

Urinary Kallikrein Excretion and Sodium Metabolism in Hypertensive Patients

MASAHIDE SEINO, KEISHI ABE, YOICHI OTSUKA, TETSUO SAITO,
NOBUO IROKAWA, MINORU YASUJIMA, SATORU CIBA and
KAORU YOSHINAGA

*The Second Department of Internal Medicine, Tohoku University
School of Medicine, Sendai 980*

SEINO, M., ABE, K., OTSUKA, Y., SAITO, T., IROKAWA, N., YASUJIMA, M., CIBA, S. and YOSHINAGA, K. *Urinary Kallikrein Excretion and Sodium Metabolism in Hypertensive Patients.* Tohoku J. exp. Med., 1975, 116 (4), 359-367 — Urinary kallikrein excretion was measured in 21 healthy subjects and 44 patients with various types of hypertension. The kallikrein activity was determined by the method of esterolytic assay. The excretion rates in normal subjects were 112.9 ± 11.1 (S.E.) EU/day. The kallikrein excretion was decreased in patients with essential hypertension, the mean estimated values were 75.2 ± 10.0 EU/day. In this disease, however, an enhancement of urinary kallikrein was observed after sodium depletion. An obvious increase in kallikrein excretion was found in the primary aldosteronism. In primary aldosteronism and renovascular hypertension, one of the secondary aldosteronisms, there was a good correlation between the urinary kallikrein output and the urinary sodium excretion. The present data indicate that the renal kallikrein-kinin system, one of the renal anti-hypertensive factors, is suppressed in essential hypertension and is under the influence of mineralocorticoid levels. — kallikrein esterolytic assay; kallikrein-kinin system; various types of hypertension

The presence of kallikrein in human urine has been known since Frey's original studies (Frey 1926). Urinary kallikrein differ distinctly from the plasma kallikrein (Webster and Pierce 1963) but they are indistinguishable from kidney kallikrein in molecular dimensions, pH optima and inhibitors behaviour (Nustad 1970). It is supposed that urinary kallikrein, produced in the kidney and excreted in urine, might be one of the renal antihypertensive factors. However, studies on the role of kallikrein-kinin system in hypertensive patients have hitherto been very scarce in the literature (Elliot and Nuzum 1934; Miwa 1965). Recently, Margolius and his coworkers (1971, 1972, 1974) described that abnormal excretion of kallikrein was observed in hypertensive patients. These reports attracted much attention of many researchers.

In our laboratory, urinary kallikrein excretion was measured in various types of hypertension, and the influence of sodium depletion or sodium load on the urinary kallikrein output was studied in the essential hypertension patients to elucidate the pathophysiological significance of urinary kallikrein in hypertension.

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MATERIALS AND METHODS

Method for the measurement of urinary kallikrein

Urine was collected for 24 hr in a bottle containing a small amount of toluene as a preservative, and kept in a refrigerator at 4°C. It was then stored at -15°C until the chemical assay. One hundred ml of each urine were taken and dialysed against running tap-water for 24 hr at 4°C, then concentrated to 1/40-1/50 volume by carbowax 6000 at 4°C. After concentration the volume was measured and 1 ml was used for assay.

Esterolytic activity was estimated by the colorimetric method described by Moriya (1964) after a slight modification by us (Fig. 1). The modifications were trichloroacetic acid (TCA) concentration (using 30% TCA instead of 0.38 M in the original method) and elimina-

