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The role of urinary kininogen in the regulation of kinin generation

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The role of urinary kininogen in the regulation of kinin generation. The kallikrein-kininogen-kinin system has been postulated to play a role in the regulation of blood pressure and modulation of renal salt and water transport. The activity of this system has usually been determined by measurements of urinary kallikrein excretion. However, urinary kallikrein rarely correlates with simultaneously measured urinary kinins. To further evaluate the factors influencing urinary kinin excretion, we evaluated the role of urinary kininogen in this system. Urines were analyzed from normal subjects and individuals with untreated essential hypertension and end-stage renal disease. Intact urinary kininogen was significantly correlated with urinary kinins in normal subjects ($r = 0.65$, $P = 0.003$) and essential hypertensives ($r = 0.52$, $P = 0.026$). In both essential hypertension and end-stage renal disease, urinary kinins were significantly decreased (8.00 ± 1.93, 0.90 ± 0.18, $P < 0.05$, respectively) compared to controls (23.73 ± 5.20). In essential hypertensives, the reduction in urinary kinins was paralleled by a reduction in intact kininogen with a normal excretion of kallikrein. In end-stage renal disease, the reduction in kinins was paralleled by a reduction in kallikrein with a normal excretion of intact kininogen. This data suggests that kininogen may be an important determinant of urinary kinin excretion in various disease states.

Le rôle du kininogène urinaire dans la régulation de la formation de kinine. Un rôle du système kallicréine-kininogène-kinine a été envisagé dans la régulation de la pression artérielle et la modulation des transports rénaux du sel et de l'eau. L'activité de ce système a habituellement été déterminée par des mesures de l'excrétion urinaire de kallicréine. Cependant, la kallicréine urinaire est rarement corrélée avec des mesures simultanées des kinines urinaires. Afin d'apprécier les facteurs influencant l'excrétion urinaire de kinine, nous avons étudié le rôle du kininogène urinaire dans ce système. Les urines de sujets normaux et de malades ayant une hypertension artérielle essentielle non traitée ou une insuffisance rénale chronique terminale ont été étudiées. Le kininogène intact urinaire était significativement corrélé avec les kinines urinaires chez les sujets normaux ($r = 0{,}65$, $p = 0{,}003$) et chez les hypertendus ($r = 0{,}52$, $p = 0{,}026$). Chez les malades hypertendus et les insuffisants rénaux, les kinines urinaires étaient significativement diminuées ($8{,}00 \pm 1{,}93$, $0{,}90 \pm 0{,}18$, $p < 0{,}05$, respectivement) comparées aux contrôles ($23{,}73 \pm 5{,}20$). Chez les hypertendus essentiels, la réduction de la kinine urinaire était accompagnée par une réduction du kininogène intact avec une excrétion normale de kallicrèine. Au cours de l'insuffisance rénale chronique, la réduction de la kinine était accompagnée d'une réduction de la kallicrèine avec une excrétion normale du kinogène intact. Ces résultats suggèrent que le kininogène peut être un déterminant important de l'excrétion urinaire de kinine dans différents états pathologiques.

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The kinins have been implicated to have several important physiologic functions, including the regulation of blood pressure, salivary gland secretion, glucose uptake in muscle, modulation of salt and water transport in the kidney and intestines, and enhancement of sperm motility [1–3]. Urine contains several components of this vasoactive peptide system; kallikrein [4], kinins [5–7], kininogen [6, 8–11], and kininases [12, 13]. Measurement of each of these components in urine may not reflect the activity of this system at physiologically important sites within the kidney. Indeed, the relative concentrations of kallikrein, kininogen, and other possible modulating factors such as pH and ions at intrarenal sites of kinin formation and activity are not known [1, 2, 14, 15]. Thus, measurement of relative concentrations in the urine after it is collected can lead only to speculation of the intrarenal activity of the kallikrein-kininogen-kinin system.

In several human diseases, alterations in urinary kallikrein excretion have been reported [1, 2]. However, with rare exception, urinary kallikrein activity is not correlated with simultaneously measured urinary kinin excretion [1, 2, 16]. This lack of correlation highlights the problems inherent in determining the physiologic importance of urinary kinins from the measurement of a single component of the system.

