

## **KALLIKREIN-KININ SYSTEM INVOLVEMENT IN CHRONIC GLOMERULAR DISEASE**

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### **Summary**

Urinary kallikrein activity of 16 normotensive and seven hypertensive patients with proven chronic glomerular disease was compared to urinary kallikrein activity of 25 healthy subjects and 24 uncomplicated essential hypertensive patients. All groups were matched for age, sex, race, urinary sodium excretion and creatinine clearance. No significant differences were observed between healthy subjects and patients with essential hypertension in urinary kallikrein activity; it was reduced only in patients with chronic glomerular disease irrespective of hypertension and histological findings. We conclude that the renal kallikrein-kinin system may be involved in a non immunological mechanism of progressive glomerular damage.

### **Introduction**

Investigations of hypertension in patients with renal parenchymal disease have emphasized the role of the renin-angiotensin system and of extracellular fluid volume. Additional studies have suggested that lack of renal vasodilator compounds may influence blood pressure in patients with systemic hypertension (see Reference [1] for a general review).

Urinary kallikrein (U-KK), an enzyme produced by the kidney, catalyses the production of small vasodilator peptide hormones called kinins. Since a decreased U-KK excretion has been shown in patients with essential hypertension, the kallikrein-kinin system has been implicated in the pathogenesis of hypertension [1]. Moreover, a reduced U-KK excretion has been reported in patients with both renal parenchymal disease and hypertension, indicating that this system may be also involved in the hypertension associated with renal parenchymal disease [2]. However, it is still unclear whether this finding is related to the hypertensive process or merely reflects the renal damage.

The present study was undertaken to evaluate U-KK excretion in patients with chronic glomerular disease with or without associated hypertension and with or without mild renal impairment.

## Methods

The seventy-two women studied included 25 healthy subjects (C), 24 patients with uncomplicated essential hypertension and 23 patients with chronic glomerular disease, of whom 16 were normotensive and seven hypertensive.

Essential hypertension was diagnosed after history, physical examination, blood chemistry studies, urinalysis and rapid-sequence intravenous pyelogram.

Twenty-one of the twenty-three patients with chronic glomerular disease were evaluated by percutaneous renal biopsy. The diagnosis in the normotensive group was: membranous glomerulonephritis (GN) (n=3), IgA nephropathy (n=3), lupus nephritis (n=2), mesangio-proliferative GN (n=2), focal GN (n=1). Alport's disease (n=1) and renal amyloidosis (n=1). The diagnosis in the hypertensive was: membranous-proliferative GN (n=3), lupus nephritis (n=2) and mesangio-proliferative GN (n=1). In 19 of the 23 patients with chronic glomerular disease urinary protein loss was over 300mg per day. Two of these presented an overt nephrotic syndrome.

No subjects had received any drugs for at least two weeks prior to the study. Each subject was hospitalized and kept on a fixed daily salt diet containing 100mEq of sodium and 80mEq of potassium. On the fourth, fifth and sixth days of sodium intake 24 hour urine collections were obtained for kallikrein activity, creatinine and sodium concentration. Urine was stored at  $-20^{\circ}\text{C}$  immediately after each voiding until it was assayed.

U-KK activity was determined by spectrophotometry. The first phase of extraction was carried out by adding 100ml of acetone to 50ml of urine taken from the 24 hour collection. The mixture was then centrifuged, the supernatant eliminated and the remaining fraction resuspended in 5ml of water and 1ml of acetone. After centrifugation, the supernatant was dialysed for 20 hours against tap water. U-KK activity was determined in the dialysate by measuring its capacity of cleaving the ester bond of a synthetic substrate (p-Tosyl-Arginine-Methyl-Ester) (TAME). To minimize possible interactions with urinary endogenous substances, a sample correction was performed as described elsewhere [3]. Purified kallikrein (Calbiochem-Behring Corp) was employed as standard. U-KK activity was expressed as Esterase Units (EU). One EU is defined as the amount of enzyme which hydrolyses 1M of TAME per minute at pH 8 at  $30^{\circ}\text{C}$ .

