pre-incubated with anti-kidney-renin antibody.

Molecular weight of renin extracted from kidney can be estimated by HPLC with a TSK G3000 SW column. With the same method, molecular weight of urinary renin was determined. The single peak of renin activity obtained corresponded to the molecular weight of 40,000 daltons, which is similar to the molecular weight of renin in the plasma and kidney. This small size renin would to some extent be filtered through glomerular capillaries and would appear in the urine. Iwao et al. reported the urinary excretion of renin in mice following intravenous injection of I-labeled submaxillary gland renin. Most of the I-labeled renin had accumulated in the renal cortex, particularly in the proximal tubules. All these data taken together suggest that the circulating renin is filtered through glomerular capillaries and some escapes from reabsorption at the proximal tubules and appears in the urine.

We reported that dog kidney renin was almost totally in the active form with little detectable inactive renin. These data have been supported by findings of other investigators. However, Kawamura et al. more recently found a small amount of inactive renin in the dog kidney. In the present experiment, urinary renin activity was not increased following acid or trypsin treatment. The existence of small amounts of inactive renin cannot be completely ruled out, because proteases other than trypsin activate inactive renin.

The RSR calculated from arterial and renal venous PRC and renal plasma flow was 76.4 ± 13.3 ng ANG I/ml-hr per min in the present experiment. Although only a small percentage of the amounts of RSR was excreted into urine, UER closely correlated with renal venous PRC (Figure 1). It may be more meaningful to relate UER to glomerular load rather than to renal venous PRC. However, the entire glomerular load of renin cannot be measured, because renin is usually secreted from afferent anterioles and is also loaded onto the glomerulus. Thereafter, renal venous PRC was used as the parameter for comparisons with UER. In any case, urinary excreted renin has a minor, quantitative role in the metabolism of renin.

Using clearance techniques, Bailie et al. altered PRC by experimental maneuvers and reported that the increased UER was associated with an increased PRC. However, maneuvers such as renal arterial pressure reduction and hemorrhagic hypotension influence renal hemodynamics and functions and may result in changes in urinary renin excretion. Therefore, in the present experiment, semipurified homologous renin was injected to increase PRC without any change in RBF and UF. Similar results were obtained in the second group in an in vivo experiment by plotting UER against renal venous PRC (Figure 3) before and after intravenous injection of semipurified dog kidney renin. The PRC increased to double that of the pre-injection levels and was accompanied by an increase in UER. On the other hand, RSR from the kidney decreased, probably due to the negative feedback mechanism of elevated plasma ANG II. Although not determined, the plasma ANG II levels would be elevated because BP increased significantly after the injection of renin.

In conclusion, the UER in anesthetized dogs is much less compared with the amounts of renin secreted from the kidney, thereby indicating a minor role in the metabolism of renin.

Acknowledgments

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