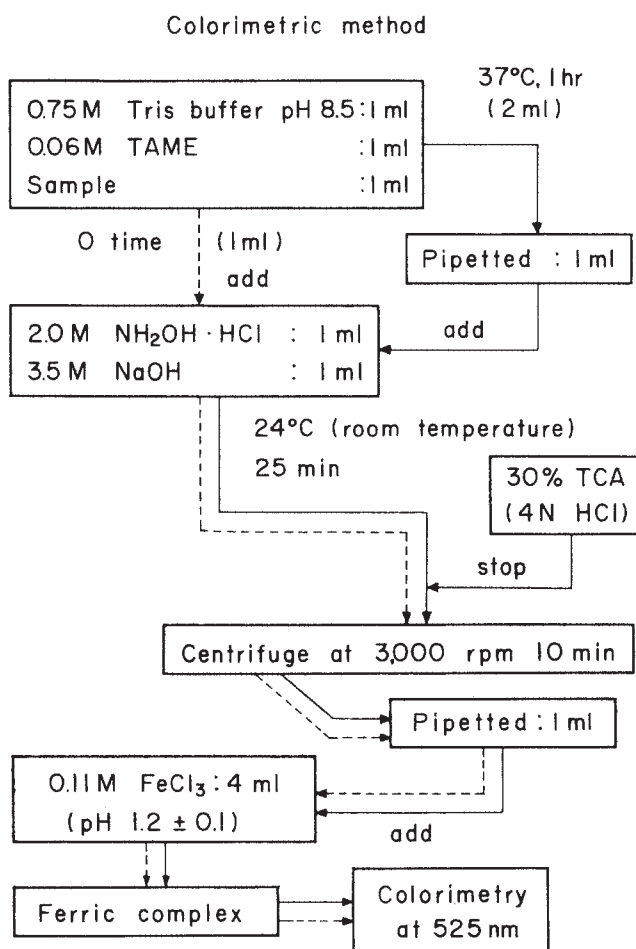


Fig. 1. Colorimetric method for the estimation of urinary kallikrein. Broken line, zero time (before incubation); solid line, incubation for one hr at 37°C. TAME, p-toluenesulfonyl-L-arginine-methylester hydrochloride; TCA, trichloroacetic acid.

tion of the precipitates by centrifugation. By this way, deproteinization was more complete. Human urinary kallikrein (39.4 FU/mg), purified by Prof. Moriya, (Sci. Univ. of Tokyo), was used as a standard. One esterase unit (EU) was defined as a dose of human urinary kallikrein corresponding to one Frey unit (FU). One esterase unit of kallikrein hydrolysed 2.6 μ moles of TAME (p-toluenesulfonyl-L-arginine-methylester hydrochloride, a product of Nakarai chemicals, Ltd., Kyoto) for one hr incubation at 37°C. Overall recovery rate by this method was 79-88%.

Plasma renin activity was measured by modification of Haber's method (Abe et al. 1972). Urinary sodium was determined by an autoanalyzer.

Subjects

Twenty-one healthy subjects and 44 patients with various types of hypertension were included in this study. Hypertensive patients consisted of 23 with essential hypertension, 9 with primary aldosteronism, 4 with pheochromocytoma, and 8 with renovascular hypertension. The control subjects consisted of 12 men and 9 women, aged from 15 to 73 years (43.6 ± 4.2). Essential hypertension consisted of 12 men and 11 women aged from 24 to 59 years (37.4 ± 1.9). Primary aldosteronism consisted of 3 men and 6 women aged from 20 to 45 years. Pheochromocytoma consisted of 2 men and 2 women aged from 31 to 45 years. Renovascular hypertension consisted of 4 men and 4 women aged from 19 to 52 years. These hospitalized hypertensive patients received no antihypertensive drugs except one patient with pheochromocytoma. Patients with essential hypertension had no albuminuria, no renal impairment, phenolsulfonphthalein test and creatinine clearance were normal.

To examine the influence of sodium depletion or sodium load on urinary kallikrein excretion, 8 cases of essential hypertension were studied. Each patient received a diet containing 150 mEq of sodium per day for a week. Thereafter, 30 mEq was given for 3 days. From the next day 7 patients took 250 mEq for a week, further 5 patients was given a high sodium diet of 340 mEq per day for additional one week. On the next morning of each period, blood samples were drawn from the cubital vein after one hour recumbency for the measurement of plasma renin activity.

