Increased Urinary Kallikrein-like Activity During
ADH-Induced Hyponatremia in Rats

KIMIO TOMITA, TATSUO SHIIGAI, HIROSHI SAITO, YASUHIKO IINO, AND JUGORO TAKEUCHI

SUMMARY The urinary kallikrein system was studied during hyponatremia associated with water and vasopressin administration in rats. Two groups of animals were studied. In the experimental group (n = 5), vasopressin (0.4 U/day) was injected intramuscularly for 7 days, and water (15%–20% body weight per day) was given via a stomach tube. The control group (n = 6) received only vasopressin. In the experimental group, plasma sodium concentration (PNa) decreased from 143.2 ± 0.5 to 130.8 ± 1.8 (m ± SEM) mmol/liter (5th day, p < 0.01) along with plasma osmolality. Urinary kallikrein-like activities (UkaV) increased from 99.1 ± 7.5 to 172.6 ± 23.5 μmol/min/day (100 g body weight) (5th day, p < 0.05; 6th day, p < 0.05; and 7th day, p < 0.05) after the administration of vasopressin. Uric acid clearance (CuA) increased from 0.153 ± 0.014 to 0.275 ± 0.041 ml/min (5th day, p < 0.05; 7th day, p < 0.05). No change was observed in urinary aldosterone excretion (UAldV), creatinine clearance, or blood pressure. UkaV correlated with CuA (r = 0.81, p < 0.01) and with the degree of change of PNa (r = -0.79, p < 0.01), respectively. In the control group, no change was observed in the above parameters. A significant relationship between UkaV and fractional Na clearance (r = 0.60, p < 0.01) was observed. We conclude that the urinary kallikrein system in rats may be stimulated during hyponatremia when induced by water and vasopressin. This increased activity is probably the result of volume expansion associated with water and vasopressin and may have some relationship to fractional Na clearance in the kidney. (Hypertension 6: 511−518, 1984)

Key Words • antidiuretic hormone • volume expansion • vasopressin • sodium clearance • kallikrein • kinin

As a possible factor in the pathogenesis of hypertension, the kallikrein-kinin system has been extensively studied after Margolius et al.1 reconfirmed the early finding by Elliot and Nuzum2 that patients with essential hypertension excreted less kallikrein in the urine than normotensive patients. Studies of urinary kallikrein,3,4 the precise localization of renal kallikrein,5−10 and kinin binding in the kidney11−14 have been reported. However, there are several points of disagreement with regards to the mechanism or physiological role of the kallikrein system. Firstly, the kallikrein-kinin system correlates with urinary sodium excretion in some conditions,15 but not in others.16−18 Secondly, aldosterone directly stimulates kallikrein activity in kidney homogenates, although urinary kallikrein increases only after several days of deoxycorticosterone administration to dogs.19 Thirdly, in two acute experiments,20,21 antidiuretic hormone (ADH) has been reported both to stimulate and not to stimulate urinary kallikrein. Therefore, it would be of interest to evaluate the chronic effects of ADH on the kallikrein-kinin system.

Similar to the syndrome of ADH excess is the syndrome of inappropriate secretion of ADH. In this situation, urinary kallikrein-like activity increased during hyponatremia.22 In addition, sodium clearance correlated with urinary kallikrein-like activity.

We studied the relationship among plasma Na concentration, urinary kallikrein-like proteolytic activity, aldosterone, and electrolyte excretion by vasopressin either alone or with water-loading in rats.

Material and Methods

Rats

Male Sprague-Dawley rats (200–280 g) were kept separate in siliconized glass metabolic cages and fed standard chow (0.28% Na). A 24-hour urine collection began at 1000 in which the rats spontaneously voided into bottles containing toluene as a preservative. The bottles were attached to cages for urine collection and were cooled to 4°C by cooling units. Before the start of the study, a control period of several days was observed.
Control Group
During the control period six rats received 0.4 ml of peanut oil, which is the solvent of vasopressin tannate in oil (Parke Davis), injected into the back muscles in two equal doses at 1000 and 2000 hours. For the next 7 days, 0.4 U (0.4 ml) of vasopressin tannate in oil was similarly administered. After vasopressin administration was stopped, peanut oil was given in the same way as during the control periods. Blood pressure was measured at 1300 hours without anesthesia. Blood samples for analyses of serum Na, K, creatinine, and uric acid were obtained at 900 (on the next morning, the 23rd hour of the period) by jugular venipuncture without surgical procedures or anesthesia. Samples were taken on the 1st day of the control period, on the 5th and 7th days of vasopressin administration, and on the 4th day after vasopressin administration had been stopped.

