Abnormal Urinary Kallikrein in Hypertension Is Not Related To Aldosterone Or Plasma Renin Activity

WILLIAM J. LAWTON, M.D., AND ANNETTE E. FITZ, M.D.

SUMMARY The relationships between urinary kallikrein (U_k), and plasma renin activity (PRA), urinary aldosterone (U_aldosterone), Na⁺ balance, Sk⁺, and renal function were studied in essential hypertensives (EHT) and normals. U_k was measured by a radiochemical esterolytic assay. We studied 18 white patients with EHT (15 men, 3 women) ages 31.6 ± 2.1 (SEM) yrs, BP 138 ± 2/95 ± 2 mm Hg. and 12 white normals (NLS) (7 men, 5 women) ages 30.2 ± 2.3 yrs, BP 112 ± 4/71 ± 2 mm Hg. All received a 5-day diet of 400 mEq Na⁺, 80 mEq K⁺/day, and 5 days of 10 mEq Na⁺, 80 mEq K⁺/day. All achieved Na⁺ balance by Day 5. On Day 5 of the low Na⁺ diet, 24 hr. U_k in EHT was 15.8 ± 2.4 (esterase units/24 hr) vs NLS, 17.0 ± 2.8. PRA was the same in EHT and NLS, but U_aldosterone was higher in NLS. (Day 5, low Na⁺, EHT, U_aldosterone - 39.4 ± 3.3 µg/24 h vs NLS 41.8 ± 4.7, p < 0.02). Analysis of individuals showed that all NLS increased U_k after salt restriction, while 3 EHT decreased U_k after salt restriction. This abnormal response in EHT was not related to abnormalities in U_aldosterone, PRA, Na⁺ balance, Sk⁺, or creatinine clearance. In 3 EHT with low-renin EHT, the U_k response was normal. In two of four patients with primary aldosteronism, U_k was normal despite increased U_aldosterone. The U_k response to salt restriction is abnormal in some EHT, unrelated to U_aldosterone or PRA, suggesting either a primary defect in U_k and/or the presence of other factors modulating U_k in EHT. (Hypertension 2: 787-793, 1980)

KEY WORDS renal function, plasma renin activity, aldosterone, urinary kallikrein, sodium balance

U RINARY kallikrein, a glandular kallikrein chemically identical with renal kallikrein, acts on kininogen to form kallidin.¹,² Kallidin promotes increased salt and water excretion due to renal vasodilation.³ Urinary kallikrein was first reported to be subnormal in patients with essential hypertension in 1934 by Elliot and Nuzum⁴ and more recently, several other groups reported similar findings.⁵-⁹ Subnormal values of urinary kallikrein suggest an abnormality in kallidin formation and an abnormality in the handling of salt and water in certain hypertensives. However, the finding of reduced urinary kallikrein in essential hypertensives is not universally reported.¹⁰ In an earlier report, we studied young men with essential hypertension and normal plasma renin activity (PRA) and found normal levels of urinary kallikrein excretion when compared to age, race, and sex matched normal subjects.¹¹ Urinary kallikrein can be influenced by aldosterone.¹²,¹³ Also the kallikrein-kinin system and the renin-angiotensin system are related, since kallikrein can activate renin¹⁴ and angiotensin I-converting enzyme acts as a kininase. In response to alterations in sodium chloride balance, plasma bradykinin and PRA change together in the same direction. In response to alterations in sodium chloride balance, plasma bradykinin and PRA change together in the same direction. In response to alterations in sodium chloride balance, plasma bradykinin and PRA change together in the same direction. In response to alterations in sodium chloride balance, plasma bradykinin and PRA change together in the same direction. Changes in sodium chloride balance are also associated with directionally similar changes in PRA, aldosterone, and urinary kallikrein.¹⁵,¹⁶,¹⁷,¹⁸ and the infusion of angiotensin into the renal artery produces an increase in urinary kallikrein. In addition, potassium can influence kallikrein¹⁹ as can alterations in renal function. In view of the differences between our previous work and others, we wished to further study urinary kallikrein in subjects with essential hypertension and normals with a careful evaluation of the known factors which influence kallikrein. We particularly wished to further characterize the relationship of urinary kallikrein to renal function, PRA, aldosterone, and sodium and potassium balance. In addition, we studied patients with primary aldosteronism to gain further insight into the regulation and interaction of aldosterone and kallikrein.
Methods