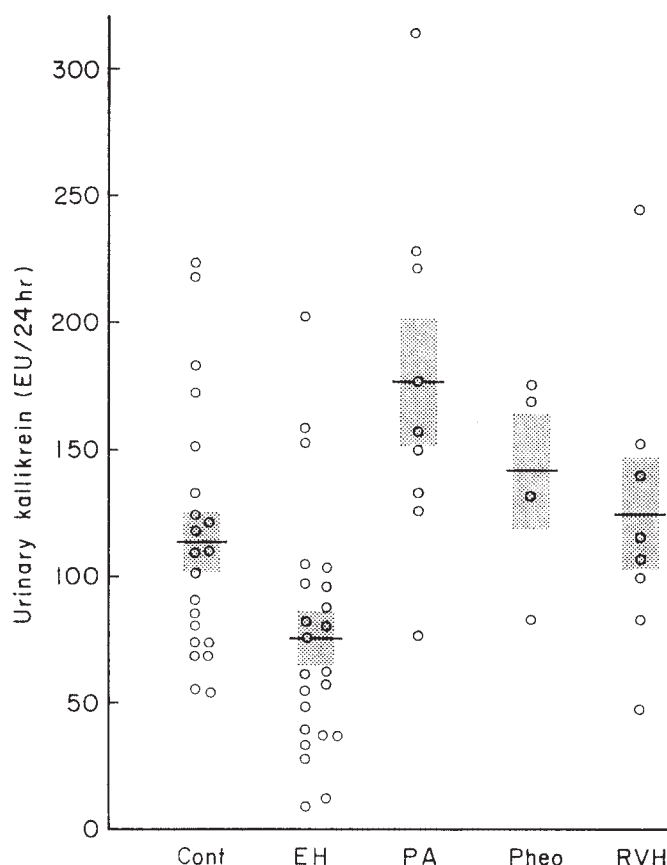


Fig. 2. Urinary kallikrein excretion in control subjects and hypertensive patients. The lines and mist columns shown are means \pm s.e. Essential hypertension differs from control values ($0.01 < p < 0.05$). Primary aldosteronism differs from control ($0.01 < p < 0.05$). Pheochromocytoma and renovascular hypertension are not significantly different from control subjects.

Abbreviations: Cont, control subjects; EH, essential hypertension; PA, primary aldosteronism; Pheo, pheochromocytoma; RVH, renovascular hypertension.

RESULTS

Kallikrein excretion in various hypertensive patients

Urinary kallikrein excretion in healthy subjects and hypertensive patients is illustrated in Fig. 2. Urinary kallikrein excretion in the control subjects ranged from 51.0 to 222.7 EU/day with a mean of 111.29 ± 11.1 (s.e.). Kallikrein output was apparently decreased in essential hypertension. In 8 cases of them, kallikrein excretions were less than lower limit of the normal values. The estimated values were from 10.0 to 204.1 EU/day with a mean of 75.2 ± 10.0 and it differed from the control with $0.01 < p < 0.02$. Twenty four hours urine volume was $1,344.7 \pm 116.9$ (s.e.) ml in healthy subjects and $1,366 \pm 97.5$ ml in patients with essential hypertension. There was no significant difference between them.

On the other hand, augmentation of kallikrein excretion was observed in primary aldosteronism. The estimated values ranged from 75.5 to 314.1 EU/day with a mean of 175.3 ± 23.4 , and it differed from control with $0.01 < p < 0.05$ and from essential hypertension with $p < 0.01$. In pheochromocytoma, kallikrein excretion ranged from 82.5 to 174.8 EU/day with a mean excretion of 140.3 ± 21.6 . Renovascular hypertension had widely distributed kallikrein excretion ranging from 47.4 to 241.9 EU/day with a mean of 121.9 ± 20.8 . In the latter two diseases urinary kallikrein excretion was not significantly different from the control subjects.

Urinary kallikrein excretion and sodium metabolism

Fig. 3 shows the relationship between urinary sodium excretion and urinary kallikrein output. In healthy subjects and essential hypertensives, there was no