Experimental Group
Five rats were water-loaded (10 ml x 4 per day; 15%-20% body weight) by stomach tube at 1000, 1100, 1900, and 2000 hours in addition to voluntary intake that started from the control period. As in the control group, vasopressin and peanut oil were injected and blood samples and blood pressure measurements obtained.

Measurements
Plasma and urinary Na, K, creatinine, and uric acid were measured by autoanalyser (Olympus, Tokyo, Japan). Plasma and urinary osmolality was measured by the freezing point method. Urinary aldosterone concentration was measured by a radioimmunoassay (RIA) kit (Midori Jyuji Ltd., Osaka, Japan). Urinary kallikrein-like proteolytic activity was measured fluorometrically by the method of Morita et al.24 A 5 μl sample was mixed with 1 ml of incubation mixture (0.1 mM prolyl-phenylalanyl-arginine-methylcoumarin amide, 100 mM sodium chloride, 50 mM Tris-HCl buffer, pH 8.0) and incubated for 10 minutes at 37° C. Reaction was terminated by adding 1.5 ml of 17% acetic acid. Fluorescence intensity was measured by fluorometer (AMINCO, Chicago, Illinois) with excitation at 380 nm and emission at 460 nm. Values are expressed as μmol of prolyl-phenylalanyl-arginine-methylcoumarin amide hydrolyzed per minute with a standard curve of the reaction product, 7-amino-4-methylcoumarin (AMC). Sensitivity for kallikrein-like activity was above 0.05 nmol-min/ml. Variation was within 4% intra-assay, within 8% between-assay. It has been reported that urinary esterase A2 has some kininogenase activity in the rat.25 However, significant correlation (r = 0.99, p < 0.01, n = 10) was observed between activities measured by this method and the rat urinary kallikrein concentrations kindly measured by John J. Pisano (National Institutes of Health, Bethesda, Maryland) by the RIA method.26 Blood pressure was measured by tail cuff method. Statistical evaluation of the data was performed with analysis of variance.

Results

Plasma Sodium, Potassium, and Osmolality
In the control group, there was no significant change in plasma Na, K, and osmolality following vasopressin (Figure 1 and Table 1). In the experimental group, plasma Na concentration levels were significantly decreased from 143.2 ± 0.5 mmol/liter (mean ± SEM) to 130.8 ± 1.8 mmol/liter on the 5th day (p < 0.01) and to 134.6 ± 1.2 mmol/liter on the 7th day (p < 0.01). Plasma osmolality levels were also decreased from 291.2 ± 3.1 mOsm/kg to 262.8 ± 8.0 mOsm/kg on the 5th day (p < 0.05) and to 277.2 ± 4.8 mOsm/kg on the 7th day (p < 0.05). There was no significant change in plasma K levels.

Body Weight and Urinary Volume, Osmolality, and Excretion of Sodium and Potassium
In the control group, there was no significant change in body weight (BW) during the study except on the 10th and 11th days (Table 1). In the experimental group, a small but significant BW increase was observed following the administration of vasopressin. After vasopressin was discontinued, the significant increase disappeared for the first 2 days. In the control group, urinary volume decreased after the administration of vasopressin except on the 1st day. In the experimental group, urinary volume decreased after the administration of vasopressin. Both in the control and experimental groups, urinary osmolality levels increased following the administration of vasopressin, and there was no significant difference in urinary Na and K excretion. There had been no significant difference in Na intake between the mean values of control days and experimental days in each group.

Creatinine Clearance and Uric Acid Clearance
In the control group, there was no significant change in the creatinine clearance and uric acid clearance during the study. In the experimental group, there was no significant change in creatinine clearance, but uric acid clearance increased significantly from 0.153 ± 0.014 ml/min to 0.275 ± 0.041 ml/min on the 5th day (p < 0.05) and to 0.251 ± 0.036 ml/min on the 7th day (p < 0.05).

Blood Pressure
Both in the control and experimental groups, no significant change in blood pressure was observed during the study.

