Urinary Kallikrein Response to Acute Saline or Water Loads in Hypertensive and Normal Humans

WILLIAM J. LAWTON, M.D.

SUMMARY Urinary kallikrein excretion during acute water or saline loading was studied in normal and hypertensive humans after chronic Na\(^+\) depletion and Na\(^+\) loading to answer the following questions. 1. Is urinary kallikrein a natriuretic or diuretic substance? 2. During acute water or saline loading, does the underlying Na\(^+\) balance influence the urinary kallikrein response? or the relationship between urinary kallikrein and renal Na\(^+\) or water handling? 1) Urinary kallikrein did not change during a 1.2 liter water load given to nine white hypertensive and five white normal men. Urinary kallikrein was significantly decreased, however, in five white hypertensive and five white normal subjects during and after 1 hour of isotonic saline infusion (30 ml/kg). In sodium-depleted hypertensive patients kallikrein excretion was decreased from 19.8 to 9.5 mEU/min, and in Na\(^+\)-depleted normal subjects it was decreased from 15.7 to 12.6 mEU/min (p = 0.003). The response in hypertensive patients was not different from normal subjects. In all Na\(^+\)-loaded subjects, kallikrein excretion was also significantly decreased during isotonic saline infusion (p = 0.01). Urinary kallikrein did not change in three other subjects given hypertonic saline. 2(a) The underlying state of Na\(^+\) balance influenced the baseline level of kallikrein excretion, but not the directional decline in kallikrein during isotonic saline. (b) In Na\(^+\)-restricted hypertensives given isotonic saline, urinary kallikrein was inversely related to the fractional excretion of Na\(^+\) (r = -0.54, p < 0.01) and the tubular reabsorption of H\(_2\)O (T\(\text{H}_2\)O/GFR; r = -0.50, p < 0.01). In Na\(^+\)-loaded hypertensives given isotonic saline, urinary kallikrein was directly related to T\(\text{H}_2\)O/GFR (r = 0.38, p < 0.05). Prior to infusion, the hypertensives who received isotonic saline showed subnormal renin and aldosterone after dietary Na\(^+\) restriction, but normal kallikrein excretion. Factors in addition to mineralocorticoids appear to regulate kallikrein excretion. Urinary kallikrein was not a natriuretic or diuretic factor in normal and hypertensive subjects who received acute water or saline loads. In Na\(^+\)-restricted and -loaded hypertensive and normal subjects, urinary kallikrein was clearly decreased by isotonic saline loading. The state of Na\(^+\) balance influenced the relationship between urinary kallikrein and renal handling of Na\(^+\) and H\(_2\)O. (Hypertension 6: 175-183, 1984)

Key Words • water loading • saline loading • renal Na\(^+\), K\(^+\), and H\(_2\)O excretion • glomerular filtration rate • renal blood flow • tubular reabsorption of water

The role of urinary kallikrein, although still incompletely defined, has been linked to the renal handling of sodium and water. Controversy abounds, however, since some investigators ascribe a natriuretic or diuretic role to urinary kallikrein, while others do not. To clarify the role of kallikrein in salt and water excretion, various investigators have acutely loaded normal subjects with sodium chloride or water and measured the urinary kallikrein response. In some studies in normal subjects, these maneuvers have been associated with increased kallikrein excretion. In others, opposite results have been reported. Urinary kallikrein has been reported to be subnormal in some hypertensives. Surprisingly, investigators have not acutely loaded hypertensive subjects with sodium chloride and water and measured the urinary kallikrein responses. It is reasonable to consider that, in hypertensives, either the kallikrein response to acute salt and water loading is abnormal or the relationship between kallikrein and salt and water excretion may be abnormal. In this study of hypertensive patients and normal subjects, I asked the following questions. 1. Is urinary kallikrein a natriuretic or diuretic factor during acute water or saline loading in

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either group? 2. During acute water or saline loading, does the underlying state of sodium balance influence (a) the urinary kallikrein response in either group or (b) the relationship between kallikrein excretion and renal sodium and water handling in either group?

In answer to Question 1, I found that urinary kallikrein is not natriuretic or diuretic, but significantly declines during acute saline loading in both normal and hypertensive subjects. In answer to Question 2, I found that the underlying state of sodium balance influences the baseline value of urinary kallikrein but not the directional change during saline loading, and that it also influences the relationship between kallikrein excretion and renal sodium and water handling.

