Mechanisms of Suppression of Renal Kallikrein Activity in Low Renin Essential Hypertension and Renoparenchymal Hypertension

Kazuaki Shimamoto, Atsushi Masuda, Toshiaki Ando, Nobuyuki Ura, Motoya Nakagawa, Yoshihiro Mori, Hidehisa Nakagawa, Toru Sakakibara, Hitoko Ogata, and Osamu Iimura

The mechanism of suppression of renal kallikrein activity in low renin essential hypertensive and renoparenchymal hypertensive patients was investigated in this study. From Sephadex G-200 column chromatography studies, a single kallikrein peak was observed in all samples from normal subjects, low renin essential hypertensive and renoparenchymal hypertensive patients, and in purified kallikrein solution. The enzyme-specific activity around the kallikrein peak in all urine samples from each group was significantly lower than that in purified kallikrein, and a significantly lower specific activity was found in both patient groups than was found in normal subjects. Moreover, it was also recognized that the specific activity of kallikrein decreased in all cases with the increase of the molecular weight of kallikrein, and this tendency was observed more obviously in the low renin essential hypertensive and renoparenchymal hypertensive patients than in the normal subjects. These results suggest the presence of a kallikrein-specific inhibitor with a low molecular weight in human urine, although the possibility of a variant form of kallikrein cannot be excluded. (Hypertension 1989;14:375–378)

Kallikrein is a specific protease that produces a kinin from a kininogen. It has been reported that the kallikrein-kinin system may have an important role in many hypertensive diseases related to sodium metabolism.1–6 Except for some reports,7,8 previous studies have revealed that in essential hypertension, enzymatic activity or immunoreactivity of urinary kallikrein were suppressed.2,5,6 Furthermore, it was reported from our laboratory9 that the slope of the regression line between urinary kallikrein quantity and activity was significantly more moderate in a low renin group than in normal subjects or in a normal renin group. As one of the possibilities to explain the lowered kallikrein activity in low renin essential hypertension, a kallikrein inhibitor may exist in human urine and play an important role in the pathogenesis and pathophysiology of this disease. Regarding the kallikrein inhibitor, only one report is available from Geiger and Mann10 who described a kallikrein inhibitor in rat kidney tubules, and no reports have been found about a kallikrein-specific inhibitor derived from the human kidney.

The aim of the present study was to determine the existence of a kallikrein inhibitor that is derived from the kidney in patients with low renin essential hypertension and those with renoparenchymal hypertension.

Materials and Methods

Twenty-four-hour urine samples were collected from 15 normal control subjects (nine men and six women, 39.7±3.9 years old, mean±SEM), six patients with low renin essential hypertension (two men and four women, 48.3±2.6 years old), and 18 patients with renoparenchymal hypertension (10 men and eight women, 49.1±4.6 years old). The patients and normal subjects, who were all of similar racial background, were admitted to our hospital and were maintained on a regular diet containing
120 meq/day sodium and 75 meq/day potassium. The patients with low renin essential hypertension were uncomplicated and renal function was within normal range, as shown by a creatinine clearance of more than 70 ml/min. The severity of the hypertension was mild to moderate (World Health Organization stage I or II). The essential hypertensive patients were divided into low and normal renin groups according to our previously reported procedure. The patients with a low value of plasma renin activity (PRA) (low renin group) were classified by both subnormal supine and 2-hour upright PRA, with supine and upright PRA, for all ages, being less than 0.3 and 0.6 ng/ml/hr, respectively. The patients with renoparenchymal hypertension consisted of 10 with chronic glomerulonephritis, four with diabetic nephropathy, two with polycystic kidney, one with Schonlein-Henoch's purpura nephritis, and one with pycnephritis. The creatinine clearance of these patients was 46.1±7.7 ml/min (mean±SEM). All patients were untreated or had discontinued their treatment for at least 1 week before the urine collection.