We studied 18 essential hypertensive patients (15 men and 3 women) ages 31.6 ± 2.1 (SEM) years, whose blood pressure was 138 ± 2/95 ± 2 mm Hg. These patients were compared to 12 age- and race-matched normal subjects (7 men and 5 women) ages 30.2 ± 2.3 years (NS), whose blood pressure was 112 ± 4/71 ± 2 mm Hg (p < 0.001). All patients and normals were white.

In the hypertensive subjects, sitting diastolic blood pressures were greater than 90 mm Hg on at least three sequential outpatient visits, 1 month apart. The hypertensives and normals underwent a complete medical history and physical examination, and laboratory studies included urinalysis, serum electrolytes, renal and liver function studies, chest x-ray and electrocardiogram. Additional studies in the hypertensives included upright PRA, creatinine clearance, rapid sequence intravenous pyelogram, and 24-hour urine determinations for catecholamines, VMA, metanephrines, sodium and potassium. The hypertensive subjects had no evidence for target organ damage, secondary causes of hypertension or other systemic disease. They were either untreated or received no antihypertensive medication for at least 2 weeks. The subjects were studied as outpatients and reported to the Clinical Research Center at the University Hospitals at least once a day. Daily weights and blood pressures in the supine, sitting, and standing positions were obtained during the diet periods. The normal subjects and hypertensives followed the same protocol receiving 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. Patients remained on these diets for a five-day period with a 1-week ad libitum diet interval between the two diet periods. Twenty-four-hour urine specimens were collected under toluene for kallikrein determination on Days 1 and 5. Twenty-four-hour urine specimens for aldosterone were collected in 33% glacial acetic acid for aldosterone. Since acidification destroys kallikrein activity, urinary aldosterone excretion was measured either one day before or after urine kallikrein collections on days 0 and 4 and in some subjects on Days 2 and 6. Twenty-four-hour urine creatinine was measured to assess the adequacy of collection, and creatinine clearances were determined. Urine sodium and potassium were measured to determine the state of sodium balance. On Days 1 and 5, after subjects were upright and ambulatory for 4 hours, blood for plasma renin activity was drawn into prechilled EDTA tubes, and serum aldosterone, sodium, and potassium were also measured.

Four additional hypertensive patients with primary aldosteronism were also evaluated in the hospital following a similar protocol. The diagnosis of primary aldosteronism was established by the presence of persistent hypokalemia, low PRA which was not stimulated during 3–5 days of salt restriction, and by the finding of elevated urinary aldosterone excretion which was not suppressed after 3–5 days of salt loading. All four patients have undergone surgery and had adrenal cortical adenomas removed which were confirmed by histopathologic study. The protocol was approved by the Committee on Research Involving Human Beings, College of Medicine, University of Iowa and informed written consent was obtained for all subjects.

Chemical Measurements

Urinary kallikrein was measured by the method of Beaven et al.27 and Margolius et al.,20 using a radiochemical esterolytic assay with the artificial substrate of p-tosyl-arginine 3H methyl ester (57 mCi/mmole) (3H-TAME) (New England Nuclear). The values of our standard human urinary kallikrein (HUK) have been previously reported,18 and urinary kallikrein excretion is expressed in esterase units (EU/24 hr).

Plasma renin activity (PRA) was measured in our laboratory by radioimmunoassay of generated angiotensin I using a modification of the method of Haber28 (I125-Angiotensin I, New England Nuclear). Twenty-four-hour urine aldosterone determinations were determined by radioimmunoassay in the Mayo Medical Laboratories, Rochester, Minn. Serum aldosterone measurements were made by radioimmunoassay at the Nichols Institute of Endocrinology, Los Angeles, CA. Sodium and potassium were measured by the flame photometer.