Serum and urinary creatinine and sodium were measured by the Jaffé chromogen reaction and flame photometry, respectively. All creatinine clearance values were corrected for body-surface area. The intrasubject coefficient of variation for creatinine clearance, measured on three consecutive occasions, was  $1.6 \pm 1.4$  per cent (mean  $\pm$  SD).

Blood pressure was obtained with a standard sphygmomanometer taking the diastolic pressure as the phase V Korotkoff sound (disappearance).

The results were evaluated by unpaired Student's 't' test.

## Results

The age ranges (reported as mean±SD) of three groups considered in this study were similar: C (37.8±9.7), essential hypertension (40.0±9.4) and chronic glomerular disease (39.7±17.3). Diastolic blood pressure values (mean±SD mmHg) were comparable in the essential hypertensives (104.2±11.1) and hypertensive (101±4.2).

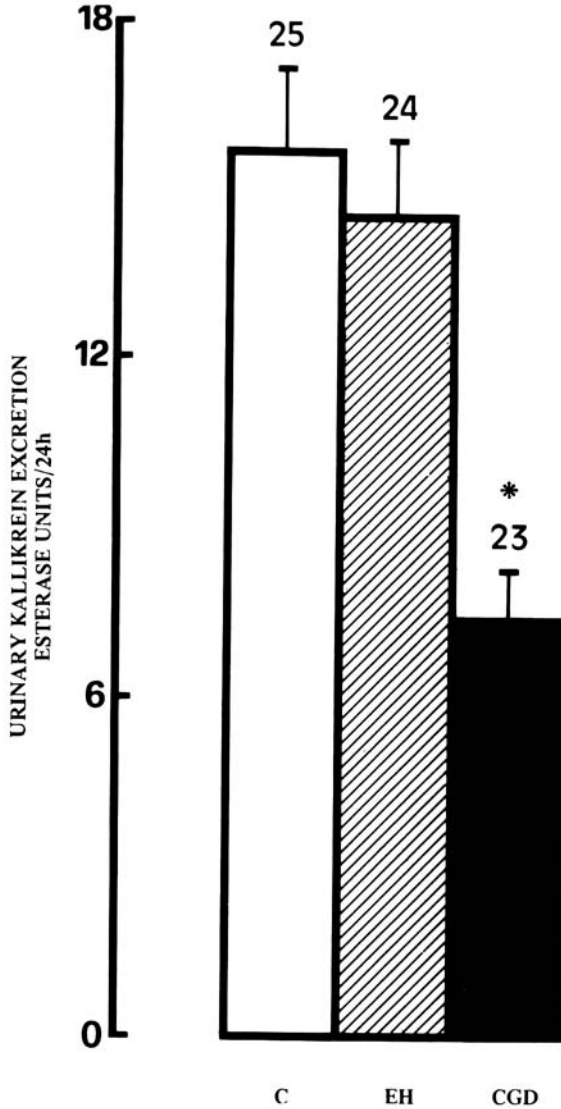


Figure 1. Urinary kallikrein excretion in controls (C), essential hypertensives and patients with chronic glomerular disease (CGD), expressed as mean±SE, \*p<0.001

Urinary excretion of sodium, expressed as mean $\pm$ SD mEq/day, was the same in all four groups: C (100.2 $\pm$ 27), essential hypertension (101.0 $\pm$ 30), normotensive (98.0 $\pm$ 20) and hypertensive (99.5 $\pm$ 15). No significant differences were observed in creatinine clearance (mean $\pm$ SD ml/min) among the four groups: C (101.0 $\pm$ 5.8), essential hypertension (99.5 $\pm$ 4.2), normotensive (93.0 $\pm$ 16.8) and hypertensive (84.8 $\pm$ 23.7).

As shown in Figure 1, U-KK activity (mean $\pm$ SE) did not differ in the essential hypertension (14.9 $\pm$ 1.3) and C (15.7 $\pm$ 1.2). It was however significantly lower ( $p<0.001$ ) in patients with chronic glomerular disease (7.3 $\pm$ 0.8) compared to both C and essential hypertension, despite similar urine volumes and urinary sodium excretion.

When the chronic glomerular disease group was subdivided into normotensive and hypertensive U-KK activity was shown to be similar (mean $\pm$ SD): 7.3 $\pm$ 3.7 and 7.07 $\pm$ 2.4, respectively (Figure 2).