Methods

All patients and normal subjects were white men and women recruited from either the Veterans Administration Medical Center or University Hospitals and Clinics, Iowa City. The hypertensive subjects had diastolic pressures greater than 90 mm Hg on at least three sequential outpatient visits, 1 month apart. All subjects underwent a complete medical history and physical examination. Laboratory studies included urinalysis, complete blood count, serum urea nitrogen and creatinine, sodium, potassium, chloride, bicarbonate, calcium, phosphorus, electrolyte determination, and chest x-ray. Additional studies for the hypertensives included 24-hour urine determinations of catecholamines, VMA, metanephrines, sodium and potassium excretion, and creatinine clearance as well as upright plasma renin activity (PRA) and rapid-sequence intravenous urogram. From the above studies, hypertensive patients had no evidence of secondary causes of hypertension nor target organ damage nor other systemic diseases. They were either untreated or had not received medication for at least 2 weeks.

All subjects achieved sodium balance as outpatients of the Clinical Research Center University Hospitals. All subjects were studied at the end of two separate diet periods and received an eucaloric protein-base formula diet containing 80 mEq potassium and either 400 mEq sodium or 10 mEq sodium. Each subject received both diets and the sequence was randomly assigned. Subjects remained on the diets for 5 days and then had a 1-week ad libitum diet interval between the two diet periods. Twenty-four-hour urine specimens were collected in glacial acetic acid for urinary aldosterone on Day 4 and under toluene for kallikrein, sodium, potassium, and creatinine on Day 5.

On Day 6 of each diet period, the subjects underwent standard inulin and para-amino-hippuric acid (PAH) clearance tests to measure glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively. Loading doses of inulin (45 mg/kg) and PAH (8 mg/kg) were given, and a constant infusion of inulin (25 mg/min) and PAH (12 mg/min) in 5 g/dl dextrose/water was maintained.

The different groups of subjects were studied according to three different protocols, which were approved by the Committee on Research Involving Human Beings, College of Medicine, University of Iowa, and the Research Committee at the Veterans Administration Medical Center. Informed consent was obtained from all subjects.

Study 1. Water Loading

Subjects were given 400 ml of water to drink initially and 50 ml every 30 minutes for a total of 750 ml by mouth over 4 hours. The inulin and PAH infusion rate was 2 ml/min for an additional 480 ml of 5 g/dl dextrose/water intravenously over 4 hours. After a 60-minute equilibration period, urines were collected by spontaneous voiding every 60 minutes for 3 hours. The protocol is summarized below.

<table>
<thead>
<tr>
<th>Equilibrium</th>
<th>Period 1</th>
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Intravenous infusion of inulin and PAH in dextrose/water

* = water given orally

Blood drawn at the midpoint of each period. Blood and urine samples were analyzed for inulin, PAH, Na+, K+, osmolality, and creatinine. An aliquot of urine was stored at 4°C under toluene for kallikrein measurement.

Study 2. Infusion of 0.9 g/dl NaCl

Subjects received 400 ml of water to drink initially and 50 ml every 30 minutes. The inulin and PAH maintenance infusion rate was 1 ml/min. All subjects received low-level subpressor infusions of antidiuretic hormone (arginine vasopressin) at 300 mU/hr. After a 1-hour equilibration period, a 30-minute baseline period (Period 1) was obtained followed by two 30-minute test periods (Periods 2 and 3) in which an acute load of 30 ml/kg 0.9 g/dl NaCl was infused. This was followed by three 30-minute postinfusion periods (Periods 4–6, as shown below).

<table>
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<tr>
<th>Equilibrium</th>
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Intravenous infusion of inulin, PAH, and ADH in dextrose/water

* = water given orally

Urine samples were obtained every 30 minutes, and blood was collected at the midpoint of each period for measurements as in Study 1.

Study 3. Infusion of 3.0 g/dl NaCl

These subjects followed a protocol similar to that of Study 2 for the oral water intake and inulin, PAH, and...
ADH infusion. However, they received 2 liters of 3.0 g/dl NaCl infused over a 3-hour period, as shown below.

<table>
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<tr>
<th>Equilibrium</th>
<th>1</th>
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<td>60 min</td>
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- Intravenous infusion of inulin, PAH, and ADH in dextrose/water
- Intravenous infusion of 3.0 g/dl NaCl

* = water given orally

Urine was obtained every 30 minutes, and blood was collected at midpoint as above.