Very little information is available regarding the role of urinary kininogen in the regulation of kinin formation. Kininogen arising from glomerular filtration of plasma kininogen synthesized by the liver or from the secretion of kininogen synthesized by the kidney [6, 10] has not been considered to be an important modulator of renal kinin formation. However, this hypothesis has not been critically evaluated and recent measurements of urinary kininogen suggests that first-order kinetics apply. The Km for the reaction between human urinary kallikrein and human low molecular weight kininogen was reported to be 9.0 to 12.5 μM [17, 18]. Human urine contains intact low molecular weight kininogen, in a concentration at least five-hundredfold below its Km for the reaction with kallikrein [6, 9–11, 19]. Although urinary kinin levels may not reflect the concentration at tissue sites, until further information is available, such measurements may provide further insight into the physiologic importance of this system.

To determine the possible physiological importance of kininogen in the formation of kinin within the kidney, various components of the renal kallikrein-kininogen-kinin system were

measured in specific disease states. These disease states were chosen because of expected alterations in either kallikrein or kininogen. Individuals with untreated essential hypertension or subjects with end-stage renal disease were compared to normal subjects. The data suggest that urinary kininogen is a determinant of urinary kinin formation. Furthermore, alterations in urinary kininogen excretion may account for disease specific abnormalities in kinin excretion.

Methods

All clinical investigations were carried out at the Roger Williams General Hospital and Providence Veterans Administration Medical Center. Human studies protocols were approved by the Institutional Review Boards of both hospitals, and all patients gave informed consent. Three groups of patients were studied. There were 21 normal volunteers (20 white and one black) with an average age of 35.3 ± 2.9 years. There were 16 men and five women. No subjects had hypertension or evidence of renal, liver, cardiac, or thyroid disease. These control subjects were allowed an ad lib sodium diet and 2 liters of fluid daily. All medications, including over-the-counter preparations, were discontinued for at least 1 week prior to study.

Twenty men with essential hypertension (19 white and one black) with an average age of 56.3 ± 1.5 years were studied. The diagnosis of essential hypertension was established by exclusion of secondary causes by history, physical examination, serum laboratory studies, urinalysis, urinary metanephrines, and an intravenous pyelogram or renogram when indicated. All subjects were studied on an ad lib diet with approximately 2 liters of fluid intake daily. Anti-hypertensive medications were withdrawn 3 weeks prior to collection of the urine.

Eleven men with end-stage renal disease of various etiologies (ten white and one black) with an average age of 56.3 ± 2.9 years were studied. Six patients received outpatient hemodialysis, while five were enrolled in an outpatient Continuous Ambulatory Peritoneal Dialysis (CAPD) program. All patients were instructed to restrict fluid intake to 1 to 2 liters and sodium intake to 2 to 4 g daily. All medications were withdrawn from these patients 24 hr prior to study.

A single 24-hr urine was collected from each subject as an outpatient. Urinary creatinine excretion was measured to estimate the completion of the 24-hr urine collection. Furthermore, the method of collection was designed to minimize the loss of kinins and kininogen without denaturing kallikrein [9, 20–22]. Subjects were encouraged to void as frequently as possible throughout the day into a graduated beaker. After measuring the volume of each void, one-half the urine was placed into a bottle containing 9 ml of 6N HCl and 1.5 mg of pepstatin, while the other half of the urine was collected untreated. Urines were stored at 4°C throughout the collection. Total volumes were subsequently measured and aliquots stored at −70°C for later analysis. We have found that collections obtained in this way are stable for at least 18 months. Following the collection of urine, blood was obtained after at least 4 hr of upright posture for the measurements of plasma renin activity, serum electrolytes, and creatinine.