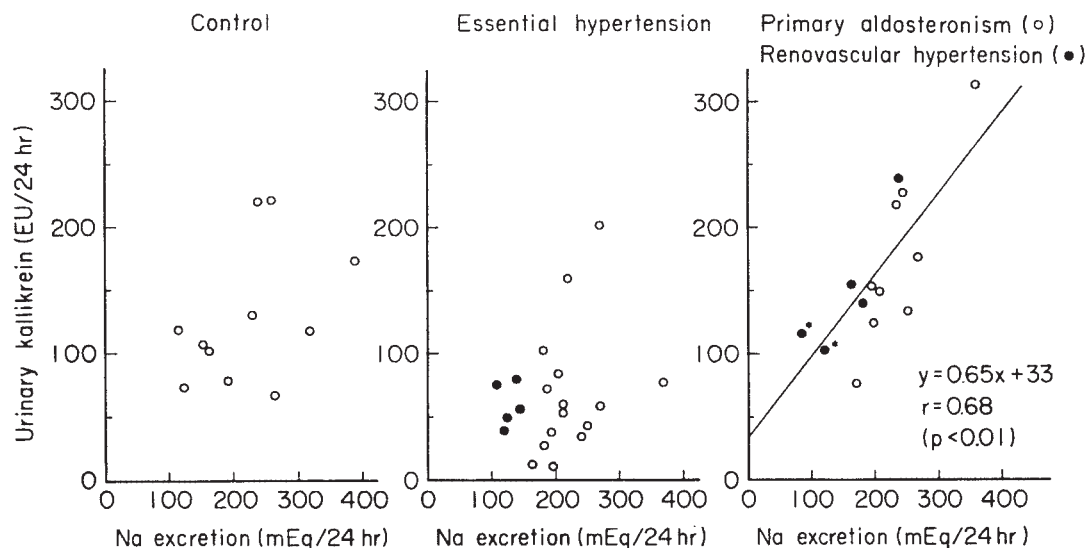


Fig. 3. The relationship between urinary sodium and urinary kallikrein excretion. Left: eleven control subjects on free-salt intake. Middle: twenty patients with essential hypertension. The closed circles indicate 100–150 mEq per day sodium diet. Right: nine cases of primary aldosteronism (○) and 5 cases of renovascular hypertension (●). 100–150 mEq per day sodium diet. There was a correlation between them ($r=0.68$, $p<0.01$).

correlation between them, while obvious correlation was observed in primary aldosteronism and renovascular hypertension.

Fig. 4 demonstrates the changes in urinary sodium excretion, plasma renin activity, urinary kallikrein excretion, and urinary volume through various sodium intakes in essential hypertension. Kallikrein excretion was 54.8 ± 7.1 EU/day during receiving a diet containing 150 mEq sodium per day. Under low sodium diet of 30 mEq per day, urinary kallikrein excretion increased to 80.0 ± 13.0 EU/day with augmentation of plasma renin activity from 11.3 ± 3.4 to 21.7 ± 6.3 ng per ml. On the contrary, high sodium intakes induced a decrease of kallikrein excretion and plasma renin activity. The excretion rates reduced to 54.0 ± 6.8 EU/day during 250 mEq sodium intake and 49.1 ± 8.1 EU/day during 340 mEq sodium intake. Plasma renin activity also decreased to 11.3 ± 3.8 ng per ml during the sodium intake of 250 mEq and 8.2 ± 3.2 ng per ml during 340 mEq.