**Chemical Methods**

Urinary kallikrein was measured by the method of Margolius et al. using a radiolabelled tracer technique with the substrate p-tosyl-l-arginine-3H-methyl ester (57 mCi/mmol) in H-TAME, New England Nuclear, Boston, Massachusetts. The value of our standard human urinary kallikrein (HUK) has been previously reported; 1 μl of our standard HUK (1:30) hydrolyzes 1.0271 x 10⁻³ μM (TAME) (EU) per minute in a titrimeic assay at 30°C, pH 8.0. Urine samples were desalted on G-25 sephadex before assay. Urinary kallikrein excretion is expressed in milliesterase units (mEU) or esterase units (EU) per unit of time. In an analysis to check possible sex differences in the 24-hour urinary kallikrein excretion, we compared five normal women to seven normal men on Day 5 of the high sodium diet and also on Day 5 of the low sodium diet. We found no significant differences between sexes in urinary kallikrein excretion.

Plasma renin activity (PRA) was measured in our laboratory by the radioimmunoassay of generated angiotensin I using a modification of the method of Haber et al. (angiotensin I, New England Nuclear). Sodium and potassium were measured by flame photometer. Osmolality was measured by the freezing-point depression method. Inulin was measured by the anthrone method and PAH by the method of Smith et al. Serum aldosterone was measured by radioimmunoassay at Mayo Medical laboratories, Rochester, Minnesota. Serum aldosterone was measured by radioimmunoassay at Nichols Institute of Endocrinology, Los Angeles, California.

Student's t test for unpaired data was used where appropriate to compare hypertensive and normal subjects. Data from Study 2 was analyzed by analysis of variance with subjects nested within groups (hypertensives and normals). Standard formulas were used to calculate fractional excretion of sodium (FESNa = GFR, potassium (FEPot/GFR), and osmolality (FEOsm/GFR), free water clearance (CfH2O = V - Csol), and tubular reabsorption of water (TmH2O = Csol - V). Selected correlation coefficients were determined. Significance was considered at p < 0.05. Group means ± the standard error of the mean (SEM) are presented.

**Results**

**Study 1. Water Loading**

Urinary kallikrein excretion in nine white hypertensive men 28 ± 3 years of age was compared to that in five normal white men 23 ± 1 years of age during acute water loading of 1230 ml (orally and intravenously) over 4 hours. Blood pressures on the clearance days and data from 24-hour urine samples collected the day before the clearance studies are shown in Table 1. There were no significant differences between the normal and hypertensive subjects for urinary Na⁺, kallikrein, aldosterone, or renin values at the end of comparable diet periods.

Data from the acute water-loading studies are shown in Table 2. Period 1 (first hour) and Period 3 (third hour) from the three 1-hour inulin/PAH clearance periods are presented. After each dietary period, the Period 1 urinary kallikrein excretion was not significantly different between normal and hypertensive subjects, nor did the water loading alter urinary kallikrein excretion. The normal and hypertensive men were also similar in the other measured parameters of renal function and Na⁺ and H₂O handling. Free water clearance (CfH₂O/GFR) was similar after both dietary sodium depletion and sodium loading, yet the urinary kallikrein excretion in sodium-loaded subjects was approximately half that of the sodium-depleted subjects. Thus, the absolute kallikrein excretion in Study 1 was determined by the chronic state of sodium balance, was not altered by a mild water load, and was not related to the absolute level of free water excretion.

Correlation coefficients were calculated for urinary kallikrein excretion vs the other parameters of renal function using all three clearance periods. Calculations were made separately for each group after each diet period. The correlations between urinary kallikrein and GFR, RPF, PRA, Na⁺, and water excretion were not significant, with two exceptions: in the sodium-depleted hypertensives during water loading, the uri-

<table>
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<th>Table 1. Study 1: Water Loading - Baseline Data</th>
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<tbody>
<tr>
<td>Baseline data</td>
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<tr>
<td>Chronically sodium-depleted subjects</td>
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<tr>
<td>Blood pressure (mm Hg)</td>
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<tr>
<td>Urine Na⁺ (mEq/24 hr)</td>
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<tr>
<td>Urine kallikrein (EU/24 hr)</td>
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<td>Urine aldosterone (μg/24 hr)</td>
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<td>Plasma renin activity (ng/ml/hr)</td>
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<td>Blood pressure (mm Hg)</td>
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<td>Urine aldosterone (μg/24 hr)</td>
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<td>Plasma renin activity (ng/ml/hr)</td>
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* p < 0.01
depleted subjects, saline infusion produced a decline in

\[ \text{Na}^+ \] infusion is shown in Figure 1. In the Na

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in normals was 2.3 ± 0.3 and in hyper-

tensive patients. Urinary kallikrein was not different

compared to five patients with essential hypertension

(37.6 ± 3.6 years).