Since urinary kinins may not be an accurate reflection of intrarenal kinins, subjects were encouraged to void as frequently as possible during the studies to minimize kinin destruction by kininases and kinin formation by the action of uropepsin and glandular kallikrein on urinary kininogen. The stability of human urinary kinins while urine dwells within the bladder for periods up to 1 hr was investigated previously (Weinberg MS, unpublished data). Urine was collected through a Foley catheter directly into HCl-pepstatin in six normal female volunteers during water diuresis. Following a 15-min baseline collection, the Foley catheter was clamped for 1 hr and then released. A second 15-min collection of free-flowing urine was obtained after the 1-hr period during which time urine was left in the bladder. Urinary kinin levels in the sample dwelling in the bladder for 1 hr were decreased 10.2 ± 6.8% compared to the mean of the two urines obtained during free flow. Since the stability of urinary kinins while urine dwells in the bladder for more than 1 hr was not evaluated, subjects were asked to void at intervals no greater than 1 hr. In addition, acid pH (5 to 6) suppressed while basic pH (6 to 8) enhanced kininase activity. We, therefore, felt justified in allowing urine to dwell in the bladder overnight because urine is usually acidic after an overnight fast.

Assays

Urinary kallikrein radioimmunoassay

Purified human urinary kallikrein and rabbit anti-human urinary kallikrein serum was generously supplied by Dr. Narendra B. Oza, Boston, Massachusetts, USA. The radioimmunoassay procedure, measuring total (active and inactive) kallikrein, has been described previously [23] and was modified by the addition of 25 μl of goat-anti-rabbit antibody on insoluble matrix following the incubation period. After 15 min of equilibration, the bound radioactivity was removed by centrifugation at 4000 rpm for 15 min in a refrigerated centrifuge. The pellet was then counted using a Hewlett-Packard 9815S system with log/logit plots. The non-specific binding, obtained by replacing anti-kallikrein serum with normal rabbit serum, ranged from 2 to 4% and was subtracted from each reaction. Urine samples and purified kallikrein for the standard curve were assayed in triplicate. The inter-assay coefficient of variation of a human urine pool was 11%. The intra-assay coefficient of variation was 6.1%. The recovery of purified human urinary kallikrein was 120 ± 15%.

Urinary kinin radioimmunoassay

Determination of kinins in urine was performed by radioimmunoassay using a highly specific rabbit antibradykinin serum generously supplied by Dr. Colin Johnston (Melbourne, Australia) as reported previously [22, 24]. Non-specific binding determined by substituting normal rabbit serum for the antibradykinin serum ranged from 2.5 to 4.0% and was subtracted from all experimental data. The standard curve ranged from 25 to 1000 pg of bradykinin. In eight experiments, the total binding was 28.4 ± 2.5% and the B/Bo values produced by addition of 50, 250, and 750 pg of unlabeled bradykinin were 82.1 ± 3.0, 57.8 ± 3.0, and 33.1 ± 3.0%, respectively. An internal standard consisting of a normal human urine pool collected in HCl-pepstatin was used in each kinin assay. The inter-assay coefficient of variation of the human urinary kinin pool was 10.4%. The intra-assay coefficient of variation of this pool was 6.5%. The recovery of synthetic bradykinin added to the human urine pool was 96.3 ± 4.3%. The cross reactivity

between bradykinin antiserum and purified human low molecular weight kininogen, generously supplied by Drs. Jack Pierce and John Pisano (Bethesda, Maryland, USA), was 3%.

Determination of kininogen excretion

Total urinary kininogen was calculated by measuring the total amount of kinins generated in urine following trypsinization. Preformed urinary kinin levels were determined in the absence of trypsin. Intact kininogen in each sample was calculated from the difference between total kinins generated after trypsinization and preformed kinins. The incubation with trypsin was performed as described previously [22]. Both total and intact kininogen were reported in μg of kinins/day. Quantitative estimates of the urinary excretion of kininogen were made by assuming that 11.2 μg of kinins are generated from the complete degradation of 1 mg of human low molecular weight kininogen [25]. These calculations were performed to compare excretory levels to the known Km between human urinary kallikrein and low molecular weight kininogen. The method of determining kininogen by measuring kinins generated following trypsinization agrees well with levels of urinary kininogen reported by others using direct radioimmunoassay with an antikininogen antibody [10, 19].