No association was found between U-KK activity and diastolic blood pressure or proteinuria in any group.

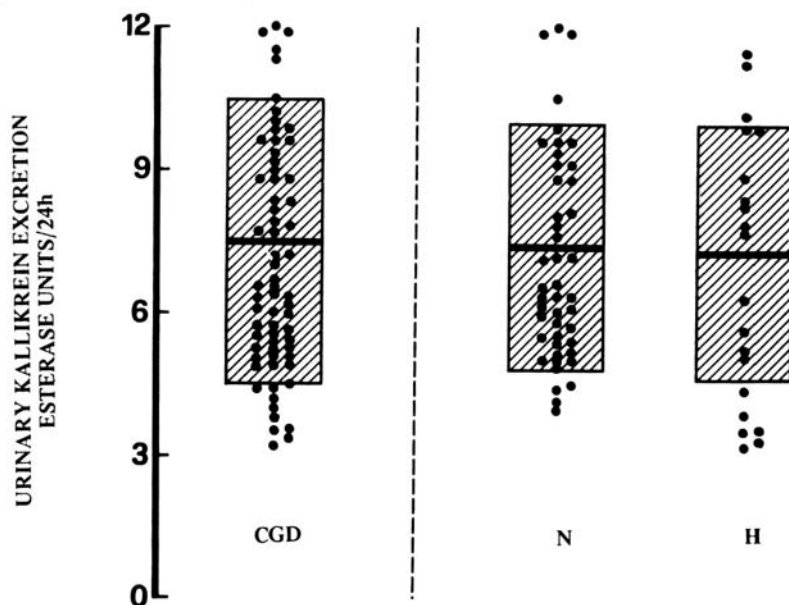


Figure 2. Urinary kallikrein excretion in patients with chronic glomerular disease (CGD). Normotensives (N) and hypertensives (H) in this group had comparable excretion rates; the shadowed areas above and below the lines represent 1 SD

## Discussion

The reduced urinary kallikrein excretion in patients with chronic glomerular disease, irrespective of blood pressure, suggests that a deficiency of the kallikrein-kinin system have been directed primarily at patients with essential hypertension.

Most of these reports, which indicate subnormal values of U-KK, are still a subject of controversy [1]. More recent works state that in essential hypertension kallikrein excretion is not significantly different from that of normotensive controls [4,5] and, secondly, that renal function is an important determinant of kallikrein excretion, since it varies directly with glomerular filtration rate [6]. In fact, Holland et al [6] found that kallikrein excretion is reduced only in those hypertensive patients with mild renal insufficiency, compared to race-matched normotensive controls. Mitas et al [2] suggest that decreased kallikrein excretion of renal parenchymal hypertensives may play a primary aetiological role in the genesis of hypertension. However, kallikrein excretion was not determined in normotensive patients with comparable renal insufficiency.

In experimental nephritis induced by anti-glomerular basement membrane antibodies and in aminonucleoside induced nephrosis a significant decrease in U-KK excretion unrelated to urinary sodium excretion and urinary volumes has been reported [7]. The parallel reduction in kallikrein excretion observed in both these experimental models suggests that it is not based upon immunological mechanisms, but may be a response to different types of injury.

Our results seem to agree with experimental data. In fact, the excretion of the enzyme was reduced only in patients with chronic glomerular disease, irrespective of histological and pressure findings. At present we do not know if the reduced enzymatic activity is linked to the pathogenesis of the disease. However we can probably exclude that the phenomenon is merely a direct consequence of the decreased renal mass, since in our patients the glomerular filtration rate was either normal or slightly reduced.

Whether renal vasodilator factors are involved in non immunological mechanisms of progressive glomerular damage is still unclear. Interestingly chronic glomerular disease seems to be characterized by a reduced renal production of prostacyclin [8], a potent vasodilator agent which plays an important part in modulating cortical events [8,9]. Thus, presuming that the activity of the renal kallikrein-kinin system is reflected by U-KK excretion, a tempting hypothesis could be that the decrease in renal prostacyclin and kinins may represent an adaptive mechanism to counteract the haemodynamic changes associated with progressive glomerular sclerosis.

## References

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