Blood pressures on the clearance day and data from 24-
hour urine samples collected the day before the clear-

ance studies. Despite this, in the

gram滤法 rate (mlln/1.73 m\(^2\)). RPF = renal plasma flow

(ml/min/1.73 m\(^2\)). \(V\) = urine flow (ml/min); \(C_{\text{Na}^+}/\text{GFR}\) = frac-
tional free water clearance (\%), \(\text{FE}_{\text{Na}^+}\) = fractional sodium excre-
tion. \(C_{\text{Na}^+}/\text{GFR}\) = fractional free water clearance (\%); PRA = plasma renin activity (ng/ml/hr).

nary kallikrein excretion rate was inversely related to the fractional excretion of Na\(^+\) (\(r = -0.53, p < 0.01\)) and directly related to plasma renin activity (\(r = 0.45, p < 0.05\)).

Study 2. Infusion of 0.9 g/dl NaCl

Five normal women aged 37.2 ± 1.2 years were compared to five patients with essential hypertension (three women and two men aged 37.6 ± 3.6 years). Blood pressures on the clearance day and data from 24-
hour urine samples collected the day before the clear-

ance studies are shown in Table 3. Hypertensive and normal subjects had achieved comparable sodium balance before the clearance studies. Despite this, in the

chronically sodium-depleted subjects, urinary aldo-

sterone and plasma renin activity were significantly higher in the normal subjects compared to the hyper-

tensive patients. Urinary kallikrein was not different

between the two groups (0.05 < \(p < 0.1\)) (Table 3). After overnight water deprivation, subjects were in a water-retaining state, as evidenced by \(U_{\text{Na}^+}/P_{\text{Na}^+} > 2\) at the start of the clearance periods. After chronic sodium depletion, \(U_{\text{Na}^+}/P_{\text{Na}^+}\) in normals was 2.6 ± 0.1 and in hypertensives, 3.0 ± 0.2. After chronic sodium loading, \(U_{\text{Na}^+}/P_{\text{Na}^+}\) in normals was 2.3 ± 0.3 and in hypertensives, 2.4 ± 0.2.

The urinary kallikrein response during and after 0.9 g/dl NaCl infusion is shown in Figure 1. In the Na\(^+\)-depleted subjects, saline infusion produced a decline in

\[ \text{Na}^+ \] depletion, \(\text{Fe}_{\text{Na}^+}\) = fractional sodium excre-
tion.
Table 4. Study 2: Infusion of 0.9 g/dl NaCl

<table>
<thead>
<tr>
<th>Period 1 Baseline</th>
<th>Period 3 31–60 min during infusion</th>
<th>Period 4 0–30 min postinfusion</th>
<th>Period 5 31–60 min postinfusion</th>
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<tr>
<td></td>
<td>Chronicity sodium-depleted subjects</td>
<td>Chronicity sodium-loaded subjects</td>
<td>Chronicity sodium-loaded subjects</td>
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<tr>
<td></td>
<td>Normals (n = 5)</td>
<td>Hypertensives (n = 5)</td>
<td>Normals (n = 5)</td>
</tr>
<tr>
<td>U_{ul}V</td>
<td>15.7 ± 2.2</td>
<td>19.8 ± 4.0</td>
<td>13.7 ± 2.0</td>
</tr>
<tr>
<td>GFR</td>
<td>90 ± 3</td>
<td>106 ± 6</td>
<td>99 ± 7</td>
</tr>
<tr>
<td>RPF</td>
<td>390 ± 66</td>
<td>436 ± 56</td>
<td>426 ± 56</td>
</tr>
<tr>
<td>V</td>
<td>0.5 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>FE_{Na}</td>
<td>0.12 ± 0.3</td>
<td>0.22 ± 0.1</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>FE_{K}</td>
<td>15 ± 1</td>
<td>20 ± 2</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>T_{iu}</td>
<td>0.9 ± 0.1</td>
<td>1.3 ± 0.3</td>
<td>1.7 ± 0.3</td>
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See Table 2 for abbreviations: FE_{Na} = fractional sodium excretion, FE_{K} = fractional potassium excretion, T_{iu} = fractional tubular reabsorption of water (5%).