Other analyses

Plasma renin activity was determined using the GammaCoat plasma renin activity radioimmunoassay kit (Clinical Assays, Cambridge, Massachusetts, USA). Urinary aldosterone excretion was measured by radioimmunoassay using the Coat-A-Count solid phase radioimmunoassay kit (Diagnostic Products, Los Angeles, California, USA). The inter-assay variabilities of plasma renin activity and urinary aldosterone were 4.9% and 6.2%, respectively. Serum creatinine was determined by routine analysis. Urinary sodium and potassium were measured by flame photometry using lithium as an internal standard.

Statistics

Differences between normal controls and subjects with essential hypertension and end-stage renal disease were tested using multiple analysis of variance (MANOVA) or multiple analysis of covariance (MANCOVA) since multiple dependent measures were used with a nominal-level factor [26]. Since differences in subject age and urinary sodium rather than group conditions might be responsible for observed differences in group means, MANCOVA was performed controlling for urinary sodium and age for urinary kallikrein [1, 27], and plasma renin activity and urinary aldosterone [28], while age alone was covaried for systolic and diastolic blood pressure [29]. In every case, the effect of the covariate was equivalent within each of the groups [30]. No covariates were used for urinary total and intact kininogen and kinins since no data is available relating their excretion with either age or urinary sodium. We, indeed, found no significant correlations using Pearson correlation test between either age or urinary sodium excretion and kininogen or kinins in any of our study groups. In addition, no correlations were found between age and urinary sodium and urinary kininogen or kinins when either urinary sodium or age were used as covariates.

After multivariate analysis established a significant overall effect between the groups, the use of univariate analysis, ANCOVA and ANOVA, then located statistically significant main effects for the group factor with all of the dependent variables [26]. Statistical tests were performed on means adjusted for the effect of covariates when covariates were used. Significantly different group means for those variables with a main effect for the group were determined by the Dunnett specialized multiple-comparison test [31].

Partial correlations with age and urinary sodium as covariates were performed within each group between the dependent variables [32].

Table 1. Correlations between urinary kinin excretion and parameters studied in normal subjects

	Urinary kinin excretion	
	r[a]	P[b]
Systolic blood pressure, *mm Hg*	0.222	NS
Diastolic blood pressure, *mm Hg*	−0.200	NS
Urinary flow, *liters/day*	−0.020	NS
Urinary potassium, *mEq/day*	0.063	NS
Urinary aldosterone, *μg/day*	0.266	NS
Kallikrein, *μg/day*	0.071	NS
Intact kininogen, *μg kinin/day*	0.645	.003

[a] Partial correlation coefficients with age and urinary sodium as covariates.
[b] Significance values, NS is $P > 0.05$.

Results

Control subjects

In normal volunteers, kallikrein, kinin, and kininogen levels were not different between males and females, and, therefore, the data were pooled. The excretion of total and intact kininogen was 36.69 ± 6.9 and 12.96 ± 2.4 μg of kinins/day, respectively. Assuming 1 mg of human low molecular weight kininogen can generate 11.2 μg/ml of kinins [25], the urinary levels of total and intact kininogen were calculated to be 2.6 ± 0.6 μg kininogen/ml and 0.9 ± 0.2 μg kininogen/ml, respectively. In view of the reported Km for the reaction between kallikrein and kininogen of 9–12.5 μM, or 0.6 to 1.0 mg/ml assuming the molecular weight of low molecular weight kininogen is approximately 68,000 daltons [25], the urinary levels of intact kininogen in normals are approximately 1000 times less than its Km. This assumes normal human urine contains low but not high molecular weight kininogen (Müller-Esterl, Werner, and Weinberg MS, unpublished observations).

Table 1 contains partial correlations controlling for age and urinary sodium for all variables studied in normal volunteers. Urinary kinins were significantly correlated with intact kininogen. No correlation was noted between urinary kinin excretion and kallikrein excretion.

