Urinary Kallikrein in Normal Renin Essential Hypertension

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

SUMMARY The relationship between urinary kallikrein, urinary aldosterone, and plasma renin activity (PRA) was studied in hypertensive patients and normal subjects. Kallikrein was measured by a radiochemical esterolytic assay. Nine white males with normal renin, mild essential hypertension (25 ± 5 [SD] yr; blood pressure [BP] 143 ± 7 / 95 ± 5 mm Hg) and six white normal males (23 ± 3 yr; BP 115 ± 15 / 70 ± 6 mm Hg) were placed on a one-week diet consisting of 400 mEq Na+, 80 mEq K+ diet and a one week diet of a 10 mEq Na+, 80 mEq K+ diet. During salt restriction, PRA, urinary aldosterone, and urinary kallikrein progressively rose. (Urinary kallikrein on day 7: normals 18.3 ± 13.7 esterase units [EU] per 24 hr; hypertensives 22.7 ± 12.5 EU/24 hrs.) There were no significant differences between the normals and hypertensives in PRA, aldosterone, or kallikrein excretion. After sodium balance was achieved, during salt loading, the PRA, aldosterone and kallikrein values were similar in both normals and hypertensives. (Urinary kallikrein on day 7: normals 5.0 ± 5.2; hypertensives 7.9 ± 4.4 EU/24 hrs.)

Abnormalities in urinary kallikrein in hypertensives were not found when young white males with normal renin essential hypertension were compared to age-matched white male normal subjects. PRA appears related to urinary kallikrein excretion in this type of patient.

THE KALLIKREIN-KININ SYSTEM as presently conceived has many facets. Plasma kallikrein, acting on plasma kininogen, produces bradykinin. Urinary kallikrein, considered to be a glandular kallikrein and similar to kidney kallikrein, acts on kininogen to produce kallidin, which although chemically slightly different than bradykinin is also a potent renal vasodilator and natriuretic agent. It is on this basis that some investigators have suggested that the kallikrein-kinin system may play a role in hypertension, particularly in conditions in which sodium metabolism is known to be abnormal.

Measurement of urinary kallikrein has been reported in human hypertension. On an ad libitum sodium diet, subnormal urinary kallikrein was reported in 43% of patients with essential hypertension. Kallikrein excretion has been related to the level of effective circulating aldosterone. Low dietary sodium and upright posture in normal man, known stimulators of the renin-angiotensin-aldosterone system, are associated with increases in urinary kallikrein. Kallikrein excretion in normals does not start to rise for at least 3–4 days after the start of fluidocortisone; however, it does increase earlier (1–2 days) after initiation of salt restriction. This suggests the possibility that other factors, in addition to mineralocorticoids, may be related to urinary kallikrein in essential hypertensives during changes in sodium balance.

In Margolis’ study plasma renin activity in the essential hypertensive patients is lower than in the normals during both salt loading and salt restriction. These hypertensives excrete less kallikrein on both the high and low salt diets as compared to normals.

Physiologic interrelationships between the kallikrein-kinin system and the renin-angiotensin system are known. Renal angiotensin I converting enzyme, known to form angiotensin II, accelerates the degradation of kallidin. In animals and in man angiotensin I converting enzyme has been reported to inactivate bradykinin.

A further interrelationship between the renin and kallikrein-kinin systems is suggested by Wong and coworkers. In sodium-loaded normal subjects, the assuming of an upright posture was associated with an increase in plasma renin activity and plasma bradykinin. In sodium deprived normal subjects an acute saline load produced a decline in plasma renin activity and bradykinin.

In addition, Johnston et al. have reported a direct relationship between urinary kallikrein excretion and plasma renin activity in normal rats on normal, high and low sodium intake. Seino and colleagues also report similar directional changes in renin and urinary kallikrein in relation to changes in sodium balance in human hypertension. Mills has also indicated that infusion of angiotensin in the renal artery of dogs causes an increase in urine kallikrein.

Two points emerge from studies previously reported: 1) The kallikrein-kinin system and renin-angiotensin system appear directly related, and 2) Some patients with human essential hypertension have subnormal urine kallikrein excretion. It is probable that the previously studied essential hypertensives were a heterogeneous group of patients with low, normal, and high renin essential hypertension and with various degrees of sodium and water balance. We, therefore, studied the relationship of the kallikrein-kinin system to the renin-angiotensin-aldosterone system in a homogeneous, group of essential hypertensive patients with normal renin during high and low sodium balance.

Study Design

Six normotensive control subjects and nine patients with mild essential hypertension were studied. In these patients, the plasma renin activity in relation to 24-hour urine sodium excretion was normal when compared to our previously established normal curve. In the hypertensive subjects, diastolic blood pressure was greater than 90 mm Hg on at least three sequential outpatient visits, one month apart. Patients were not receiving antihypertensive therapy and underwent a complete medical history, physical examination, laboratory data base including CBC, urinalysis, serum electrolytes, liver function studies, chest X-ray, electrocardiogram, upright plasma renin activity, creatinine clearance,
rapid sequence intravenous pyelogram, and 24-hour urine determination for catecholamines, VMA, and metanephrines. The study subjects had no evidence for target organ damage nor evidence for secondary causes of hypertension.