*One patient (C.M.) demonstrated an exaggerated natriuresis, kaliuresis, and diuresis, and his values for FE_{Na}, FE_{K}, and T_{iu} are not included in Periods 3, 4, or 5.

The table shows the study results for the infusion of 0.9 g/dl NaCl in chronic sodium-depleted and loaded subjects. The table compares the baseline values to those during and after the infusion. The changes in urine flow, GFR, RPF, and fractional sodium and potassium excretion are tabulated. The data show significant increases in urine flow and fractional sodium excretion during the infusion period in both groups, with the hypertensive patients showing greater increases. The fractional potassium excretion also increases significantly in the hypertensive patients, but not in the normotensive subjects.

Data from the baseline period and the two periods that showed the maximum natriuresis are shown in Table 4 for infusion studies in both chronically sodium-depleted and loaded subjects. The GFR and RPF did not change. In the chronically sodium-loaded subjects, the RPF during saline infusion was significantly higher in the hypertensives compared to the normals during Periods 3 through 6 (Period 3: p = 0.001; Period 4: p = 0.03; Period 5 and 6: p = 0.0006). (Only Periods 3 and 4 are shown in Table 4.) In the sodium-depleted subjects, the RPF was not different between the hypertensives and normals (Table 4).

Infusion of 0.9 g/dl NaCl produced changes in salt excretion, as shown in Table 4. In the sodium-depleted normal subjects, saline infusion doubled the urine flow from baseline, increased fractional sodium excretion (FE_{Na}+) approximately 7 times and fractional potassium excretion (FE_{K}+) 1½ times, and doubled the tubular reabsorption of water (T_{iu}/GFR).

During acute saline infusion in the sodium-depleted hypertensives, four patients showed changes in urinary sodium and potassium excretion that were almost identical to the normal subjects (Table 4). Only one hypertensive patient (C.M.) responded to the acute saline infusion with an exaggerated natriuresis, kaliuresis, and diuresis, and his values for salt and water handling are not included in Table 4 (see below).

In the chronically sodium-loaded normal subjects, saline infusion produced a 50% increase in urine flow and led to a doubling of fractional sodium excretion, little change in fractional potassium excretion, and a doubling of tubular reabsorption of water.

During the acute saline infusion in the chronically sodium-loaded hypertensives, four patients showed changes in sodium and potassium excretion that were similar to those in the normal subjects (Table 4), and the same hypertensive patient (C.M.) again demonstrated an exaggerated natriuresis, kaliuresis, and diuresis.

C.M. was one of the older subjects (44 years) and the most hypertensive patient studied (170/106 mm Hg, supine). In the chronically sodium-restricted state, C.M.'s baseline urinary kallikrein was the lowest of all urinary kallikrein from the baseline values by the first or second postinfusion period, Periods 4 or 5 (Figure 1 and Table 4). Changes in urinary kallikrein excretion during the six clearance periods were tested by analysis of variance, with subjects nested within groups (hypertensives and normals). In all the sodium-depleted subjects, the decrease in urinary kallikrein was highly significant, p = 0.003. In all chronically sodium-loaded subjects, the decrease in urinary kallikrein was significant, p = 0.01. When the variability between individual subjects was considered, the group differences between the hypertensives and normals were not significant.
the sodium-depleted hypertensives and normals; it further declined during saline infusion and remained the lowest of all subjects studied (Table 5). In the chronically sodium-depleted state, C.M. had one of the lowest values for PRA (0.36 ng/ml/hr), serum aldosterone (15 ng/100 ml), and urinary aldosterone (15 μg/24 hrs). In the chronically sodium-loaded state, C.M.’s exaggerated salt and water loss to isotonic saline infusion was associated with a slight decrease in his urinary kallikrein excretion. C.M. also showed only a slight increase in baseline urinary kallikrein after chronic dietary sodium depletion compared to dietary sodium loading, while in all other subjects chronic sodium depletion produced kallikrein values 2 to 4 times the chronic sodium-loaded values.