In Table 2, the observed means and statistically significant group differences are shown for normals and subjects with essential hypertension and end-stage renal disease. Hypertensives were significantly older and had greater urine flow than controls, but had no differences in plasma renin activity and creatinine, or urinary potassium, sodium, and aldosterone. Diastolic blood pressure was significantly elevated, while systolic blood pressure was arithmetically, but not significantly, increased. Subjects with end-stage renal disease were also older

Table 2. Group characteristics in normals, essential hypertensives, and end-stage renal disease subjects

	Normal (N = 21)	Hypertensive (N = 20)	Renal disease (N = 11)
Age, *years*	35.3 ± 2.9	56.3 ± 1.5[a]	56.6 ± 3.1[a]
Systolic blood pressure, *mm Hg*	127 ± 5	146 ± 5	140 ± 6
Diastolic blood pressure, *mm Hg*	78 ± 4	97 ± 3[a]	66 ± 4[a]
Plasma			
Renin, *ng/ml/min*	2.41 ± 0.53	2.25 ± 0.48	2.76 ± 0.71
Creatinine, *mg%*	0.78 ± 0.04	0.90 ± 0.30	12.98 ± 1.34[a]
Urinary			
Flow, *liters/day*	1.27 ± 0.15	1.76 ± 0.14[a]	0.55 ± 0.19[a]
Aldosterone, *μg/day*	7.14 ± 1.55	9.88 ± 1.40	3.81 ± 2.08
Potassium, *mEq/day*	50.0 ± 7.3	65.8 ± 9.8	25.1 ± 5.1
Sodium, *mEq/day*	99.3 ± 13.2	118.4 ± 12.6	36.7 ± 6.8[a]
Creatinine, *g/day*	1.53 ± 0.09	1.60 ± 0.08	0.63 ± 0.19

Data are mean ± SE.
[a] $P < 0.05$ compared to Normal group.

Table 3. Components of the urinary kallikrein-kininogen-kinin system in normals, essential hypertensives, and end-stage renal disease subjects

	Normal (N = 21)	Hypertensive (N = 20)	Renal disease (N = 11)
Total kininogen, *μg kinin/day*	36.69 ± 6.99	11.34 ± 2.42[a]	22.75 ± 9.90
Intact kininogen, *μg kinin/day*	12.96 ± 2.40	3.34 ± 0.76[a]	21.85 ± 9.90
Kinins, *μg/day*	23.73 ± 5.20	8.00 ± 1.93[a]	0.90 ± 0.18[a]
Kallikrein, *μg/day*	116.56 ± 29.58	175.59 ± 26.70	11.84 ± 39.70[a]

Data are mean ± SE.
[a] $P < 0.05$ compared to Normal group.

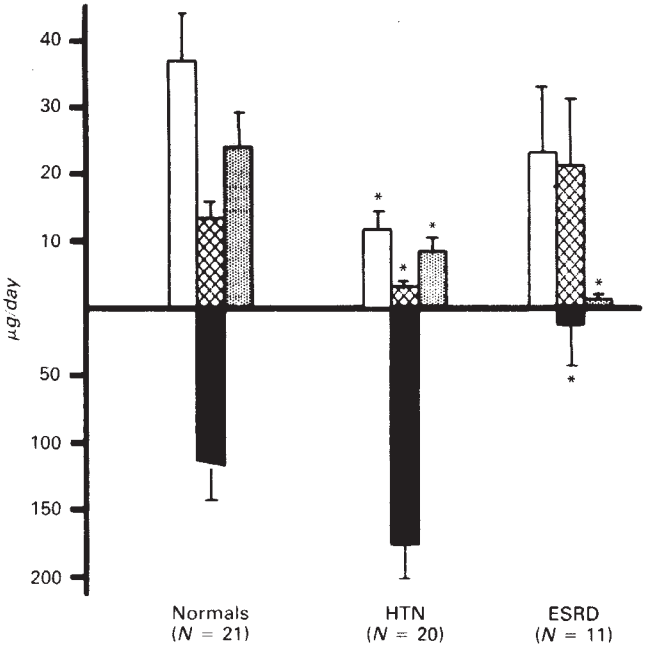

Fig. 1. *Components of the kallikrein-kininogen-kinin system in normals, essential hypertensives (HTN), and end-stage renal disease (ESRD) subjects.* N is number of subjects in each group. Bars represent the 24-hr urinary excretion of total kininogen (▭), intact kininogen (▩), kallikrein (■), and kinin (▤). *$P < 0.05$.

than controls, had significantly elevated plasma creatinine levels, and significantly decreased urinary flow, sodium excretion, and diastolic blood pressure. There were no differences in plasma renin activity or urinary potassium and aldosterone excretion. Systolic blood pressure was arithmetically, but not significantly, increased.