Student's t test for unpaired data was calculated where appropriate, or the Wilcoxon rank sum test for nonparametric data was used.29 Significance was considered to be at p < 0.05. Group means ± SEM are presented.

Results

All hypertensive patients and normals achieved sodium balance by Day 5 of both the low and high sodium diets. The 24-hour-urinary sodium excretion on Day 5 of the high sodium diet was 343 ± 17 mEq/24 hr for the hypertensives and 366 ± 21/24 hr for the normals. On Day 5 of the low sodium diet, hypertensives excreted 11 ± 2 mEq/24 hr sodium while the normals = 11 ± 3.

Urinary kallikrein and aldosterone excretion, PRA, and serum aldosterone are shown in Table 1. The urinary kallikrein excretion was similar in the hypertensive group compared to the normals at the end of both the high salt diet and low salt diet.

Since there was a sex disparity between the hypertensives (EHT) and normals (NLS), urinary kallikrein excretion/g creatinine/24 hr was calculated. The results were similar in both groups after the high Na+ diet (EHT: 5.0 ± 0.6 EU/g creat vs NLS: 5.6 ± 1.2) and after the low Na+ diet (EHT: 10.0 ± 1.4 vs. NLS: 14.8 ± 2.8; NS). Within the normal group, the 24 hr urinary kallikrein excretion in the five women was not significantly different from the seven men. In addition, Spearman correlation coeffi-
RELATIONSHIP OF KALLIKREIN TO ALDOSTERONE AND PRA/Lawton and Fitz

TABLE 1. Urinary Kallikrein, Aldosterone, and Plasma Renin Activity in Essential Hypertensive Patients and Normal Subjects

<table>
<thead>
<tr>
<th></th>
<th>400 mEq Na⁺ Diet, Day 5 values</th>
<th>10 mEq Na⁺ Diet, Day 5 values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypertensives n</td>
<td>Normals n</td>
</tr>
<tr>
<td>Urinary kallikrein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E.U./24 hr)</td>
<td>8.1 ± 1.0</td>
<td>17</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>2.0 ± 0.4</td>
<td>18</td>
</tr>
<tr>
<td>Urinary aldosterone (Days 4 and 6)</td>
<td>3.9 ± 0.8</td>
<td>17</td>
</tr>
<tr>
<td>Serum aldosterone (m ng/100 ml)</td>
<td>7.9 ± 1.3</td>
<td>7</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM.

*Hypertensive vs Normals during same diet period: p < 0.02.

...solute levels of kallikrein varied after sodium-loading, all normal subjects increased their urinary kallikrein after salt restriction. In contrast, three of the hypertensives decreased their urinary kallikrein excretion after salt restriction while all other hypertensives increased their values. Urinary kallikrein excretion after salt restriction in the three abnormal hypertensives (3.0 ± 1.1 EU/24 hr) was significantly lower than the other hypertensives (18.4 ± 2.4 EU/24 hr, n = 15; p < 0.02) and lower than the normals (17.0 ± 2.8, p < 0.05). On Day 5 of the low Na⁺ diet two of the hypertensives had urinary kallikreins lower than the lowest value in normals. After salt loading, there were no hypertensive values lower than the normals.

To further assess the abnormality in the three hypertensives with suppressed kallikrein excretion after salt restriction, we plotted urinary kallikrein excretion...
vs. urinary aldosterone excretion for each subject on Day 5 of both diets. Both urinary kallikrein and urinary aldosterone increased in each normal subject in response to salt restriction, although maximally stimulated values varied over a wide range. Aldosterone and kallikrein in hypertensives also had an appropriate directional response after salt restriction in most subjects and mean values ± SEM for the hypertensives are shown in figure 2. However the three hypertensives in whom kallikrein excretion was abnormally suppressed after salt restriction are of particular interest. These patients all responded with normal increases in aldosterone excretion, indicating a dichotomy between the response of aldosterone and kallikrein (fig. 2).