Thus, in both normal subjects and hypertensives, acute isotonic saline loading produced the expected increases in salt and water excretion, and kallikrein excretion significantly decreased. The underlying state of sodium balance did not alter the directional response of urinary kallikrein to saline loading. In one patient with exaggerated natriuresis, there was a subnormal increase in renin, aldosterone, and kallikrein levels in response to dietary sodium restriction, and a decline in urinary kallikrein levels from baseline during the isotonic saline infusion.

To further analyze the relationship between kallikrein excretion and both renal hemodynamics and salt and water excretion during the six periods, correlation coefficients were calculated for the hypertensives and for the normals separately. Significant correlations were detected only in the hypertensives. The underlying state of sodium balance did influence the relationship between urinary kallikrein and the renal tubular handling of Na⁺ and H₂O. Figure 2 shows the log of the kallikrein excretion (in EU/min) vs the fractional sodium excretion. In the chronically sodium-depleted hypertensives undergoing isotonic saline infusion, an inverse relationship was present between kallikrein excretion and fractional sodium excretion (r = −0.54, p < 0.01). In the sodium-loaded hypertensives, there was no significant correlation, nor were there correlations in the normal subjects between urinary kallikrein and sodium excretion. Figure 3 shows the log kallikrein excretion vs the fractional tubular reabsorption of water (TW/HVR/GFR). In chronically sodium-depleted hypertensives, r = −0.50, p < 0.01, while in the chronically sodium-loaded hypertensives, the relationship became positive (r = 0.38, p < 0.05). In normal subjects, there were no significant correlations between urinary kallikrein and TW/HVR/GFR. In the chronically sodium-depleted hypertensives, urinary kallikrein excretion was also inversely related to the fractional potassium excretion (C₉⁺/GFR), r = −0.43, p < 0.05 and fractional osmolar excretion (C₉/O₂/GFR), r = −0.40, p < 0.05. Urinary kallikrein excretion was not significantly related to GFR, RPF, or filtration fraction in hypertensive or normal subjects. Thus, the strongest correlations were found in sodium-depleted hypertensives in whom the decrease in urinary kallikrein excretion was associated with increased sodium and potassium excretion and lumen reabsorption of water. The underlying state of sodium balance influenced these relationships in hypertensives.

### Study 3. Infusion of 3.0 g/dl NaCl

Three subjects, one normal (BP 104/70 mm Hg) and two with borderline hypertension (BP 135/90; 130/90

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Fractional sodium excretion (CN₉⁺/GFR) vs the log transformation of urinary kallikrein excretion rate in EU/min (log U kallikrein V) for the same chronically sodium-depleted and sodium-loaded normal and hypertensive subjects who received acute infusions of 0.9 g/dl NaCl after each diet period.
pertensive patients who received acute infusions of 0.9 g/dl NaCl during a 3-hour period. The results obtained during the infusion were similar in all chronic sodium-loaded subjects. By Period 6 (the sixth and last half-hour of infusion), they all showed an increase in GFR and RPF of 30% to 40% from Period 1, a sevenfold increase in urinary sodium excretion, a fivefold increase in FE\(_{\text{Na}^+}\), a fourfold increase in C\(_{\text{osm}}/\text{GFR}\), and a threefold increase in T\(_{\text{H}_2\text{O}}\). Despite these changes, urinary kallikrein excretion did not increase during the infusion periods. U\(_{\text{Na}^+}\)V during the first half-hour was 3.2 ± 1.2 mEU/min, and during Period 6 was 2.5 ± 0.6 mEU/min. In one salt-depleted normal subject, the hypertonic sodium infusion produced the following: U\(_{\text{Na}^+}\)V increased 20-fold, FE\(_{\text{Na}^+}\) increased 30-fold, urinary volume increased ninefold, C\(_{\text{osm}}/\text{GFR}\) increased fivefold, and T\(_{\text{H}_2\text{O}}\) increased threefold. Urinary kallikrein excretion, however, did not change in this normal individual. It seemed clear that, despite marked changes in salt and water excretion, urinary kallikrein excretion did not increase during hypertonic saline loading and in this setting was not a natriuretic factor.