Components of the urinary kallikrein-kininogen-kinin system are shown in Table 3 and Figure 1. Compared to normal subjects, patients with hypertension and end-stage renal disease had significantly reduced urinary kinin excretion. Total and intact kininogen were decreased in hypertensives but not end-stage renal disease patients, while kallikrein was significantly decreased in end-stage renal disease but not hypertension.

Correlations among components of the kallikrein-kininogen-kinin system

To gain insight into the relationship between components of the kallikrein-kininogen-kinin system and other parameters studied, as well as to compare our study population to previous investigations, partial correlations controlling for age and urinary sodium were performed for all variables in the three groups. In normal volunteers, preformed kinins were significantly correlated to intact kininogen, as can be seen in Table 1. Furthermore, intact kininogen was correlated to kallikrein (r = 0.52, P = 0.025) and urinary aldosterone (r = 0.57, P = 0.011), while kallikrein and aldosterone were also related (r = 0.52, P = 0.024). Furthermore, intact kininogen was significantly correlated (r = 0.41, P = 0.045) to aldosterone even when kallikrein, age, and urinary sodium were used as covariates. In hypertensives, urinary kinins were correlated to intact kininogen (r = 0.52, P = 0.026) (similar to the findings in control subjects). Kallikrein excretion was significantly correlated to aldosterone (r = 0.65, P = 0.004) and urinary potassium (r = 0.69, P = 0.001), while intact kininogen and urine flow (r = 0.50, P = 0.036), and aldosterone and urinary potassium (r = 0.67, P = 0.003) were related. In end-stage renal disease, diastolic blood pressure was correlated to total kininogen (r = − 0.72, P = 0.014), intact kininogen (r = − 0.73, P = 0.013), and aldosterone (r = 0.68, P = 0.022). Aldosterone was

correlated to total kininogen ($r = -0.58$, $P = 0.05$) and intact kininogen ($r = -0.59$, $P = 0.47$), while urine flow was correlated to kallikrein ($r = 0.66$, $P = 0.026$).

Discussion

Kinins are potent vasoactive peptides. Their ability to produce vasorelaxation has resulted in speculation about a role for kinins in the physiologic regulation of blood pressure and renal sodium excretion [1, 2]. Specific kinin receptors have been identified in vascular endothelial cells as well as in the distal tubular cell of the kidney [33]. Kinins might influence physiologic processes in these tissues by means of a change in the concentration of kinins at the receptor site (determined by the balance between kinin formation and degradation) and/or an alteration in kinin receptor number or affinity. In addition, any factors that affect the intracellular events that follow the kinin-receptor interaction might also determine the physiologic importance of kinins.

To date, most studies of the physiologic role of renal kinins have focused upon the concentration of kinins in urine and/or urinary kallikrein, which catalyzes the release of active kinins from inactive substrate, kininogen. It is important to note, however, that most studies have failed to find any correlation between urinary excretion of kallikrein and kinins. While this lack of correlation in urine may not reflect activities at tissue sites, it is consistent with the hypothesis that other factors are important in the regulation of kinin formation in urine.

Three major findings of this study support the hypothesis that urinary kininogen is a physiologically important determinant of urinary kinin excretion. First, using a highly specific antibody to bradykinin and a method for collecting urine designed to minimize kinin degradation, we determined urinary excretion of kinins to be 23.7 ± 5.2 μg/day. These values compare favorably to the range of 8 to 42.3 μg/day reported by others [5–7, 9, 10, 21, 34–36]. Utilizing this sensitive assay and a standard technique for releasing kinins from kininogen, we were then able to calculate kininogen excretion. Intact kininogen levels were approximately 0.91 μg/ml (0 to 3.3 μg/ml range), almost one-thousandfold less than the Km for the reaction between urinary kallikrein and urinary kininogen. These are the precise conditions in which substrate concentrations become a significant determinant of product formation. Our calculation of total urinary kininogen is based upon total kinins generated after trypsinization and therefore detects functionally intact kininogen as well as endogenous kinins already released from des-kinin-kininogen. While these calculations may be affected by destruction of kinins at sites proximal to the bladder, the fact that these calculated values for total kininogen compare remarkably well with the values reported using bispecific antibodies to total kininogen argues for the utility of this method [10, 19].