Urinary kallikrein excretion was measured for both the kallikrein quantity by direct radioimmunoassay and the kallikrein enzymatic activity by kininogenase assay reported previously. Purified human urinary kallikrein prepared by Dr. J. Chao (Chao and Margoulis) was used in direct radioimmunoassay of kallikrein. The purification factor from the crude urine concentrates to purified kallikrein was 2,114-fold. Direct radioimmunoassay of kallikrein was performed according to the following procedure. One to ten microliter samples or fractionated samples, Iodine-125-labeled kallikrein, and free kallikrein were divided by the specific antibody for human urinary kallikrein, and 0.01 M phosphate-buffered saline, pH 7.0, containing 0.1% bovine serum albumin were incubated at 20° C. After 24 hours of incubation, bound kallikrein and free kallikrein were divided by the polyethylene glycol method. The supernatant was aspirated, and the radioactivity in the unwashed precipitates was counted by gamma spectrometer (Wallack Co.). Kininogenase assay was performed with the method previously reported. The reaction of kinin generation from bovine low molecular weight kininogen (Seikagaku Kogyo Co., Tokyo, Japan) in each sample was carried out in an incubation buffer (0.02 M phosphate buffer, pH 8.5, containing 0.003 M phenanthroline and 0.03 M disodium EDTA). A 10 µl sample of diluted urine and 20 µl of incubation buffer were mixed and preincubated for 5 minutes at 37° C. To keep pH equal in each incubation solution, all urine samples were diluted with an incubation buffer. After the preincubation, 50 µg kininogen dissolved with 20 µl incubation buffer was added and incubated at 37° C for 30 minutes. Then 450 µl cold ethanol was added to the assay tubes to stop the reaction, and these tubes were centrifuged at 4° C. After these procedures, generated kinin in 2.5 µl supernatant was measured by using the kinin radioimmunoassay method established in our laboratory. Kallikrein-specific activity was calculated from the enzyme activity divided by the immunoreactivity.

**Column Chromatography Study**

Urine samples of six control subjects (four men and two women, 39.8±4.5 years old), four low renin essential hypertensive patients (two men and two women, 49.5±3.7 years old), and five patients with renoparenchymal hypertension (three men and two women, 44.0±9.1 years old) were selected at random from all subjects. Pure kallikrein solution was applied to a 1.0×28.0 cm Sephadex G-200 column, and eluted by phosphate-buffered saline, pH 7.0, containing 0.1% bovine serum albumin. One milliliter of every fractionated sample was collected. Kallikrein quantity, enzymatic activity, and specific activity of each fraction were studied by the same method described previously.

**Statistical Analysis**

Results are expressed as mean±SEM. The evaluation of statistical probability was carried out with analysis of variance and with Student’s t test, where appropriate. The 5% probability level was used as a criterion for significance.

**Results**

**Kallikrein-Specific Activity in Each Group**

As shown in Figure 1, the daily excretion of both urinary kallikrein quantity and activity were significantly lower in both the low renin essential hypertensive patients (71.9±21.2 µg/day and 341.9±94.7 µg kinin/min/day, respectively) and the patients with renoparenchymal hypertension (45.6±11.1 µg/day and 208.8±51.8 µg kinin/min/day, respectively) than in normal control subjects (248.6±56.2 µg/day and 2,386.9±530.0 µg kinin/min/day, respectively). Significantly lower kallikrein-specific activity was observed in both the low renin essential hypertensive patients (4.7±0.3) and the patients with renoparenchymal hypertension (4.7±0.4) compared with normal subjects (9.2±0.4). No significant difference was found in urinary kallikrein excretion between men and women in these groups.

**Column Chromatographic Study of Each Sample**

The daily excretion of both kallikrein quantity, kallikrein activity, and kallikrein-specific activity were 188.5±70.3 µg/day, 1,585±691 µg kinin/min/day, and 8.6±0.8, respectively, in six control subjects; 106.8±29.5 µg/day, 433.6±109.8 µg kinin/min/day, and 4.1±0.4, respectively, in four low renin essential hypertensive patients; and 77.4±22.0 µg/day, 251.7±79.0 µg kinin/min/day, and 3.3±0.5, respectively, in five renoparenchymal hypertensive patients. The specific activity of kallikrein was
FIGURE 1. Bar graphs showing excretion of urinary kallikrein quantity (KAL-q) and activity (KAL-act) and kallikrein-specific activities (SPEC) in normal subjects (NC), low renin essential hypertensive patients (LRH) and renoparenchymal hypertensive patients (RPH).

significantly lower (p<0.01) in both the low renin group and renoparenchymal hypertensive group.