**Discussion**

The function of urinary kallikrein remains unclear. However, a number of studies have linked kallikrein to renal sodium and water handling and have found urinary kallikrein to be subnormal in some hypertensive patients. Since kallikrein forms the putative vasodilator kallidin, it has been attractive to postulate a natriuretic and diuretic role for kallikrein, and in hypertensives, a deficiency of an endogenous natriuretic factor. This hypothesis is controversial and not well supported. Since a specific kallikrein inhibitor is not available for direct studies, investigators have used indirect methods, usually by increasing salt and water intake acutely and measuring the kallikrein response. Surprisingly, even these indirect methods of water and salt loading have not been applied to hypertensives. I therefore set out to determine if urinary kallikrein is a natriuretic or diuretic factor in either normal subjects or hypertensive patients during acute loading with water, isotonic saline, or hypertonic saline. Since dietary sodium restriction increases kallikrein excretion, I also wished to determine if the underlying state of sodium balance influences the urinary kallikrein response to acute water or saline loading in normal or hypertensive subjects or if sodium balance influences the relationship between kallikrein excretion and the renal handling of sodium and water. All subjects were studied twice, after 5 days each of low Na\(^+\) and high Na\(^+\) diets, randomly assigned.

It is clear from these studies that urinary kallikrein is not a natriuretic or diuretic factor. In Study 1 in which normal and hypertensive subjects received 1.2 liters of water over a 4-hour period, urinary kallikrein, measured hourly for 3 hours, did not change. The state of sodium balance did not influence the kallikrein response to water loading. Free water clearance and urinary sodium were also not related. Study 1 differs from that of Levy et al., in which only normal subjects were studied, water load was given intravenously, and increased kallikrein excretion was observed in 6-hour collections over 24 hours. In my study, I used one-third of the volume Levy used, gave it orally and intravenously, and collected urine hourly. Levy et al.'s greater water load, longer collection periods, and intravenous administration may be related to the increased urinary kallikrein observed. My results also differ from those of Adetuyibi and Mills who gave 1 liter of water to 10 fasting normal subjects and reported an increase in 24-hour kallikrein excretion compared to control collections. Again, my study only measured acute changes over 3 hours. However, my results are compatible with those of Margolius et al. who found that 2 liters of water given acutely to four normal subjects failed to alter kallikrein excretion.

In Study 2, normal and hypertensive subjects received isotonic saline intravenously over a 1-hour period. The expected natriuresis and diuresis were seen and were accompanied by a highly significant decrease in kallikrein excretion (\(p = 0.003\) in all sodium-deplet-
ed subjects and \( p = 0.01 \) in chronically sodium-loaded subjects). The state of sodium balance did not influence the direction of kallikrein response and the response did not differ between normal and hypertensive subjects. This decreased kallikrein response to acute saline infusion has not been reported before for both normal and hypertensive subjects studied after high- and low-sodium diets.

Study 2 results are in contrast to those of Margolius et al., who only studied normal subjects and found no change in kallikrein excretion after acute isotonic saline loading, despite a threefold increase in sodium excretion. Although we both used comparable volumes of isotonic saline, Margolius et al.'s subjects were studied after a 59 mEq Na\(^+\) diet, the infusion lasted almost three times longer than mine, and postinfusion collections were not made. Thus, our different findings may be due to the different length of saline infusion, differences in beginning sodium balance, or the absence of postinfusion collections by Margolius et al. Levy et al. also studied the effect of acute infusions of up to 3.5 liters of isotonic saline during 3 to 4 hours in salt-repleted normals and found no change in kallikrein excretion. They collected urine samples during four consecutive 6-hour periods and may have obscured acute changes in kallikrein excretion by the relatively long collection periods.

In Study 3, three subjects received 2 liters of hypertonic saline by infusion over a 3-hour period. Despite a marked natriuresis and diuresis, kallikrein excretion did not increase. The hypertonic saline continually altered and expanded the extracellular fluid compartment while reducing intracellular fluid water. Nevertheless, the fluid compartment shifts did not increase kallikrein excretion.

Thus, during conditions of acute salt, isotonic saline, or hypertonic saline loading, urinary kallikrein either declined or remained unchanged and was not directly related to the attendant increases in sodium and water excretion. Our results in humans also differ from those of acute salt- or water-loading studies in rabbits, rats, and dogs, in which urinary kallikrein excretion increased. The potential causes for the differences are numerous and include obvious species differences, the route of administration of fluid, the extent of volume expansion and fluid compartment shifts, underlying sodium balance, or the effects of anesthesia in the animal studies. The latter point is addressed in an extensive study in conscious rats in which infusions of water or saline produced significant increases in kallikrein excretion.