Urinary Aldosterone Excretion and Kallikrein-like Proteolytic Activity
Both in the control and experimental groups, there was no significant change in urinary aldosterone excretion. In the control group, there was no significant change in urinary kallikrein-like proteolytic activity during the study. In the experimental group, however, kallikrein-like proteolytic activity increased from 99.1 ± 7.5 μmol-min/day (100 g BW, mean value of the control days) to 172.6 ± 23.5 μmol-min/day (100 g BW) on the 5th day (p < 0.05), to 154.0 ± 24.8
Increased urinary kallikrein by ADH and water/Tomita et al. 513

**Figure 1.** Biochemical parameters of control and experimental groups. Posm = plasma osmolality, UAldV = urinary aldosterone excretion, UKaV = urinary kallikrein-like proteolytic activity. UAldV and UKaV are expressed as the values per 100 g body weight. The values of the experimental days and those of the after-experimental days were compared with the mean values of the control days. *p < 0.05; **p < 0.01.

\[ y = 1.50x + 84 \]

\[ R = 0.71 \]

\[ p < 0.05 \]

\[ m \pm SEM \]

\[ UAldV = \text{urinary aldosterone excretion} \]

\[ UKaV = \text{urinary kallikrein-like proteolytic activity} \]

Relationship Between the Urinary Kallikrein-like Proteolytic Activity and Urinary Aldosterone Excretion

We studied the relationship between urinary kallikrein-like proteolytic activity and urinary aldosterone excretion in the control period and in the experimental period on the days in which urinary kallikrein-like activity increased. As shown in Figure 2, there was a significant relationship between urinary kallikrein-like proteolytic activity per 100 g BW and urinary aldosterone excretion per 100 g BW. The regression line obtained from the relationship was shifted upward during vasopressin administration.

Relationship Between Urinary Kallikrein-like Proteolytic Activity and Uric Acid Clearance and Change in Plasma Sodium Concentration in the Experimental Group

A significant relationship was observed between urinary kallikrein-like proteolytic activity and uric acid

\[ y = 1.08x + 55.4 \]

\[ R = 0.47 \]

\[ p < 0.05 \]

\[ \text{URINARY ALDOSTERONE EXCRETION (100g B.W.)} \]

\[ \text{URINARY KALLIKREIN-LIKE ACTIVITY (100g B.W.)} \]

\[ 0 \]

\[ 50 \]

\[ 100 \]
### TABLE 1. Biochemical Parameters of Control and Experimental Groups

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<th>Parameter</th>
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<td>106</td>
<td>±3</td>
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<td>Na intake (mmol/day)</td>
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<td>2014*</td>
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<td>2.56</td>
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<td>±15.8</td>
<td>121.0</td>
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<td>UkaV (μmol·min·day/100 g BW)</td>
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<td>121.7</td>
<td>±8.9</td>
<td>102.3</td>
<td>±12.4</td>
<td>114.3</td>
<td>±15.6</td>
<td>123.3</td>
<td>±11.6</td>
<td>134.1</td>
<td>±16.1</td>
<td>104.6</td>
<td>±15.8</td>
<td>121.0</td>
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BP = blood pressure; BW = body weight; Uosm = urinary osmolality; UNaV = urinary sodium excretion; UkV = urinary potassium excretion; Cua = uric acid clearance; Ccr = endogenous creatinine clearance; UkaV = urinary kallikrein-like proteolytic activity. Values are means ± SEM.

*P < 0.05.
†P < 0.01.
<table>
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<td>3.73 ± 0.20</td>
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<td>2193* ± 0.3</td>
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<td>1.54 ± 0.22</td>
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<td>245* ± 1.9</td>
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<td>13.0† ± 13.0†</td>
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<td>21362* ± 1280</td>
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<td>1.28 ± 0.13</td>
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<tr>
<td>2.09 ± 0.28</td>
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<tr>
<td>0.28* ± 0.28*</td>
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<td>126.2 ± 20.4</td>
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clearance \( r = 0.81, p < 0.01 \) and between urinary kallikrein-like proteolytic activity and plasma sodium concentration \( r = -0.79; p < 0.01 \) (Figure 3).

**Relationship Between Urinary Kallikrein-like Proteolytic Activity and Fractional Sodium Clearance**

In the experimental group, a significant relationship was observed between urinary kallikrein-like proteolytic activity and fractional Na clearance \( (\text{Na clearance/creatinine clearance}) \ (r = 0.60, p < 0.01, \text{Figure 4}) \). No significant relationship was observed between fractional Na clearance and urinary aldosterone excretion.