In the gel filtration profile of urine from normal subjects, low renin essential hypertensive and renoparenchymal hypertensive patients, and a pure kallikrein solution, both kallikrein quantity and activity appeared as a single peak in all samples. No kallikrein quantity peak without kallikrein activity was found in this column chromatography study. Furthermore, we examined the kallikrein-specific activity of the fractionated samples (fraction numbers 14–18). As shown in Figure 2, no significant change among these fractions (5.2±0.2, 5.6±0.1, 5.4±0.2, 5.5±0.3, and 5.6±0.2, respectively) was observed in the purified kallikrein solution. The specific activity in normal subjects (3.4±0.3, 3.8±0.3, 4.2±0.5, 4.5±0.4, and 4.1±0.3, respectively), low renin hypertensive patients (1.8±0.2, 2.3±0.1, 2.6±0.2, 3.0±0.2, and 3.1±0.2, respectively), and renoparenchymal hypertensive patients (1.9±0.4, 1.8±0.4, 2.4±0.4, 2.6±0.3, and 2.8±0.3, respectively) is also shown in Figure 2. It was observed that the specific activity decreased in all cases with the increase of kallikrein molecular weight (Figure 2). In the fractionated samples of this column chromatography study, the kallikrein-specific activity from normal subjects and low renin essential hypertensive and renoparenchymal hypertensive patients were significantly lower compared with that from the pure kallikrein solution. Furthermore, the samples from the low renin essential hypertensive and the renoparenchymal hypertensive patients showed significantly lower kallikrein-specific activity than that from the normal control subjects (Figure 2).

Discussion

It has been reported that urinary kallikrein quantity and activity are lower in essential hypertensive patients than in normal subjects.3-6 It was also reported from our laboratory7,12 that both kallikrein quantity and kallikrein activity were suppressed, and the specific activity of kallikrein more obviously lower in low renin essential hypertensive patients than in normotensive subjects.

In this study, the mechanism of the lowered specific activity in low renin essential hypertensive patients was investigated. In column chromatogra-
phy, a single peak was observed at fraction number 16 in both kallikrein radioimmunoreactivity and kininogenase activity in all urine samples from each of the groups and the purified kallikrein solution. Furthermore, no other peak of kallikrein radioimmunoreactivity without the peak of kininogenase activity could be found in this study. When Iodine-125-labeled kallikrein solution was eluted under the same conditions, the peak of radioactivity appeared at fraction number 16, as did the peak of kallikrein radioimmunoreactivity of urine samples. These results suggest that there is no high molecular weight inhibitor bound with kallikrein and that a very low molecular weight inhibitor might exist in human urine.

Then, to investigate the low molecular weight inhibitor, the kallikrein-specific activity of fraction numbers 14–18 was studied in each urine sample and in the purified kallikrein solution. Significantly lower specific activity was found in normal subjects and low renin essential hypertensive and renoparenchymal hypertensive patients than in the purified kallikrein, and a significantly lower specific activity of kallikrein was found in the low renin essential hypertensive and the renoparenchymal hypertensive patients than in the normal control subjects. It was also recognized that the specific activity decreased in all cases with the increase of the molecular weight of kallikrein. Furthermore, this tendency was observed more in the low renin hypertensive and the renoparenchymal hypertensive patients than in normal subjects. These findings suggest that a kallikrein inhibitor with very low molecular weight exists in human urine derived from the kidney. This inhibitor might exist not only in low renin essential hypertensive and renoparenchymal hypertensive patients but also in normal subjects, and the proportion of inhibitor to kallikrein should be higher in patients with low renin essential hypertension and in those with renoparenchymal hypertension. On the other hand, the possibility cannot be excluded from our study that a variant kallikrein produced by a different kallikrein gene might exist in these subjects, as suggested by Berry et al.15

Therefore, further investigation is needed to clarify the mechanism of the suppressed specific activity of kallikrein.

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