Subjects were studied while outpatients in the Clinical Research Center at the University Hospital. The normals and hypertensive subjects followed the same protocol and received a protein base, eucaloric formula diet containing 80 mEq potassium and either 10 mEq sodium or 400 mEq sodium. Each subject received both diets, the sequence being randomly assigned, and the patients remained on these diets for a 7-day period, with a one-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, 3, 5, and 7. Twenty-four hour urine specimens were collected in acetic acid for aldosterone on days 2 and 6. Twenty-four hour urine creatinine determinations were made on each of the urine collections to assess the adequacy of collection, and creatinine clearances were determined. Plasma renin activity, serum sodium and potassium, were measured on days 1, 3, 5, and 7.

Chemical Methods

Urinary kallikrein was measured by the method of Margolius et al.,5 a radiochemical esterolytic assay utilizing the artificial substrate of p-tosyl-l-arginine (H) methyl ester (57 mCi/mmmole) (H-TAME) (New England Nuclear). The tritiated methanol released by the esterase activity of urinary kallikrein is extracted and counted in a liquid scintillation counter. The activity of our human urinary kallikrein (HUK) standard in esterase units (EU) has been separately determined in our laboratory. One esterase unit is defined as that amount of enzyme which hydrolyzes 1 μM TAME per minute at pH 8 at 30°C in a titrimetric assay. One μl standard HUK (1:30) hydrolyzes 1.0271 × 10⁻⁴ μM TAME (EU) per minute at a titration assay at 30°C pH 8.0. The standard HUK used in this study has been compared to a standard HUK preparation supplied by the NIH. A standard curve is generated for each assay, each urine sample is assayed in triplicate, and recoveries are run in duplicate on the unknown samples. Results are expressed as esterase units (EU) per 24 hours.

Plasma renin activity (PRA) is performed by radioimmunoassay of angiotensin I. The 24-hour urine aldosterone determinations were determined by radioimmunoassay. Sodium and potassium were measured by the flame photometer. Statistical analysis was performed using the Student's t-test for unpaired data, and correlation coefficients were determined.

Results

Six normotensive white male subjects, aged 23 ± 3 (SD) years, blood pressure 115 ± 15 / 70 ± 6 mm Hg, and nine normal renin white male essential hypertensives, aged 25 ± 5 years, blood pressure 143 ± 7 / 95 ± 5 mm Hg, were studied. Sodium balance was achieved on either the high or low sodium diet by the fifth day and data are presented in table 1. On the low sodium diet, urinary sodium excretion was less than 10 mEq/24 hours by the fifth day in both hypertensives and normal renin essential hypertensives. Urinary sodium was in excess of 300 mEq/24 hours by day 5 in both groups on the high sodium diet.

During the period of salt restriction, urinary kallikrein excretion progressively rose (fig. 1), compared to initial levels which partially reflect the period of ad lib sodium intake. The increase in kallikrein in the normals and in the essential hypertensives was nearly identical, and absolute kallikrein levels following sodium restriction were not different (table 1). During salt loading in both normal subjects and hypertensives, urinary kallikrein decreased modestly as compared to the period of ad lib sodium intake.

Urinal kallikrein was slightly higher in the patients with essential hypertension on each day during both diet periods. Most important, however, kallikrein levels on day 7 of both periods were not different when normals and essential hypertensives were compared. In addition, the changes in kallikrein from peak suppression (day 7 high sodium diet) to peak stimulation (day 7 low sodium diet) were not different when normals (13.3 EU) and hypertensive subjects (14.0 EU) are compared.

Plasma renin activity increased during the period of salt restriction and on days 5 and 7, when sodium balance was achieved, the renin levels were similar in both normals and

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<th>TABLE 1. Sodium Balance Data in Essential Hypertension (EHT)</th>
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<td>10 mEq Na⁺</td>
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<td>UAldo (µg/day)</td>
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All values are mean ± standard deviation.

Abbreviations: EHT = essential hypertension; U = urinary; KAL = kallikrein; PRA = plasma renin activity; Aldo = aldosterone; EU = esterase units.

*Human urinary kallikrein standard supplied courtesy of Dr. J. V. Pierce and Dr. J. J. Pisano, National Institutes of Health. The NIH HUK standard was prepared to a dilution of 2 × 10⁻⁴ E.U./μl. In our radiochemical assay system, 1 μl of the NIH HUK hydrolyzed 1.9880 × 10⁻⁴ μM TAME (EU).
hypertensives. During the week of salt loading, renin values on days 5 and 7 were also similar.

