Urinary Kallikrein
in Normal Renin Essential Hypertension

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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 Margolius' 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.

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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 deter-
mination 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 determina-
tions were made on each of the urine collections to assess the
adequacy of collection, and creatinine clearances were deter-
mined. 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, 6 a radiochemical esterolytic assay utilizing
the artificial substrate of p-tosyl-l-arginine 3H methyl ester
(57 mCi/mrnole) (H-TAME) (New England Nuclear). The
tritiated methanol released by the esterase activity of
urinary kallikrein is extracted and counted in a liquid scinti-
tillation 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 mM
TAME per minute at pH 8 at 30°C in a titrimetric assay.
One μl standard HUK (1:30) hydrolyzes 1.0271 × 10-μM
TAME (E.U.) per minute in 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. 6 A standard
curve is generated for each assay, each urine sample is

*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 × 104 E.U./μl. In our radiochemical assay
system, 1 μl of the NIH HUK hydrolyzed 1.9880 × 10-μM TAME (EU).

| TABLE 1. Sodium Balance Data in Essential Hypertension (EHT) |
|------------------|------------------|------------------|------------------|------------------|
|                  | 10 mEq Na+       | 80 mEq K+ Diet   | 400 mEq Na+      | 80 mEq K+ Diet   |
|                  | Normals (N = 6)  | Normal Renin EHT | Normals (N = 6)  | Normal Renin EHT |
| UNa+ (mEq/24h)  |                |                  |                  |                  |
| Day 5            | 6 ± 4           | 9 ± 3            | 364 ± 72         | 324 ± 64         |
| Day 7            | 7 ± 4           | 4 ± 3            | 323 ± 83         | 273 ± 85         |
| UKAL (E.U./24h)  |                |                  |                  |                  |
| Day 5            | 17.1 ± 11.5     | 19.6 ± 11.1      | 5.5 ± 3.5        | 8.8 ± 3.8        |
| Day 7            | 18.3 ± 13.7     | 22.7 ± 12.5      | 5.0 ± 5.2        | 7.9 ± 4.4        |
| PRA (ng/ml/hr)   |                |                  |                  |                  |
| Day 5            | 11.7 ± 3.1      | 14.3 ± 12.9      | 2.6 ± 2.1        | 2.4 ± 1.3        |
| Day 7            | 18.5 ± 13.5     | 12.3 ± 7.6       | 1.9 ± 1.5        | 2.4 ± 1.6        |
| UAldo (μg/day)   |                |                  |                  |                  |
| Day 6            | 36.3 ± 14.7     | 34.7 ± 12.9      | 2.5 ± 2.3        | 2.2 ± 1.1        |

All values are mean ± standard deviation.

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

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. They have also shown a positive relationship between the levels of sodium retaining steroids and urinary kallikrein. In Margolius' study, plasma renin activity in the essential hypertensives was lower than the normals during high and low sodium periods. 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' study 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. 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 Margolius 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. 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 others 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

![Figure 1. Urinary kallikrein excretion during high and low sodium diets.](image-url)
previously reported\(^3\) 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.

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References

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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 close 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.\(^1\)

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normal,\(^1\)\(^,\)\(^9\)\(^,\)\(^4\) moderately diminished,\(^4\) or faint.\(^3\) It should be noted, however, that such descriptions of the intensity of the second heart sound are based upon the results of physical examination or uncalibrated phonocardiograms, a fact which may account for the wide variations observed. Previous work in this laboratory related to ejection murmurs has shown a diminished second sound in one patient with pure aortic insufficiency.\(^3\) In order to minimize such factors which depend on personal judgment and rule out extracardiac factors which may affect the apparent intensity of the second heart sound, an intracardiac phonocardiographic study of

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