**Discussion**

Vasopressin had no effect on urinary kallikrein-like activity during normonatremia but increased during hyponatremia, a result compatible with that observed clinically in patients with inappropriate secretion of ADH. Several reports describe an interaction between kallikrein-kinin and ADH, renin-angiotensin-aldosterone, volume expansion, and prostaglandins. Vasopressin induced an increase in kallikrein excretion in conscious dogs and in anesthetized rats. However, kallikrein excretion did not increase during arterial vasopressin infusion in water-loaded dogs. Both of the above experiments were short-term experiments. In our long-term experiment, vasopressin without water-loading did not increase the kallikrein-like activity. The combined administration of water with vasopressin, however, clearly increased urinary kallikrein-like activity. It is probable that factors other than vasopressin itself are important in the increase in urinary kallikrein excretion in our experiment.

There are several reports that aldosterone stimulates urinary kallikrein excretion. Conflicting data have also been reported. It is important to observe urinary aldosterone and kallikrein excretion in a state of volume expansion associated with water and vasopressin. In our experiment, there was no apparent change in urinary aldosterone during volume expansion. The reason for this is not known but may relate to an interaction between the suppressive effect of volume expansion and stimulative effect of hyponatremia on the aldosterone system. Our results showed a significant relationship between kallikrein-like proteolytic activity and urinary aldosterone excretion both during the control period and during increased kallikrein-like proteolytic activity. The regression line obtained on the days of increased kallikrein-like proteolytic activity was shifted upward compared to the value obtained on the control days. Two possible reasons may be considered: the responsiveness of the kallikrein system to the aldosterone system may have been augmented, or the volume expansion may have stimulated the kallikrein system.

Many reports have dealt with the relationship between kallikrein and volume expansion. Kallikrein excretion increased during saline infusion in dogs and rats. Saline infusion had no effect on kallikrein excretion in humans. Kallikrein excretion was increased by water-loading in reports of studies in humans and animals, but not in other reports. It has been suggested that since urinary volume was increased at least three to four times, the increased kallikrein excretion may be due to a concomitant washout phenomenon associated with the increased urine volume. This is unlikely in our case,
because urine volume was decreased by the effect of vasopressin in the period of higher kallikrein excretion. We examined the relationship between kallikrein-like activity and volume factors. Plasma Na concentration is a function of the water intake and of the accompanying fluid retention in a normal subject given vasopressin. A low plasma uric acid level is generally observed in the syndrome of inappropriate secretion of ADH. Uric acid excretion is increased after extracellular fluid volume expansion. In our experiments, the decreases in plasma Na concentration were correlated with increases in urinary kallikrein-like proteolytic activities. Further, uric acid clearances were correlated with the changes in urinary kallikrein-like proteolytic activities. Thus, there may be a positive relationship between volume expansion and urinary kallikrein excretion.

With regard to the relationship between kallikrein and urinary sodium excretion, some data suggest a positive correlation but other data do not. It is important to examine sodium excretion in the light of plasma sodium concentration. The amount of glomerular-filtered sodium has a large effect on urinary sodium excretion because the glomerular-filtered sodium is the main source of urinary sodium. We examined fractional sodium excretion as an index of the capacity of kidney tubules to excrete sodium. Fractional sodium excretion was significantly correlated with kallikrein-like activity, although the total amount of sodium excretion was not. No change was observed in glomerular filtration rate, urinary aldosterone excretion, and blood pressure. The effect of vasopressin itself on fractional sodium excretion can be excluded, because in the control group there was no significant change in plasma Na concentration nor in urinary sodium excretion. The urinary kallikrein system does seem to have some effect on fractional sodium excretion during hyponatremia associated with water and vasopressin administration.

In conclusion, kallikrein-like proteolytic activity increased during hyponatremia associated with waterloading and vasopressin in rats. The increase in activity may be induced by volume expansion but not by vasopressin itself. The increase may have some effects on fractional sodium excretion in the kidney.

Acknowledgments
We thank Dr. John J. Pisano and Patricia Herring (National Institutes of Health, Bethesda, Maryland) for their measurements of rat urinary kallikrein concentration, and Dr. Leonard B. Berman (Director of Medical Education of Saint Joseph Hospital, California) for his valuable criticism and help in preparing the manuscript:

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