The other major question addressed is: Does the underlying state of sodium balance influence the response of urinary kallikrein to water or sodium loading, or the relationship between kallikrein excretion and the renal handling of sodium and water in normal subjects and/or hypertensive patients?

The directional response of urinary kallikrein to acute saline was not altered by the underlying state of sodium balance; after both dietary sodium restriction or loading, acute saline infusion produced a significant decline in kallikrein excretion. However, the relationship between kallikrein excretion and renal sodium and water handling was related to the state of sodium balance.

In sodium-restricted hypertensives undergoing isotonic saline loading, significant inverse correlations were found between kallikrein excretion and the following: fractional sodium excretion, fractional potassium excretion, and tubular reabsorption of water. In addition, in the sodium-restricted hypertensives undergoing water loading, an inverse relationship was seen between kallikrein excretion and fractional sodium excretion. In the chronically sodium-loaded hypertensives undergoing isotonic saline loading, the correlation between kallikrein excretion and tubular reabsorption of water became positive. Kallikrein may be an antinatriuretic and antidiuretic factor in sodium-depleted hypertensives. Since tubular reabsorption of water is a complicated function that involves sodium chloride transport along the loop of Henle, ADH, and water reabsorption from the collecting duct, the specific effect of kallikrein on these tubular processes cannot be determined from these studies. A "biphasic" relationship between kallikrein excretion and sodium balance has been proposed by Mills to explain the very high kallikrein levels during sodium restriction, decreased levels during acute or chronic sodium loading, and high levels observed during chronic sodium loading in conjunction with increased renal artery pressure.

In one older, more severely hypertensive patient, isotonic saline infusion produced an exaggerated natriuresis and was associated with pre- and postinfusion urinary kallikrein values that were the lowest of all those in the sodium-depleted hypertensive and normal subjects. The low kallikrein is compatible with reports that kallikrein excretion in hypertensives may decrease with age or the severity of hypertension. The hyporesponsive kallikrein excretion may be related to the hyporesponsive renin and aldosterone which have been reported in some patients with exaggerated natriuresis.

Two other points need to be addressed. First, the hypertensive subjects evaluated in Study 2 interestingly had subnormal aldosterone responses to the low sodium diet. Since urinary kallikrein is, in part, regulated by aldosterone, it might be expected that these patients would have subnormal kallikrein excretion. This was not the case (Table 3), and thus other factors presumably modulate kallikrein excretion. Other investigators have reported abnormalities in the renin-angiotensin-aldosterone axis in hypertensive patients. My Study 2 patients do not fit the recent description by Williams et al. of essential hypertensive patients with subnormal aldosterone and possibly hyposensitive adrenal and vascular receptors to angiotensin II. Their patients had normal renin values and abnormally high renal blood flow after both salt depletion and repletion, while my patients had subnormal renin values after salt restriction and high renal blood flow only after salt repletion. This abnor-
mality in renal blood flow in my salt-repleted hypertensives is not related to an abnormality in kallikrein excretion, and the cause is not clear.

Second, my use of ADH in this study requires further comment. One purpose of this study was to assess the relationship of urinary kallikrein to the renal tubular handling of water. In the antidiuretic state, the tubular reabsorption of water is calculated from measurements of osmolar clearance and urine flow rate (\(T_{\text{H2O}} = C_{\text{osm}} - V\)). This is a complicated function, as discussed, but depends on optimal levels of ADH. Because the subjects were studied as outpatients with potentially varying levels of endogenous ADH, all subjects, both hypertensive and normal, were given identical low-level subpressor infusions of ADH to establish a basal level for ADH. The influence of subpressor amounts of ADH on kallikrein excretion is not clearly defined and is controversial. Mills and Newport have infused ADH into the dog kidney and produced no change in kallikrein excretion. In contrast, Fejes-Toth and others infused subpressor levels of ADH intravenously and elevated urinary kallikrein in rats and dogs. The effect of ADH on kallikrein excretion in humans is not known. Since all normal subjects and hypertensive patients in my studies received the same low-level ADH infusion, comparisons between these two groups should be valid.

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