In a similar manner, we plotted urinary kallikrein vs. PRA for each subject on Day 5 of both diets. Urinary kallikrein and PRA increased in all normals after salt restriction. The three hypertensives with suppressed kallikrein responses to salt restriction had appropriate increases in PRA to salt restriction. In addition three other hypertensives failed to normally stimulate their PRA after 5 days of salt restriction (PRA = 0.28, 0.36, and 1.26 ng/ml/hr) and are therefore "low-renin"-essential hypertensives. In these three low renin patients, urinary kallikrein excretion increased after salt restriction by 3.1, 1.3, and 3.9 EU/24 hr (fig. 2). Although the magnitude of change in kallikrein in the low-renin hypertensives was modest, it was similar to changes seen in two normal-renin hypertensives, and in two normal subjects. Thus six hypertensives, three low kallikrein and three low renin, show an abnormal relation between PRA and urinary kallikrein in response to salt restriction as compared to the normals, and as compared to other hypertensives.

We also examined the relationship of urinary kallikrein and urinary sodium, GFR and serum potassium (S_{K+}) on Day 5 of each diet period in individual subjects. The three hypertensives with suppressed urinary kallikrein responses to salt restriction achieved appropriate salt balance on the low salt diet (U_{Na+} = 6.8 and 14 mEq/24 hr) and had normal creatinine clearances. Two of these three hypertensives showed normal increases in S_{K+} after salt restriction, and in one patient S_{K+} was unchanged.

Four patients with primary aldosteronism were studied. All patients followed a similar protocol in the hospital with 3–5 days of both salt restriction and salt loading. At the end of the high salt period, 24-hour urine samples were collected sequentially for aldosterone and kallikrein. Preoperative lab data is shown in table 2. FH, a 50-year-old white female with BP 138/94 mm Hg, had a right adrenal cortical adenoma, 1.6 × 1.9 cm removed at surgery. During high salt intake, her urinary kallikrein was clearly elevated compared to normal subjects. CK is a 33-year-old white female, BP 148/94, who underwent a left adrenalectomy and removal of a 1 cm adrenal cortical adenoma. Her urinary kallikrein was collected during a no-added salt diet and for her level of sodium excretion, the urinary kallikrein is in the upper normal range compared to normal subjects. BS is a 49-year-old white female with BP 178/105, in whom a left adrenal cortical adenoma, 2.5 × 2 × 1.5 cm was removed. Her urinary kallikrein was normal on the high salt diet. Urinary kallikrein was also measured during salt restriction and failed to stimulate (UKAL = 6.02 EU/24 hr; U_{Na+} = 38 mEq/24 hr). AP, a 50-year-old white female with BP 198/112 mm Hg, underwent a left adrenalectomy and an adrenal cortical adenoma, 3 × 3 × 2 cm, was removed. Her PRA does not stimulate on salt restriction, the urinary aldosterone is elevated and does not suppress on salt loading, and urinary kallikrein is elevated during salt loading (table 2).
Discussion

Some forms of human hypertension may result from deficiencies of vasodilator and natriuretic substances, or an imbalance between naturally occurring vasoconstrictor and vasodilator substances. The kallikrein-kinin system has been shown to possess hypotensive and natriuretic properties and urinary kallikrein has been reported to be subnormal in human essential hypertensives by Elliot and Nuzum and more recently by Margolius et al., and recently by Margolius et al., by Levy et al., by Lechi et al., and Mersey et al. The finding of subnormal urinary kallikrein in normals, racial differences could explain lower kallikrein levels in the hypertensive group. A number of important factors may account for our findings compared to those studies in which urinary kallikrein is low in hypertensives. In Margolius et. al.'s study the normals included 25 white and two black subjects while the hypertensive group included three whites and eight blacks. Since more blacks were present in the hypertensive group compared to the normals, racial differences could explain lower kallikrein levels in the hypertensive group. A number of differences exist between our studies and those studies in which urinary kallikrein is low in hypertensives. The eight essential hypertensives who were hypertensive, and more responsive. Urine specimens were frozen at -30°C for later analysis, and were not desalted while ours were stored at 4°C and desalted. Our studies also differ from Lechi et al.'s study. Their hypertensives were hospitalized, older and blood pressures probably higher. In reviewing the work by Seino et al. sodium intake is not specified for the larger group of hypertensives. The eight essential hypertensives who were studied on various sodium intakes were not compared to normal Japanese subjects on known Na⁺ intakes and thus, differences in the level of sodium balance between normals and hypertensives may be a confounding variable. In comparing our results to Mersey's et al., their hypertensives were somewhat older, but the exact level of blood pressure elevation is not given.