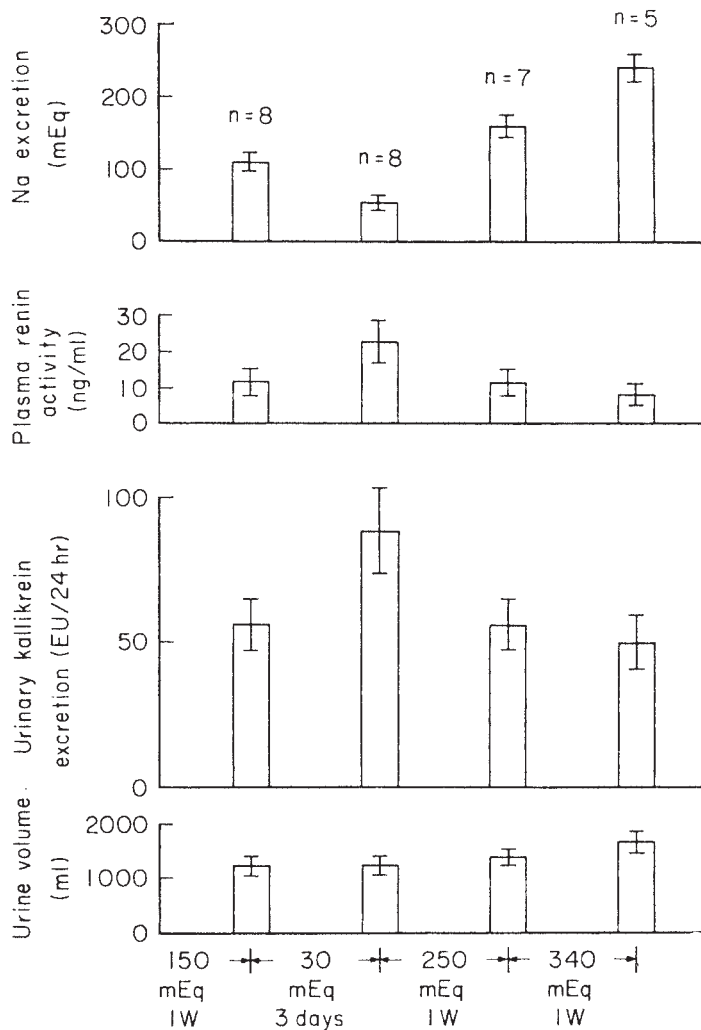


Fig. 4. The changes in urinary sodium excretion, plasma renin activity, urinary kallikrein excretion and urinary volume during various sodium intakes in essential hypertension. Values shown are means \pm s.e. Urinary kallikrein increased under low sodium diet of 30 mEq per day with augmentation of plasma renin activity than that of 250 mEq sodium diet ($0.01 < p < 0.05$).

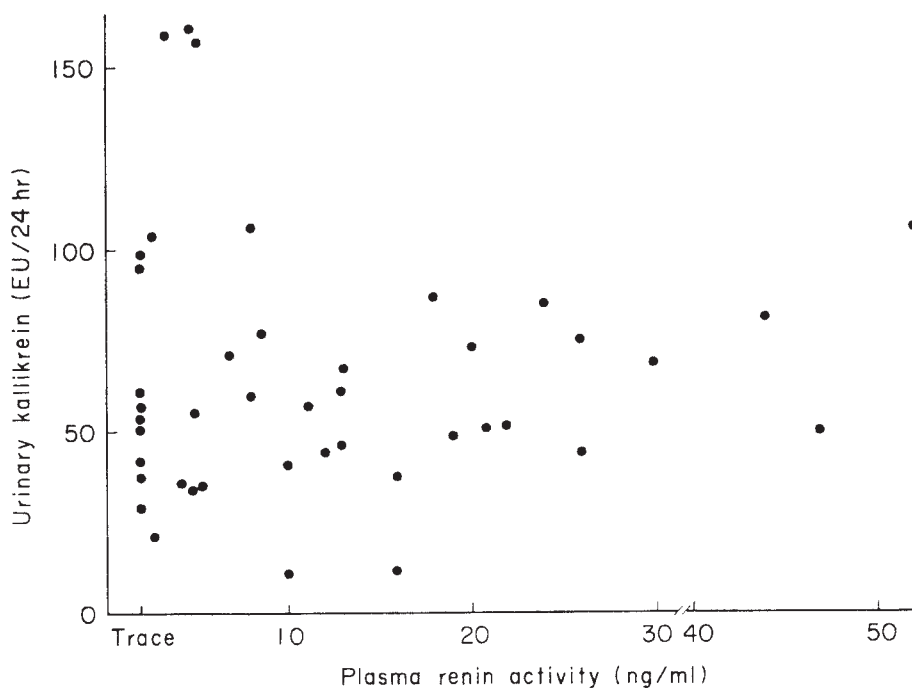


Fig. 5. The relationship between plasma renin activity and kallikrein excretion in 44 collections of 23 patients with essential hypertension adjusting to 150–250 mEq per day sodium intake. The values of PRA ranged from 5 to 30 ng/ml in normal subjects (Abe et al. 1972).

Fig. 5 illustrates the relationship between the plasma renin activity and the kallikrein excretion in essential hypertension. There was no correlation between the two enzymes.