A second finding was the highly significant correlations between the excretion of urinary intact kininogen and urinary kinins. This positive correlation was found in both normal subjects and in those with hypertension. Furthermore, in both these groups, there was no correlation between urinary kallikrein and urinary kinins. This observation is consistent with the hypothesis that urinary kininogen is a physiologic regulator of urinary kinin excretion.

The third major finding and the one of greatest physiologic interest is the observation that a deficiency in urinary kinin excretion is seen in hypertensives and patients with end-stage renal disease. In hypertensives, the kinin deficiency is associated with a deficiency of urinary kininogen excretion with normal excretion of kallikrein. In contrast, in patients with end-stage renal disease, kinin excretion is depressed in association with a decrease in urinary kallikrein excretion while kininogen excretion is normal. The use of covariate analysis indicates that the alterations in kininogen excretion between the three groups cannot be attributed to differences in age, systolic pressure, urinary excretion of sodium, potassium, or aldosterone, or plasma renin activity. Thus, these two pathophysiologic conditions support the hypothesis that urinary kininogen excretion might be important in the regulation of urinary kinin excretion. The physiologic importance of this hypothesis might be tested by altering kininogen excretion through experimental maneuvers.

Previous studies in hypertensives have focused upon urinary kallikrein excretion. A deficiency of either total kallikrein excretion [21, 37, 38] or kallikrein activity [37, 39–49] or the ratio of active to total kallikrein [24] has been reported by some but not all studies [24, 27, 50–57]. Kinin secretion was found to be depressed by Shimamoto [21], but no studies have reported simultaneously on all three components of this system in hypertensive patients.

In our hypertensive patient population, total kallikrein excretion was similar to our control group. Nevertheless, a marked reduction in kinin excretion was found (23.7 vs. 8.0 μg/day) compared to the normal group. We do not believe that these differences can be explained by group differences in age, sodium or potassium excretion, urinary aldosterone, plasma renin activity, or systolic blood pressure. The reduced excretion of kinins might be the result of either increased degradation or decreased formation. A marked reduction in urinary kininogen excretion in these patients was found. While we cannot exclude changes in kallikrein activity as a contributor to the reduced kinins in urine, measurements of total kallikrein by radioimmunoassay have been found to correlate significantly with active kallikrein in both normals and hypertensives [37, 50]. In view of the kinetics of the kallikrein-kininogen-kinin interaction, it is attractive to attribute the decrease in kinin excretion to the deficiency of kininogen excretion and therefore decreased kinin formation.

A deficiency of kinin excretion was also noted in subjects with end-stage renal disease, and this was found in the presence of a markedly depressed urinary total kallikrein excretion. Although decreases in urinary kallikrein excretion have been noted in some studies of end-stage renal disease [53, 58], elevated [59–61], and normal levels [62, 63] of kallikrein activity have also been reported. Since our assay measures total kallikrein protein, it is likely that a reduction in activity would also be found in our patients. The reason for the different kallikrein excretions in subjects with renal disease reported by other investigators may relate to differences in severity of disease, study population, and methodology. Although reduction in kinin excretion might result from an increase in degradation in the urine, it is again attractive to speculate that a reduction in formation of kinins resulting from a deficiency of kallikrein played the major role.

The precise role of urinary kininogen in the physiologic regulation of the renal kallikrein-kininogen-kinin system remains speculative. The data presented suggest that urinary

kininogen may be an important regulator of kinin excretion. Furthermore, in certain disease conditions, kininogen deficiency may be of pathophysiologic significance.

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