Since many of the studies which differ from ours evaluated older, more hypertensive subjects, it is possible that we may be describing an earlier phase of the disease in which kallikrein excretion decreases with advancing age, or severity or duration of hypertension. This concept is supported by Shkhvatsabaya et al. and the need for age and race matched studies is clear.

In our subjects, after salt restriction, we find significantly lower urinary aldosterone levels in our
hypertensives compared to our normals, yet urinary kallikrein levels are similar between the two groups (table 1). Since aldosterone is one of the factors thought to regulate renal kallikrein, 6, 7, 10-14 our data suggest that other modulating factors must be present or that the kallikrein response to aldosterone in hypertensives may be abnormal. Our finding of lower urinary aldosterone levels in hypertensives after salt restriction has also been reported by Margolius et al.12 and Mitchell et al.13 Mitchell and co-workers found somewhat lower aldosterone excretion rates in normal-renin and low-renin hypertensives compared to normals after 10 mEq Na+ diet. In contrast, Williams and Dluhy14 review the evidence reporting increased urinary aldosterone excretion in hypertension. Nowaczynski et al.15 have reviewed the complexities of assessing aldosterone in hypertensives and report decreased metabolic rates and secretory rates, elevated plasma aldosterone levels, and decreased urinary metabolites in hypertensives compared to normals.

We also noted lower potassium excretion in our hypertensives compared to our normals, yet both groups were receiving the same dietary potassium intake and the serum K+ values were not different between the hypertensives and normals. Nevertheless, our data does not suggest a direct relationship between kallikrein excretion and potassium metabolism since kallikrein excretion was similar in both hypertensives and normals.

In evaluating individual hypertensives, three patients showed an abnormal response to salt restriction and decreased their urinary kallikrein excretion. A decrease in kallikrein excretion after salt restriction has not been described before. In the three abnormal hypertensives, the urinary aldosterone response to salt restriction was appropriate, showing a dichotomy between kallikrein and aldosterone. When urinary kallikrein and PRA were characterized in the three abnormal hypertensives, PRA increased normally to salt restriction. Thus, neither PRA nor aldosterone can account for the abnormality seen in the three patients with suppressed kallikrein responses to salt restriction. In similar analyses, we found that the suppressed kallikrein response in the three hypertensives was not related to abnormalities in sodium balance,SCR or creatinine clearance. Three low renin hypertensive patients were also studied. In each of these patients after salt restriction, urinary kallikrein increased in the appropriate direction again demonstrating a dichotomy between PRA and kallikrein responses to salt restriction.

Our observations in four patients with primary aldosteronism also showed a dichotomy between aldosterone and kallikrein. In two patients, increased non-suppressible urine aldosterone and increased urinary kallikrein were found during salt loading. Increased kallikrein in primary aldosteronism has been reported by others and supports a role for aldosterone as a regulator of urinary kallikrein. 8, 9, 11 However, in our two other patients despite elevated urinary aldosterone levels, urinary kallikrein excretion was normal. Margolius et al.9 and Seino et al.8 have also found that some patients with primary aldosteronism excrete normal values of urinary kallikrein. The finding of normal kallikrein in the presence of increased aldosterone raises the possibility that other factors, as yet unknown, modulate urinary kallikrein.

Our study shows that most mild hypertensives have normal kallikrein excretion. An abnormality in urinary kallikrein excretion occurs in some hypertensives and is detected during sodium restriction. This kallikrein abnormality appears to not be related to deficits in plasma renin activity or aldosterone and may therefore be either a primary defect or secondary to another abnormality. If the kallikrein-kinin system has a role to counter-balance the renin-angiotensin-aldosterone system, defects in kallikrein may be important in certain hypertensives.

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