DISCUSSION

For the estimation of urinary kallikrein, colorimetric (Moriya 1964), isotopic (Beaven et al. 1971), and bioassay (Horton 1959; Miwa 1965; Marin-Grez and Carretero 1972) methods have been used. Among these the bioassay method is the most reliable. But concerning the human urinary kallikrein, it may be measured by a chemical assay using synthetic substrate (Webster and Pierce 1961; Moriya 1964). Many samples can be estimated exactly in a short time without using experimental animals. For this reason the urinary kallikrein excretion was estimated by a colorimetric method in the present study.

Elliot and Nuzum (1934) found that the excretion of urinary kallikrein was reduced in patients with essential hypertension. Recently similar results were reported by Margolius et al. (1971). Elliot reported that mean urinary kallikrein in patients with essential hypertension was 55% of that in healthy persons, and in Margolius's report it was 47%. In the present data, the mean level was reduced to 67% of control subjects. What is the reason for this reduction of urinary kallikrein in essential hypertension? Until recently, the question has not been answered. The present data suggest the possibility that an antihypertensive mechanism

through renal kallikrein is suppressed in essential hypertension, although it has not been ruled out that the occurrence of kallikrein inhibitor (Jenner and Croxatto 1973) is a cause for the low kallikrein level in this disease.

Recent reports (Margolius et al. 1971; Miyashita 1971) that urinary kallikrein output is increased in primary aldosteronism are very attractive. In our study, a similar result was obtained. What is the mechanism which induces the augmentation of urinary kallikrein excretion in this disease? It may be related to the escape phenomenon from the sodium retaining effect of mineralocorticoid (August et al. 1957; Marin-Grez et al. 1973). However, it could not be explained why urinary kallikrein excretion did not increase in patients with renovascular hypertension, since they are known to have secondary aldosteronism. In the latter disease, it may be considered that there is an increase of kallikrein excretion in the non ischemic kidney and a decrease in the ischemic kidney (Keiser et al. 1974), resulting in a normal range of excretion in total.

Margolius et al. (1971) reported that urinary kallikrein excretion in pheochromocytoma was also increased. We did not observe any increase in kallikrein in pheochromocytoma as compared with healthy controls, but found significantly higher values than those in essential hypertension ($0.02 < p < 0.01$).

Augmentation of urinary kallikrein was observed under low sodium diet in essential hypertension. In this state, an increase in plasma renin activity was also observed. Under low sodium diet, aldosterone secretion is known to increase. The increase in aldosterone or renin may be a stimulus to increase the excretion of kallikrein. Geller et al. (1972) found a similar finding in animal experiments in which an increase in urinary kallikrein resulted from the elevation of mineralocorticoid levels. In the present data, there was no relation between the plasma renin activity and the urinary kallikrein excretion in essential hypertension. This result indicates that plasma renin activity does not regulate the urinary kallikrein directly. Margolius et al. (1973, 1974) described that sodium depletion in normal subjects increased markedly the urinary kallikrein, but there was a dull response in essential hypertension. Unfortunately, we did not make comparison between the two groups.

Marin-Grez et al. (1972) revealed the positive correlation between urinary kallikrein excretion and extracellular fluid volume. He thought of the possibility of the physiological role for the system in the regulation of body fluid. Adetuyibi and Mills (1972) reported that there was a relation between urinary kallikrein and sodium excretion in normal, essential hypertension, and patients with renal failure. On the contrary, we found no relationship between the urinary kallikrein and the sodium excretion in both normal control subjects and patients with essential hypertension. In our data, however, obvious correlation was observed in primary aldosteronism and renovascular hypertension, the latter being known as a form of secondary aldosteronism. The present results may suggest a possibility that the urinary kallikrein excretion is modulated by mineralocorticoid which is closely related to sodium balance.

The localization and physiological meaning of renal kallikrein is not clear, but the present study revealed an abnormal excretion of kallikrein in some forms of hypertension. It seems that the urinary kallikrein might play a great role in the regulation of blood pressure as one of the renal antihypertensive mechanisms.

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