Correlation coefficients were determined for urinary kallikrein and plasma renin activity in both normals and in hypertensive patients on days 1, 3, 5, and 7 of both diet periods. A log transformation of PRA was determined to adjust for the known hyperbolic relationship of PRA and urinary sodium excretion.10 Fisher transformation of \( r \) to \( z \) was made in determining significance. Weak correlations were found for both the normal subjects \((r = 0.477; N = 43)\) and normal renin essential hypertensives \((r = 0.377; N = 62)\). Urinary aldosterone excretion on day 6 of each period, when subjects were in sodium balance, was similar in both normals and hypertensives.

**Discussion**

Margolius and colleagues have reported suppressed levels of urinary kallikrein in human essential hypertension.4,5 They have also shown a positive relationship between the levels of sodium retaining steroids and urinary kallikrein.5,6,8 In Margolius' study, plasma renin activity in the essential hypertensives was lower than the normals during high and low sodium periods.9 These hypertensives excreted less kallikrein than normals on both high and low sodium diets.

In reviewing Margolius' data in the essential hypertensives, the differences in kallikrein between the hypertensives and normals seem more closely related to renin activity than to aldosterone excretion. It is also likely that the hypertensive patients studied by Margolius included low, normal, and high renin patients.

In our study of normal renin essential hypertensives, lower urinary kallikrein levels were not detected in the patients when compared to matched control subjects, a finding in marked contrast to that of Margolius. The urinary kallikrein levels in our hypertensives are normal or slightly increased as compared to the controls at comparable levels of sodium balance. In addition, in our subjects the urinary kallikrein levels increased similarly in both hypertensives and normals during salt restriction. Urinary kallikrein showed comparable decreases in both groups during salt loading. These findings are also in contrast to Margolius' study7 in which his hypertensives showed an attenuated response in urinary kallikrein during sodium restriction and sodium loading.

Our hypertensive subjects form a homogeneous group based on plasma renin activity, with all patients classified as normal renin essential hypertensives. The plasma renin activity was similar in both hypertensives and normals during high and low sodium balance. In addition, the urine aldosterone values were similar in our normal and hypertensive subjects. This is in contrast to Margolius' data comparing essential hypertensive subjects to normals, ages 19-29 years.8 During sodium restriction, Margolius' hypertensives excreted less aldosterone than normals.

Our hypertensive population and that of Margolius differ in race, age, and level of blood pressure. The hypertensive subjects reported by Margolius8 were predominantly black (8 black and 3 white) with a mean age of 45 years, and blood pressure range of 120/98 to 170/110. Age and race are known to influence renin. Black subjects and older patients are reported to have lower renin levels.14 Our hypertensives were all white and were younger, with milder elevations of blood pressure. Although they cannot be classed as labile since outpatient diastolic screening blood pressure levels were all 90 mm Hg or greater, they may be at an earlier stage in the spectrum of hypertensive disease.

It is possible that the suppression of kallikrein seen by others6-8 is related to age, sex, race, or lower levels of plasma renin or urinary aldosterone. Our white male subjects, studied in the ambulant state, were matched for age, sex and race, and had normal renin essential hypertension. The elevation of blood pressure was milder than in those
previously reported³, ⁵ and they also had no evidence of end-organ disease. These data raise the possibility that suppressed kallikrein observed by others occurs as a result rather than cause of hypertension. Our data show that young mildly hypertensive male patients with normal plasma renin activity levels have normal urinary kallikrein levels, and that their kallikrein levels respond normally to sodium restriction. This suggests that classification of patients with regard to plasma renin activity, as well as to other factors, is essential in trying to determine the role of the kallikrein-kinin system in hypertension. This study does not demonstrate that the renin-angiotensin system directly influences the kallikrein-kinin system but further investigation of the interrelationships between the two systems is needed.

Acknowledgment

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References


The Aortic Closure Sound in Pure Aortic Insufficiency

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SUMMARY The second sound in aortic insufficiency has been described as accentuated, normal, or moderately diminished. A study of intracardiac phonocardiograms was performed to evaluate its intensity and to eliminate extracardiac factors. Pressure and intracardiac sound measurements were made in 28 patients undergoing diagnostic cardiac catheterization. Recordings were obtained above the aortic valve and within the left ventricle in 14 patients with normal aortic valves and 11 patients with aortic insufficiency uncomplicated by aortic stenosis. The amplitude of the aortic closure sound in the patients with pure aortic insufficiency, 1000 ± 100 dynes/cm², was significantly lower than in those patients with normal aortic valves, 3100 ± 200 dynes/cm² (P < 0.001).

The results indicate, therefore, that the presence of aortic insufficiency causes a diminished amplitude of the aortic closure sound. These results are supportive of the theory that the second heart sound is caused by diastolic vibrations of the closed aortic cusps. Diminished valvular vibrations and sound would occur in pure aortic insufficiency if the valve is unable to properly tense during diastole, or if the rate of development of the driving pressure is diminished.

ALTHOUGH it is well known that the aortic component of the second sound is diminished or absent in acquired aortic stenosis, little attention has been given to the intensity of the second sound in aortic insufficiency. The intensity of the second sound has previously been described as accentuated,¹ ⁴

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